THE UNIVERSITY OF ADELAIDE
SCHOOL OF AGRICULTURE, FOOD & WINE

"PHYSIOLOGICAL ATTRIBUTES OF DROUGHT-ADAPTATION AND ASSOCIATED MOLECULAR MARKERS IN THE SERI/BABAX HEXAPLOID WHEAT (*Triticum aestivum,* L.) POPULATION"

THESIS PRESENTED FOR THE AWARD OF THE PH.D. DEGREE TO

JUAN JOSÉ OLIVARES-VILLEGAS

DEDICATORY

To Sofía, my wife, for your love, everlasting trust and wholehearted support through the marvelous adventure of having embarked into the luminosity of scientific and spiritual discovery.

A mi Mamá Juanita, porque con tu luminoso amor, ejemplo, cariño y cuidados me has enseñado la dicha de la vida y has educado para aprender, respetar, amar y soñar.

A mi Mamá Eva, porque con tu inmenso amor, apoyo, ejemplo y sabios consejos me has dado educación, cuidados y alas para volar en pos de mis sueños.

A mi Tío Víctor (†), quien como un noble padre y amigo me proveyó de cariño, cuidados y enseñanzas para que nunca dejara de ser un explorador.

To Norman E. Borlaug, lifelong mentor, friend and guide on the moral obligation of contributing through scientific work to the betterment of humankind.

TABLE OF CONTENTS

RESULTS

DISCUSSION

CONCLUSIONS

APPENDICES

LIST OF TABLES

PAGE

LIST OF FIGURES

ABSTRACT

Agronomic and physiological traits associated with drought adaptation were assessed within the Seri/Babax recombinant inbred line population, derived from parents similar in height and maturity but divergent in their sensitivity to drought. Field trials under different water regimes were conducted over three years in Mexico and under rainfed conditions in Australia.

Under drought, canopy temperature (CT) was the single-most drought-adaptive trait contributing to a higher performance (R^2= 0.71, p<0.0001), highly heritable (h^2= 0.65, p<0.0001) and consistently associated with yield phenotypically (r= –0.75, p<0.0001) and genetically [$R(g)$= –0.95, p<0.0001]. CT epitomises a mechanism of dehydration avoidance expressed throughout the growing season and across latitudes, which can be utilised as a selection criteria to identify high-yielding wheat genotypes or as an important predictor of yield performance under drought.

Early response under drought, suggested by a high association of CT with estimates of biomass at booting (r= –0.44, p<0.0001), leaf chlorophyll (r= –0.22, p<0.0001) and plant height (r= –0.64, p<0.0001), contrast with the small relationships with anthesis and maturity (averaged, r= –0.10, p<0.0001), and with osmotic potential (r= –0.20, p<0.0001). Results suggest that the ability to extract water from the soil under increasing soil water deficit is a major attribute of drought adaptation.

Ample genetic variation and significant transgressive segregation under drought suggested a polygenic governance feasible of dissection via molecular markers of CT and associated physiological and agronomic traits. Bulked segregant analysis of selected secondary traits was utilised as an alternative to complete genome mapping, due to a low polymorphism (27%) within the cross and limited chromosomic linkage of loci. The assessment of the extremes of expression in a genotypic subset with a composite molecular database of 127 markers (PCR-based and AFLPs) allowed evaluation of the three hexaploid wheat genomes and coverage of all chromosomic groups, except 3D. One-way analysis of variance indicated significant associations of loci explaining phenotypic variance under drought and rainfed conditions, of 20-70% in Mexico and 20-45% in Australia ($F \geq 5.00$, p<0.05). Significant loci were established in both latitudes for all physiological and agronomic

traits assessed via BSA, with CT being the trait with the most numerous associations (in Mexico, 34 loci; in Australia, 24).

Results demonstrate an efficient development of molecular markers associated to physiological traits under specific soil water conditions in Mexico and Australia, and suggest further genomic and transcriptomic studies be conducted for unravelling the complex relationship between drought adaptation and performance under drought.

DECLARATION

This Ph.D. Thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been stated in the text.

I hereby give consent to this copy of my Ph.D. Thesis being made available in the University Library.

Juan José Olivares-Villegas

24th June 2007

ACKNOWLEDGEMENTS

The author is deeply grateful to the peoples of Australia for supporting the unique opportunity of international researchers to pursue higher degrees at world-recognised institutions in Australia via the granting of scholarships and the establishment of collaborative research endeavours at the national and international levels for the furthering of science and technology.

The author largely acknowledges the inestimable opportunity granted by the Cooperative Research Centre for Molecular Plant Breeding (CRCMPB), Australia, so as to having provided the financial resources for developing the research for pursuing a Ph.D. degree from The University of Adelaide, South Australia. The CRCMPB is a unique world-class research and industry framework in where scientific knowledge and technology can be collaboratively developed in benefit of Australia and of developing nations around the globe. Great appreciation goes to all personnel of the former CRCMPB (currently MPBCRC) for their kind support throughout the present Ph.D. degree candidature, particularly to Dr. Bryan Whan, CEO, and to Dr. Peter Langridge, Education Programme Leader.

The author appreciates the support of all past and present authorities of the International Maize and Wheat Development Center (CIMMYT, INT: Directorate General, Wheat Programme and Applied Biotechnology Centre) and of The University of Adelaide on developing the work presented in this Ph.D. Thesis.

The author is indebted to the supervisors' support at various stages of the Ph.D. candidature: Drs. Matthew P. Reynolds, Glenn K. McDonald, Manilal William, David A. Hoisington and Jean-Marcel Ribaut. The author appreciates their scientific guidance and support at various stages of the research. In particular, the author is in deep gratitude with the first three, without which the cristalisation of this Ph.D. Thesis would have been jeopardised in various orders of magnitude: Thank you for your kind generosity, understanding and irrestricted trust.

Grateful recognition goes to all personnel at the Waite Institute, The University of Adelaide, and at CIMMYT, INT, at El Batan Headquarters (former Applied Biotechnology Centre, Wheat Screenhouses, Wheat Programme, Library and Administrative division) and at the Ciudad Obregon Experimental Station. The author is particularly indebted to the field assistants' efforts at both sides of the

Pacific Ocean, in particular to Eugenio Pérez Dorame and to CIMMYT's Wheat Physiology Team. Many thanks go to Gricelda Palacios and Margarita Crosby, for their valuable world-class laboratory assistance.

Many thanks are due to CIMMYT's Biometrics and Statistics Unit, in particular to Dr. Francisco J. Crossa, and to Mateo Vargas and Juan Burgueño for their inestimable advice.

The author is utterly grateful to Drs. C. Lynne McIntyre, Scott C. Chapman and Ky L. Mathews at CSIRO-Plant Industry for performing and sharing the molecular endeavours that complemented those at CIMMYT, INT. Appreciation goes to Drs. Ray Shorter and Anthony van Herwaarden, as well as to the various authorities at CSIRO for supporting the expansion of the scientific efforts leading to furthering the genomic knowledge on the Seri/Babax population.

INTRODUCTION

Drought stress is a permanent constraint to wheat (*Triticum aestivum*, L.) production on at least 40 million ha in the developing world and in *ca.*25 million ha in industrialised nations (Morris *et al.* 1991; Byerlee & Moya 1993). Modelling exercises suggest that yield in marginal wheat growing environments is typically reduced by between 50 and 90% of their theoretical irrigated yield potential due to factors associated with water-limited environments (Morris *et al.* 1991). As water resources are likely to decline in the coming decades (World Meteorological Organisation 1997), the areas devoted to wheat production will be increasingly threatened by water availability. Hence, improving wheat adaptation to drought will acquire a greater socioeconomic importance across the globe than it currently has.

Breeding for drought adaptation in wheat has been largely empirical to date, based on drought escape (phenology modification for hydration maintenance) or selection for traits contributing to improved water use efficiency indirectly, such as enhanced tolerance to soil toxicities or deficiencies, or resistance to root diseases (Richards 1996; Trethowan & Pfeiffer 2000). More strategic approaches have been advocated which target one or more specific drought-adaptive (physiological) traits consistently related to yield (Morgan 1983; Ludlow & Muchow 1990; Loss & Siddique 1994; Richards 1996).

Even though drought escape is recognised as an effective strategy in some wheat cultivars for overcoming the pernicious effects of drought stress by phenophasic modification (Ludlow & Muchow 1990; Richards 1991; Loss & Siddique 1994), it compromises yield potential to an extent dependent on the timing of the stress during the crop cycle (Blum 1996). In contrast, adaptation to a drought-stressed environment via a number of bioenergetic (efficiency of the photosynthetic and respiratory systems for carbon fixation and energy consumption), metabolic (nutrient utilisation and assimilates distribution) and physiological (complex regulatory networking and environmentally responsive systems) mechanisms is conducive to maintaining a high level of hydration in varying moisture environments (Bálint 1984; Blum 1988; Goggin & Setter 2004; Xue *et al.* 2006). This is achieved either by reducing transpiration rate (thereby increasing water use efficiency) or by managing transpiration via attributes that sustain *in planta* availability of water resources

(Turner & Begg 1981; Blum 1988). When transpiration is reduced by stomatal closure, gas exchange is affected and carbon fixation is reduced (Turner & Begg 1981; Molnár *et al.* 2004). In contrast, when hydration is maintained through water expenditure, the plant is known to be characterised by a lower canopy temperature, a more open stomata and a higher carbon isotope discrimination ($\Delta^{13}C$) (Araus *et al.* 2002), altogether associated with increased carbon fixation, biomass and yield (Condon *et al.* 1987).

Physiological traits that are integrative, either in time or at an organisational level (Araus *et al.* 2002), constitute ideal selection criteria for drought adaptation. In recent years they have acquired increased importance in breeding programmes largely due to a greater understanding of their relative contribution to yield (Blum *et al.* 1982; Richards *et al.* 2001; Araus *et al.* 2002; Rebetzke *et al.* 2002; Reynolds *et al.* 2005). An ample portfolio of novel indirect selection methodologies that assist in evaluating such integrative traits (Araus 1996; Araus *et al.* 2002) are not only practical, but increasingly cost-efficient tools that can support breeders in screening, early generation or advanced-line selection (Blum *et al.* 1982; Araus *et al.* 2001, 2002; Richards *et al.* 2001; Reynolds *et al.* 1994, 2005). However, there has been little systematic evaluation of these traits in large populations of sister lines over a range of environments and varying drought intensities.

While conventional or physiological breeding strategies have utilised genetic diversity at its various levels of expression for developing drought-adapted genotypes for increased crop productivity, they have encountered limitations in dissecting the complex polygenic interactions associated to the quantitative genotypic response through phenotype selection (Cushman & Bohnert 2000; Ribaut *et al.* 2001). With the advent of an increased diversity of molecular markers (Liu 1998), it has been possible to develop comprehensive genetic maps of virtually any crop species, permitting the location of the genetic factors responsible for subtle, quantitative differences (Sax 1923) or quantitative trait loci (QTLs) (Geldermann 1975), in specific genetic pools. However, the application of QTL mapping to the dissection of drought-adaptive physiological traits in hexaploid wheat has been limited not solely because of a restricted access to markers for map saturation, but as a consequence of the difficulty of obtaining a low repeatability when phenotyping under stressed conditions and, mainly, on the virtual inexistence of suitable genetic pools in where the response is amply diverse and significantly inherited.

AIMS

i. To investigate and understand the physiological and molecular basis for genotypic differences in wheat performance under drought.

ii. To evaluate a number of traits linked to drought adaptation in the Seri/Babax recombinant inbred line population, which is characterised by a relatively low variation for phenology and height, factors that might obscure those drought-adaptive traits that directly contribute to an increased performance under drought.

iii. To identify genome regions in selected Seri/Babax recombinant inbred lines associated with physiological traits controlling drought adaptation via molecular markers.

iv. To develop a better understanding of the genetics and physiology of drought adaptation in the Seri/Babax hexaploid wheat population.

OBJECTIVES

i. To evaluate the genetic diversity for drought adaptation in the Seri/Babax population under drought stress in a number of environments in terms of yield, phenology and physiological attributes.

ii. To establish the traits best associated with yield under drought.

iii. To discern the drought-adaptation strategy and inherent mechanisms contributing to a higher performance under drought.

iv. To assess the feasibility of utilising secondary selection criteria to identify high-yielding hexaploid wheat genotypes.

v. To examine the possibility of efficiently develop molecular markers for locating genomic regions explaining the phenotypic variation of selected traits in a number of environments.

LITERATURE REVIEW

2.1 CONSTRAINTS FOR CROP PRODUCTION

The plant productive system- An introduction

Agriculture is the primary economic human activity which sustains civilisation, aimed at satisfying metabolic requirements of the human species, thus providing the energy means for the social networking to interchange a flow of diverse resources.

Dynamic a system (Altieri 1995), agriculture is an activity that relies on particular conditions, which either enhance or hinder the metabolic (and economic) outcome: good quality, high-yielding crop resources. By optimising the resources implemented to obtain the crop, agriculture can be organised as an industry, and plants, to be visualised as factories. The rate and time at which the efficiency of resources (productivity) is optimised by the human improvement of the domesticated plants is highly dependent on both endogenous and exogenous factors. Some exogenous factors are, namely, *stresses* that affect the stability of production (Harlan 1975) and hinder yield potential (Welsh 1981; Evans & Fischer 1999).

The stresses categorised as *abiotic* include hydric, thermal and eolic limitations, as well as edaphic variations. Of all stresses, drought is the most consequential, as plants necessitate the water molecule to operate as dynamic aqueous solutions (Voet & Voet 1990). Their responses to the absence of water are complex and dependent on their genetic architecture, involving a number of climatic, edaphic and agronomic factors whose dynamic interaction varies depending on the time of duration, occurrence and intensity of hydric resources.

Of the products obtained from domesticated plants, food is an essential one that is largely affected by drought stress, and the effect of such a limitation varies between the regions or latitudes in where the crop plants are disseminated (Turner 1979; Blum 1988; McWilliam 1989). Widely distributed around the globe, drought stress occurs in either periodic, semi-permanent or permanent mode, in both underdeveloped or industrialised agricultural systems. Consequently, any productivity increase on the performance of domesticated or crop plants under drought stress can have a dramatic effect on the human welfare.

2.2 DROUGHT STRESS

2.2.1 Conceptual definition

Drought[1] stress implies the absence of hydric resources to the plant system (Kramer 1983), to which it responds via a complex conglomerate of mechanisms that operate at various levels (cellular, tisular, organismical). It is considered an stress when the absence of water occur for a period of time prolonged enough to affect the metabolic machinery of the plant, disrupt its development and growth, and/ or affect the overall agronomic performance.

For domesticated plants, drought stress may ensue from a shortage of rain or irrigation that varies depending on the cultivated soil characteristics (composition) that determine a capacity of retaining water upon the circumstance(s) that impose a larger stress to the water flux –*i.e.*, solar radiation, temperature, eolic dynamics, season, plant density. Hence, when the water supply in the soil is sufficiently less than the maximum tendency of the plants to lose water, as is determined by the evaporative demand of the atmosphere, a water deficit occurs (Turner & Begg 1981).

2.2.2 Drought and plant systems

Plants have evolutionarily adapted to the environmental conditions that alter their ability to occupy a habitat (Curtis 1986). Their responses to an insufficient availability of water resources involve strategies in the domesticated plants that differ from those of the wild species. A development dependent on a series of external inputs (under conventional agricultural systems) make cultivated crops more fragile to variations, especially to that of water.

The different responses exerted by domesticated plants for adapting or withstanding drought stress involve mechanisms that have been comprehended as strategies (Turner 1986; Blum 1988): *drought escape*, *dehydration avoidance*, or *dehydration tolerance*, from which a notion of *adaptation* is derived:

1. *Drought escape*. Ability of a plant to complete its life cycle previous to the development of plant water deficits. It is expressed via phenology: rapid plant development or by adapting the length of the developmental phases to the climate and soil conditions for optimising the water resources. It is observed in early-maturing genotypes in agroecological regions where the limitations of water occur

iii

[1] Term that refers to a *dryland*, *dryness* (Soanes & Hawker 2005)

late in the growing season or in late-maturing genotypes grown in regions where drought occurs early in the season.

2. *Dehydration avoidance (high plant water potential)*. Ability of a plant to sustain a high water status or a relatively higher level of *hydration* under conditions of soil or atmospheric water stress, seemingly unaffected by the water limitation. The avenues of expression that reflect the different genotypic responses to high rates of evapotranspiration are: a) reduction of water loss by an increase in stomatal and cutilar resistance, absorbed radiation, or a reduction in exposed leaf area; b) ability to access and maintain water uptake either by increased root density and depth, or liquid phase conductance.

3. *Drought tolerance (low plant water potential)*. Ability of a plant to endure periods with low-tissue water status (Levitt 1980) or to postpone dehydration (Kramer 1980). It is exerted via maintenance of turgor through osmotic adjustment, increase in cell elasticity or a decrease in size. The operation of molecular mechanisms to withstand water loss and its resulting effects, effect: a) the production and dispersion of hormones and secondary metabolites at the subcellular, cellular and tisular levels; and, b) the regulation of osmolyte concentration in the cellular compartment as a result of loss of water.

4. *Drought adaptation*. Considering the previous conceptual framework, the adaptation to drought depends on the relative yield or survival of a genotype compared to others subjected to the same drought. Thus, plants are classified into: those that postpone dehydration and those that adapt to dehydration through morphological or physiological characteristics that either reduce water loss by transpiration or increase water absorption (Levitt 1980). However, during prolonged drought periods, the usefulness of the various mechanisms that contribute to the postponement of dehydration is fully exhausted, and plants are subjected to severe dehydration that often results in irreversible injury and death. Thus, it is vital that the mechanism of drought adaptation operates throughout the crop developmental stages until the crop plant reaches physiological maturity.

Breeding for optimum productivity of any crop aims at obtaining high yield. Hence, understanding the nature and avenues of expression of drought adaptation and the mechanisms to increase them in the domesticated plants constitute, *per se*, a pivotal field of interest to physiologists, agronomists and breeders to sustain agricultural systems.

2.3 WHEAT AND DROUGHT STRESS

2.3.1 The crop species

Cultivated hexaploid wheat (*Triticum aestivum*, L.) is an allopolyploid (2n = 6*x* = 42, AABBDD), diploid through hexaploid. Donors for the hybridisation of an AA diploid into a AABB tetraploid are still of a conjectural origin --*A. speltoides* is the species most closely related to the donour of the B genome--, while the D genome progenitor is *Aegilops tauschii* (Coss.) Schmal (Kihara 1944; McFadden & Sears 1946). A tough rachis (with a larger retention of kernels in the ear under threshing) and a larger kernel size were consequences of the first two hybridisations, while the integration of the D genome conferred the gluten protein attributes and an increased adaptive range (Evans *et al.* 1975).

Bread wheat is one of the oldest domesticated plants –*ca.*10,000 years ago in the Fertile Crescent of the Middle East (Harlan & Zohary 1966). At present, it is the most extensively grown crop –*ca.*17% of the total global arable land (Slafer & Satorre 1999)--, and the one crop with the highest global production –*ca.*500 m ton (FAO 2006)--.

While wheat is grown across a wide range of latitudes covering a considerable diversity of conditions, the optimum productivity in the plant is attained in regions with cool, moist climate during a long period followed by dry, warm climate to enable the kernels to adequately mature. The distribution of the species is so ample, that a crop of wheat is harvested somewhere around the globe during every month of the year (Hanson *et al.* 1982; Briggle & Curtis 1987).

Uses

Wheat is utilised mainly as a human food. It is nutritious, readily facile to be stored, transported and processed into various types of food. Unlike any other plant-derived food, that made from wheat contains gluten protein, which enables leavened dough

to rise by forming minute gas cells that hold carbon dioxide during fermentation –a process that produces light textured bread.

Wheat supplies about 20% of the food calories for the world's people and is a national staple in many countries (Hanson *et al.* 1982; Briggle & Curtis 1987), being the most important source of carbohydrates in the temperate zone (Leonard & Martin 1963). In addition to being a high-carbohydrate food, wheat contains valuable protein, minerals and vitamins. When balanced by other foods that supply certain amino acids such as lysine, wheat becomes an efficient source of protein. Processed, wheat is used in baby foods or as a common thickener in soups, condiments and sauces. Additional types of wheat products are germ, bran and malt.

Although much of the wheat used for livestock and poultry feed is a by-product of the flour-milling industry, it can serve a dual purpose as green forage or as silage. As for industrial uses, wheat straw is used for newsprint, paperboard and other paper products, while the wheat kernel is used as starch for gluten, paste, oil and alcohol.

2.3.2 Impact of drought in global wheat production

Drought stress is the major constraint to wheat production, affecting both the condition and stability of the land resource from which it is derived (McWilliam 1986). Drought occurs, permanently or intermittently, on at least 40 million ha in the developing world and in *ca.*25 million ha in industrialised agroecological systems (Morris *et al.* 1991; Byerlee & Moya 1993). Modelling exercises suggest that yield in marginal wheat growing environments is typically reduced by between 50 and 90% of their theoretical irrigated yield potential due to factors associated with water-limited environments (Morris *et al.* 1991). As water resources are likely to decline in the coming decades (World Meteorological Organisation 1997), the areas devoted to wheat production will be increasingly threatened by water availability and demand (Borlaug & Dowswell 2005; FAO 2006). Hence, improving wheat adaptation to drought will acquire a greater socioeconomic importance across the globe than it currently holds.

2.3.3 Relevance of drought to the Australian wheat industry

Australia is a drought-prone continent in where wheat production is primarily performed on dryland conditions --the irrigated area constitutes a mere 5% of the

total arable land--, with large environmental interactions (McWilliam 1989). Spring habit wheat is sown in late fall (May-June) and harvested in early summer (November-December), with the crop depending on winter rainfall (May through October). Constraints to production include low soil fertility, poor water-holding capacity, mineral deficiencies and toxicities, and various fungal and viral diseases and parasites, interactions that result in highly variable crop yields from year to year (Fischer 1999).

As of 2000, Australian wheat production was 22 m tn^{-1}, harvested from an area of 11.5 m ha (GRDC 2006). As Australia's total arable land is 50 m ha (FAO 2006), the area is the highest devoted to any other crop grown in the continent. Considered within the soft commodity category of Australian winter crops (wheat, durum, barley, oats, triticale and rye), wheat constitutes an industry with an estimated gross value of production of A$7000 m in 2004 (GRDC 2006). Annually, strong investment is placed to research and technological developments to obtain higher-quality and better-yielding wheat varieties that can withstand the effects of a stressed environment, as *ca.*80% of the crop is exported (GRDC 2006).

2.3.4 South Australian wheat industry and drought stress

The Australian wheat industry has been classified (GRDC 2006) into three wheat production regions in where the Southern one –where South Australia is included– is characterised by: temperate climate, relatively low soil fertility, a yield dependency upon reliable spring rainfall, an smaller enterprise size than that of the Northern region, diverse production patterns and opportunities in large and diverse domestic markets, phase farming innovation, and increases in intensive livestock production and demand for feed grains.

The Mediterranean climate (long, hot, dry summers and short, mild, wet winters) under which the wheat is produced (Loss & Siddique 1994; Turner & Asseng 2005) is subject to water-limiting situations that are aggravated by the extension of the hot conditions and the resulting unavailability of soil micronutrients. The intermittent, high-rainfall brief periods are interspersed with long, dry periods (droughts). Such conditions make South Australia the driest wheat-growing region of the Commonwealth of Australia (Australian Bureau of Statistics[2]).

vii

[2] Australian Bureau of Statistics, Australian Government (www.abs.gov.au)

Whilst the history of wheat production in Australia had a dramatic beginning (Macindoe 1975), the 2006 growing season has been considered the worst –in a century of statistical records– for the South Australian wheat industry and to other Australian wheat-growing regions (Australian Bureau of Statistics and Bureau of Meteorology[3]). Large (>75%) or complete crop losses occurred due to the extent and intensity of drought stress (Fig.1). Hence, investigating drought-adaptive traits in hexaploid wheat that are expressed under highly variable hydric conditions is paramount for the productivity and sustainability of rainfed (dryland) agroecological systems and for the stability and growth of the Australian economy.

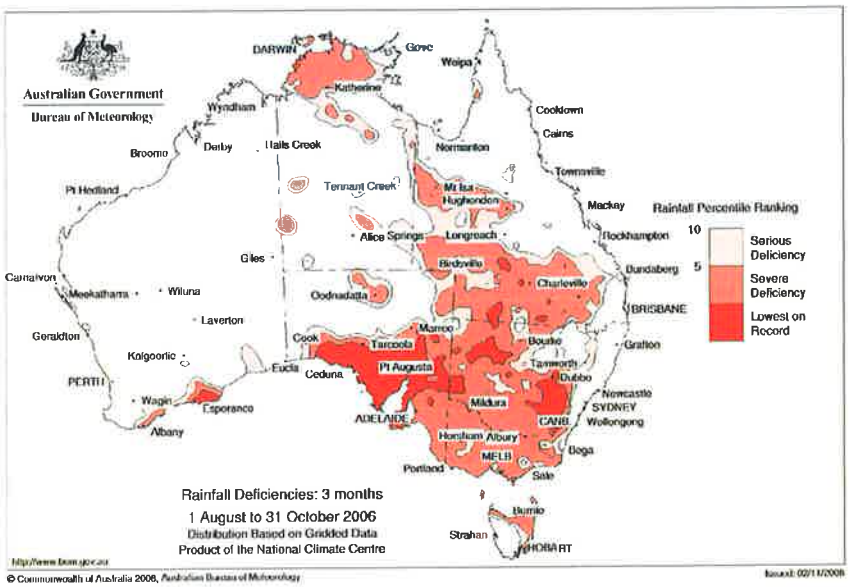


Figure 1. Rainfall deficiencies in Australia for the late-winter to mid-spring 2006 growing season (Australian Bureau of Meteorology, Australian Government).

2.4 AVENUES FOR ASCERTAINING THE DROUGHT ADAPTATION OF WHEAT

Effective breeding of drought-adaptive wheat varieties requires defining the target environment and type and time of drought stress, for which a number of attributes are exerted or integrated in an ideal plant type, or ideotype (Engledow & Wadham 1923; Donald 1968).

viii

[3] Australian Bureau of Meteorology, Australian Government (www.bom.gov.au)

2.4.1 Breeding strategies

The adaptation to drought in wheat is a major factor in the crop performance stabilisation in drought-prone environments. Breeding work for droughted or moisture-stressed environments has been largerly empirical to date (Richards 1996; Pfeiffer & Trethowan 1999), with dychotomous breeding philosophies that govern strategies: narrow or broad adaptation.

Effective wheat breeding for dryland conditions has been performed by simultaneously selecting for yield responsiveness (and stability) and broad adaptation (Rajaram & Nelson 1982; Rajaram *et al.* 1996). It has been documented (Rajaram *et al.* 1996) that selecting for highly-responsive, widely-adapted genotypes under optimum conditions with non-limiting nutrients and water warrant the best selection efficiency –as yield potential is maximised– for suitable germplasm to be assessed under various types of moisture–stressed conditions. However, there are alternative breeding views. Breeding for targetted crosses and multilocation testing allows for selection of early segregating genotypes with wide adaptability to unpredictable environmental fluctuations of rainfed agriculture in dry areas (Ceccarelli *et al.* 1987). While both strategies appear to be effective in both philosophy and results, they seem to be more a result of their general research policies (for mandated, specific objectives, and agroecological regions) rather than opposite efforts *per se*.

Coincidences seem to occur in various established, systematic programmes in Australia, where there are focusses on either broad adaptability (Finlay & Wilkinson 1963) and multilocation yield selection so as to account for genotype-by-environment interactions for a target region (Hollamby & Bayraktar 1996), on multienvironment assessments for broad adaptation (Brennan *et al.* 1981; Cooper *et al.* 1995; Chapman *et al.* 2001), or genetic diversity for selecting for attributes contributing to narrow or broad adaptations (Rathjen *et al.* 1999).

Types of stress

As drought stress effects a plant response, the time at which it occurs impact differently the various developmental phases (Fig.2) and, ultimately, the yield components (Slafer *et al.* 1996).

Different classifications have been pondered for targetting different agroecological systems for wheat production, from agroecological regions (Rajaram

et al. 1984) to specific megaenvironments (ME, Rajaram *et al.* 1993). A simplistic classification of three major drought types can be outlined as (Rajaram *et al.* 1996): late drought (common to the Mediterranean region), early drought (found in Latin America) and residual soil moisture (Indian subcontinent). Within the ME involving drought conditions (<500 mm), the South Australian environment (Mediterranean drought) is classified as ME4B, as it is characterised by winter rainfall early in the crop season (preanthesis), with little rainfall occurring during the post anthesis phase.

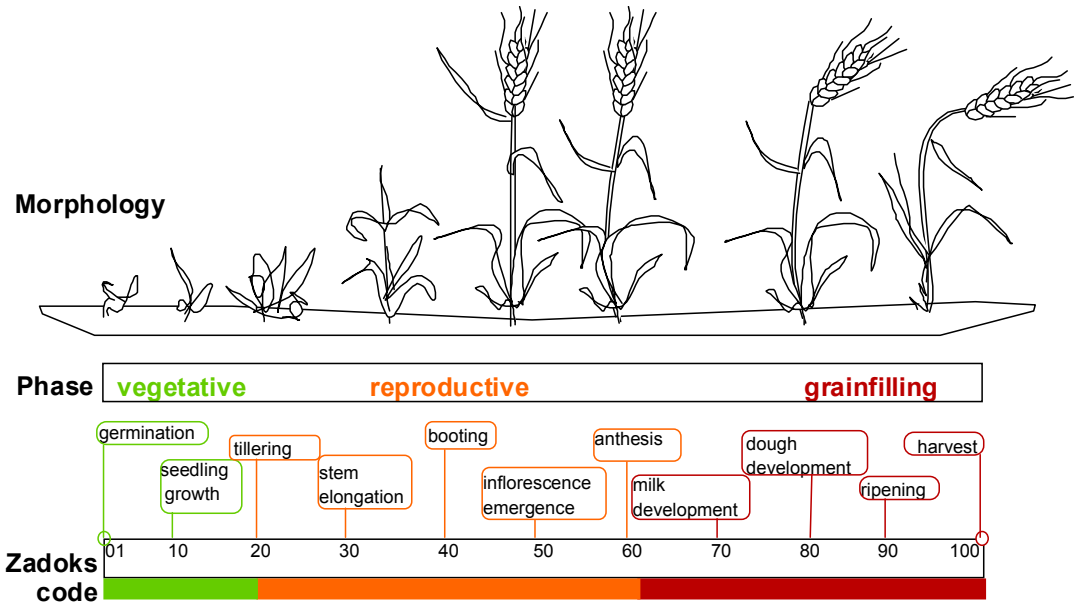


Figure 2. Wheat (*Triticum aestivum*, L.) plant life cycle (adapted from Zadoks *et al.* 1974 and from Slafer *et al.* 1996).

2.4.2 Conceptual model for the investigation of drought stress

Breeding for wheat adaptation to drought stress is performed not only by selecting for yield *per se*, but also by introgressing attributes that render a higher performance under stress in terms with an ideotype or conceptual model that allow to integrate them.

The different plant responses to overcome the pernicious effects of drought have been amply studied and classified in light of an increased understanding of the strategies derived from scientific observations and experiments performed in different agroecological conditions with varying plant materials, under specific drought conditions (Hurd 1968; Turner & Begg 1981; Ceccarelli 1984; Innes *et al.* 1984; Passioura 1986; Turner 1986; Ludlow & Muchow 1990; Chapin 1991; Araus *et al.* 1993;

Loss & Siddique 1994; Richards 1996; Blum 1998; Reynolds *et al.* 1998; van Ginkel *et al.* 1998; Richards *et al.* 1999, 2001; Rebetzke *et al.* 2001; Turner & Asseng 2005). However, they vary in their specificity or *trait for genotype restrictiveness*, as the plant ideotype or described genotype exerts traits within a single strategy (collection of traits). Recent integrative views (Araus *et al.* 2002, 2004; Reynolds *et al.* 2005) are orchestrating the anatomical, morphological, agronomic, physiological and biochemical traits in a framework of phasic-related traits that interact and intervene at various stages of plant development, and dynamically operate at various levels within the plant, depending on the time and intensity of drought. Instead of assessing particular traits, or advocating one strategy, it would be more effective to consolidate a genotype with a conglomerate of traits that operate as required by the plant water status. Within that consolidation framework, to target secondary traits (Araus *et al.* 2002), such as physiological ones related to yield, is to select for integrative traits either in time or at a level of organisation that reveal a myriad of interactions that underlie complex processes that are ultimately providing a higher performance under drought stress.

Depending on the response at the organisational level, the various effects of stress can be classified in nine different categories (forthcoming A to I), namely:

A. Growth, development and yield

Water stress at certain stages of growth is more injurious than at other stages. In Mediterranean-type environments (Turner & Asseng 2005), the time of sowing is critical, as it determines the response of the plant to the occurrence of drought. In wheat, the critical period is usually before the reproductive organ formation (Bingham 1961) and after pollination (Wardlaw 1971), where most of the yield components are established (Slafer *et al.* 1996; Miralles & Slafer 1999).

The effects of drought stress on growth, development or yield are, as follows (Barlow *et al.* 1971; Wardlaw 1971; Quarrie & Jones 1977; Morgan 1980*a*, *b*; Keim & Kronstad 1981; Munns & Weir 1981; Saini & Aspinall 1981; Blum *et al.* 1983): significant reduction in leaf area due to reduced cell division and cell expansion; size decrease of stomata; marked increase in the frequency of trichomes on both leaf surfaces; increase in total number of cells per unit leaf area; reduction in spikelet number; inhibition of leaf elongation, apical growth and spikelet formation; depression in dry weight accumulation in stems and roots; reduction in top growth; decrease in tiller number; possible root growth affected or stimulated (depending on

the genotype and severity of water stress); effect on kernel yield (if stress coincides with reduction division period in the reproductive organs); reduction on kernel number per spike via male sterility induction when stress occurs during early stage of development –male sterility may be mediated through an increase in the endogenous concentration of ABA, which in turn causes inviability of pollen grains.

Biomass and assimilates partitioning

In a post-anthesis drought scenario, vigorous growth prior to heading can, in theory, provide two advantages: a) partitioning of assimilates to a better root system for the relief of stress; and, b) partitioning of assimilates to stems (from jointing onwards) for remobilisation during grain filling to supply assimilates generated in the post-anthesis period. Provided that favourable conditions exist prior to heading, the fast rate of fructan storage becomes an essential attribute for drought-adaptive genotypes, for they can readily contribute to grain filling or when the stress occurs – especially when canopy photosynthesis is inhibited by the post-anthesis drought (Blum 1998).

B. Morphological adaptation

One general response in plants to drought stress is a marked reduction in leaf area, resulting from reduced growth and senescence of older leaves. Leaf growth is restricted in stressed plants due to the sensitivity of cell division and expansion to water deficits (Hsiao 1973). When drought stress occurs pre-anthesis, it also reduces leaf area by enhancing senescence of the physiologically older leaves. However, for some genotypes, an important advantage of reduction in leaf area in response to water deficit is the associated reduction in water loss, delaying the onset of more severe stress (Gaff 1980).

In wheat, leaf area is fixed at anthesis. Under drought, yield is inversely related to the rate of post-anthesis leaf senescence. Once leaf area development is complete, one of the main mechanisms for adapting to stress is through changes in leaf angle. This includes passive wilting response, active leaf movements, and leaf rolling (Gaff 1980). In grasses, leaf rolling is a common response to stress and results in a 50-70% reduction in water transpired (Sirault *et al.* 2004), through both a marked reduction in effective exposed leaf area and a more vertical leaf orientation.

A disadvantage of the morphological adaptations that occur during growth and development of crops is that they have no scope for compensation through an increase in leaf number. Thus, the growth or yield potential that has been lost cannot be fully recovered upon return to a more favourable water supply.

C. Plant-water relations

Water potential (ψ) is referred as a fundamental measure of plant water stress. Water potential of the living plant cell is the sum of the component potential arising from the effect of pressure (pressure, or ψ_p), solutes (osmotic potential, or ψ_s), and matrix (matrix potential, or ψ_m). Unless the tissue is severely dehydrated (loss of >50% water), ψ_m can be ignored since it is negligible in tissues of well-watered plants (Kramer 1969, 1983).

Pressure potential is usually decreased more than the osmotic potential during the early stages of water stress (Hsiao 1973). Water potential is generally measured by thermocouple psychrometers or pressure chambers, while osmotic potential is measured on the expressed sap with a psychrometer or osmometer (Turner 1981; Kramer 1983).

Osmotic adjustment

Many plants accumulate high levels of compatible solutes –such as, sugar alcohols, quaternary ammonia compounds, proline and tertiary sulfonic compounds– in response to osmotic stress. High concentrations of these substances are thought to stabilise some macromolecules or molecular assemblies, thus decreasing the loss of either enzyme activity of membrane integrity that occurs when water is limiting (Schwab & Gaff 1986, 1990). Water alcohols, because of their hydroxyl groups, might mimic the structure of water and maintain an artificial sphere of hydration around macromolecules (Schobert 1977; Kameli & Dosel 1995). Sugar alcohols may also function as scavengers of activated oxygen species thereby preventing peroxydation of lipids and resulting cell damage (Smirnoff & Cumbes 1989; Bianchi *et al.* 1991). Experiments with model systems have established that sugars are able to protect the structural integrity of membranes during dehydration by preventing membrane fusion, phase transition and phase separation (Crowe *et al.* 1988).

Osmotic adjustment refers to the lowering of osmotic potential arising from the net accumulation of solutes in response to water deficits (Turner & Jones 1980). Osmotic adjustment facilitate critical growth functions (root growth, meiosis and pollen development) and metabolic operations (when they require fluxes of water or solutes between cells and organelles). Degrees of adjustment varies with species, cultivars, and different organs of the same plant --at the leaves, hypocotyls, roots, apices and reproductive organs (Morgan 1980*a*,*b*). The degree of osmotic adjustment is influenced by factors such as the rate of development of water deficit, the degree of water deficit *per se*, genotype *per se* and other environmental conditions (temperature and light) (Turner & Jones 1980).

Mechanisms such as stomatal closure or a reduction in leaf area, are effective in reducing water loss, but result in a loss in productivity and yield. In contrast, osmotic adjustment provides the potential for maintaining photosynthesis and growth, as water deficit increases (Turner & Jones 1980; Morgan & Condon 1986; Ludlow & Muchow 1990; Blum *et al.* 1999; Subbarao *et al.* 2000).

Transpiration

In most plants, water stress results in a decrease in transpiration, due to stomatal closure (Hsiao 1973). In general, leaf permeability is well correlated with the rate and size of stomatal aperture. Thus, it is utilised as an index of stomatal closure. Transpiration is directly proportional to the vapour-pressure gradient from the leaf to the air, and inversely proportional to the total resistance to water vapour transport of the air boundary layer and the leaf (Hsiao 1973).

Respiration

Water stress may increase, decrease or have no effect on respiration (Hsiao 1973). Although respiration frequently increases with a moderate water stress, rate of respiration decline as stress becomes severe (Gaff 1980). It is generally observed that dark respiration and photorespiration of wheat leaves are essentially unaffected with moderate water deficits.

Roots

A root system that extracts water available in the soil profile under stress is, *per se*, constitutive of a drought adaptive trait (Hurd 1968), but difficult to measure directly.

An increase in hydraulic conductivity of root membranes (Powell 1978) or an increment in root growth are traits (Steudle 2000) indicative of a plant's ability to satisfy evaporative demand through the ability to explore and extract soil water.

Stomatal aperture-related traits

Water extraction patterns can be estimated from the instantaneous measurement of traits affected by the plant water relations –such as relative leaf water content, stomatal conductance and canopy temperature. Of such traits, the easiest to measure is the latter (Condon *et al.* 2006), which has been associated with other water relations parameters (Blum *et al.* 1982), as well as with performance under drought (Reynolds *et al.* 2000). As water evaporates from the surface of a leaf, it loses thermal energy (cooling) whose rate (of evaporative cooling) is directly affected by stomatal conductance –also influenced by the feedback mechanisms of photosynthetic metabolism and vascular transport. Leaf cooling contributes to improvement of the photosynthetic activity of leaves and prevents premature ageing. Thus, canopy temperature is a good indicator of a genotype's physiological fitness, as a low canopy temperature value is indicative of good expression of trait integration, carbon fixation, biomass and yield (Condon *et al.* 1987) under a given set of environmental conditions. As the measurement integrates the temperatures of plant organs over a small area of the canopy, the *interplanta* error is abated (Blum 1988; Blum *et al.* 1989).

D. Water use efficiency-related traits

Water use efficiency (WUE, Richards *et al.* 2001) is defined by the ratio of total dry matter to the sum of water evaporation and plant transpiration. An increase in the efficiency of transpiration (dry matter/ transpiration) and/or a reduction in soil evaporation increases WUE.

Carbon isotope discrimination

Measurement of carbon isotope discrimination is a reliable estimate of a crop WUE, as it integrates plant water status over a period of time (Condon *et al.* 1987). However, higher-performance wheat cultivars under drought have been associated with lower WUE (Condon *et al.* 1992; Araus *et al.* 1998, 2002; Rebetzke *et al.* 2002) and viceversa (Ehdaie *et al.* 1991). The trait reveals the amount of photosynthetic machinery per

unit leaf area and the rate of transpiration –the efficiency of the stomatal conductance (Richards *et al.* 2001).

Harvest index

A greater partitioning of assimilates to reproductive and non-reproductive organs results in a higher harvest index and better performance under drought. Dependent on WUE, harvest index is largely influenced by phenology and when the stress occurs (Richards *et al.* 2001).

E. Photosynthesis

Water stress severely reduces net photosynthesis in the flag leaf, top internode, and ear of wheat (Wardlaw 1971; Burbank 1995). This reduction in CO_2 assimilation may be due to stomatal closure, which restricts the inward diffusion of CO_2 into the leaf. However, there is also evidence of non-stomatal effects on net CO_2 assimilation in leaves subjected to a moderate water stress (Hsiao 1973).

The relative importance of stomatal and non-stomatal effects on CO_2 fixation is controversial. It appears that the impairment of the photosynthetic apparatus occurs at a higher water stress than that which results in stomatal closure (Shimshi *et al.* 1982). Nonstomatal effects of water stress on photosynthesis include an increase in mesophyll resistance to CO_2 transport, and an increase in the CO_2 compensation point. There is a reduction in the activity of RuBP carboxylase, PEP carboxylase, electron transport, photophosphorylation, chlorophyll and protein synthesis (Mayoral *et al.* 1981; Loggini *et al.* 1999).

F. Protein synthesis

During water stress, plants experience a number of physiological and metabolic changes that impact protein synthesis (Dhindsa & Cleland 1975; Valluri *et al.* 1989; Crowe *et al.*, 1992; Gehlot *et al.* 1998) and gene expression (Skeriver & Mundy 1990; Bray 1993). Protein synthesis in rapidly growing tissue is extremely sensitive to water stress and decreases swiftly (Hsiao 1973; Mayoral *et al.* 1981).

Tissues subjected to water deficit generally show a reduction in protein synthesis as measured by amino acid incorporation (Dhindsa & Cleland 1975; Valluri *et al.* 1989) or by polyribosome analysis (Mason *et al.* 1988). Aside the quantitative effect, water stress usually causes qualitative changes in protein patterns (Heikkila *et*

al. 1984; Valluri *et al.* 1989), resulting in the synthesis of stress-polypeptides (stress proteins) (Mason *et al.* 1988; Valluri *et al.* 1989; Lacerenza *et al.* 1995). Along with the appearance of stress proteins, the synthesis of those constitutively produced prior to water stress is drastically suppressed.

Some of the best characterised genes expressed in response to drought stress are the late embryogenesis abundant (LEA) genes (Dure *et al.* 1989; Ried & Walker-Simmons 1993). Expressed in late seed development, the expression of LEA genes is correlated with increased ABA levels and tolerance of embryos to dessication (Dure *et al.* 1989).

Proteins related to the LEA family, abscisic acid-regulated (RAB) (Skriver & Mundy 1990) and cold-regulated proteins (Hajela *et al.* 1990) are considered *dehydrins* (Campbell & Close 1997; Close *et al.* 1999). Several dehydrin genes and proteins have been identified in a wide range of herbaceous plant species (Bartels *et al.* 1990). The fundamental biochemical role of dehydrins is yet unknown, and several hypotheses have been proposed: structural (Baker *et al.* 1988) due to osmolyte presence in the cytoplasm, surface stabilisers (against deformation) (Carpenter & Crowe 1988; Csonnka & Hanson 1991), related to nuclear matrix or envelope, nuclear transport (Goday *et al.* 1994), sequestration of ions and binding of water (Pelah 1999).

G. Hormonal changes

Most plants experience dramatic and rapid modifications in their endogenous hormone levels when subjected to water deficit (Walton 1980). The reduction in cellular turgor might be the major factor responsible for triggering changes in the hormonal balance of plant.

H. Abscisic acid and ethylene

Abscisic Acid plays a central role in stress responses by enhancing adaptation to salt, high osmoticum (Close *et al.* 1989), wounding (Wright 1969) cold and drought (Wright & Hiron 1969). Water stressed plants show substantially enhanced ABA (Wright & Hiron 1969; Cohen & Bray 1990) or redistribution of existing ABA. Current models suggest that the stress is first perceived by cells as plasmalemma perturbations: loss in turgor pressure is followed by an increase in cytosolic and apoplastic ABA due to *de novo* synthesis and/or release of the hormone sequestred in organelles. The major mechanisms that govern stress-ABA response: rapid synthesis

of ABA; release of stored ABA by farnesol; message cancelation when the cellular ABA levels are to reach equilibrium; and, destruction or cessation of ABA synthesis when turgor is regained.

Functions of ABA in response to water stress (Davis & Mansfield 1983) include: reduction in transpirational water loss by stomatal closure and inhibition of stomatal aperture; rapid stomatal closure; increase of water flux into the root system; inhibition of shoot/root growth; and, rapid accumulation of proline and betaine.

ABA mediates embryo maturation (during late seed development), which involves various morphogenic and biochemical changes, including the programming of embryo dormancy and dessication tolerance (Bartels *et al.* 1988; Dure *et al.* 1989). Genetic differences in the ability to accumulate ABA in response to water stress in wheat are heritable (Quarrie 1981). The absence of a large maternal influence on the level of ABA accumulation in wheat implies that plastid genes are not critical in determining differential ABA accumulation.

Ethylene acts as a plant hormone and is involved in growth, development, ripening, abscission and senescence (Abeles 1973). Increased ethylene production occurs in response to drought, mechanical wounding and radiation stresses. Hormonal balance at the onset of water stress determines and regulates the amount of ethylene produced: while ABA inhibits its synthesis, indolacetic acid and the conversion of S-adenosyl methionine to 1-aminocylo-propane-1-carboxylic acid estimulate its production.

I. Prolines and polyamines

Total free amino acids increase in water-stressed leaves, with proline being the most pronounced (Hsiao 1973; Levitt 1980). Accumulation of proline in water-stressed plants is found to be mediated by both ABA-dependent and ABA-independent signalling pathways (Hare *et al.* 1999), and induced by (Stewart & Hanson 1980): synthesis stimulation due to loss of feedback inhibition; oxidation inhibition (probably of effects on mitochondria); imparing of protein synthesis; and, rates of proline exchange via the phloem.

While polyamines are important for DNA replication, cell differentiation and growth regulation (Galston & Sawhney 1990), their levels and biosynthesis are modified in response to environmental stresses, particularly drought (Ye *et al.* 1997). They act as free- or bound- radical scavengers that induce a biosynthesis cascade of

antioxidant molecules (ascorbate, glutathione, α-tocopherol and carotenoids) and of associated enzymes that, in the drought resistant biotypes, are constitutively elevated.

2.4.3 Dissection of drought adaptation

Whichever adaptation strategy is exerted in the wheat plant as a response to drought, it is paramount to elucidate the central element of control –from the atomistic, reductionistic view. The dissection can be conducted at the translational, transcriptional or genetic levels for furthering the manipulation of the components of a genotypic response.

During the past three decades, various biotechnological, genetic and information tools have been developed (and adopted) to reveal basic features in the genetics and expression of different species. Some are presented.

2.4.3.1 Architecture and organisation

A. ***Linkage mapping***

Linkage maps provide indirect information about the structure and organisation of an organism at the genetic level. From the outlining of their relative positions along the genome, the elucidation of gene function, gene evolution and gene isolation can be attained (Griffiths *et al.* 2000).

Any attribute or character at the morphological, biochemical or DNA (molecular) levels, can be observed in its segregation within a progeny (backcross, F_2, recombinant inbred lines, doubled-haploid), derived from two parental organisms divergent in their expression for such a character (González-de-León *et al.* 1995). As characters are useful in establishing indirect genetic information of other traits of interest in the organism of study, they are termed *markers* –DNA markers exhibit sequence polymorphism in different individuals within a species (Liu 1998).

While during the past decade there was a plethora in the types of molecular markers available (Liu 1998), such a diversity can be classified in only two categories: hybridisation-based (RFLPs) and PCR-based markers (Çakir & Karakousis 2000). For the former, DNA is digested with restriction enzymes, and the restricts are electrophoresed for a posterior hybridisation with specific DNA probes. For the latter,

the polymerase chain reaction (PCR, Mullis 1989) is used to allow the specific enzymatic amplification of DNA regions with primers under particular thermal and ionic conditions; microsatellites (a type of tandem di-, tri- nucleotide sequence repeats), are highly polymorphic and codominant, whose flanking regions are used for PCR amplification (Tautz & Renz 1984; Tautz 1989; Litt & Luty 1989; Senior & Heun 1993; Powell *et al.* 1996; Taramino & Tingey 1996). In a conceptually synthesis of the two previously described systems (AFLPs, Vos *et al.* 1995), DNA is digested with restriction enzymes, but DNA primers amplify the polymorphic fragments; thus, a large number of dominant genomic loci can be monitored for segregation.

Within the span of the past thirty years, molecular markers have been considered important biotechnology tools for enhancing the magnitude of plant breeding. From the conceptualisation and delineation of perspectives in their use in breeding programmes (Tanksley 1983; Tanskley *et al.* 1989) to their usefulness and efficacy proposals (Lee 1995; Sorrells 1998), the methodologies have ample applications: characterisation of genetic diversity (Appels & Dvorak 1982; Caetano-Anollés *et al.* 1991; Dreisigacker *et al.* 2005), introgression of exogenous genetic material for diversity increment (Tanksley *et al.* 1989; Beckmann & Soller 1986), advancement in novel varieties release (Eathington 2006), diagnostics (D'Ovidio & Anderson 1994) or selection tools (Ribaut *et al.* 1997).

While not all of the molecular efforts require the mapping of genetic factors, for studying the genomic genetics (Beckmann 1991) a saturation of linkage maps has been established as a necessary condition for the effective molecular dissection of quantitative traits. The marker saturation facilitates the location of QTLs closer to the multiple genetic factors controlling such traits (Liu 1998). Quantitative trait loci (QTL) mapping is a means to estimate the locations, numbers, magnitude of phenotypic effects and modes of action, of individual determinants that contribute to the inheritance of continuously variable traits (Paterson 2002). For the assessment of relevant QTL associations, a number of statistical methodologies have been proposed to identify the minimum number and likely location of genetic factors involved in the governance of the trait (Quarrie 1996; Liu 1998): simple t-test, simple linear regression, multiple linear regression, non-linear regression, interval test using partial regression, and QTL-by-environment interactions. Whichever methodology is employed, it is important to consider: experimental design, mode and saturation of

the genetic linkage associations, trait information and germplasm (species and genetic pool utilised for the mapping and QTL assessment).

In several cereal species, genetic linkage maps have allowed the identification of regions controlling some traits related to the response to drought. Different segregating populations from maize, rice, sorghum, barley, durum (tetraploid) wheat and sugar cane (amongst others) have been studied for many different criteria or quantitative characters, such as phenology, plant architecture, metabolic pathways, water-use efficiency or carbon isotope discrimination. In contrast, developments in molecular genetics in wheat have been relatively slow and exiguous. Explanations are ample: wheat´s ploidy level (2n = 6*x* = 42, AABBDD); genome size (estimated to contain 18.1 picograms of DNA per haploid nucleus (Bennett 1972), equivalent to *ca.*16x10^6 kilobase pairs); and, genomic complexity (>75% consists of repeated DNA sequences of varying degrees of reiteration and length, with a lesser proportion (*ca.*20%) of low-copy number or unique sequences (Smith & Flavell 1975)).

The wheat hexaploid nature and its amenity to cytogenetic manipulation do offer unique tools to geneticists, allowing to determine evidence of major chromosome rearrangements (Devos *et al.* 1995; Nelson *et al.* 1995) and the comparison of linkage maps among related species (Ahn *et al.* 1993; Devos *et al.* 1994). The low number of quantitative traits dissected into their QTL is a reflection of the focus given to simply inherited traits (Parker *et al.* 1998; Taylor *et al.* 1998; William *et al.* 1998; Somers *et al.* 2003) and the difficulty of building comprehensive genetic linkage maps for wheat.

B. *Bulked segregant analysis*

Bulked segregant analysis (Michelmore *et al.* 1991) has been successfully applied to wheat for monogenic traits (Eastwood *et al.* 1994; Hartl *et al.* 1995, 1998; Hu *et al.* 1997; Goodwin *et al.* 1998; William *et al.* 1997, 1998) as an alternative to complete linkage mapping. The methodology has also been used to saturate linkage maps and to screen for markers linked to QTLs (Liu 1998). The strategy consists of screening two bulks of DNA samples from individuals identified in the two opposite tails of a segregating population for a target trait. Thus, a specific target allele will occur in one bulked sample, but not in the other (Liu 1998). Such pattern of frequency difference would have to obliguedly be observed for any marker or gene tightly linked to the target genes or nearby markers. Hence, the success of bulking by

phenotypes is dependent on the correspondence of genotype and phenotype (*i.e.*, heritability).

C. *Comparative mapping*

The study of the similarities and differences in structure and function of hereditary information across taxa, genera and species (Gale & Devos 1998; Devos & Gale 2000; Paterson *et al.* 2000) has been allowed by the production and saturation of different linkage maps, gene sequencing and function characterisation of the *Triticeae*, rice, maize and other species.

Crop species of the *Poaceae* display a remarkable level of genetic similarity despite their evolutionary divergence 65 million years ago (Bennetzen & Freeling 1995; Paterson *et al.* 1995). Molecular markers have been used to develop comparative chromosome maps for several members of the *Gramineae* and these have been used to study genes of agronomic importance across species. Large segments of the genomes of maize, sorghum, rice, wheat, and barley conserve gene content and order (Ahn & Tanksley 1993; Ahn *et al.* 1993; Gale & Devos 1998; Hulbert *et al.* 1990; Kurata *et al.* 1994; Van Deynze *et al.* 1995*a*,*b*,*c*), although the correspondence has been modified by duplications, inversions, and translocations. For the domesticated grasses, the conserved linkage blocks and their relationships with rice linkage groups provides the insight into the basic organisation of the ancestral grass genome (Moore *et al.* 1995; Wilson *et al.* 1999). This allows the transfer of information from species with small diploid genomes, such as rice, to species with more complex genomic structures, such as that of wheat (Appels *et al.* 1989; Weining & Langridge 1991; Gale & Devos 1998; McCouch 1998). Successful linkage relationships have been achieved between the *Triticeae*, allowing to elucidate the benefits of comparative mapping (Sharp *et al.* 1988; Quarrie *et al.* 1994; Dubcovsky *et al.* 1995; Sherman *et al.* 1995). However, the integration of genomic maps to dissect the shared markers at specific loci and to elucidate their use has shown it to be more complicated than expected. Thus, microsynteny analysis for similar QTL regions and the identification of orthologous genes within them is also pursued (Killian *et al.* 1995; Dunford *et al.* 1995; Bennetzen 2000; This *et al.* 2000).

2.4.3.2 Dynamics and expression

Gene discovery

Rapid discovery of genes by large-scale partial sequencing of selected cDNA clones or expressed sequence tags (ESTs) is the initial step towards characterisation and categorisation of genetically complex abiotic stress responses (Adams *et al.* 1991; Stack *et al.* 2000). Expressed sequence tag (EST) analysis was proposed for efficient sampling of a genome for information about genes that could be useful in searching at databases (Adams *et al.* 1991). By searching online databases for similar genes with known function, one can determine if a specific gene (or gene motif) has been found in the same or other organisms and if its function has been detremined. These ESTs can also be useful for further laboratory work in gene expression, mapping and direct alteration of the organism (Newman *et al.* 1994; Cooke *et al.* 1996; Rounsley *et al.* 1996). EST information can be merged with that of a protein database to provide information on patterns of gene expression. For the long term, EST information will be a critical resource for crop improvement and will be used extensively for locating genes, understanding changing patterns of gene expression, and biotechnological modification of traits. However, identifying and mapping all the expressed genes in a species without sequencing the entire genome is a complex task.

Extensive EST collections and databases already exist for *Arabidopsis* (Bevan *et al.* 1999) and rice (Goff 1999) while large-scale ESTsequencing initiatives for various crop species is undercourse (Walbot 1999), including that of wheat[4]. However, such collections are biased towards high to moderate abundance studies that are derived from different tissues, organs or cells; different developmental stages; various external stimuli; and treatments with plant growth regulators. In contrast, relatively few studies have focused specifically on ESTs from plants that have been exposed to environmental stresses (Holappa & Walker-Simmons 1995).

Candidate genes

A candidate gene is such that is associated with the variation in a trait, involved with the development or physiology of the trait. Frequently, candidate genes are sequenced genes of known or suspected function and may belong to biochemical or

xxiii

[4] wheat.pw.usda.gov/genome

regulatory pathways (Rothchild & Soller 1997). Identifying the genes involved in complex trait governance derived from QTL analysis can provide different kinds of genetic information, regularly over a broader range of germplasm. Because there may be large numbers of genes located in the region of a QTL, the odds of identifying the gene that actually controls the expression of the trait appear to be quite low; however, a number of factors can increase the odds of success, specially as the number of genes sequenced increases (Sorrells 1999; Zhou *et al.* 2000).

Functional Genomics

Using *DNA chips* (Lemieux *et al.* 1998), it may be possible to determine the relative importance (contribution) of each gene to some of the studied physiological traits involved in drought adaptation at different phenological stages under different water regimes (Hieter & Boguski 1997; Liu *et al.* 2000). Some potential uses include the ability to search for clones directly or indirectly that are related to major-gene differences, mutations, QTLs and for genes showing changes in gene expression during a developmental time-course or in a tissue basis (Schena *et al.* 1995; Ruan *et al.* 1998; Desprez *et al.* 1998; Lemieux *et al.* 1998; Kehoe *et al.* 1999; Baldwin *et al.* 1999). In grain crops, time-course studies of seed development, gene expression during meiosis, and responses to specific environmental stimuli will identify expressed genes. This will assign function to ESTs that will serve as potential candidates for mapped qualitative or quantitative loci affecting important traits. Characterisation of the expression patterns of genes involved in genotype-by-environment interactions may eventually help unravel the complexities of the phenomena (Schena *et al.* 1995; Shalon *et al.* 1996).

The elucidation of genomic regions associated to the expression of traits involved in drought adaptation, the novel genes discovery or the determination of their expression patterns in response to drought stress, will provide the basis of effective engineering strategies leading to enhanced hexaploid wheat germplasm for specific agroecological niches. For any molecular assessment to be performed, it is paramount to firstly establish the plant adaptation strategy to overcome drought.

In the present study (forthcoming chapters), a series of experiments will be described on the assessment for the expression of key traits in a wheat population

across different hydric conditions and latitudes, with a preliminary screenhouse evaluation of the population's parental genotypes. The genetic diversity (for the expression of the traits) will be determined, as well as the identification of those traits best associated with yield under drought. These, in conjunction with the assessment of the feasibility of utilising secondary selection criteria for identifying high-yielding hexaploid wheat genotypes. Finally, an alternative to linkage mapping will be utilised for the development of molecular markers for locating those genomic regions explaining the phenotypic variation of those selected traits in a number of environments.

MATERIALS AND METHODS

3.1 GERMPLASM- PLANT MATERIAL

3.1.1 Seri/Babax population

3.1.1.1 Parental genotypes

Hexaploid semi-dwarf wheat (*Triticum aestivum*, L.) varieties were used for the *ex profeso* development of a population suitable for the study and genetic mapping of physiological traits. Parental genotypes were identified from a series of diallel cross experiments conducted at CIMMYT Ciudad Obregon Experimental Station [*see* **3.2.1.2.1 Environments**]. The three-year experiments encompassed 90 crosses from the genotypes (M.P. Reynolds, *pers.comm.*): Altar 84/Ae.Sq.//Opata, Attila, Babax, Borlaug M95, Chil/2*Star, Lucero Mexicano, Kauz//Kauz/Star, Seri M82, Super Kauz and UP 2338. Selected parental genotypes, identified as differing significantly in yield performance under drought while both showing high yield potential (Reynolds *et al.* 2000), were:

- Seri M82 (IWIS CODE (Fox *et al.* 1996), selection history: M31 IBWSN S-1 MXI96-97). Veery 'S'-derived variety conveying the 1BL/1RS translocation (Rajaram *et al.* 1983; CIMMYT 1986). Widely adapted semi-dwarf (CIMMYT 1986), is moderately susceptible to severe drought stress (Pfeiffer 1988), but considered tolerant under reduced irrigation (Villareal *et al.* 1995).
- Babax (IWIS CODE (Fox *et al.* 1996), selection history: CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y). Baviacora 92 variety sister line, an *Lr19*-derived Veery without the 1BL/1RS translocation (R.P. Singh, *pers.comm.*). Widely adapted semi-dwarf (CIMMYT 1986), is highly-tolerant of severe drought stress.

3.1.1.2 Population development

Experimental population consisted of 167 recombinant inbred lines (RILs) derived from a Seri/Babax cross. The Seri/Babax population included sister lines from reciprocal crosses: current genotypes 1-139 (*Babax/Seri*) and 140-167 (*Seri/Babax*). Original F_2 populations of *ca.*1000 plants were advanced to F_3 by choosing 200 plants at random from Babax/Seri and 50 plants from Seri/Babax. Approximately 200 seeds from each F_2 plant were sown as an F_3 plot. Single spikes from each F_3 plot were sown as small F_4 plots. From F_4 to F_6, plots were bulk-harvested until the F_7 generation at a rate of approximately 200 seeds per plot. All of the above

environments were irrigated and well-managed such that selection pressure for yield under stress among genotypes was negligible. Single plants were taken from selected F_7 plots to generate seed for the first evaluation of $F_{7:8}$ lines (194) in small yield plots under drought and irrigated conditions (1999/2000). Selection in the $F_{7:8}$ plots (posterior to cycle 1999/2000) removed 27 lines due to substantial differences in phenological development (either in anthesis or maturity timing), leaving material which had a 15 day maturity range. Subsequent field cycles in Mexico (2000/2001 & 2001/2002) and Australia (2001 and 2002) considered a subset of 167 lines with a relative narrow range of height, with a majority of RILs being within acceptable agronomic limits in the experimental field environment.

3.2 EXPERIMENTAL METHODS

3.2.1 PHENOTYPIC EVALUATION

3.2.1.1 Screenhouse experiments

Prior to evaluating the Seri/Babax population in a series of field experiments, a number of traits were measured in the parental genotypes in two screenhouse experiments at CIMMYT's Greenhouse/Screenhouse facilities, El Batan Headquarters, Mexico (summer 1999) for their possible evaluation in the field:

Experiment A. Chlorophyll content (SPAD), osmotic potential, flag leaf thickness, spike length, stem length, plant height, spike weight, root weight (per plant), kernel weight (per plant), kernel number (per plant) and kernel length (per plant).

Experiment B. Spike length, stem length, plant height, root length, spike weight, root weight (per plant), kernel weight (per plant) and kernel number (per plant).

3.2.1.1.1 Methodology/ Design

A randomised complete block design was utilised, with twelve plastic containers (15 cm in diameter, 20 cm in height pots) and twelve plastic pipetubes (1 m long, 10 cm in diameter polyvinyl chloride pipes). Two water regimes were used in each of the two-replicate experiments: watered (continuously irrigated until physiological maturity) and droughted (watering stopped at 30 days after sowing). After initial watering, six seeds per genotype per treatment were sown in each pot and pipe.

Pots and pipes were filled with a soil mixture resembling the consistency of a sandy loam without organic matter, mixing alluvial soil and sand (3 : 1) for a 40%

holding capacity. Fertiliser was applied every 20 days after sowing (1.25 grams of urea/ triple phosphate, 2 : 1, by volume); once per month, 250 and 500 ml of Peters NPK (20-20-20) soluble fertiliser (2 g L^{-1} of water) were applied for the pots and pipes, respectively.

All experiments were conducted under a controlled thermal environment (23^{o}C), with the provision of an additional hour of supplementary light at both dawn and dusk.

3.2.1.1.2 Traits

All data collection was performed according to the Zadoks' phenological stages classification (Zadoks *et al.* 1974), graphically depicted in Figure 2. Traits assessed were, as follows:

Chlorophyll content

Assessed at grain filling (Zadoks 70-85) on the adaxial side of flag leaves with a self-calibrating chlorophyll meter (Minolta model SPAD 502, Minolta Camera Co., Ramsey, NJ, USA). Three data points per flag leaf were collected twice a week during the aforementioned stages.

Osmotic potential

To measure osmotic potential (OP), flag leaves segments (*ca.*1 cm) on three plants per genotype per treatment were harvested 13 h after watering at Zadoks 70. At time of collection, samples were kept in 25 ml polypropylene tubes (Corning©) and freeze-killed when placed in a container filled with liquid nitrogen, and subsequently maintained at –20^{0}C (Babu *et al.* 1999) until OP was measured. Prior to measurement with a vapour pressure osmometer (Wescor model 5500, Logan, Utah, USA), samples were left to thaw for 40 min and dried with a paper blot. After this, a drop of cell sap was extracted using a glass rod and placed on the sampling cuvette of the psychrometer. To reduce the errror in readings, instrument standarisation was done using osmolality standards (100, 290, 1000 mmol kg^{-1}). Osmolality measurements (in mmol kg^{-1}) were adjusted according to those of control samples (at full turgor) for their conversion (to MPa).

Leaf thickness

Three measurements were performed on the central adaxial side of flag leaves of three plants per genotype per treatment, using a precision (reading error: 1/200 mm) digital micrometer (Mitutoyo Minolta, Japan), soon after anthesis (Zadoks 65).

Spike and stem length, and plant height

Estimated (in cm) on the spikes and stems of physiologically-matured plants with a flexible meter (Truper S.A. de C.V., model A45, Mexico).

Root length

Fully-elongated primary roots were measured (in cm) with the previously described flexible meter, at Zadoks 89.

Spike weight

At Zadoks 95-100, all spikes were collected, excised from the stem and left to dry in an oven at 65% for 24 h. Weight was estimated with a three digit weight balance (Ohaus model GT 480, Pine Brook, NJ, USA).

Root weight

After collection in plastic bags, roots were immersed in water, eliminating soil particles attached to primary roots, to which overnight dehydration (*ca.*20 h at 65°C) followed. Dry weight was recorded using the previously described balance.

Kernel weight

Total kernels per plant were collected, to which threshing and cleaning followed previous to weighting (in g) with the previously described balance.

Kernel size

Width and length measurements of all kernels per plant were performed with the previously described digital micrometer.

Kernel number

Total plant kernels were counted manually.

3.2.1.2 Field experiments

3.2.1.2.1 Environments

Field trials were conducted in Mexico (CIMMYT Obregon Experimental Station, Yaqui valley, Sonora, Northwestern Mexico, 27°25'N, 109°54'W, 38 masl) and Australia (Charlick Experimental Station, Strathalbyn, 35°17'S, 138°53'E, 58 masl; Roseworthy Agricultural College, Gawler, 34°30'S, 138°40'E, 65 masl; Minnipa Agricultural Centre, Minnipa, 32°50'S, 135°09'E, 162 masl; all three sites located in South Australia). Localities are geographically located within traditionally-intensive wheat agro-ecosystems. At Obregon, semi-dry, stable environmental conditions are dependent on irrigation. In contrast, at Charlick climate conditions are dependent on irregular residual moisture from intermittent rainy periods at the beginning of the agricultural cycle and of increased drought stress, due to rising temperatures and declining rainfall, as the crop cycle develops until harvest. At Obregon, sowing normally occurs from mid-November and harvest occurs in March; at Charlick, Roseworthy and Minnipa, sowing occurs from early June and harvest occurs in early to mid-December.

3.2.1.2.2 Soil characteristics

The soil at Obregon is a coarse sandy clay mixed with montmorillonitic clay, classified as Typic Caliciorthid (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture[5]), low in organic matter (0.76%) and slightly alkaline (pH 7.7). Due to observed soil heterogeneity on the experiment station, at the end of the 1999/2000 cycle, an area of 6 ha (260 m North-South, 307 m West-East) was evaluated for pertinent soil characteristics [*see* **3.2.1.2.2.1 Soil mapping**]. The maps produced aided in identifying a sowing area at Obregon, suitable for conducting all subsequent experiments with no significant soil limitations.

The soil at Charlick is alkaline clay loam to loam, classified as Calcarisol (Isbell 2002) with a surface pH (0-10 cm) of 7.5-8.0 and an alkaline trend with depth. Salinity is low. The organic C of the surface soil is 1.8-2.0%, while the surface EC_e range is 1.6-

xxxi

[5] http://soils.usda.gov/technical/classification/scfile/index.html

2.0 dSm^{-1}. Free lime is present through the profile. The effective rooting depth is 0.6-1.0 m, depending on the variation of subsoil properties.

The soil at Roseworthy is a non-sodic type with a loam topsoil over a Calcareous clay subsoil (Liu *et al.* 2000), classified as Calcarosol (Isbell 2002).

The soil at Minnipa is alkaline sandy to sandy loam in the topsoil, classified as a Calcarosol (Isbell 2002) with an increasing content of clay with depth and higher levels of carbonate with depth (sodic subsoil). High to toxic concentrations of Boron are commonly observed as well as micronutrient deficiencies (especially Zinc).

3.2.1.2.2.1 Soil mapping

For the purposes of determining soil characteristics in the 6 ha area, soil samples (3) were collected in sixty mapping points, at various depths (0-30 cm, 30-60 cm, 60-90 cm). All soil samples were taken with a Giddings hydraulic soil probe (Giddings Machine Company, Ft. Collins, CO).

Soil analyses were performed at the Soil and Plant Nutrition Laboratory (CIMMYT Headquarters, El Batan, Mexico), particularly for: electric conductivity (mmhos cm^{-1}), sodicity (Sodium absorption ratio), texture (percentage of sand), Boron (ppm), DPTA micronutrients (Zn, Mn, Cu), Ammonium acetate elements (Ca, Mg, Na, K), Sulphur, Phosphorus (Olsen and Bray methods), pH in water, and organic matter (%). The spatial variability in soil properties for the various depth segments was mapped utilising a geostatistical analysis software package, (GS+, GeoStatistics for the Environmental Sciences, v2.1, Gamma Design Software, Plainwell, Michigan, USA).

3.2.1.2.3 Climatic conditions

Climatic data for Obregon are presented in Table 1A. Virtually no rain was registered throughout any cycle (0-0.22 mm per month).

While the climate at Charlick (Table 1B) is typically Mediterranean with a historical average annual rainfall of 493 mm (concentrated between autumn and spring), *ca.*353 mm fell in winter and spring (June-November) in 2001 while *ca.*227 mm fell in 2002. At Roseworthy and Minnipa, the rainfall pattern is similar to that of Charlick, but in relation to it, Roseworthy's historical annual average is higher (440 mm) while Minnipa's is smaller (327 mm). Rainfall in 2001 at Roseworthy was 471 mm, while at Minnipa only 256 mm were recorded in 2002.

Table 1A. Environmental conditions under which experiments were undertaken on the Seri/Babax population in the Yaqui valley, Mexico

YEAR	MONTH	TEMPERATURE		RAIN	SOLAR	WATER	SOLAR
		MAX (°C)	MIN (°C)	(mm)	INCIDENCE (h day^{-1})	EVAPORATION (mm day^{-1})	RADIATION (MJ m^{-2} day^{-1})
1999-2001	NOVEMBER	30.4	12.6	0.22	8.73	3.73	16.5
1999-2001	DECEMBER	26.0	7.4	0.97	8.15	2.79	14.5
2000-2002	JANUARY	25.7	6.6	0.48	8.46	2.86	15.1
2000-2002	FEBRUARY	25.8	7.9	2.19	8.31	3.10	18.3
2000-2002	MARCH	26.9	8.7	0.35	9.32	4.18	23.1
2000-2002	APRIL	31.4	11.9	0.00	9.03	6.13	24.9
MEAN	*ALL CYCLES*	27.7	9.20	0.70	8.67	3.80	18.8

NOTE:
All data are averaged daily measurements. Prevalent climate during cycles 1999/2000, 2000/2001 & 2001/2002. Data collected at CIMMYT's Obregon Field Station, Sonora, Mexico.

Table 1B. Environmental conditions of the Australian sites in where studies were undertaken on the Seri/Babax population

	CHARLICK								*ROSEWORTHY*				*MINNIPA*			
	2001				2002				2001				2002			
	TEMPERATURE			WIND SPEED	TEMPERATURE			WIND SPEED	TEMPERATURE			WIND SPEED	TEMPERATURE			WIND SPEED
MONTH	MAX (°C)	MIN (°C)	RAIN (mm)	PM (km h^{-1})	MAX (°C)	MIN (°C)	RAIN (mm)	PM (km h^{-1})	MAX (°C)	MIN (°C)	RAIN (mm)	PM (km h^{-1})	MAX (°C)	MIN (°C)	RAIN (mm)	PM (km h^{-1})
JUNE	16.2	8.30	37.8	18	16.1	7.60	54.6	22	17.0	8.20	56.6	18	17.2	7.50	47.8	23
JULY	15.2	5.80	29.0	17	16.3	7.40	41.6	24	15.6	5.80	49.4	16	18.3	7.70	52.4	23
AUGUST	16.7	7.60	75.2	23	16.3	5.50	34.2	19	15.9	6.60	62.2	20	18.0	5.10	24.2	20
SEPTEMBER	19.5	8.10	79.6	20	18.6	7.70	23.6	26	18.8	8.20	77.7	20	22.0	7.30	12.0	27
OCTOBER	18.3	8.70	41.8	25	21.0	8.20	18.8	26	18.9	7.10	54.6	24	24.8	10.1	17.4	25
NOVEMBER	21.9	10.6	50.2	25	25.3	11.2	28.6	23	24.0	8.80	42.4	22	30.3	12.5	24.4	22
DECEMBER	22.3	10.9	39.0	25	26.3	13.1	4.40	27	25.6	9.60	14.2	23	32.3	14.4	24.2	24
MEAN	18.6	8.57	50.4	21.9	20.0	8.67	29.4	23.9	19.4	7.76	51.0	20.4	23.3	9.2	28.9	23.4

NOTE:
All data are averaged daily measurements (Climate Services Section, South Australia Office, Bureau of Meteorology, Australian Government)..

3.2.1.2.4 Experimental layout and management

In Obregon, all experiments were sown with an α-lattice design with two replicates. A bed-planting system (Limón-Ortega *et al.* 2000) was utilised for the field plots, consisting of alternating raised beds and furrows with a repeat distance of 80 cm between furrows. Each set of beds (two per genotype) had three rows of plants, except for the 1999/2000 cycle (one bed per genotype), which had four.

Plot size and irrigation treatments varied between years due to seed availability. In 1999/2000, plot sizes were 1.5 x 0.8 m, with a 10 g m^{-2} seed rate. Two irrigation regimes were used: full irrigation (IRR) and soil drying conditions simulating a drought stress (DRT) experienced in megaenvironments where spring wheat is sown with ≤500mm of rainfall (Rajaram *et al.* 1994, 1996). For IRR, plots were irrigated when approximately 50% of available soil moisture was depleted, estimated gravimetrically. Previous estimates in the same environment suggested that a crop thus irrigated would use *ca.*700 mm of water during a typical crop cycle. DRT treatment consisted of a single irrigation at planting. Approximately 200 mm of water was available to the plant down to 1.2 m depth based on measurements in adjacent plots. Plant emergence dates were estimated on 26th November (DRT) and 19th December (IRR).

In 2000/2001 & 2001/2002, plot size was 5x1.6 m, with a seed rate of 15 gm^{-2}. Three irrigation regimes were used: IRR, DRT, and a reduced irrigation scenario (RED) comprising one irrigation at planting and a second irrigation during jointing stage. Water availability estimated on adjacent plots down to 1.2 m showed that approximately 350 mm of water was available. Emergence dates for cycle 2000/2001 were 8th (DRT) and 25th (RED and IRR) December. Cycle 2001/2002 emergence dates were 9th (DRT and RED) and 23th (IRR) December.

At Charlick, 2001 and 2002 experiments were sown with an α-lattice design with two replicates; plots were 6-row wide, 5 m long, with 15 cm between rows. The 2001 experiment was sown on 26th June, at 150 seeds per m^2; emergence ocurred by 9th July. In 2002, experiment was sown on 25th June, at the same seed rate as in 2001; emergence ocurred by 5th July.

At Roseworthy in 2001, seed rate and sowing layout were the same as described for Charlick, with emergence estimated by 16th July. At Minnipa in 2002, field experiment was sown following the same layout as in the Charlick trial, emerging on 25th July.

3.2.1.2.5 Soil preparation and irrigation

For experiments in Mexico, all plots were fertilised for optimum irrigated conditions (200:40:0, N:P:K), at sowing. Pesticides and fungicides were applied prophylactically. Available soil water in the experiments was estimated from gravimetric sampling of neighbouring plots after irrigation, and considering rainfall. During the three consecutive cycles under DRT, water was estimated at *ca.*175 mm, *ca.*185 mm, and *ca.*195 mm, respectively. For RED, an additional 150 mm was estimated to have been applied with the booting stage irrigation. For IRR treatment unlimited water availability was assumed.

In the Charlick and Roseworthy experiments, a basal fertiliser dressing of 24 kg N ha^{-1} and 13 kg P ha^{-1} was drilled with the seed at sowing, while at the Minnipa experiment ca.50 kg ha^{-1} diammonium phosphate plus Zinc was applied. In all Australian experiments, weeds were controlled using a combination of pre- and post-emergence herbicides. No fungicides or insecticides were applied to the experiments as there was no foliar disease or insect damage evident in any growing season.

3.2.1.2.6 Traits

3.2.1.2.6.1 Agronomic and physiological

All agronomic and physiological analyses on the Seri/Babax population included the parents. They were, as follows:

Anthesis and maturity dates

Both anthesis and physiological maturity dates were estimated from 50% seedling emergence to when 50% of the spikes had reached anthesis (Zadoks 65), and when 50% of the spikes had lost their chlorophyll (Zadoks 87), respectively.

Chlorophyll content

Assessed on the adaxial side of flag leaves at the booting stage (Zadoks 40-53) and during grain filling (Zadoks 70-85) with the same self-calibrating chlorophyll meter described in described in **3.2.1.1.2 Traits**. Two measurements per leaf from five randomly chosen plants per plot were taken and the average reading recorded.

Canopy temperature

Canopy temperature (CT) was measured with a portable infrared thermometer (PIT; Mikron M90 Series, Mikron Infrared Instrument Co., Inc., Oakland, NJ, USA) at a field view angle of 2.5°. The PIT apparatus permits the operator to have a photographic view of the part of the plot being measured, enabling the angle of measurement to be adjusted to avoid viewing bare soil between plants. Two measurements per plot were taken standing close to the two corners of the plot (with the sun behind and avoiding shadows). Data were means of five to seven measurements taken between late tillering and booting (Zadoks 30-50) and post-flowering stages (grain filling, Zadoks 70-85). Measurements were taken in the morning (10:00-12:00h) and afternoon (14:00-16:00h) on clear (cloudless), windless and sunny days, as outlined in Reynolds *et al.* (2001).

Normalised difference in vegetative index

Spectral response of light reflected from the canopy was determined using a prototype hand-held radiometer (K. Freeman, *pers.comm.*), which measured incoming radiation in 8 spectral bands. Each waveband was *ca.*25 nm wide, with centres at 460, 507, 558, 613, 661, 706, 760 and 813 nm. Incident and reflected light were simultaneously measured and percentage reflectance was recorded in each waveband. Readings were always taken within 2 h either side of solar noon to reduce the effect of azimuth angle, at 40-50 cm above canopy. Readings were converted to a normalised difference in vegetative index (NDVI) using 661 nm and 813 nm reflectance values. Calculations for near infrared readings considered an overall value of 671 nm for red spectrum, and the NDVI estimate resulted from: $\text{NDVI RED} = (\text{NIR-RED})/(\text{NIR+RED})$

Osmotic potential

To measure osmotic potential (OP) of leaves, leaf tissue was collected early in the morning while dew was still on the leaves at Zadoks 70. A 1 cm section from the middle of the flag leaf from three different bordered plants were excised with scissors and immediately placed in a tube containing distilled water. Leaf samples were left to fully hydrate for 3 h at *ca.*5^{o}C before being quickly dried of all surface moisture and placed in a 2 ml propylene tube, sealed with the lid and placed in a deep freeze to rupture the cells. Measurement of OP was performed as described in **3.2.1.1.2 Traits**.

Leaf rolling

Plants were visually evaluated for the degree of leaf rolling two to three times during each treatment, soon after anthesis (Zadoks 65). Leaf rolling was visually assessed using a '1 to 5' scale (O'Toole & Cruz 1980).

Plant height

Two measurements (in centimetres) per plot were made during late grain filling (Zadoks 85) on representative plants in each of the plot, from the ground to the top of the ear.

Yield

In the 1999/2000 experiment, yield was measured hand-harvesting 1 m^2 of plot. In 2000/2001 and 2001/2002 cycles, yield was estimated by machine harvesting 3.6 m^2. At Charlick, Roseworthy and Minnipa, yield was estimated by machine harvesting 3.5 m^2 of the plots. In all Obregon experiments, the thousand kernel weight (ATKW) was measured by counting out two samples of 100 entire kernels at random, followed by drying at 70°C for 48 h and weighing; kernel number (KNO) was calculated by dividing yield (g m^{-2}) by kernel weight (mg) (Hobbs & Sayre 2001).

Boron Tolerance[6]

Boron tolerance assessment of the Seri/Babax recombinant inbred line population was performed adapting the method of Campbell *et al.* (1998). A selection of 10 uniform seeds was done from a subset of entries (every five entries), prior to experimentation in order to assure even seed germination. Subsequent sterilisation was done by immersing seeds in a 1% $HClO_4$ solution, followed by ddH_2O rinsing and UV-radiating for 20 h. Germination of seeds was initiated by placing them for 48 h at 4-5°C and 24 h at 20°C. Posterior to this, seeds were placed in a grid located in the experimental apparatus[7], immersed in a Boron solution (100ppm H_3BO_3, 5mM $ZnSO_4.7H_2O$, 0.1M $Ca(NO_3)_2.4H_2O$) and kept in the dark. Root lengths were measured after 7 days.

xxxvii

[6] Method assayed in 2001 at the Waite Agricultural Research Institute, School of Agriculture and Wine, The University of Adelaide, Australia.

Nematode Screening

The presence of nematode (*Pratylenchus thornei*) was visually screened soon after harvest of DRT treatment for cycle 1999/2000, where a distinguishable plot pattern was observed similar to the characteristic nematode infested-plot type (J.M. Nicol, *pers.comm.*). The method (Nicol *et al.* 2001) involved the collection of 0.5 kg soil samples at two different depths (0-20 and 20-40 cm) in the 58 selected plots (24 in replicate 1, 34 in replicate 2), coded by entry number (in terms of the assigned Seri/Babax genotype). Three grams of a dried soil (24 h at 105^{o}C) subsample were dissolved in 10 ml of water, after which nematodes were counted under a stereomicroscope on a 250 μm sieve.

3.2.1.2.6.2 Biochemical

Screening evaluations for assessing constitutively elevated levels of polyamines (putrescine, and the putrescine-generating enzymes spermidine and spermine) were performed on five samples of both parental genotypes and on two five-line subsets of the Seri/Babax population in terms of the extremes (low and high) in yield data for DRT (2000/2001 cycle). The laboratory protocol followed an adaptation (Ye *et al.* 1997) of the Flores & Galston (1984) original methodology. Sampling of one flag leaf per genotype was performed at grain filling (Zadoks 70) in the 2001/2002 cycle under DRT and RED. After placing each sample in an sterile plastic vial in an ice container, they were subsequently placed in deep freeze. For laboratory analyses, samples were kept in a sealed ice container for 5 h, after which they were immediately stored at –20^{o}C.

3.2.2 MOLECULAR CHARACTERISATION OF DROUGHT ADAPTATION

For molecularly dissecting the genetic factors that govern the segregation of the genotypic response in the Seri/Babax population, the original strategy considered the development of a full map of the Seri/Babax population. Problems with resource allocation at CIMMYT (D.A. Hoisington, *pers.comm.*) and the low levels of polymorphism found between the parents [*see* **4.6.2.2 Parental polymorphism assessment**] obliged to pursue an alternative strategy [*see* **3.2.2.2.2 Bulked segregant analysis (BSA)** section] and to establish a collaborative endeavour between CIMMYT, INT and CSIRO-

[7] *Experimental apparatus*. Plastic trays (40x20x10cm; Decor Item 154, Australia) with uniform 0.5cm wide perforations for connection of rubber hoses of an air pump (SilentFlow Item SA002) to allow constant oxygenation. Plastic grid fitted to base area, served as seed receptacle.

Plant Industry for generating genotypic information and advancing the research, based on the physiological attributes, to a full QTL mapping of the Seri/Babax population (McIntyre *et al.* 2006). Hereby, only the research performed at CIMMYT is presented, and solely the CSIRO molecular genotyping relevant to the alternative strategy is described. Hence, herein after, all molecular assessments termed "CIMMYT" refer to those conducted at CIMMYT's biotechnology laboratory (El Batan Headquarters, Texcoco, Estado de Mexico, Mexico), while "CSIRO" designate the efforts undertaken at CSIRO-Plant Industry (St.Lucia, Brisbane, Queensland, Australia).

3.2.2.1 DNA substrate

CIMMYT

Fresh plant tissue was harvested from a field trial sown during the 1999-2000 winter cycle at CIMMYT's Tlaltizapan Field Experimental Station, Morelos, Central Mexico (18°N, 99°W). The trial consisted of a 5-row bed-planting (two-tier bordered) for all plots (1.5 m long with 10 cm between rows).

At Zadoks 41, five healthy flag leaves were carefully selected from each genotype. Leaves were harvested from those plants grown in the inner rows of each plot, avoiding potential plot-to-plot contamination. Six to seven 10 cm leaf portions were cut and placed in fiberglass screen bags. All sample bags were tagged and placed in a previously prepared ice container at 4°C immediately after harvest and transported for final storage at –80°C. Frozen leaf tissue was lyophilised and ground in a mechanical mill (Foss Tecator Cyclotec Sample Mill model 1093), after which was placed at –20°C.

DNA extraction followed a modified version of a reported CTAB method of DNA extraction (Saghai-Maroof *et al.* 1984), according to laboratory protocols (Hoisington *et al.* 1994) used at CIMMYT. Quality of isolated DNA was determined based on the pattern of the electrophoresed molecules on 1% (w/v) agarose gel (SEAKEM:NUSIEVE (1:1) agaroses (Biowhittaker Molecular Applications, Rockland, ME, USA)), whereas a spectrophotometer (Beckmann model DU-65) was used for DNA quantification. All DNA samples were diluted to various concentrations (5, 10 and 100 ng μl^{-1}) and kept at 4°C (5 and 10 ng μl^{-1}) and at –20°C (100 ng μl^{-1}) for short and long term storage, respectively.

CSIRO

DNA was extracted following, in general, the aforementioned procedure from plants grown in field trials at the Gatton Campus, The University of Queensland, Queensland, during the 2002 growing season by CSIRO-Plant Industry personnel (C.L. McIntyre & S.C. Chapman, *pers.comm.*).

3.2.2.2 Genetic characterisation

3.2.2.2.1 Genetic evaluation of parents

For the purposes of DNA quality evaluation and allele segregation verification, as well as for assessing genotypic consistency, ten samples of each parental genotype, collected from individually grown plants, were evaluated with molecular markers. Two GWM microsatellites (GWM6 and GWM135) and 4 different AFLPs enzyme/primer assortments (*Pst*IAAG*Mse*ICTT, *Pst*IACC*Mse*ICGA, *Pst*IACG*Mse*ICAA, *Pst*IACG*Mse*ICGT) were used on DNA from individual genotypes.

3.2.2.2.2 Bulked segregant analysis (BSA)

3.2.2.2.2.1 Procedure

Bulked segregant analysis (BSA) (Michelmore *et al.* 1991) was performed using the genotypes that were selected from the tails of the distributions for various quantitative traits analysed under varying soil water regimes in Mexico (DRT, RED and IRR) and Australia (AUS, averaged trait data of the 2001 South Australian trials, Charlick and Roseworthy). As the BSA presented in this report was performed on the totality of the regimes and latitudes in where the Seri/Babax population was evaluated, it was termed "INTEGRAL BSA".

For the INTEGRAL BSA, two different set of bulks were considered for each trait, consisting (each) of ten genotypes (at each of the tails of the distribution): highly resistant (high tail) and highly susceptible (low tail). Careful selection for the bulks was performed on the averages of the combined datasets of the spatially-adjusted means of the genotypic response of a subset (127 genotypes) of the Seri/Babax RILS [*see* **3.2.2.2.2.1.1 Preliminary evaluation**]. In order to perform the INTEGRAL BSA molecular assessments, in preparing the bulks 1 µg of DNA from each selected genotype was mixed in a plastic vial; equal volumes of each genotype's DNA (diluted at equal concentrations) were evenly and carefully mixed for molecular analyses.

3.2.2.2.2.1.1 Preliminary evaluation

Prior to conducting the molecular characterisation of traits via BSA, four (related) preliminary experiments were conducted (consecutively) after the polymorphism screening with molecular markers of the parental genotypes:

1. Polymorphism consistency. An analysis of 22 random Seri/Babax RILs with sixteen selected polymorphic PCR-based markers in order to observe the segregation at various loci.

2. Selection of bulks. An attempt [*see* **4.6.2.3.1 Preliminary evaluation** section] to utilise the bulked segregant analysis for some traits (Table 14), considering four bulks, each consisting of five genotypes: highly resistant, resistant, susceptible and highly susceptible. The careful genotype selection for each bulk was based on the averages of the DRT and AUS data (combined datasets of the spatially-adjusted means of the genotypic response) for the complete population (167 RILs). Bulks were screened with the same PCR-based markers used in the Polymorphism consistency evaluation.

3. Debulking. Amplification of genomic loci with the same set (sixteen) of polymorphic markers of each genotype DNA used for producing the four bulks (Table 14).

4. Population analyses for each class of bulks. Assessment of the genomic loci amplification in all of the population genotypes (167 RILs) with the aforementioned set of polymorphic markers.

3.2.2.2.2.2 Traits

Traits analysed for the INTEGRAL BSA [**4.6.2.3.2 INTEGRAL BSA**] were selected due to their relatedness to the drought-adaptive mechanism in the RILs [*see* **5.2 RILs ADAPTATION AND PERFORMANCE**]. The complete trait information for the 127 RILs evaluated under DRT, RED, IRR and AUS, that were formally characterised via INTEGRAL BSA, is described in (Tables 15 A-D) [*see* **4.6.2.3.2 INTEGRAL BSA**].

As previously stated, a preliminary assessment was performed with PCR-based markers, involving the bulking for eight traits of the overall genotypic response. The rationale of such selection was that they reflected the diverse genotypic response to drought in the genetic pool. The latter was done (following suggestions of J.-M.Ribaut) by averaging values of only two (of the three) cycles (2000/2001 & 2001/2002) of the DRT dataset with AUS (averaged values of the 2001 Australian trials). To illustrate the

pertinence of using such combined dataset of traits, their significant (p<0.01) correlations with yield are described, as follows: osmotic potential at grain filling (r= –0.27), chlorophyll at booting (r= 0.22) and at grain filling (r= 0.11), CT at booting in the morning (r= –0.74) and in the afternoon (r= –0.41), CT at grain filling in the morning (r= –0.47) and in the afternoon (r= –0.51).

3.2.2.2.2.3 Markers

Two different set of markers were utilised at CIMMYT (PCR-based and AFLPs), while only one type at CSIRO (PCR-based).

At CIMMYT, the evaluation between parental samples involved four different collections of PCR-based (microsatellite primers and sequence-specific) markers: 232 Gatersleben wheat microsatellite primers (GWM, Röder *et al.* 1998); 149 wheat microsatellite clone primers (WMC, International Wheat Microsatellite Consortium[8]); 56 Simple Tagged Sequence (STS) markers (Cornell University, NY, USA); and 196 DuPont microsatellite sequences (DUP, DuPont de Nemours Co. Inc., DE, USA) (Eujayl *et al.* 2002; Dreisigacker *et al.* 2005; DuPont, *unpublished data*).

At CSIRO, parental screening involved *ca.*300 markers from three collections (C.L. McIntyre, *pers.comm.*): Gatersleben wheat microsatellite collection, International Wheat Microsatellite Consortium collection and Gatersleben D-genome microsatellite primers (GDM, Pestsova *et al.* 2000).

3.2.2.2.2.3.1 Marker methodologies

There was an optimisation procedure at CIMMYT exclusively for the PCR-based markers, as it was considered important to establish a standardised set of experimental conditions for the various different collections prior to conducting any molecular assessment. A description of the optimisation procedure is included in the subsection related to the PCR-based markers. At CSIRO, there was no optimisation as the protocol used is standard. The latter applies for the AFLP methodology at CIMMYT.

xlii

[8] www.wheat.pw.usda.gov

CIMMYT

i. PCR-based markers

Methodology optimisation

A number of experimental modifications based on selected references (Saiki *et al.* 1985, 1988; Saiki 1989; Higuchi 1989; Gelfand 1989; Mullis 1989; Newton and Graham 1995) were conducted to optimise the polymerase chain reaction (PCR) for the sequences amplifications (Table 2). Various PCR volumes (15, 20 and 30 µl) were tested to evaluate the most efficient final volume of sample preparations.

In all molecular analyses (*i.e.*, polymorphism screening studies, bulk segregant analysis, debulking and population studies) a single set of PCR conditions were utilised: 5 µl ([25 ng final]) genomic DNA; 5 µl ([0.4 µM]) combined primers; 0.4 µl ([2 U final]) *Taq* (*Thermus aquaticus*) DNA polymerase; 1.6 µl ([0.2 mM final]) of Deoxyoligonucleotides mix (dATP, dGTP, dCTP, dTTP); 1.5 µl (1X final) *Taq* Buffer; 1 µl ([3 mM final]) Magnesium Chloride, 1.5 µl (10% final) glycerol; ddH_2O for a total volume of a 20 µl PCR reaction.

Table 2. Optimisation for the final reagent concentration in an standardised PCR mixture

REAGENT	INITIAL/ STOCK CONCENTRATION	FINAL CONCENTRATION
Genomic DNA	5 ng μl^{-1}	20, 25 ng μl^{-1}
Primers (combined *forward* and *reverse*)	5 µM	0.25 µM, 0.4 µM
Taq (*Thermus aquaticus*) DNA polymerase	5 U μl^{-1}	0.6 U, 1 U, 2 U
Taq Buffer	10X	1X
Magnesium Chloride	50 mM	2.5 mM, 3 mM, 5 mM, 5.5 mM
Glycerol	100%	10%
Deoxyoligonucleotides mix (*dATP, dGTP, dCTP, dTTP*)	10 mM*	50 µM, 100 µM, 200 µM

* Concentration for each dNTP.

A thermocycler (Peltier Thermal Cycle PTC-225, DNA Engine Tetrad with 4 α-unit block assembly, 96 wells each; MJ Research, USA) was used for all PCR amplifications. The PCR cycling conditions are described in Table 3.

Table 3. Thermocycler program (detailed) for the PCR-based marker amplification

STEP	TEMPERATURE	TIME
1.	94°C	2 min
2.	94°C	1 min
3.	64°C	1 min
4.	-1°C per cycle	
5.	72°C	1 min
6.	Go to step 2 (6 cycles)	
7.	94°C	1 min
8.	57°C	1 min
9.	72°C	5 min
10.	Go to step 6 (34 cycles)	
11.	72°C	5 min
12.	10°C	*ad infinitum*

All PCR amplifications were done in sterilised 96-well polycarbonate microplates. Amplicons (PCR products) (10-12 µl PCR sample volume + 3 µl 5XSGB) and a standard reference marker (20 µl ΦX174/*Hae*III + 3 µl 5XSGB[9]) were electrophoretically separated in 3% agarose gels (Sambrook *et al.* 1989; Voet & Voet 1990; Hoisington *et al.* 1994). SEAKEM and METAPHOR (1:1) agaroses (Biowhittaker Molecular Applications, Rockland, ME, USA) were used to prepare the 5x20x1 cm gels in 1X TBE Buffer[10]. Electrophoresis was conducted at constant content (120V) (Saghai-Maroof *et al.* 1984; Hoisington *et al.* 1994) for 4 h.

Selected amplicons with a 1-10 base pair-polymorphism were separated in 6% polyacrylamide (PAGE) 22x20cmx1mm gels (acrylamide:*bis*acrylamide (29:1), polymerised with 25%(w/v) ammonium persulfate and 65 µl N,N,N′,N′-Tetraetilendiamine) (Sambrook *et al.* 1989; Voet & Voet 1990). Gels were run for 4 h at 150 V (constant). Agarose and PAGE gels were stained in ethidium bromide for 15 min and washed for 30 min in ddH_2O (Hoisington *et al.* 1994). Amplicons were visualised through a Fotodyne transilluminator (Fotodyne, Madison, WI, USA) and captured with a Kodak photographic camera (DC290 camera, KS 120, 3.5.4 Kodak 1D). A selection of PAGE gels was silver-stained following Sanguinetti *et al.* 1994.

xliv

[9] Sample Gel Buffer: 50mM Tris pH 8.0; 5mM EDTA pH 8.0; 25% Sucrose; 2 mg/ ml Bromophenol Blue, sodium salt; 2 mg/ ml xylene cyanole; ddH_2O.

[2] 5X TBE: 0.45 mM Tris-Borate; 10mM EDTA.

Selection of polymorphic primers was based on amplification repeatability, allele patterns, allele/ band quality, ease of evaluation and allele segregation.

ii. AFLP markers

A selected subset of genotypes from the population were analysed with AFLPs (Vos *et al.* 1995), after an analysis of segregation patterns for PCR-based loci. Forty-eight primer combinations of *Pst*1/*Mse*1 were used in the AFLP analysis as described in Vos *et al.* (1995) with some modifications (Hoisington *et al.* 1994). The restricted DNA was ligated with double-stranded adaptors in the presence of T4- DNA Ligase (GIBCO BRL, Invitrogen, USA) in a 60 µl volume. The sequence of the *Pst*1 adaptor was 5′-GAC TGC GTA GGT GCA-3′; 3- GAG CAT CTG ACG CAT CC-5′ and that of the *Mse*1 adaptor was 5′-GAC GAT GAG TCC TGA G-3′; 3′-TAC TCA GGA CTC AT-5′. Five microliters of the above ligation reaction were amplified using pre-amplification primers in a total PCR reaction volume of 25 µl, containing 200 µm dNTPs (Boehringer, Mannheim, Roche), 0.75 mM $MgCl_2$, 560 nM of each primer in 1 X PCR buffer (GIBCO BRL, Invitrogen, USA) and 2 U of *Taq* DNA polymerase (GIBCO BRL, Invitrogen, USA). The PCR conditions included 25 cycles of 94^{o}C for 30 sec, 56^{o}C for 1 min, and 72^{o}C for 1 min.

A sample of the digested DNA and pre-amplified DNA was verified on a 1% neutral agarose (SEAKEM) to determine the quality of the digestion and of the pre-amplification. The pre-amplification primer for *Pst*1 adaptor was 5′-GAC TGC GTA GGT GCA GA-3′, whereas the primer for *Mse*1 adaptor was 5′-GAT GAG TCC TGA GTA AC-3′. The remainder of the pre-amplified DNA (20 µl) was diluted with 75 µl of ddH_2O. Three µl of the diluted pre-amplified DNA was used in the second PCR for selective amplification. Such selective-amplification PCR, was performed in a volume of 20 µl, consisting of 200 µm dNTPs (Boehringer, Mannheim, Roche), 1.5 mM $MgCl_2$, 250 nM of *Mse*1 selective amplification primer and 100 nM of dig-labeled *Pst*1 selective amplification primer in 1 X PCR buffer and 3 U of *Taq* DNA polymerase (GIBCO BRL, Invitrogen, USA). The selective amplification primers had the same sequence as the pre-amplification primer except for two additional nucleotides. The *Pst*1 selective amplification primers were end-labeled with Digoxigenin-labelling (DIG, a chemilluminiscent methodology[11]). The PCR conditions for the selective amplifications included a *Touch Down* thermocycler

xlv

[11] The use of digoxigenin technology to label nucleic acids is licensed under patents (EP 0 324 474, US 5.344.757, US 5.702.888, US 5.354.657, JP 1999884 and HK 1169) owned by Roche Diagnostics GmbH.

program with an initial 94°C for 1 min, followed by a starting annealing temperature of 65°C that decreased by 1°C for 10 cycles with a 90 sec extension step after each cycle. This was followed by another 23 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min. The selective amplification products (5 µl) were verified on a 1% neutral agarose (SEAKEM) for the explicit purpose of quality, and separated on polyacrylamide gels under denaturing conditions in 1 X TBE (0.09 M Tris-borate and 0.002M EDTA). The gels were transferred overnight onto Hybond nitrocellulose filters and were detected with CSPD chemiluminescent substrate according to the protocols of Hoisington *et al.* (1994). The components of the digestion and ligation reactions are described in Tables 4 and 5, respectively.

Table 4. Details for the DNA digestion reaction utilised in the AFLP methodology

REAGENT	FINAL CONCENTRATION	50µL REACTION
ddH_2O	-	39.9 µl
10X Buffer for *Mse*I	1 X	5.0 µl
*Mse*I (5U/µl)	2.5 U μg^{-1}	0.5 µl
Genomic DNA (0.3µg μl^{-1})	1 µg	3.35 µl
*Pst*I (10 Uμl^{-1})	2.5 μg^{-1}DNA	0.25 µl
NaCl	50 mM	1 µl

Table 5. Details for the AFLP fragment-adaptor ligation utilised in the AFLP methodology

REAGENT	INITIAL/ STOCK CONCENTRATION	FINAL CONCENTRATION	10 µL REACTION
ddH_2O	-	-	5 µl
Ligase Buffer	5 X	1 X	2 µl
*Mse*I adaptor	0.5 µM	50 ρM	1 µl
*Pst*I adaptor	0.5 µM	50 ρM	1 µl
T4 DNA Ligase	1 Uμl^{-1}	1 U	1 µl

CSIRO

i. PCR-based markers

At CSIRO, the amplification of microsatellite loci was performed in radiolabelled 10 µL PCR reactions containing 1X PCR buffer (FisherBiotech International Ltd), 2.5 mM $MgCl_2$, 0.2 mM low C dNTPs (10 mM dATP, 10 mM dTTP, 10 mM dGTP, 1 mM dCTP), 9 µCi 33P-dCTP, 0.32 µM of each of the forward and reverse primers (Roder *et al.* 1998; Pestsova *et al.* 2000; Varshney *et al.* 2000; Gupta *et al.* 2002), 0.04 U *Tth* (*Thermus thermophilus*) Plus DNA polymerase (FisherBiotech International Ltd) and 25-50 ng of genomic DNA. Thermocycle conditions were: 95°C for 1 min 15 sec; followed by 35 cycles of 95°C for 15 sec, annealing temperature (primer dependent) for 30 sec, 72°C for 30 sec. PCR was performed in a Perkin-Elmer GeneAmp(r) 2700/9700 thermocycler.

Amplicons were run on a 5% PAGE gel (6 M urea, 5% acrylamide:*bis*acrylamide 19:1, 1 X TBE Buffer), for *ca.* 2 h at 90 W. After electrophoresis, PAGE gels were transferred to blotting paper, dried using a gel drier at 80°C and exposed to Kodak X-Omat X-ray film for 3-4 days.

3.3 ANALYSES

3.3.1 Phenotypic data

Screenhouse experiments

Analyses considered the randomised complete block design utilised for the experimental layout. All data analysed was averaged per plant or per genotype for every treatment. Specifically-designed programmes for the analysis of variance (ANOVA) and general linear model (GLM) were run in SAS® environment –using PROC ANOVA and PROC GLM, respectively (SAS Institute, Cary, NC, USA; version 6.12 for MacIntosh® System & version 8 for Windows®)–, and results/outputs were verified with Systat® (version 5.2) and StatviewSE+Graphics® statistical applications for MacIntosh®.

Field experiments

All field analyses considered the α-lattice design in the randomised complete block experiments. Data points and allocated replicates were examined as neighbouring plots within rows or columns (Cullis & Gleeson 1991).

3.3.1.1 Phenotypic and genetic correlations

Linear correlations were calculated according to Steel & Torrie (1980) using spatially-adjusted means of all data points, after an standarisation procedure (to zero mean and standard deviation of one); values were obtained using the correlation procedure (PROC CORR) for the SAS system. Genetic correlations between measured variables were calculated using the methods of Falconer (1989).

3.3.1.2 Regressions

Linear regressions and graphic representations were calculated with spatially-adjusted means using Systat® and StatviewSE+Graphics®. Multiple linear regression values were calculated following PROC REG with a stepwise selection method for SAS.

3.3.1.3 Heritability

Broad-sense heritabilities were calculated using the mixed model procedure (PROC MIXED) in SAS, to obtain the variance components. Prior to estimating heritabilities, all variables were standardised (to mean zero and standard deviation to one) (M.Vargas, *pers.comm*.). Broad-sense heritability was defined as the ratio (proportion) of genotypic ($\sigma^2 g$) to phenotypic variance ($\sigma^2 p$) under the influence of environmental pressure/interaction, attributable to the intrinsic genetic background, or:

$$H = \frac{\sigma^2 g}{\sigma^2 p} = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e}$$

3.3.1.4 Genotype-by-Environment interactions

For the GxE interactions (GEI) dissection, the raw data of 167 RILs plus parents under the DRT, RED and IRR treatments were analysed utilising the method of Crossa & Cornelius (1997) for the site regression model with one multiplicative term (SREG). In such genotype-discriminative model, the main effects of genotypes are absorbed into bilinear terms. The SREG linear-bilinear model is given by:

$$\overline{Y}_{ij}. = \acute{\imath} + \ddot{a}_j + k^{t}=1 \ddot{e}_k \acute{a}_{jk} \hat{a}_{jk} + \bar{e}_{ij}.$$

where $\overline{Y}_{ij}.$ is the mean of the ith genotype in the jth environments; *í* is the overall mean; äj is the site effect: ëk (ë1,ë2,…,ët) are scaling constants (singular values) that allow the imposition of orthonormality constraints on the singular vectors for genotypes, αik=(α1k,…,αgk) and sites, γik=(γ1k,…,γek), such that ₁á²jk=ⱼâ²jk=1 and ₁ájkájk,=ⱼâjkâjk,=0 for k≠k´; ájk and âjk for k=1,2,3,…are called "primary", "secondary", "tertiary", …, *et cetera*.effects of genotypes and sites, respectively; $\bar{e}_{ij}.$ is the residual error assumed to be NID (0, ó²/*r*) (where ó² is the pooled error variance and *r* is the number of replicates). Least squares estimates of the multiplicative (bilinear) parameters in the kth bilinear term are obtained as the kth component of the deviations from the additive (linear) part of the model.

The two sources of variation revealed via the SREG model (G, genotype main effect, plus GEI, genotype by environment interaction) were graphically displayed as biplots. In such representations, coordinates for environments, genotypes and environmental co-variables corresponding to the first two factor components are simultaneously depicted by vectors in a Cartesian space with starting points at the origin (0, 0) and end points determined by the value of the coordinate. Environments and genotypes having the same direction reveal positive interactions, and *vice versa*. The relationships between any pair of environmental variables, genotypes or years is defined by the angle formed by the vectors. The more acute the angle, the larger their relatedness. Angles of 90^{o} indicate no relationship.

3.3.2 Genotypic data

3.3.2.1 Input datasets

At CIMMYT, molecular data (microsatellites and AFLPs) were evaluated following Sankoff *et al.* (1986) and manually-entered in HyperMapData, a Hypercard software application (1993-1995, Apple Co.), designed *ex profeso* (Hoisington & González-de-León 1993) to handle genetic segregation datasets for linkage mapping.

At CSIRO, molecular data was manually scored and entered in Excel® spreadsheets (Office® suite of programs, Microsoft Corp., Redmond, WA, USA) (C.L. McIntyre & K.L. Mathews, *pers.comm*.).

Both molecular datasets (generated at CIMMYT and CSIRO) were integrated in the Excel® application into a compiled version (composite CIMMYT-CSIRO database), after carefully aligning the data with respect of the genotype number, authenticating the alignment with the pedigree history classification per genotype.

3.3.2.2 Linkage mapping

Linkage association was performed at CIMMYT using MAPMAKER v2.0 (Lander *et al.* 1987). Two-point linkage mapping (Lorieux 1994) was declared when –log(*P* value) (LOD score, or *log of the odds* ratio) ≥ 3.0 and $\theta \leq 0.40$. Recombination frequencies and the related genetic distances between linked markers, were defined by means of the Haldane mapping function (Haldane 1919).

Resulting linkage groups and loci position were verified with published maps (Röder *et al.* 1998; Pestsova *et al.* 2000; Paillard *et al.* 2003; Somers *et al.* 2003, 2004) and publicly available information (*grain genes* database[12]).

3.3.2.3 Phenotypic and genotypic associations

At CIMMYT, association analysis between genotypic and phenotypic datasets was performed using Q-Gene software for genomic analysis (Nelson 1997). Simple linear regression at every locus was utilised to calculate the coefficient of determination (r^2), as a measure of the proportion of the phenotypic variation explained by the markers. The computer application calculated LOD score, *F* test value, and associated significance levels for every marker in all set of observations for every trait analysed.

[12] www.wheat.pw.usda.gov

RESULTS

4.1 SCREENHOUSE SCREENING

While the deprivation of water in the confined, thermally-controlled environment induced significant responses in both the Seri and Babax genotypes (Tables 6A & B), there was some intergenotypic variation in a few traits and a large combined variation between treatments and genotypes for all traits. There were no effects of repetitions versus genotype, nor of treatment. As no pests nor diseases were detected in the facility, the combined effect of uneven electric light and solar radiation depending of the pots or pipes positions remains a possible cause that the layout design was intended to have reduced, if not obviated. Nonetheless, the significance of effect in water deprivation was significant, as the ranges in Experiment A and B were 10-72% and 15-67%, respectively.

When comparing both evaluatory experiments, there were small trait differences between the two semi-dwarf genotypes in the pipes, which contrasted with the large differences observed in the pots. Also, for the significantly different traits evaluated in both experiments (spike and stem lengths, plant height, root weight, kernel weight and number), there were also divergencies in their magnitude, suggesting that the container shape would have influenced water retention and, in consequence, the genotypic response to water stress. As it was not the objective of the present study to investigate the causes of differences between standardised experiments under screenhouse conditions, the screenhouse experience was examined in light of its evaluatory circumstance for the field experiments in where the Seri/Babax population was evaluated.

The response of the parental genotypes in the experiments was, as follows:

Experiment A. The effect of water deprivation was significant in all traits evaluated, except for stem length and kernel number (Table 6A). In terms of their response in the droughted treatment, both parents were significanly different, even though there were distinctive genotypic divergencies between them (watered treatment). The former was evident in all of the traits assessed (except for spike, kernel and root weights, and for kernel number) with sufficient distinction of Babax being a genotype with a better overall response, reflected in a number of attributes (higher chlorophyll and less osmolyte concentration at grain filling, a thicker flag leaf, a taller posture with both longer spike and stem, and a larger kernel), than Seri.

Experiment B. All traits were significantly influenced by water deprivation (Table 6B). Only plant height was significantly different between the parents, with the Babax genotype being taller than Seri.

Table 6A. *Experiment A*. Screenhouse assessment of parental genotypes in pots (Central Mexico, summer 1999)

TRAIT	DROUGHTED		WATERED		*F*	*F*	R^2	CV
	MEAN	SE	MEAN	SE				
	Seri, Babax	Seri, Babax	Seri, Babax	Seri, Babax	TTMT	GNO		
CHLO-gf (SPAD)	17.7, 22.9	3.29, 10.3	37.7, 40.1	8.92, 2.93	15.6***	32.8***	0.72	21.1
OP-gf (MPa)	-1.95, -1.33	0.88, 0.61	-1.10, -0.09	0.05, 0.04	16.3***	26.4***	0.69	9.12
LTHICK (mm)	0.16, 0.17	0.01, 0.02	0.17, 0.21	0.01, 0.01	14.2***	20.9***	0.67	8.93
SPKLGTH (cm)	7.98, 10.6	1.55, 1.38	8.03, 10.2	1.00, 0.93	0.14^{ns}	22.2***	0.53	13.5
STEMLGTH (cm)	53.6, 61.7	10.7, 12.4	56.7, 64.9	7.02, 8.32	0.61^{ns}	4.15*	0.19	16.6
HEIGHT (cm)	61.4, 72.3	11.8, 13.3	63.9, 75.1	6.83, 8.83	6.66**	7.8**	0.26	15.4
SPKWT (g)	0.63, 0.84	0.32, 0.21	1.62, 2.20	0.45, 0.92	27.7***	0.74^{ns}	0.61	41.4
ROOTWT (g)	2.39, 4.74	1.85, 2.59	2.65, 6.61	2.39, 4.74	8.35**	0.95^{ns}	0.33	65.3
KWT (g)	0.48, 0.50	0.08, 0.20	1.17, 1.77	0.41, 0.78	36.6***	0.58^{ns}	0.68	50.4
KNO (units)	34.2, 32.0	7.80, 13.2	33.1, 41.5	15.1, 14.6	0.63^{ns}	0.35^{ns}	0.09	36.9
KLGTH (mm)	5.32, 8.76	0.11, 0.8	7.11, 10.4	0.30, 0.39	16.6***	13.3***	0.55	73.1

NOTE:
ANOVA (SAS); GENERAL LINEAR MODEL (GLM), Type 3 *SS*.
***, $p<0.0001$; **, $p<0.01$; *, $p<0.05$; ^{ns}, non-significant.
TTMT, Treatment; GNO, Genotype; CHLO-gf, Chlorophyll SPAD measurements at grain filling; OP-gf, Osmotic potential at grain filling; LTHICK, Leaf thickness; SPKLGTH, Spike length; STEMLGTH, Stem length; HEIGHT, Plant height; SPKWT, Spike weight; ROOTWT, Root weight; KWT, Kernel weight; KNO, Kernel number; KLGTH, Kernel length.

Table 6B. *Experiment B*. Screenhouse assessment of parental genotypes in pipes (Central Mexico, summer 1999)

TRAIT	DROUGHTED		WATERED		*F*	*F*	R^2	CV
	MEAN	SE	MEAN	SE				
	Seri, Babax	Seri, Babax	Seri, Babax	Seri, Babax	TTMT	GNO		
SPKLGTH (cm)	7.26, 7.37	1.02, 2.02	9.06, 10.4	0.67, 2.48	10.2***	0.70^{ns}	0.42	20.1
STEMLGTH (cm)	45.3, 51.2	9.53, 11.4	57.2, 57.8	3.85, 4.39	6.73**	0.83^{ns}	0.34	15.1
HEIGHT (cm)	52.7, 58.6	9.11, 10.7	66.3, 68.2	3.41, 6.33	11.1***	4.13*	0.44	12.9
ROOTLGTH (cm)	93.1, 102.3	0.33, 0.37	140.2, 141.6	0.24. 0.30	13.8***	1.03^{ns}	0.50	27.4
SPKWT (g)	0.99, 1.01	0.43, 0.39	2.40, 2.15	0.75, 0.32	32.2***	0.28^{ns}	0.67	30.5
ROOTWT (g)	4.76, 4.82	4.19, 3.78	18.1, 22.3	16.7, 8.04	12.6***	0.23^{ns}	0.15	77.6
KWT (g)	0.66, 0.74	0.65, 0.50	1.59, 1.76	0.22, 0.58	23.1***	0.08^{ns}	0.60	38.0
KNO (units)	22.4, 20.1	11.7, 10.4	44.9, 46.8	3.82, 3.92	26.9***	0.19^{ns}	0.63	31.7

NOTE:
ANOVA (SAS); GENERAL LINEAR MODEL (GLM), Type 3 *SS*.
***, $p<0.0001$; **, $p<0.01$; *, $p<0.05$; ^{ns}, non-significant.
TTMT, Treatment; GNO, Genotype; SPKLGTH, Spike length; STEMLGTH, Stem length; HEIGHT, Plant height; SPKWT, Spike weight; ROOTLGTH, Root length; ROOTWT, Root weight; KWT, Kernel weight; KNO, Kernel number.

4.2 ENVIRONMENT AND SOIL MOISTURE CONDITIONS

For the three years of experimentation in Obregon, climate conditions (Table 1A) were those of a typical temperate, highly-radiated, spring-wheat growing environment (Rajaram *et al.* 1994). Rainfall averaged 0.7 mm per month during the cycle, a tiny fraction of the evaporative demand, that averaged 3.8 mm per day. Solar radiation increased coincidentally with the normally warmer months, March and April, resulting in increased evaporative demand during grain filling for all treatments.

In 2001, South Australia experienced an unusually wetter and cooler climate during the wheat growing season (Table 1B), and higher yields than average. While an intense wind activity was notably prevalent throughout the crop cycle, temperatures increased and rain decreased as the cycle progressed. However, in 2002, severe dry conditions and markedly warmer temperatures, resulted in a lower average yield, which was not different from that obtained in the DRT treatment in Mexico.

At all Australian sites, severe eolic activity was recorded (Table 1B), particularly in the July-October period in the 2002 sites (Charlick and Minnipa). The notably prevalent and severe wind activity was coincident (in 2001) with the advent of the rain season at the beginning of the cycle, and (in 2002) with heat waves occurring at the end of the crop cycle.

The soil characterisation conducted in a six-hectare field site in Obregon (May 2000) assisted with the selection of a suitable location for the subsequent field experiments, mainly to avoid potentially confounding soil factors (mineral toxicities and soil nutritional deficiencies) that might have impacted on the genotypic performance under the different moisture regimes. Since there was an area identified with high concentrations of Boron (4.5 mg kg^{-1}) and Sodium (>2000 mg kg^{-1}) at the 60-90 cm depth (Fig.3), subsequent experiments were located in a specific area chosen away from these problems –in reference to the thresholds established in the International Soil Fertility Manual (1995). In general, the selected site showed an alkaline pH at all depths (on average: 0-30 cm, 8.7; 30-60 cm, 8.73; 60-90 cm, 8.5) with high levels of ammonium acetate elements; DPTA micronutrients concentrations were close to critical values in the high-Boron area; the clay, lime and sand ratio (*i.e.*, soil texture) ratio was 45 : 20 : 35. No salinity problems were detected (averaged gradient, 9-14 meq L^{-1}) and electric conductivity levels were low (on average, 2.7 dSm^{-1}).

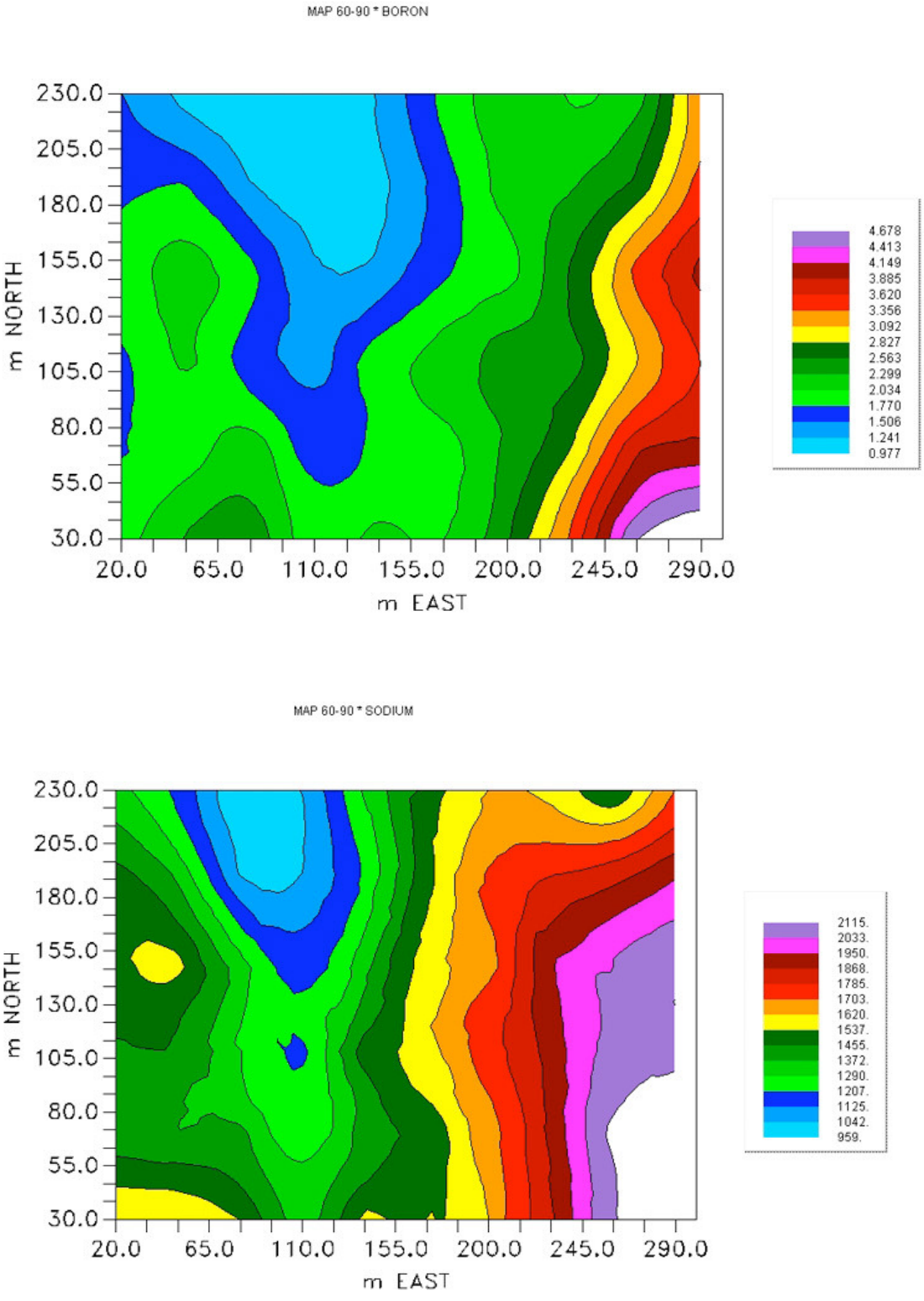


Figure 3. Soil profile maps for Boron and Sodium (depth: 60-90 cm) in a six-hectare sector of the CIMMYT Obregon Experimental Field Station, Yaqui valley, Northwestern Mexico at the end of the 1999/2000 cycle. Ranges are indicated in mg kg^{-1}.

No soil characterisation analyses were performed in the South Australian sites for this study, although there have been previous detailed efforts in this respect (Northcote & Skene 1972; Naidu & Rengasamy 1993; Naidu *et al.* 1995; Liu *et al.* 2000; Isbell 2002). Thus, it is known that deficiencies in Zinc and Copper occur intermittently at those locations, with Boron toxicity occurring in dry years. However, there were no visual symptoms of these nutritional problems in the experiments at Charlick nor at Roseworthy. In contrast, there were indications of Boron toxicity at the Minnipa site [*see* **4.5 INFLUENCE OF ENVIRONMENT IN RESPONSE**]. At all Australian sites, relatively low soil-borne diseases occurrence were estimated, as no soil nor plant confirmation analyses were performed.

4.3 INFLUENCE OF DROUGHT UNDER FIELD CONDITIONS

4.3.1 Seri/Babax population

4.3.1.1 Agronomic traits

Average yield for all genotypes under IRR was 656 g m^{-2} (Table 7A). Yield reduction associated with drought treatments resulted in an average yield loss of *ca.*55% under DRT and *ca.*23% under RED. Comparing both parents in Mexico (Fig.4), Babax was less affected by drought stress than Seri, yield reductions being 52% and 61% under DRT and 23% and 30% under RED, respectively. Under DRT, there was clear evidence of transgressive segregation within the population, with over 20 RILs showing higher yield under DRT than the better parent, and nearly 50 genotypes with drought susceptibility greater than either parent (Fig.5). Yield reduction varied from 35% to 82% under DRT (Fig.5), while the absolute median reduction was 44%. Although yield under IRR and DRT were correlated, irrigated yield potential only explained approximately half of the variation in yield under DRT (Fig.5). Consequently, the highest yielding group of lines (*i.e.*, 750-800 gm^{-2} range) did not include most of the best performing lines under DRT. These are mainly encompassed in a group with average irrigated yield in the 650-750 gm^{-2} range.

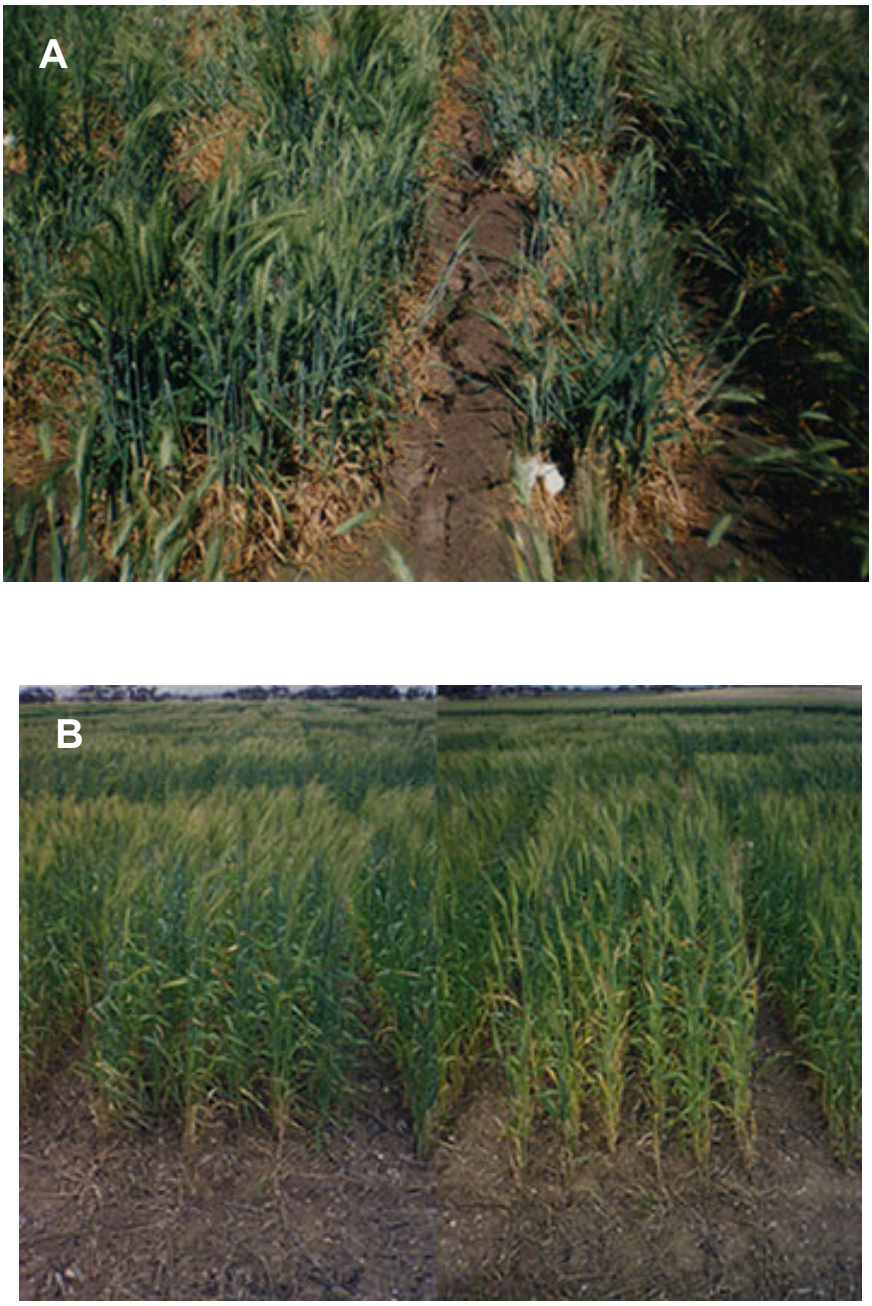


Figure 4. Parental genotypes Babax (*left*) and Seri (*right*) at grain filling: (A), under drought stress at the Obregon field station, Northwestern Mexico during cycle 1999/2000; and (B), under rainfed conditions at Charlick, South Australia during the 2001 growing season.

Table 7A. Selected agronomic traits studied on the Seri/Babax population under various hydrological conditions (Yaqui valley, Mexico)

	MEXICO											
	DROUGHT STRESS[A]				*REDUCED IRRIGATION*[B]				*FULL IRRIGATION*[A]			
TRAIT	SERI	BABAX	MEAN[C]	SD	SERI	BABAX	MEAN[C]	SD	SERI	BABAX	MEAN[C]	SD
ANTHESIS[1]	80.5	77.6	75.7	1.81	86.1	83.5	84.0	2.38	90.0	88.8	86.3	3.22
MATURITY[2]	109.2	108.0	108.7	2.75	117.2	117.3	118.7	3.12	124.5	123.3	125.6	3.27
HEIGHT (cm)	66.9	82.4	73.4	2.83	81.8	100.5	86.6	3.87	92.4	97.5	95.6	4.45
ATKW(g)	30.4	34.5	32.3	3.10	31.8	35.4	32.4	3.18	39.6	43.6	41.2	3.10
KNO	8000	10100	9200	1986	14000	15900	16600	2039	16000	16800	16000	1598
YIELD (gm^{-2})	244.5	348.8	297.8	95.1	444.9	562.4	504.6	72.3	633.5	732.5	655.5	53.0

NOTE:

[A], Mean and standard deviation (SD) of RILs data from cycles: 1999/2000, 2000/2001 & 2001/2002; [B], mean and standard deviation (SD) of RILs data from cycles: 2000/2001 & 2001/2002.

[C], n=167.

1, Days to anthesis; 2, Days to maturity. HEIGHT, Plant height; ATKW, A thousand kernels weight; KNO, Kernel number.

Table 7B. Selected agronomic traits studied on the Seri/Babax population under various hydrological conditions (different South Australian locations)

	AUSTRALIA															
	CHARLICK 2001				*CHARLICK* 2002				*ROSEWORTHY* 2001				*MINNIPA* 2002			
TRAIT	SERI	BABAX	MEAN[A]	SD	SERI	BABAX	MEAN[A]	SD	SERI	BABAX	MEAN[B]	SD	SERI	BABAX	MEAN[A]	SD
ANTHESIS[1]	111.2	108.5	108.7	3.60	.	.	.	.	125.9	126.6	126.2	1.80	.	.	.	.
MATURITY[2]	148.5	147.5	147.9	2.70	.	.	.	.	.	.	.	.	.	.	.	.
HEIGHT (cm)	85.8	96.2	87.6	4.90	.	.	.	.	93.7	107.8	96.6	6.20	.	.	.	.
ATKW(g)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KNO	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
YIELD (gm^{-2})	373.1	338.4	340.7	35.1	185.9	180.8	211.7	0.17	488.0	469.3	485.6	80.9	66.2	43.5	54.7	0.07

NOTE:

[A], Mean and standard deviation (SD) of RILs: n=144; [B], n=125.

All data averaged over years/cycles evaluated.

1, Days to anthesis; 2, Days to maturity. HEIGHT, Plant height; ATKW, A thousand kernels weight; KNO, Kernel number.

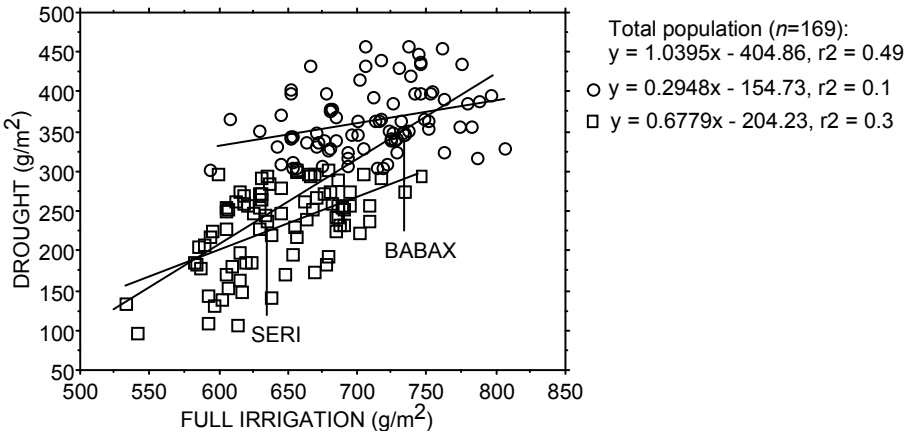


Figure 5. Association between yield under full irrigation and drought stress for Seri/Babax recombinant inbred line (RILs) population in Yaqui valley, Mexico (average of cycles 1999/2000, 2000/2001 and 2001/2002). Regressions are shown for 167 RILs and both parents, for the top yielding 85 lines (302.4-459.6 gm-2), ○, and for the bottom yielding 84 lines under drought stress (96.9-302.0 gm-2), □.

At all Australian sites, Seri was less affected by moisture stress (on average, *ca.* 11%) than Babax (Table 7B). At Charlick in 2001 (Fig.4) and 2002, the differences in yield reduction (compared to their perfomance under IRR) between the parents were of 12.7 and 13.1%, respectively, while at Roseworthy in 2001 was of 12.9%. At Minnipa, however, the difference between them was the smallest recorded at all sites (4.5%). Significant reductions in yield were observed in the RILs across all Australian sites (Table 7B; Fig.6), with Minnipa the largest (*ca.*92%) and Roseworthy the smallest (26%). The difference between the Charlick growing seasons was of 20%, with the largest impact of drought in 2002 (*ca.*68%). At Charlick, the RILs average yield was 341 g m^{-2} and 211 g m^{-2} in 2001 and 2002, respectively. At Roseworthy and Minnipa, the RILs average yield was 485.6 g m^{-2} and 54.7 g m^{-2}, respectively. RILs average yield across the cycles at Charlick was correlated with the Obregon treatments, as follows: under DRT, r=0.41 (p<0.01); with RED, r=0.30 (p<0.01); and, with IRR, r=0.50 (p<0.01). In contrast, there were no correlations in the averaged yield of Roseworthy nor of Minnipa with any Obregon treatment. When comparing the RILs averaged yield within the Australian sites, the highest correlated combination was Charlick (averaging both seasons) with Roseworthy (r= 0.39, p<0.01), while the lowest was that of Charlick with Minnipa (r= 0.33, p<0.01), but not significantly different than that of Roseworthy with Minnipa (r= 0.35, p<0.01).

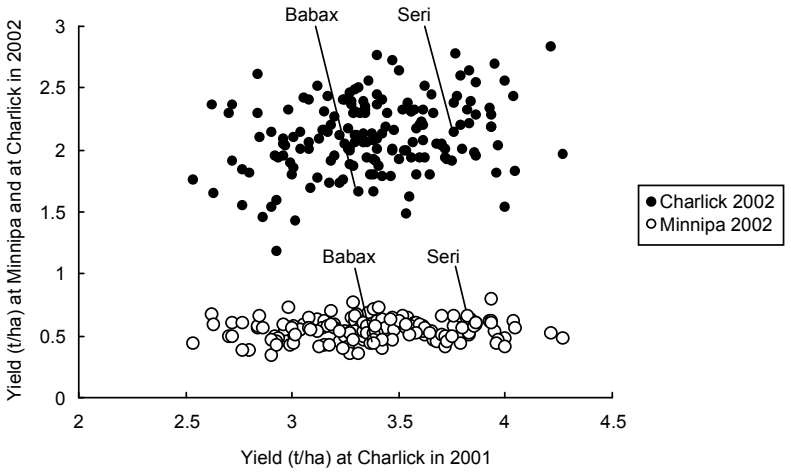


Figure 6. Yield (t ha^{-1}) of the Seri/Babax population at Charlick in 2001 relative to those at Charlick and Minnipa in 2002.

Crop development was significantly hastened by drought stress in Obregon (Table 7A). Under DRT, parents and RILs experienced a *ca.*10% reduction in the duration of both phenological phases, emergence to anthesis and grain filling. When comparing the parents, in general they did not differ in phenological pattern except for anthesis date under DRT, where Seri reached anthesis approximately 3 days after Babax. However, there was significant variation among RILS (Fig.7). In IRR, the average anthesis date was 3 days earlier than the parents, while phenological variation among RILs showed a range of 80-94 days to anthesis and 119-131 days to maturity. Under DRT, the average anthesis date among the RILS was similar to the earlier parent Babax, while the range of days to anthesis and to maturity were 71-81 and 105-112 days, respectively. Under both IRR and DRT average maturity date of RILS was the same as that of both parents.

Relationships between anthesis and maturity for RILS under IRR and DRT are shown in Figure 7; clearly, a better association of anthesis with physiological maturity is shown under IRR than under DRT. The tight relationship between anthesis and maturity dates normally observed under IRR is reduced significantly under DRT. However, the relationship between days to anthesis and duration of grain filling remains strong in all soil moisture conditions (DRT, r^2= 0.51; RED, r^2= 0.69; IRR, r^2= 0.43 p<0.0001). Considering RILs, the reduction in phenophases duration was only weakly associated with yield (r= *ca.*0.2, p<0.0001).

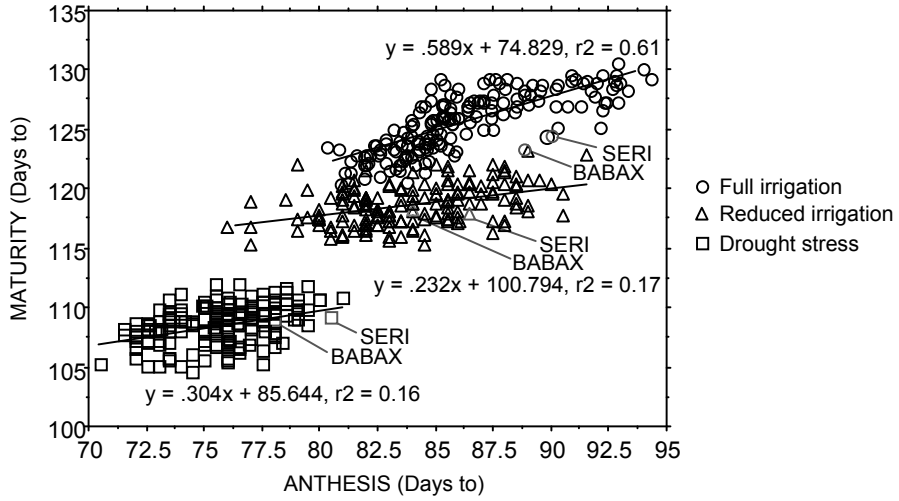


Figure 7. Relationships between anthesis and maturity dates, under different hydrological regimes. Seri/ Babax population (167 RILs and both parents) in Yaqui valley, Mexico (average of cycles 1999/2000, 2000/2001 and 2001/2002).

In contrast with the effect on the reduction in plant development observed in the Northern Hemisphere, there were important increases in duration in the two Australian sites in where observations were performed (Table 7B). At Charlick and Roseworthy in 2001, both parents reached anthesis 22 and 61 days, respectively, later than when under DRT.

There was a large difference in the height of parental lines in response to reduced soil moisture availability. Seri was reduced by 28% and 12% under DRT and RED, respectively, while Babax was unaffected by RED and only showed a 15% reduction under DRT. RILs average height was reduced by 23% and 10% under DRT and RED, respectively. However, the range in reduction in height under DRT varied from just 10% to 53%, a reduction paralleled by a yield decrease (r=0.45, p<0.0001).

At Charlick in 2001, Seri's height was reduced by 7% relative to the height in IRR, while Babax's was nearly unaffected (1%). RILs average height reduction was of 8%, while the overall reduction ranged from 4% to 14%. In contrast with DRT, there was no association between yield and height. At Roseworthy, Babax was higher than Seri by 4%, although both parents were unaffected when compared to their height in IRR (Babax and Seri being 11 and 1 % higher, respectively). RILs increase was identical to Seri's, but the range in reduction was 19-29%. At Roseworthy, the association of height with yield was small (r= –0.23, p<0.0001).

4.3.1.2 Physiological traits

Irrespective of developmental stage or time of day, Seri had a warmer canopy than Babax under DRT, while under RED and IRR, the difference was not significant (Table 8A). Differences in canopy temperature (CT) between Seri and Babax were greater during grain filling under DRT (average difference of 1.5°C) than at the booting stage (average difference, 0.8°C). Under DRT, differences in the CT of the parental genotypes was larger in the morning (1.9°C) than in the afternoon (1.0°C), even though mean temperatures were generally warmer in the afternoon. Under DRT, the RILs showed a larger range of morning CT values during grain filling (5.2°C range) than during booting (3.7°C range). The average CT for RILs was higher in the afternoon than in the morning by 3.8°C and 5.2°C in booting and grain filling, respectively. A comparison in thermal foliage response to the impact of the various experimental moisture conditions is illustrated in Figure 8.

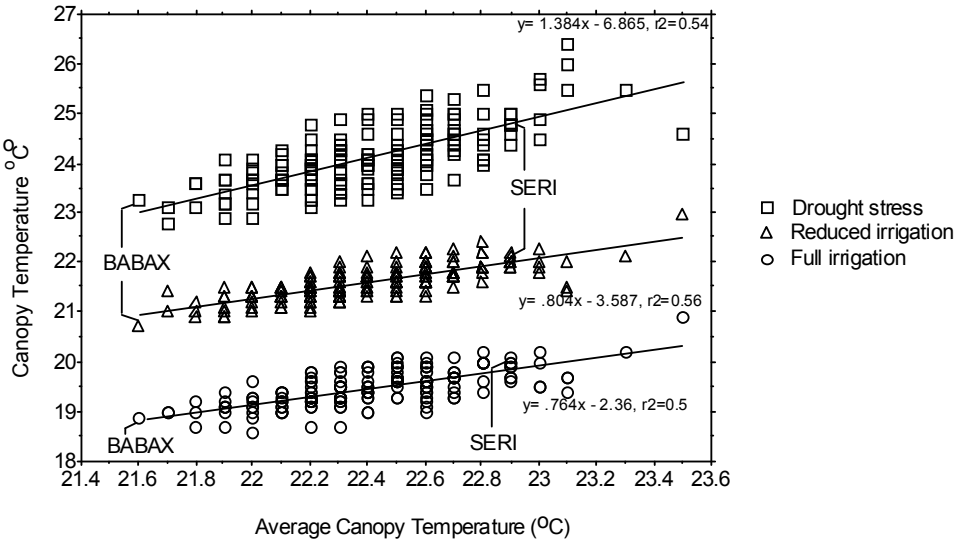


Figure 8. Comparison of canopy temperature (°C) at grain filling under different hydric conditions. Seri / Babax population (167 RILs and both parents) in Yaqui valley, Mexico (cycle 2001/2002).

CT exhibited a transgressive segregation when subjected to DRT (Fig.9) with the coolest RILs under DRT at booting and grain filling showing on average 1°C lower than Babax and the warmest RILs 2°C higher than Seri. At Charlick, the CT data were very consistent with that observed in DRT: Seri had a warmer canopy than Babax (0.6°C) throughout the cycle, while the RILs showed a larger range of CT values at booting (2.6°C) than during grain filling (1.8°C). As under DRT, transgressive segregation was observed, with the coolest genotypes –0.3°C and –0.7°C lower than Babax and the warmest genotypes 1.7°C and 0.5°C higher than Seri at booting and grain filling, respectively.

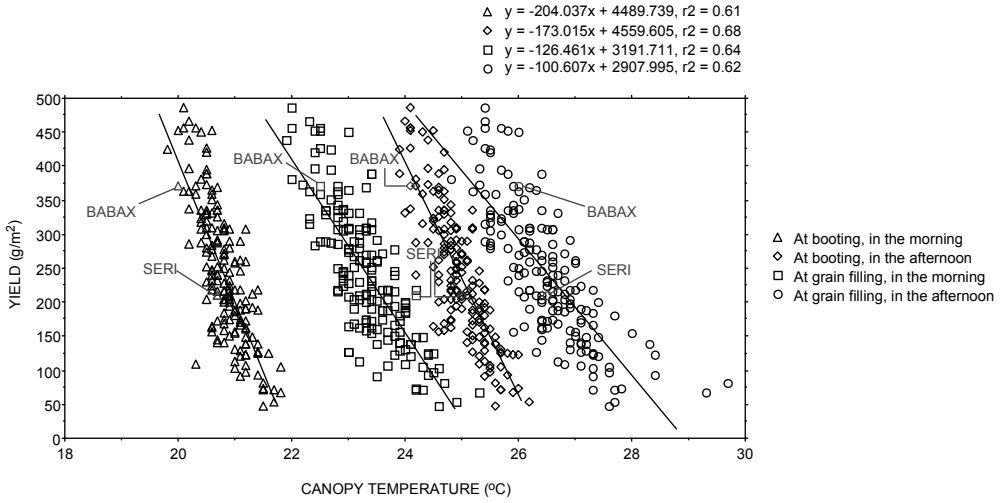


Figure 9. Association of yield performance (gm^{-2}) and canopy temperature (^{o}C) under drought stress, Seri/Babax population in Yaqui valley, Mexico (cycle 2001/2002).

Table 8A. Selected physiological traits studied on the Seri/Babax population under various hydrological conditions in Yaqui valley, Mexico

TRAIT	*DROUGHT STRESS*[A]				*REDUCED IRRIGATION*[B]				*FULL IRRIGATION*[A]			
	SERI	BABAX	MEAN[C]	SD[C]	SERI	BABAX	MEAN[C]	SD[C]	SERI	BABAX	MEAN[C]	SD[C]
CHLO-boot (SPAD)[A]	47.9	47.9	47.8	1.24	46.1	45.9	45.5	1.67	49.5	49	49.9	1.69
CHLO-gf (SPAD)[A]	44.0	48.8	45.7	1.78	53.6	49.7	49.7	2.14	53.8	51.8	50.1	1.45
CTAM-boot (oC)[D]	22.2	21.5	22.2	0.42	.	.	.	.	.	.	.	.
CTPM-boot (oC) [D]	26.3	25.2	26	0.75	.	.	.	.	.	.	.	.
CTAM-gf (oC)[E]	23.4	21.5	22.3	0.59	22.8	21.5	22.2	0.57	21	20.5	21.4	0.35
CTPM-gf (oC) [E]	27.7	26.7	27.5	0.56	26.1	25.5	25.7	0.41	25.6	25.2	25.4	0.30
OP-gf (MPa)[F]	-2.2	-1.53	-2.11	0.18	-1.93	-1.51	-1.88	0.1	-1.71	-1.47	-1.55	0.08
RNDVI-boot [F]	0.76	0.79	0.75	0.03	.	.	.	.	.	.	.	.
RNDVI-gf [F]	0.62	0.58	0.55	0.05	.	.	.	.	.	.	.	.
LROLL-gf [F]	3.4	4.2	4.17	0.46	3.3	3.7	3.4	0.53	2.5	3	2.37	0.47

NOTE:

[A], mean and standard deviation (SD) of RILs data from cycles: 1999/2000, 2000/2001 & 2001/2002; [B], mean and standard deviation (SD) of RILs data from cycles: 2000/2001 & 2001/2002.

[C], *n*=169.

[D], mean air temperatures (cycles: 1999/2000, 2000/2001 & 2001/2002) were 24^{o}C in the morning and 27.9^{o}C in the afternoon.

[E], mean air temperatures (cycles: 1999/2000, 2000/2001 & 2001/2002) were 27.2^{o}C in the morning and 30.5^{o}C in the afternoon.

[F], traits measured in cycles: 2000/2001 & 2001/2002.

CHLO-boot, Chlorophyll SPAD measurements at booting; CHLO-gf, Chlorophyll SPAD measurements at grain filling; CTAM-boot, Canopy temperature at booting, in the morning (9:00-11:00h); CTPM-boot, Canopy temperature at booting, in the afternoon (14:00-16:00h); CTAM-gf, Canopy temperature at grain filling, in the morning (9:00-11:00h); CTPM-gf, Canopy temperature at grain filling, in the afternoon (14:00-16:00h); OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised Difference in Vegetative Index (red spectrum), at booting; RNDVI-gf, Normalised Difference in Vegetative Index (red spectrum), at grain filling; LROLL-gf, Leaf rolling at grain filling.

Table 8B. Selected physiological traits studied on the Seri/Babax population under different hydrological conditions in South Australian locations

TRAIT	CHARLICK[A]				ROSEWORTHY[A]			
	SERI	BABAX	MEAN[B]	SD	SERI	BABAX	MEAN[C]	SD
CHLO-boot (SPAD)	39	42.3	40.2	2.2	.	.	.	.
CHLO-gf (SPAD)	37.3	37.4	37.6	2.7	46.2	42.1	44.2	2.77
CTPM-boot (oC)	17.5	16.9	17.7	0.4	.	.	.	.
CTAM-gf (oC)	18	17.4	17.8	0.3	17.4	16	16.4	0.38
CTPM-gf (oC)	21	20.4	20.5	0.5	21.2	18.1	20.5	0.60
OP-gf (MPa)	-1.39	-1.35	-1.23	-0.1	-0.19	-0.16	-0.17	0.54
LROLL-gf	2	4	3.6	0.8	3.0	4.5	3.8	0.70

NOTE:

[A], mean and standard deviation (SD) of RILs data of 2001 growing season.

[B], *n*=146; [C], *n*=125.

CHLO-boot, Chlorophyll SPAD measurements at booting; CHLO-gf, Chlorophyll SPAD measurements at grain filling; CTAM-boot, Canopy temperature at booting, in the morning (9:00-11:00h); CTPM-boot, Canopy temperature at booting, in the afternoon (14:00-16:00h); CTAM-gf, Canopy temperature at grain filling, in the morning (9:00-11:00h); CTPM-gf, Canopy temperature at grain filling, in the afternoon (14:00-16:00h); OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised Difference in Vegetative Index (red spectrum), at booting; RNDVI-gf, Normalised Difference in Vegetative Index (red spectrum), at grain filling; LROLL-gf, Leaf rolling at grain filling.

Osmotic potential (OP) for Seri was progressively lower in successively drier environments, while the values for Babax were higher than Seri's and were stable across all environments (Tables 8A & B). RILs showed a large range of OP values under all environments but the range and absolute values of OP were generally larger with increased moisture stress (Fig.10).

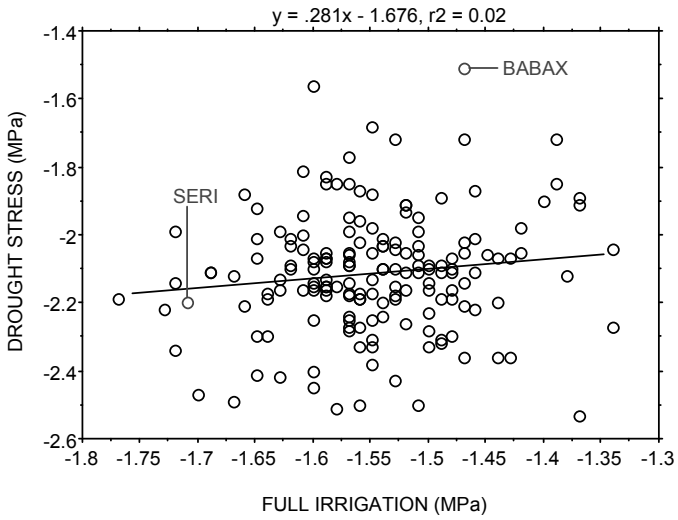


Figure 10. Osmotic potential at grain filling under contrasting hydric conditions. Seri/Babax population in Yaqui valley, Mexico (average of cycles 1999/2000, 2000/2001 and 2001/2002).

Leaf rolling was less pronounced in Seri than in Babax (Tables 8A & B). There was a large range in the degree of leaf rolling in RILs under DRT and at Charlick, but not at Roseworthy. However, the average value for the population was the same as that for Babax in all three latitudes.

A reduction in the chlorophyll content was observed in both parents when under DRT (Fig.11), as early as booting (Seri, 3%; Babax, 2%). By mid-grain filling, Seri had lost 18% of its flag-leaf chlorophyll while Babax only 6% (Table 8A). Among the RILs, the average decrease in chlorophyll was 4% and 9% at the booting and grain filling stages, respectively. Within RILs, the chlorophyll loss ranged from no loss to 20% loss of chlorophyll as severity of drought stress increased. At Charlick, chlorophyll content (compared to the response in IRR) was also significantly reduced

in both parents at booting (Seri, 21%; Babax, 14%) and at grain filling (Seri, 31%; Babax, 28%), but as the cycle progressed, Babax was more affected (14%) than Seri (9%). The between stages reduction in RILs was smaller (6%) than either parent (booting, 19%, grain filling, 25%). At Roseworthy, the decrease in chlorophyll at grain filling compared to IRR was greater in Babax (19%) than in Seri (14%) or the RILs (12%).

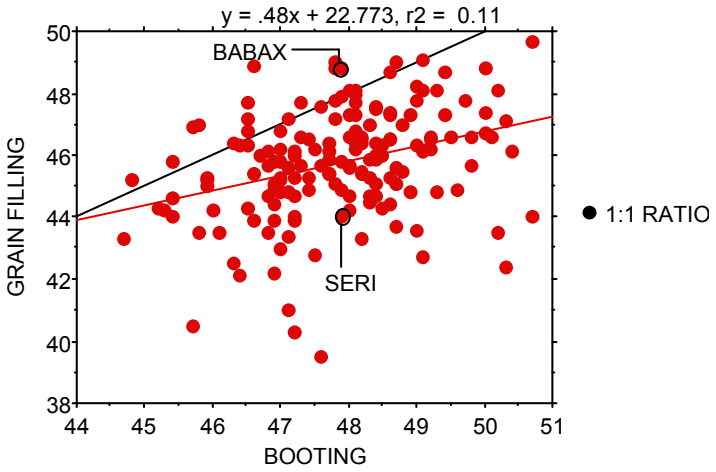


Figure 11. Chlorophyll content (SPAD) at different phenological stages under drought stress, Seri/Babax population in Yaqui valley, Mexico (average of cycles 1999/2000, 2000/2001 and 2001/2002).

NDVI was only measured under DRT, at booting and mid-grain filling stages in two cycles (2000/2001 & 2001/2002). Values of NDVI (red spectrum) were lower during grain filling than at booting (Table 8A), reflecting the progressive loss in photosynthetic tissue with intensification of moisture stress. Differences between the parents were not significant, while RILs showed a range of values from 0.68 to 0.83 in the booting stage and 0.39 to 0.66 in grain filling. There was no correlation between NDVI measured on RILs in the booting stage and later on in the grain filling stage, indicating that large changes in genotypic effects occurred over time. The change in NDVI between booting and grain filling showed a weak association with flag leaf chlorophyll loss between the same stages (r= 0.3, p<0.0001).

4.3.1.3 Biochemical traits

At grain filling, a significant difference (F= 123.6, p<0.05) in putrescine concentration was only observed between the parental genotypes Seri (114.4±5.05) and Babax (51.2±5.80). As the opposite has been established (Ye *et al.* 1997), no further assessments were conducted in any subsequent cycle. In addition, it was found no segregation for the metabolite production as there were no differences between the contrasting selected population subsets, which showed an small intersample variation (4%).

4.3.2 Alternative populations: Frontana/Inia66 and International Triticeae Mapping Initiative (ITMI)

As in the Seri/Babax RILs under DRT (1999/2000), small associations were observed between the assessed physiological traits and yield (chlorophyll at booting, r= –0.11; CT at booting in the morning, r= –0.37; and CT at booting in the afternoon, r= –0.49; p<0.0001), alternative segregating genetic pools were sought. Two possible single-seed descent candidate populations, already genetically mapped, were available within CIMMYT's wheat germplasm programme endeavours: Frontana/ Inia66, FXI (Ayala *et al.* 2002; William *et al.* 2005) and the International Triticeae Mapping Initiative, ITMI, population (Nelson *et al.* 1995*a*,*b*; Van Deynze *et al.* 1995; http://wheat.pw.usda.gov/).

Thus, in cycle 2000/2001, the two alternative populations were concurrently sown with the original target one, following the same experimental layout and under the previously described water regimes. Both alternative populations were evaluated for a number of traits (chlorophyll at grain filling, CT, NDVI, height and phenology). However, under DRT the large ranges in phenology and height of the 118 FXI (24 days, 110 cm) and of the 98 ITMI lines (36 days, 59 cm), prevented a proper assessment of the physiological traits, leading to null segregation of the traits (FXI) and of confounding effects of canopy architecture in this particular experience (NDVI at grain filling with CT: $covar$=0.002, r= 0.07; p<0.0001) –although there is evidence (Reynolds *et al.* 2000) of association of this trait with yield.

As significant trait associations with yield (chlorophyll at booting, r= 0.43; CT at booting, r= –0.78; CT at grain filling, r= –0.71; p<0.0001) were found under DRT in the Seri/Babax RILs, it was evident that the original target population was the most adequate genetic pool for the dissection of drought adaptation.

4.4 ASSOCIATION OF PHYSIOLOGICAL TRAITS AND YIELD UNDER DROUGHT UNDER FIELD CONDITIONS

CT consistently showed negative phenotypic and genotypic correlations with yield under DRT (Table 9), across phenological stages and time of day in Mexico (Fig.9). However, CT measured under RED and IRR in Mexico was not as strongly associated with yield either in Mexico or Australia in comparison with CT measured under DRT (Table 10). CT measured at Charlick in 2001 showed association with yield *in situ* as well as with Mexican DRT yields (Table 10). At Charlick in 2001, the moderate phenotypic correlations between CT and yield increased as the cycle progressed (Table 10). At Roseworthy, only the afternoon measurements at grain filling paralleled those at booting in the Charlick experiment. However, at the two South Australian sites, the conditions for taking canopy measurements were less than ideal: there was strong and consistent eolic activity and the occurrence of constant cloudiness (on average, 11 days per month), which limited the number of CT measurement rounds performed. This possibly explained such moderate association. No physiological measurements were taken at Charlick nor at Minnipa in 2002.

Table 9. Phenotypic (r) and genetic correlations (r(g)), contribution (R^2) and broad-sense heritabilities (h^2) between selected traits and yield in the Seri/Babax population under different hydrological conditions (Yaqui valley, Mexico)

	DROUGHT STRESS[A]				*REDUCED IRRIGATION*[B]				*FULL IRRIGATION*[A]			
TRAIT	r	R^2	r(g)	h^2	r	R^2	r(g)	h^2	r	R^2	r(g)	h^2
CHLO-boot (SPAD)[A]	0.25	0.06	0.62	0.45	0.05	0.00	0.24	0.12	0.17	0.03	.	0.19
CHLO-gf (SPAD)[A]	0.23	0.05	0.48	0.20	-0.16	0.03	-0.56	0.07	0.02	-	-0.04	0.11
CTAM-boot (°C)[A]	-0.73	0.54	-0.99	0.64	.	.	.	.	.	.	.	.
CTPM-boot (°C)[A]	-0.78	0.61	-0.98	0.66	.	.	.	.	.	.	.	.
CTAM-gf (°C)[A]	-0.72	0.51	-0.91	0.61	-0.05	0.00	-0.43	0.35	-0.23	0.05	-0.17	0.21
CTPM-gf (°C)[A]	-0.74	0.54	-0.90	0.67	-0.35	0.12	-0.47	0.32	-0.05	0.00	-0.11	0.25
OP-gf (MPa)[A]	-0.16	0.03	-0.85	0.04	-0.08	0.01	-0.10	0.23	-0.05	0.00	0.12	0.14
RNDVI-boot (nm)[B]	0.55	0.30	0.76	0.47		.	.	.	.	.	.	.
RNDVI-gf (nm)[B]	0.21	0.05	0.43	0.17		.	.	.	.	.	.	.
LROLL-gf[B]	-0.31	0.10	0.00	0.00	0.04	0.00	.	.	0.10	0.01	.	.
YIELD (gm^{-2})	.	.	.	0.85	.	.	.	0.82				0.74

NOTE:
n=169, in all experiments; p<0.0001.
[A], Calculated from cycles: 1999/2000, 2000/2001 & 2001/2002.
[B], Calculated from cycles: 2000/2001 & 2001/2002.
CHLO-boot, Chlorophyll SPAD measurements at booting; CHLO-gf, Chlorophyll SPAD measurements at grain filling; CTAM-boot, Canopy temperature at booting, in the morning (9:00-11:00h); CTPM-boot, Canopy temperature at booting, in the afternoon (14:00-16:00h); CTAM-gf, Canopy temperature at grain filling, in the morning (9:00-11:00h); CTPM-gf, Canopy temperature at grain filling, in the afternoon (14:00-16:00h); OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised Difference in Vegetative Index (red spectrum), at booting; RNDVI-gf, Normalised Difference in Vegetative Index (red spectrum), at grain filling; YIELD, yield.

Table 10. Phenotypic associations of canopy temperature (°C) and yield (gm^{-2}) under various hydrological conditions (Yaqui valley, Mexico & Charlick, South Australia)

	CANOPY TEMPERATURE											
	MEXICO										*AUSTRALIA*	
	DROUGHT STRESS					*REDUCED IRRIGATION*		*FULL IRRIGATION*			*CHARLICK01*	
YIELD	BOOTING Y9900[A]	BOOTING Y0001[B]	BOOTING Y0102[C]	GRAIN FILLING Y0001[B]	GRAIN FILLING Y0102[C]	GRAIN FILLING Y0001[B]	GRAIN FILLING Y0102[C]	GRAIN FILLING Y9900[A]	GRAIN FILLING Y0001[B]	GRAIN FILLING Y0102[C]	BOOTING 2001[D]	GRAIN FILLING 2001[D]
DROUGHT STRESS-Y9900[A]	-0.48	-0.51	-0.32	-0.46	-0.35	-0.28	-0.15	-0.19	-0.21	0.03	-0.07	-0.07
DROUGHT STRESS-Y0001[B]	-0.37	-0.86	-0.67	-0.69	-0.68	-0.54	-0.07	-0.25	-0.28	-0.05	-0.21	-0.05
DROUGHT STRESS-Y0102[C]	-0.18	-0.63	-0.84	-0.47	-0.83	-0.37	-0.05	-0.30	-0.16	-0.06	-0.22	0.02
CHARLICK-2001[D]	-0.15	-0.35	-0.24	-0.26	-0.24	-0.33	0.03	-0.15	-0.06	-0.27	-0.21	-0.35
MEAN	*-0.30*	*-0.59*	*-0.52*	*-0.47*	*-0.53*	*-0.38*	*-0.06*	*-0.22*	*-0.18*	*-0.09*	*-0.18*	*-0.11*

NOTE:

[A], Mexican cycle (Yaqui valley, Northwestern Mexico, Mexico): 1999/2000; n=169; p<0.0001.

[B], Mexican cycle (Yaqui valley, Northwestern Mexico, Mexico): 2000/2001; n=169; p<0.0001.

[C], Mexican cycle (Yaqui valley, Northwestern Mexico, Mexico): 2001/2002; n=169; p<0.0001.

[D], Australian cycle (South Australia, Australia): 2001; n=146; p<0.0001.

Table 11. Phenotypic associations between selected traits and yield under drought stress (Yaqui valley, Mexico)

	ANT	MAT	CHLO -boot	CHLO -gf	CTAM –boot	CTPM –boot	CTAM -gf	CTPM -gf	RNDVI -boot[A]	RNDVI -gf[A]	OP-gf	LROLL -gf[A]	dNDVI[A]	dCHLO	HEIGHT	ATKW	KNO
MAT	0.40																
CHLO-boot	-0.12	-0.12															
CHLO-gf	0.02	0.20	0.34														
CTAM-boot	0.10	0.04	-0.22	-0.31													
CTPM-boot	0.12	-0.05	-0.24	-0.31	0.80												
CTAM-gf	0.16	0.09	-0.24	-0.24	0.61	0.66											
CTPM-gf	0.15	-0.01	-0.16	-0.20	0.62	0.72	0.76										
RNDVI-boot[A]	-0.08	-0.18	0.15	0.13	-0.47	-0.44	-0.45	-0.40									
RNDVI-gf[A]	0.26	0.61	0.05	0.34	-0.34	-0.41	-0.30	-0.35	0.16								
OP-gf [A]	-0.03	-0.03	0.07	-0.07	0.13	0.15	0.19	0.22	-0.13	-0.16							
LROLL-gf[A]	-0.13	-0.35	-0.12	-0.26	0.37	0.40	0.40	0.40	-0.24	-0.62	0.05						
dNDVI[A]	-0.29	-0.67	0.03	-0.26	0.10	0.17	0.06	0.14	0.34	-0.87	0.09	0.47					
dCHLO	-0.10	-0.27	0.36	-0.76	0.16	0.14	0.07	0.09	-0.02	-0.30	0.12	0.18	0.28				
HEIGHT	-0.20	-0.06	0.25	0.21	-0.62	-0.65	-0.69	-0.64	0.42	0.36	-0.14	-0.39	-0.14	-0.04			
ATKW	-0.09	-0.18	0.16	0.26	-0.38	-0.37	-0.41	-0.39	0.23	-0.04	-0.10	-0.11	0.15	-0.15	0.38		
KNO	-0.26	-0.24	0.23	0.18	-0.69	-0.75	-0.67	-0.70	0.54	0.25	-0.14	-0.31	0.03	-0.02	0.63	0.20	
YIELD	-0.26	-0.28	0.25	0.23	-0.73	-0.78	-0.72	-0.74	0.55	0.22	-0.16	-0.31	0.07	-0.06	0.67	0.43	0.97

NOTE:

n=169; p<0.0001.

All data calculated from the mean of data obtained during cycles 1999/2000, 2000/2001 & 2001/2002, except: [A], Mean calculated using datasets corresponding to cycles 2000/2001 & 2001/2002.

ANT, Days to anthesis; MAT, Days to maturity; CHLO-boot, Chlorophyll SPAD measurements at booting; CHLO-gf, Chlorophyll SPAD measurements at grain filling; CTAM-boot, Canopy temperature at booting, in the morning (9:00-11:00h); CTPM-boot, Canopy temperature at booting, in the afternoon (14:00-16:00h); CTAM-gf, Canopy temperature at grain filling, in the morning (9:00-11:00h); CTPM-gf, Canopy temperature at grain filling, in the afternoon (14:00-16:00h); RNDVI-boot, Normalised difference in vegetative index (red spectrum), at booting; RNDVI-gf, Normalised difference in vegetative index (red spectrum), at grain filling; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; dNDVI, average NDVI loss under drought stress; dCHLO, average chlorophyll loss under drought stress; HEIGHT, Height at maturity; ATKW, A thousand kernels weight; KNO, Kernel number; YIELD, Yield.

OP in Mexico showed a small, negative association with yield at the phenotypic level (r= –0.16, p<0.0001; Table 11) and a large negative genetic correlation (on average, R(g)= –0.85, p<0.0001) under DRT (Table 9). Similarly, in Australia there was a negative phenotypic correlation with yield (r= –0.23 p<0.0001).

Leaf rolling showed low phenotypic association with yield (r= –0.31, p<0.0001) in the two Obregon cycles (2000/2001 & 2001/2002) in which it was measured (Table 11), but it was not genetically correlated (Table 9). At Charlick, leaf rolling was less associated to yield than at Obregon (r= –0.24, p<0.0001).

Chlorophyll content showed a small phenotypic association with yield under DRT (r=0.24, p<0.0001), even though it was negligible under RED or IRR (Table 9). At both phenological stages, the trait was genetically correlated with yield under DRT (Table 9). When comparing chlorophyll between stages there was only a weak association (r=0.34, p<0.0001), while the loss of chlorophyll between these two stages showed no association with yield under DRT (Table 11). At Charlick, chlorophyll content was moderately correlated with yield at both booting and grain filling (averaged, r=0.24, p<0.0001), as in Obregon, and when comparing chlorophyll content between stages, the association was moderately high (r=0.56, p<0.0001). At Roseworthy, association with of chlorophyll at grain filling with yield was smaller (r= 0.19, p<0.0001) than any at Charlick.

NDVI measured at the booting stage showed a stronger phenotypic and genetic correlation with yield than when measured during grain filling under DRT, although both were significant (Table 9). When comparing NDVI between stages there was no association and change in NDVI between stages was not associated with yield under DRT (Table 11).

4.5 INFLUENCE OF ENVIRONMENT IN RESPONSE

As the genotypic response under varying soil moisture conditions is strongly influenced by the environment, varying from cycle to cycle (*i.e.*, year to year) even in stable environments (as Obregon), different evaluations were performed attempting to discriminate the effects of alternate major factors from drought *per se* in a site (Obregon) without soil-confounding factors.

Nematodes

Even though a visual screening on the presence of the soil-borne pathogen *P.thornei* was performed in soil samples to explain the significant decrease in yield under DRT in the 1999/2000 cycle, it was found that the association with yield decrease was low at the two depths in where the screening was estimated (0-20 and 20-40 cm depths, where r=0.13 and r=0.10 at p<0.0001, respectively; no covariance was observed either). The number (3.12±2.0) of *P.thornei* in a gram of the soil sampled where the selected RILs grew was similar to that reported (Nicol *et al.* 2001) for resistant germplasm –defined as a reduction in the multiplication of the pathogen. Similarly, the presence (in number) of *P.thornei* in the soil samples where both parents grew in the field was not significantly different, although it was found that in both depths in the Babax samples (0-20 cm, 2.09±0.9; 20-40 cm, 2.44±0.07) the figure was lower than that in Seri's (0-20 cm, 2.72±0.91; 20-40 cm, 3.74±0.76). The latter suggest that both parents (particularly Babax) are resistant to a pathogen that is known to be a major pathogen to wheat in Australia (Vanstone & Taylor 1992), in Mexico (Nicol & Ortiz-Monasterio 2004; Nicol 2000) and in other arid world regions (Orion *et al.* 1984; Nicol *et al.* 2001), significantly decreasing its yield (*ca.*30%).

As the preliminary results suggest that the Seri/Babax germplasm response in the DRT treatment in the 1999/2000 cycle was not affected by the pathogen, the most likely reason for the significant impact of drought on the genotypic response would be the number of seeds per square meter sown in what was considered an evaluatory field experiment. The main objective of such evaluatory experiment, was: a) to evaluate (under field conditions) the diverse avenues of expression of the adaptation to drought, and b) the increase of seed for subsequent field cycles.

Boron toxicity and mineral deficiencies

Free of areas with high concentrations of Boron, the selected site at Obregon obviated the need of screening for Boron tolerance. However, as the occurrence of subsoil toxicity is observed in South Australian sites under periods of high transpiration, there were incentives for evaluation for the attribute. It was found that there was no significant difference between both parents, as they were both susceptible (*ca.*8% for Seri and *ca.*9% for Babax in root reduction at 50 μM B). The latter was confirmed by a grain nutrient analyses (Plant Nutrition Laboratory, Waite Agricultural Research Institute) on parental samples from the Minnipa site and at Charlick in 2002. While

the Boron concentration (mg kg^{-1}) in Charlick was the same in both parents (<2.0), at Minnipa it was higher in Babax (5.9±0.50) than in Seri (5.4±0) but not significantly different (p<0.05).

The analyses also showed that there was no defficiency for Phosphorus at neither site, but at Minnipa the uptake in Babax was 8% lower than that of Seri, being the opposite at Charlick in 2002 by *ca.*5%. Copper doubled its critical value (200 mg kg^{-1}) at both sites, but the concentration in Babax was lower than in Seri in ten percentage points at Minnipa, while the opposite (by *ca.*5%) was observed at Charlick in 2002. Calcium was moderately low (*ca.*400 mg kg^{-1}) at both sites, but in Babax the concentration was consistently lower than Seri's, with Minnipa 5% lower than the figure at Charlick (*ca.*17%). Although the concentration of Sodium was low (<58 mg kg^{-1}) and medium (range 34-129 mg kg^{-1}) at Minnipa and Charlick in 2002, respectively, the concentration in Babax was, on average, 17% lower than Seri's. At Charlick in 2002, Manganese concentration was marginally higher in Babax (25±4.24) than in Seri (24±4.24), being the opposite at Minnipa (Seri, 47.5±0.71; Babax, 42.5±3.5) but by a non-significant (p<0.05) 11%.

4.5.1 GxE interactions

Having obviated some environmental limitations --nematodes, toxicities or deficiencies-- in the Obregon agroecological site, an exercise was conducted on dissecting the principal factors influencing the ample genotypic response to drought stress, seemingly being exerted via physiological adaptation. Table 12 summarises the influence of GxE in the expression of the traits in the environment, while --except otherwise stated forthcomingly-- the complete biplots are found in APPENDIX 1. No GxE examination was performed for the Australian environments, as the statistical methodology requires more than two cycles in the same environment to be ascertained (F.J.Crossa, *pers.comm.*).

Table 12. Genotype-by-environment interactions for various traits under different hydrological conditions (Yaqui valley, Mexico)

TRAIT	TOTAL GxE (%)	FACTOR 1 (%)	FACTOR 1 TREATMENT(R^2)[A]	FACTOR 2 (%)	FACTOR 2 TREATMENT(R^2)[B]
ANT	68	52	IRR1 (0.95)/IRR3(0.95)	16	IRR2 (0.45)
MAT	85	68	DRT2 (1.00)	17	DRT1 (0.60)/IRR1 (0.60)
CHLO-boot	58	33	DRT3 (0.78)	25	DRT3 (0.40)
CHLO-gf	47	29	RED3 (1.00)	18	DRT2 (0.60)
CTAM-boot	92	56	DRT1 (1.00)	36	DRT2 (0.82)
CTPM-boot	92	66	DRT2 (1.00)	26	DRT1 (0.82)
CTAM-gf	66	44	DRT2 (1.00)	22	IRR3 (0.71)
CTPM-gf	67	51	DRT2 (1.00)	16	DRT3 (0.59)
OP-gf	81	66	DRT3 (1.00)	15	DRT2(0.65)
LROLL-gf	64	41	RED3 (1.00)	23	RED2 (0.65)
HEIGHT-gf	75	57	DRT3 (1.00)	18	IRR3 (0.67)
ATKW	71	57	IRR3 (0.90)	14	DRT1 (1.00)
KNO	74	60	DRT2 (0.85)/RED2(0.85)	14	IRR1 (1.00)
YIELD	73	63	DRT2 (1.0)	10	IRR1 (0.95)

NOTE:
n=169; p<0.0001.
TRAITS: ANT, Days to anthesis; MAT, Days to maturity; CHLO-boot, Chlorophyll SPAD measurements at booting; CHLO-gf, Chlorophyll SPAD measurements at grain filling; CTAM-boot, Canopy temperature at booting, in the morning (9:00-11:00h); CTPM-boot, Canopy temperature at booting, in the afternoon (14:00-16:00h); CTAM-gf, Canopy temperature at grain filling, in the morning (9:00-11:00h); CTPM-gf, Canopy temperature at grain filling, in the afternoon (14:00-16:00h); RNDVI-boot, Normalised difference in vegetative index (red spectrum), at booting; RNDVI-gf, Normalised difference in vegetative index (red spectrum), at grain filling; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; dNDVI, average NDVI loss under drought stress; dCHLO, average chlorophyll loss under drought stress; HEIGHT, Height at maturity; ATKW, A thousand kernels weight; KNO, Kernel number; YIELD, Yield.
TREATMENTS: DRT, drought stress; RED, reduced stress; IRR, full irrigation. 1, 1999/2000; 2, 2000/2001; 3, 2001/2002.

The influence of the GxE interaction on phenology seemed to be mostly influenced by one factor across treatments, with a greater interaction at maturity (85%) than at anthesis (68%). In the 1999/2000 cycle under both DRT and IRR, the expression of both traits was closely related (APPENDIX 1).

When graphically (APPENDIX 1) dissecting the GxE influence (Table 12) on the expression of chlorophyll at booting (58%), it was observed that it varied strongly when compared to its measurement at grain filling (47%), with close relatedness under all treatments in all cycles except for RED (APPENDIX 1): No relatedness was found at booting and at grain filling in the 2000/2001 and 2001/2002 cycle, respectively.

Of all of the traits measured, CT at booting was the trait with the highest influence by the GxE interaction (92%, Table 12). In both the morning and afternoon in 2000/2001 and 2001/2002, the expression of CT was closely related under DRT –an observation with no relation to the trait expression in the 1999/2000 cycle (Fig.12). A similar expression was observed at CT at grain filling in the morning –there was no measurement of the trait in the afternoon in the 1999/2000. In such stage in the afternoon, the GxE strongly influenced the trait expression, with the observation of no relatedness between the 2000/2001 and 2001/2002 DRT measurements. The low seed number in the experimental 1999/2000 is a likely cause of the large GxE interaction observed (Fig. 12).

As observed with Chlorophyll at grain filling, OP was the trait with the least ample expression, and with important dicotomous influence by the two factors in the two cycles when the trait was measured.

Leaf rolling at grain filling showed the least GxE interaction (64%, Table 12), a consistent influence when comparing the very closely-related influence in treatments, except for RED in the 2002/2002 cycle (close-to-zero relatedness).

With height at grain filling, the GxE interaction discriminated the expression of the trait under all treatments from the DRT ones, which were related.

While the influence of the effect of the GxE interaction was large in the expression of a thousand kernels weight (ATKW) (Fig.12), of kernel number (KNO) and of yield (YLD), relationships were complex, from ATKW having the least across treatments and YLD with the most. GxE discriminated all three DRT treatments from the other two (RED and IRR), which were related. With KNO, DRT in 1999/2000 and in 2000/2001 were closely related, but only positively discriminated by Factor 1; in contrast, the influence of Factor 2 was evident in discriminating all the RED and IRR treatments, but particularly the was in the 1999/2000 cycle from the rest. The latter was observed with the GxE interaction for yield, but the overall influence of GxE with the other treatments (RED and DRT) across the three cycles was intricated. There was a highly closely-related expression in the 2001/2002 cycle between DRT and RED, with RED closely related to the IRR in 2001/2002, which, in turn was related to the RED in 2000/2001. Overall, their expression is negatively influenced by Factor 2, which discriminates the expression in the other two DRT and the remaining IRR.

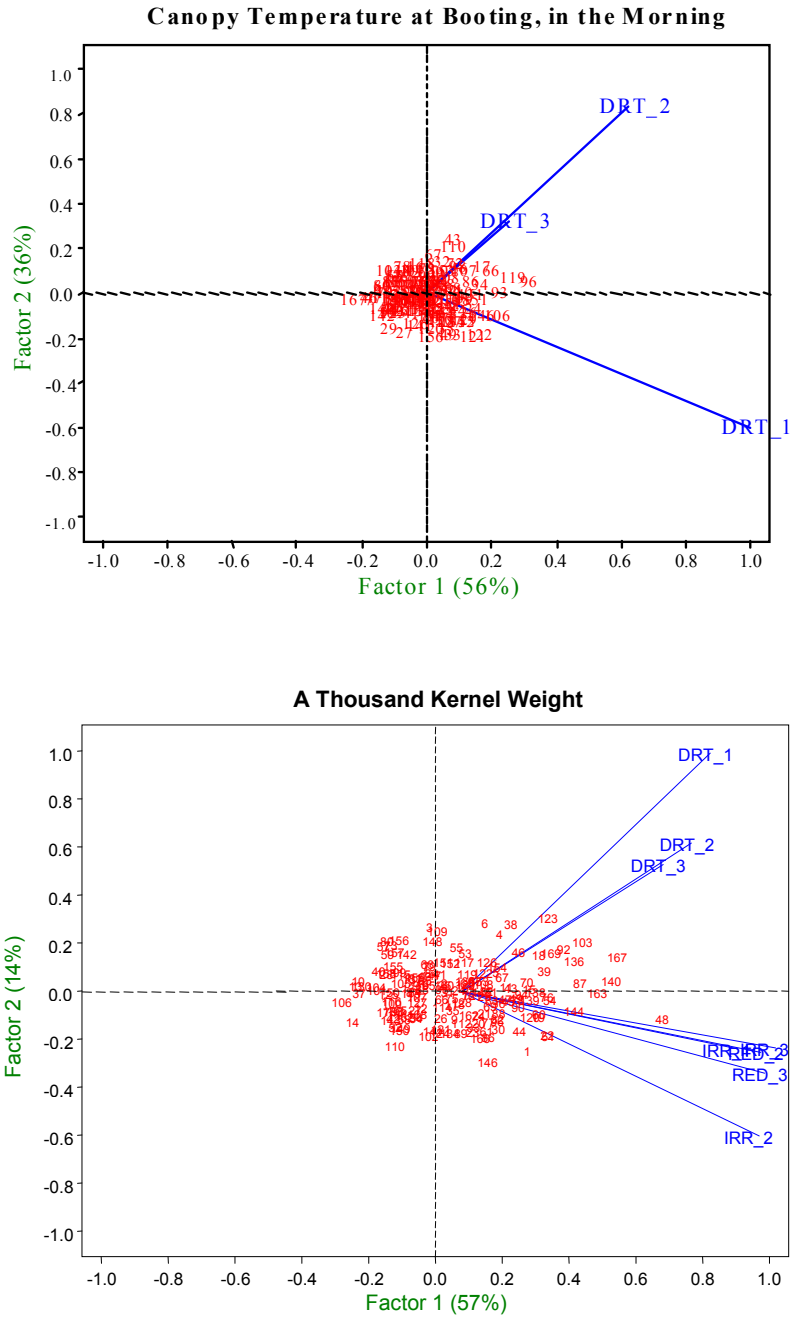


Figure 12. Genotype-by-environment interactions for two selected traits under various hydric conditions (DRT, drought stress; RED, reduced irrigation; and, IRR, full irrigation). Seri/Babax population, Yaqui valley, Mexico (cycles: 1, 1999/2000; 2, 2000/2001; 3, 2001/2002).

4.6 MOLECULAR CHARACTERISATION OF DROUGHT ADAPTATION

4.6.1 DNA substrate and marker methodologies

The same DNA extraction methodology (Hoisington *et al.* 1994) was used in both laboratories (CIMMYT and CSIRO) in order to ensure the uniformity in the quality of the DNA.

The assay optimisation for the PCR-based markers allowed to establish an standard set of experimental conditions outlined for all PCR reactions (Table 13), aiming at reducing (at the most) the experimental errors derived from the preparation of reaction mixtures. Thus, it allowed to establish that the only two varying elements in the methodology was: a) thermal cycling (dependent on a primer sequence basis for its PCR amplification) and, b) the detection methodology (chemifluorescence at CIMMYT and radiolabelling at CSIRO).

Table 13. Reagent optimisation for the standardised PCR assessments

REAGENT	INITIAL/ STOCK CONCENTRATION	FINAL CONCENTRATION
Genomic DNA	5 ng μl^{-1}	25 ng μl^{-1}
Primers (combined *forward* and *reverse*)	5 μM	0.25 μM
Taq (*Thermus aquaticus*) DNA polymerase	5 U μl^{-1}	1 U
Taq Buffer	10X	1X
Magnesium Chloride	50 mM	2.5 mM
Glycerol	100%	10%
Deoxyoligonucleotides mix (dATP, dGTP, dCTP, dTTP)	10 mM*	200 μM

* Concentration for each dNTP.

As for the AFLPs, optimum experimental conditions were carefully assessed in every quality assurance step of the methodology, contributing to obtaining limpid, definable results in the exposure films (as shown in Figure 13), and a prompt scoring of AFLP products.

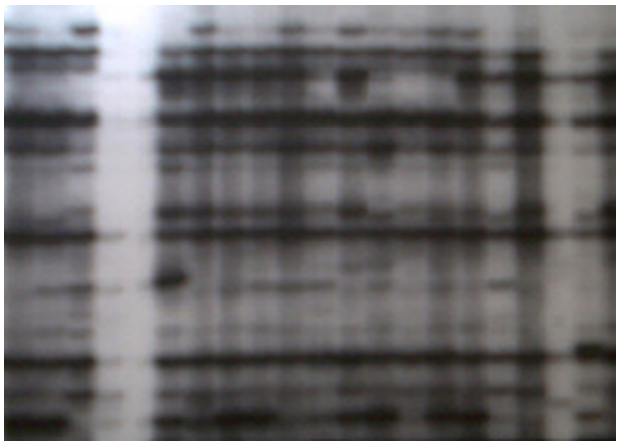


Figure 13. Luminograph (*detail*) with chemiluminiscently-exposed AFLP products (PstIAAGMseICGT) on the Seri/Babax RILs.

4.6.2 Genetic characterisation

4.6.2.1 Genetic scrutiny of parents

While the allelic variation study between samples of parents with two microsatellites showed consistencies in polymorphism patterns, the AFLP evaluation did not (Fig.14). One sample of parental genotype Seri (sample number 7) showed significant differences in the AFLP products amplification with three of the four enzyme/primer assortments (*Pst*IACC*Mse*ICGA, *Pst*IACG*Mse*ICAA, *Pst*IACG*Mse*ICGT) when compared to the rest of the samples –which shared identical AFLP product patterns in all four assays. With the *Pst*IACC*Mse*ICGA assortment, seven additional differing AFLP products were observed in sample number 7, while with *Pst*IACG*Mse*ICAA a total of 43 major AFLP products were not amplified, and with *Pst*IACG*Mse*ICGT any AFLP product was amplified. In contrast, no differences were observed between Babax parental samples in any of the AFLP products.

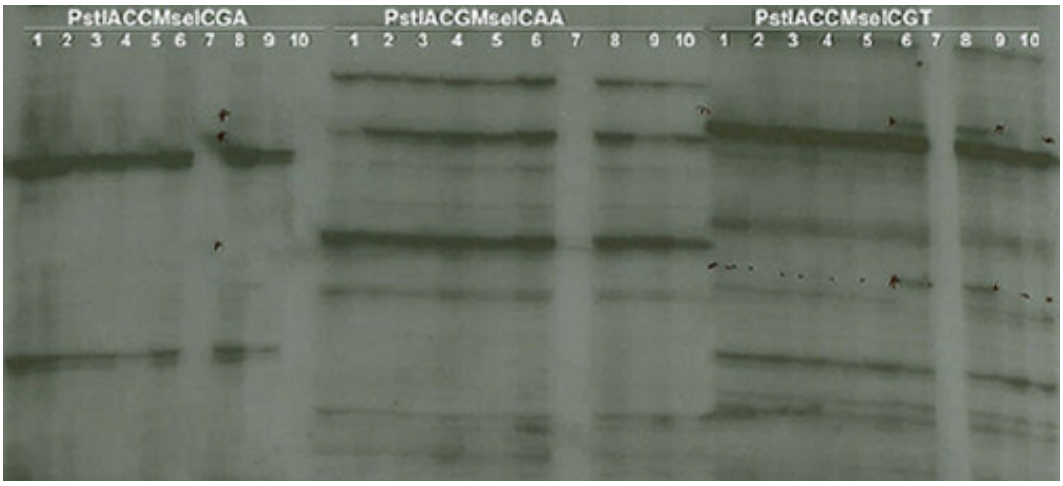


Figure 14. Homozygosity screening of parental genotypes. Luminograph (*detail*) showing an allelic variation of ten samples of parental genotype Seri when analysed with various AFLP enzyme/primer assortments.

4.6.2.2 Parental polymorphism assessment

CIMMYT

A low polymorphism was observed in the parental screening on 3% agarose gels: 64 GWM microsatellites were amplified (28% of total assayed), 5 WMC (3%), 4 STS (7%) and 15 DUP (8%). Concurrently, varying amplification qualities were observed, being classified as *optimum* (*i.e.*, clearly definable) and *medium* (*i.e.*, close) polymorphisms (Fig.15). Only 25 GWM were *optimum*. All other primers (remaining GWM, WMC, STS and DUP) rendered *medium* quality polymorphisms. Of all *medium* primers subsequently electrophoresed on PAGE gels, only an additional subset of four *medium* GWM polymorphisms was distinguished, despite some PAGE gels were silver-stained for attempting to increase amplicon differentiation.

CSIRO

In terms of polymorphism for the PCR-based markers, the experimental results at CSIRO were similar to CIMMYT's, as 25% of the radiolabelled primers assessed were polymorphic. From these, 51 were GWM and 10 were STS, while 8 and 5 were from the GDM and WMC collections, respectively.

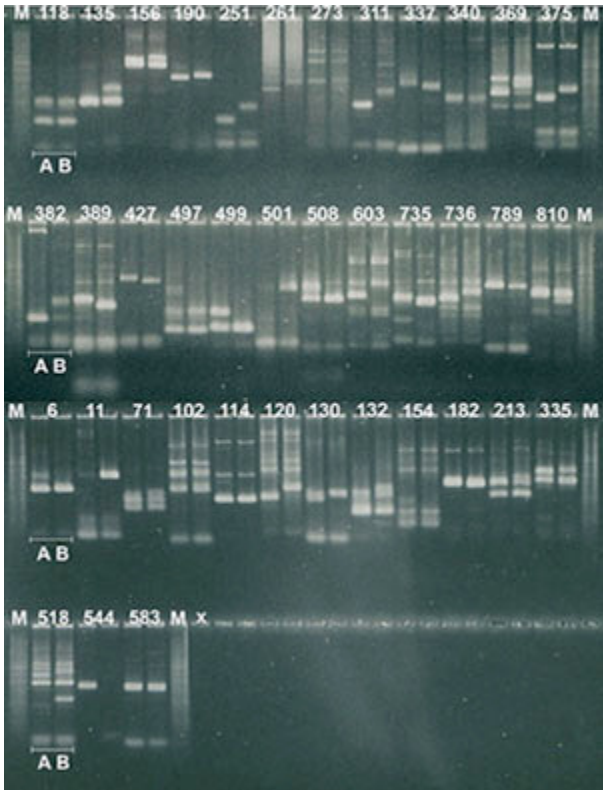


Figure 15. Example of the verification of an initial parental screening, prior to the BSA assessments, with a pre-selected set of polymorphic GWM markers (CIMMYT collection), as electrophoresed in a 3% agarose (SEAKEM : METAPHOR, 1 : 1) gel. GWM marker identification is specified for every pair of parental samples (A, Seri; B, Babax) evaluated. Previous GWM amplicon classification considered two amplification/ polymorphism qualities to be verified in the markers confirmatory evaluation in the shown gel (top to bottom): "optimum", tiers 1 and 2; "medium", tiers 3 and 4. Standard molecular reference (ϕX174/*Hae*III), M, and end of test, x, are indicated.

4.6.2.3 Bulked segregant analysis strategy

4.6.2.3.1 Preliminary evaluation

The four preliminary experiments conducted prior to the molecular characterisation were decisive in the development (and efficacy) of the posterior INTEGRAL BSA, as they provided valuable information on the polymorphic allele segregation within the population genotypes and important considerations for outlining the bulks for the INTEGRAL BSA:

1. Polymorphism consistency. Based on the results obtained from assessing the parents with sixteen polymorphic markers, the evaluation (with the same 22 RILs) was expanded with the other markers that were polymorphic at CIMMYT. While only 23 GWM amplicons (21 single-allele markers[13] and one, GWM149 with two amplicons) showed consistent allele segregation (with that of the parents) within the 22 RIL subset, none of the WMC or STS amplicons amplified as expected –and thus, were not used for the INTEGRAL BSA. Although reduced in number, the selected set of 22 polymorphic primers covered all 7 chromosomic groups in all of the three genomes of hexaploid wheat (subset in Table 16).

Albeit this assay was deemed essential within the experimental strategy at CIMMYT –for performing the subsequent set of experiments, as financial resources were scarce–, at CSIRO no pre-screening with the resulting polymorphic primers was performed in the population for segregation verification. Any marker that gave a clear polymorphism was run across the progeny set (C.L. McIntyre, *pers. comm.*).

2. Selection of bulks. About half of the preliminary bulks showed a consistent amplification pattern with that of the parents when assessed with sixteen PCR-based markers (Table 14). Based on their pattern consistency, the bulks were categorised (categories: *promising*, *interesting*, *to be considered*) for prioritising the subsequent debulking examinations.

3. Debulking. After assessing the genotypes selected for producing each bulk with the same sixteen PCR-based markers, their segregation ratios were combined for each major class –the four bulks allele ratios (Fig.16) were consolidated in two (Fig.17): resistant and susceptible– and each bulk was classified according to such segregation ratio (Table 14). It was expected that for any phenotypic class (either a resistant or susceptible bulk), a higher number of alleles was to be found in the debulked samples that shared a position in the bulk representing a distal portion for their corresponding extreme phenotypic class. Overall, it was observed that such was the tendency. However, when debulking the corresponding genotypes, the allele pattern was not clearly reflecting their phenotypic class in *ca.*40% of the bulks. Further, a large proportion of heterozygotes were found to have been used for preparing the bulks (*ca.*30%).

lxxxi

[13] GWM markers codes (Röder *et al.* 1998): 6, 113, 120, 135, 190, 251, 261, 311, 337, 340, 369, 375, 382, 389, 427, 480, 499, 508, 518, 566 and 617.

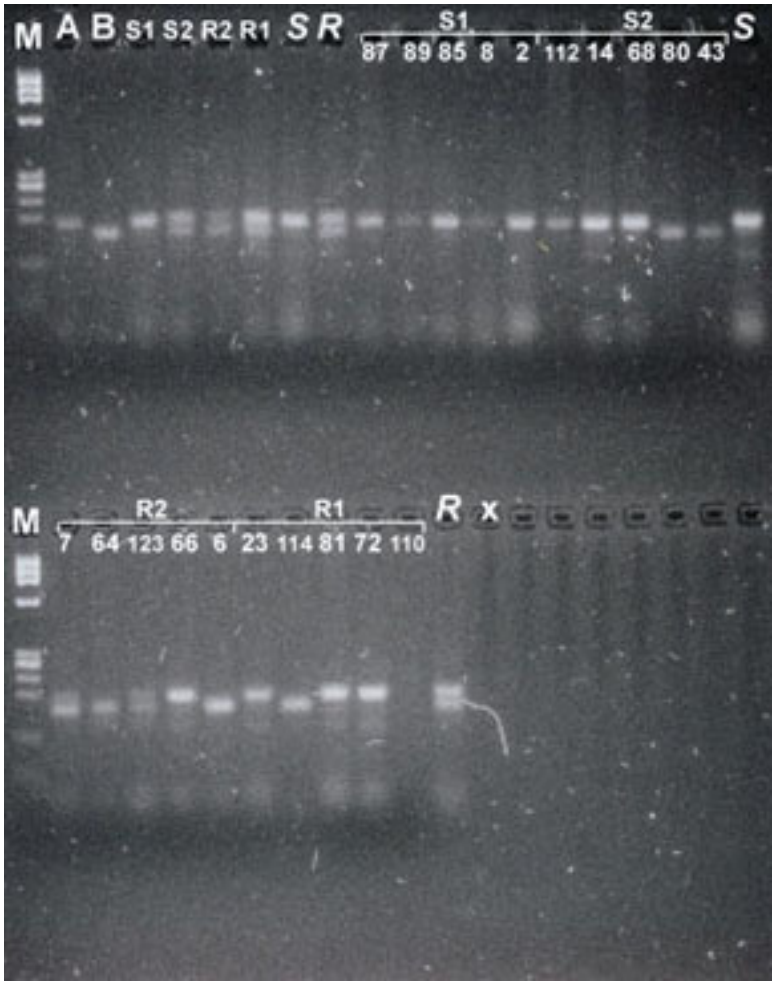


Figure 16. Example of preliminary BSA of Osmotic Potential at Grain Filling with GWM251 marker, as electrophoresed in a 3% agarose (SEAKEM : METAPHOR, 1 : 1) gel. Parents (A, Seri; B, Babax), 5-genotype *highly* susceptible bulk (S1, selected from distal position in averaged data), 5-genotype *moderately* susceptible bulk (S2,selected immediately adjacent to S1) 5-genotype *moderately* resistant bulk (R2,selected immediately adjacent to R1), 5-genotype *highly* resistant bulk (R1, selected from distal position in averaged data), 10-genotype bulks (*S*, *susceptible*: S1 and S2; *R*, *resistant*: R1 and R2) and corresponding debulked RILs (genotypes of 5-genotype bulks), standard molecular reference (ϕX174/HaeIII), M, and test end, x, are indicated.

Table 14. Preliminary BSA on the trait expression of the Seri/Babax population (167 RILs) under drought. Segregation ratios per trait bulk

PRIMER[1]	K[2]	OP-gf S[3]/R[4]	CHLO-boot S/R	CHLO-gf S/R	CTAM-boot S/R	CTPM-boot S/R	CTAM-gf S/R	CTPM-gf S/R	YIELD S/R
GWM135	1A	8 : 1, 1no / 1 : 8, 1no▲	2 : 7 / 6 : 4✦				6 : 2, 2no / 4 : 6●	6 : 4 / 2 : 7, 1no✦	2 : 8 / 5 : 5✦
GWM 337	1D	2 : 6, 2H / 6 : 3, 1no✦	4 : 4, 2no / 8 : 2✦	4 : 6 / 7 : 3✦	2 : 7, 1no / 5 : 5✦	10no / 4 : 5, 1no✦			5 : 3, 1H / 4 : 6✦
GWM 382	2A; 2B; 2D	4 : 6 / 8 : 2✦	7 : 3 / 3 : 7▲	5 : 5 / 3 : 7●				6 : 4 / 5 : 5✦	
GWM 130	2B; 7A, 7B, 7D	4 : 5, 1no / 4 : 6●			6 : 3, 1no / 5 : 5●				5 : 3, 2no / 4 : 5, 1no✦
GWM 261	2D	5 : 3, 2no / 4 : 5, 1H●		6 : 4 / 4 : 4, 1H●			3 : 6, 1H / 7 : 2, 1H✦		6 : 3, 1no / 3 : 7●
GWM 369	3A	5 : 3, 2no / 4 : 6✦	4 : 4, 2no / 5 : 5✦						
GWM 389	3B								
GWM 251	4B	7 : 3 / 3 : 4, 2H●		6 : 4 / 6 : 3●			4 : 4, 1H, 1no / 3 : 7●	4 : 5, 1H / 3 : 7●	8 : 2 / 4 : 5, 1no●
GWM 375	4B	7 : 3 / 4 : 4, 2H✦	2 : 6, 2no / 3 : 5, 2H✦				4 : 4, 1H, 1no / 3 : 6, 1no✦	5 : 5 / 2 : 8●	8 : 2 / 5 : 4, 1no●
GWM 499	5B	5 : 4, 1H / 4 : 6●	6 : 4 / 6 : 4●			7 : 3 / 4 : 6▲	5 : 4, 1H / 5 : 4✦	4 : 6 / 2 : 8●	
GWM 190	5D	5 : 4 / 2 : 8●	2 : 5 / 4 : 6✦				4 : 5, 1no / 4 : 5✦	6 : 4 / 6 : 4✦	7 : 3 / 8 : 2●
GWM 508	6B				2 : 7, 1no / 7 : 3✦				
GWM 518	6B	4 : 5, 1no / 6 : 4●							6 : 3, 1no / 5 : 5✦
GWM735	unknown	6 : 3, 1no / 4 : 3, 2H✦		6 : 4 / 6 : 4●	4 : 5 / 3 : 7●	6 : 3, 1H / 4 : 6●	4 : 4, 1H, 1no / 3 : 7●	4 : 5, 1H / 3 : 6, 1H✦	8 : 2 / 6 : 4●
GWM736	unknown	5 : 4, 1no / 5 : 5✦	10no / 2 : 5, 1H, 2no✦				4 : 6 / 3 : 7●	4 : 6 / 3 : 7●	

NOTE:
Trait information is the averaged phenotypic data collected in Mexico (cycles 2000/2001 & 2001/2002) and at two localities in Australia (Charlick in 2001)
Traits are, as follows: OP-gf, Osmotic potential at grain filling; CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; and, Yield.
[1], *Gatersleben* wheat microsatellite (Röder *et al*. 1998)
[2], K, chromosome
[3], S, Susceptible bulk.- Consists of ten genotypes from the low extreme of the response.
[4], R, Resistant bulk.- Consists of ten genotypes from the high extreme of the response.
To interpret the bulk segregation ratio, the "/" divides the two sets of bulks. The segregation ratios (:) describe the occurrence of one Seri (susceptible) or Babax (resistant) parental allele amplified in the debulked genotype –*i.e.*, (Seri : Babax). The number of Seri alleles is on the left side of the ratio, while the Babax alleles are on the right side. When heterozygous (H) alleles were found in the debulking, their notation followed the segregation ratio corresponding to the Seri : Babax allele segregation ratio. When no amplification occurred in the samples, the number of samples were noted and annotated as "no" after the allele segregation ratio for both bulks.
Segregation ratios, depending to their proximity to the theoretical ratio for the bulks (10 : 0 / 0 : 10), were classified as promising (▲), interesting (●) or to be considered (✦) for subsequent analyses.

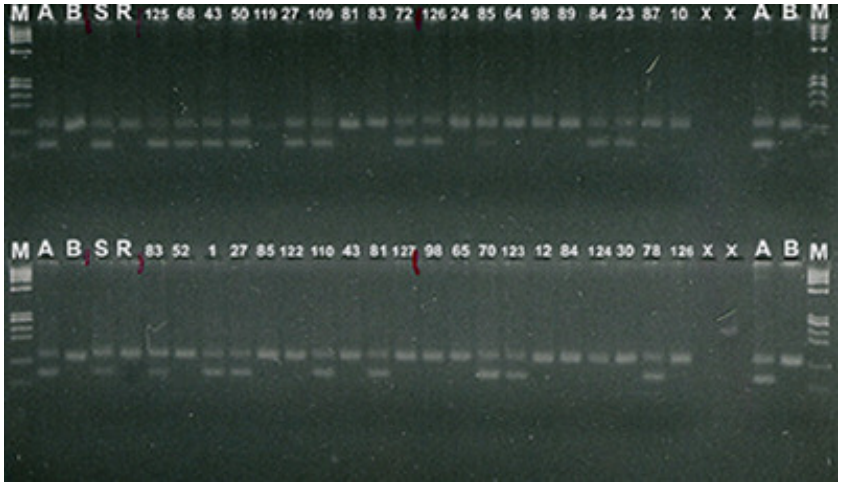


Figure 17. Example of preliminary BSA with GWM382 marker. Analysed traits are (top to bottom): Chlorophyll at Booting (tier 1) and Chlorophyll at Grain Filling (tier 2). Parental samples (A, Seri; B, Babax) are followed (from left to right) by two ten-genotype bulks (S, *susceptible*; R, *resistant*), the corresponding debulked RILs (genotypes denoted) for each bulk (*susceptible* followed by *resistant*), the standard molecular reference (ϕX174/HaeIII), M, and the absence of loaded sample (no sample), x.

4. Population analyses with polymorphic markers for each class of bulks. As the number of markers was small, it was decided to screen the totality of the progeny (167 RILs) plus the parents, for such loci. Remarkably, it was found that 40 genotypes[14] were heterozygous in a large proportion of the amplified loci (>90%). Such an ubiquitous pattern in the genotypes was unrelated to possible sample-to-sample cross-contamination when loading gels and independent from PCR handling and pipetting. Thus, it was pondered that those genotypes were heterozygous at the amplified loci in question, for which a closer analysis revealed that in 70% of all occuring heterozygous loci, the PCR-based markers were specifically amplifying B genome loci, with only 30% of the total markers

lxxxiv

[14] Population genotypes: 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 159, 160, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 190, 191, 192, 193 and 194.

amplifying D genome sequences. Hence, the heterozygous species were deemed to be producing the misleading allele association results of the preliminary debulking and were discarded for subsequent analyses (INTEGRAL BSA). The final list of 127 genotypes was established, all of which were, coincidentally, derived from the *Babax/Seri* cross according to their pedigree –the discarded genotypes were derived from the other reciprocal cross, *Seri/Babax* [*see* **3.1.1.2 Population development**].

4.6.2.3.2 INTEGRAL BSA

The preliminary results provided valuable insight in some genetic aspects of the Seri/Babax population –constituted by the progenies from two crosses: *Babax/Seri* and *Seri/Babax*. The subset of 127 *Babax/Seri* cross-derived RILs were used for preparing the INTEGRAL BSA as described in **3.2.2.2.2.1 Procedure**. Careful database curation from the results at CIMMYT and CSIRO on the genotypes debulking was performed on a pedigree basis, allowing to perform INTEGRAL BSA with a high degree of confidence.

For the INTEGRAL BSA, firstly, a reduced set of genotypes (127) was used (Tables 15A-D). Secondly, instead of averaging the data of selected DRT cycles (2000/2001 & 2001/2002) with AUS (as in the preliminary results), individual bulks for every trait were outlined separately for every treatment in Mexico (DRT, RED and IRR) and in Australia (AUS, averaged data of Charlick and Roseworthy localities) (Figures 18A&B; description in APPENDIX 2). The data from AUS was separated from the DRT bulks in order to avert conclusions confounded by large environmental interactions with the assessed genotypes (S.A. Quarrie, *pers.comm.*), which were confirmed through phenotypic correlations. Using data for the 127 genotypes and their parents, the correlations between datasets of DRT and AUS were significantly (p<0.0001) low, if not zero (for CT at grain filling, in the morning; and, leaf rolling at grain filling): yield (r= –0.23); chlorophyll at booting (r= –0.07); osmotic potential at grain filling (r= –0.06); chlorophyll at grain filling (r= –0.04); CT at grain filling, in the afternoon (r= 0.04); height at grain filling (r= 0.06); and, CT at booting, in the afternoon (r= 0.11). Further, within the AUS dataset (averaged data from the trials at Charlick and Roseworthy in 2001), the relationships between the two datasets were close in the significant (p<0.0001)

association that the traits showed with yield at Charlick and Roseworthy, respectively: chlorophyll at grain filling, (r= 0.25 and r= 0.20), CT at grain filling in the morning (r= –0.10 and r= –0.05); osmotic potential at grain filling (r= –0.10 and r= –0.05), leaf rolling at grain filling (r= –0.24 at both sites). The overall variation, in its ultimate relationship to yield in the AUS dataset (yield between Charlick and Roseworthy in 2001, r= 0.39) was similar to that observed within the treatments (DRT, RED and IRR: r=0.53, RED, r= 0.70, IRR, r=0.40) in Mexico (on a cycle per cycle basis). Hence, by separating the AUS data from DRT, a single agroecological region –different from DRT– was to be targeted for specific marker/ trait associations via INTEGRAL BSA.

Table 15-A. Trait information of the 127 *Babax/Seri* RILs and parents, assessed via INTEGRAL BSA. Drought stress in Yaqui valley, Northwestern Mexico (average of cycles 1999/2000, 2000/2001& 2001/2002)

TRAIT	SERI	BABAX	MEAN	SD	CV
CHLO-boot (SPAD)	47.9	47.9	47.9	1.34	2.79
CHLO-gf (SPAD)	44.0	48.8	45.7	1.72	3.76
CTAM-boot (°C)	22.2	21.5	22.2	0.44	1.97
CTPM-boot (°C)	26.3	25.2	26.2	0.80	3.07
CTAM-gf (°C)	23.4	21.5	22.3	0.62	2.78
CTPM-gf (°C)	27.7	26.7	27.5	0.57	2.07
OP-gf (MPa)	-2.20	-1.53	-2.14	0.17	-8.14
RNDVI-boot	0.76	0.79	0.75	0.03	3.71
RNDVI-gf	0.62	0.58	0.54	0.05	9.77
LROLL-gf	3.40	4.20	4.26	0.48	11.2
HEIGHT-gf (cm)	60.7	78.8	65.6	7.90	12.0
KNO	7029.2	8174.4	13524.4	3731.9	27.6
ATKW (g)	34.4	42.4	37.0	2.41	6.52
YIELD (gm^{-2})	244.5	348.8	503.5	149.7	29.7

NOTE: CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised difference in vegetative index at booting (red spectrum); RNDVI-gf, Normalised difference in vegetative index at grain filling (red spectrum); LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 15-B. Trait information of the 127 *Babax/Seri* RILs and parents, assessed via INTEGRAL BSA. Reduced irrigation in Yaqui valley, Northwestern Mexico (average of cycles 2000/2001& 2001/2002)

TRAIT	SERI	BABAX	MEAN	SD	CV
CHLO-boot (SPAD)	46.1	45.9	45.5	1.71	3.74
CHLO-gf (SPAD)	53.8	49.7	49.7	2.25	4.52
CTAM-gf (°C)	18.8	17.5	18.1	0.53	2.93
CTPM-gf (°C)	27.1	25.7	26.4	0.42	1.58
OP-gf (MPa)	-1.93	-1.49	-1.90	0.09	-4.80
LROLL-gf	3.30	3.70	3.44	0.51	14.7
HEIGHT-gf (cm)	90.1	102.4	94.8	5.48	5.79
KNO	13938.4	15972.2	15624.8	1946.9	12.5
ATKW (g)	31.8	35.4	32.4	2.78	8.57
YIELD (gm^{-2})	444.9	562.4	500.6	68.9	13.8

NOTE: CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 15-C. Trait information of the 127 *Babax/Seri* RILs and parents, assessed via INTEGRAL BSA. Full irrigation in Yaqui valley, Northwestern Mexico (average of cycle 1999/2000, 2000/2001& 2001/2002)

TRAIT	SERI	BABAX	MEAN	SD	CV
CHLO-boot (SPAD)	49.5	49.0	50.0	1.69	3.39
CHLO-gf (SPAD)	53.6	51.8	50.2	1.45	2.89
CTAM-gf (°C)	21.0	20.5	21.4	0.36	1.66
CTPM-gf (°C)	25.8	25.2	25.4	0.33	1.29
OP-gf (MPa)	-1.71	-1.47	-1.56	0.08	-5.16
LROLL-gf	2.50	3.00	2.38	0.44	18.5
HEIGHT-gf (cm)	92.5	93.6	94.2	3.61	3.83
KNO	16709.8	18735.8	16519.8	1714.2	10.4
ATKW (g)	39.6	43.6	41.2	2.62	6.36
YIELD (gm^{-2})	633.5	732.5	673.8	54.1	8.04

NOTE: CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 15-D. Trait information of the 127 *Babax/Seri* RILs and parents, assessed via INTEGRAL BSA. Drought stress at Charlick and Roseworthy, South Australia in 2001

TRAIT	SERI	BABAX	MEAN	SD	CV
CHLO-boot (SPAD)	39.0	42.3	40.2	2.20	5.47
CHLO-gf (SPAD)	41.8	39.8	40.5	2.62	6.45
CTPM-boot (°C)	17.5	16.9	17.7	0.43	2.42
CTAM-gf (°C)	17.7	16.7	17.2	0.34	1.98
CTPM-gf (°C)	21.1	19.3	20.5	0.43	2.11
OP-gf (MPa)	-1.63	-1.47	-1.42	0.12	-8.50
LROLL-gf	2.50	4.30	3.73	0.68	18.1
HEIGHT-gf (cm)	89.8	102.0	91.7	5.35	5.84
YIELD (gm^{-2})	430.6	403.9	404.0	46.9	11.6

NOTE: CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

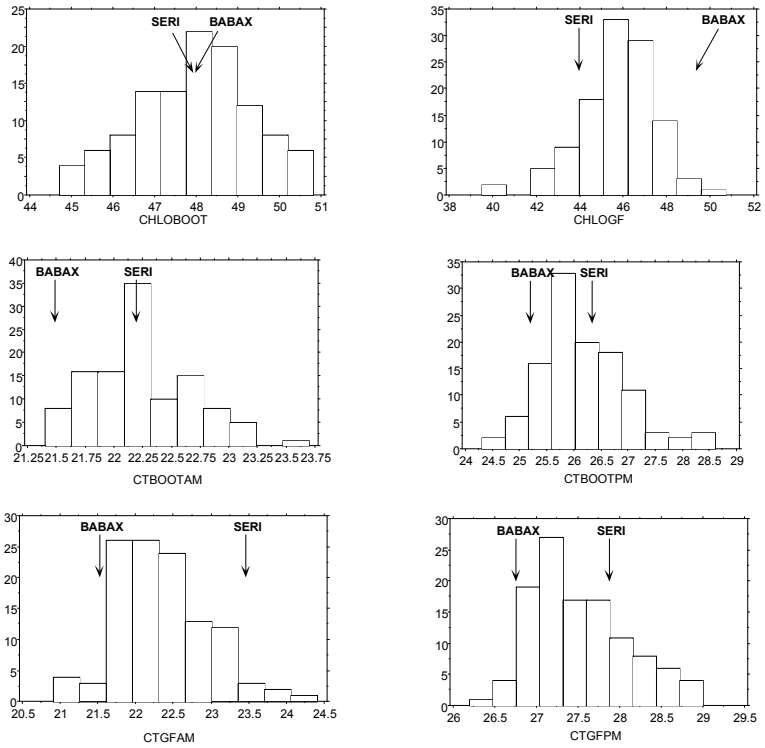


Figure 18A. Histograms of complex traits utilised for the INTEGRAL BSA assessment under drought stress in Mexico (DRT, averaged phenotypic data of three cycles: 1999/2000, 2000/2001 & 2001/2002), utilising 127 *Babax/Seri* RILs. Parental genotypes (Seri and Babax) are indicated.

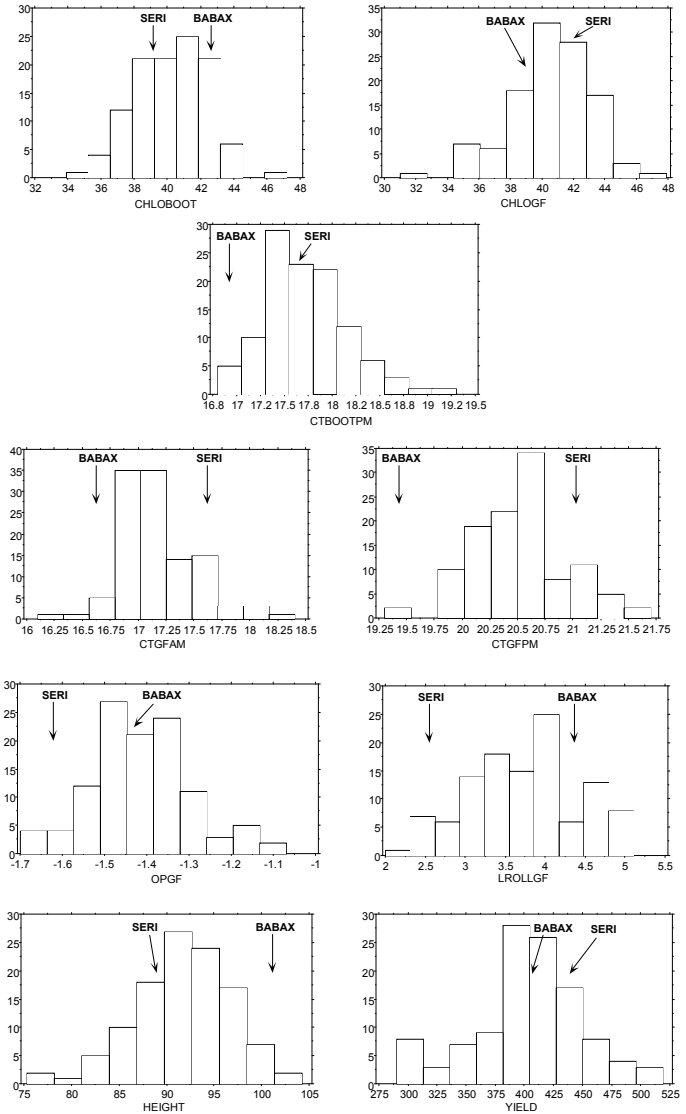


Figure 18B. Histograms of complex traits utilised for the INTEGRAL BSA assessment under rainfed conditions in Australia (AUS, averaged phenotypic data of Charlick and Roseworthy in 2001), utilising 127 *Babax/Seri* RILs. Parental genotypes (Seri and Babax) are indicated.

4.6.2.3.2.1 Composite CIMMYT-CSIRO molecular assessment

Polymorphic PCR-based markers were assessed at CIMMYT and CSIRO with the bulks of the INTEGRAL BSA strategy. In addition to this, AFLPs were also used. A 48 ALFP enzyme/primer assortment analysis on the bulks was conducted without prior parental screening, as reasonable polymorphism was expected in the custom laboratory procedure (William *et al.* 2003). As with the GWM collection efficiency in detecting polymorphisms, a 10% selection efficiency was obtained through the AFLPs polymorphism alternative strategy –*i.e.*, *ca.*5 primer combinations gave polymorphisms between the bulks.

The 107 markers used in the INTEGRAL BSA assessments are listed in Table 16. Combined, the markers from CSIRO (74 PCR-based markers) and CIMMYT (33: 23 PCR-based allele-markers and 10 AFLPs) covered the seven chromosomic groups in every one of the three hexaploid wheat genomes (A, B, D).

When analysing the CIMMYT-CSIRO composite molecular database derived from the INTEGRAL BSA debulking and verifying those with data from CSIRO-Plant Industry (S.C. Chapman, *pers.comm.*), the combined proportions of the parental alleles in all of the 20 *Babax/Seri* RILs subsets for the assessed traits were, on average (Table 17): 42% for the Seri allele (A) and 45% for the Babax allele (B). Even though the minor, non-significant difference in the allele proportions (3%) might be partly explained by maternal effects, it was congrous with that expected in the genetic mosaic of a recombinant inbred population (1 : 1). However, when graphing the parental allele proportions separatedly, the Babax (B) allele showed a normal distribution (Fig.19B), while the Seri (A) allele showed a transgressive segregation (Fig.19A).

Table 17. Allelic proportions in a *Babax/Seri* RILs subset used in molecular assessments

	ALLELE	
STATISTIC	A	B
MEAN	0.42	0.45
SD	0.10	0.09
C.V.	23.3	19.5
MINIMUM	0.08	0.19
MAXIMUM	0.63	0.65

NOTE: A, Seri; B, Babax.

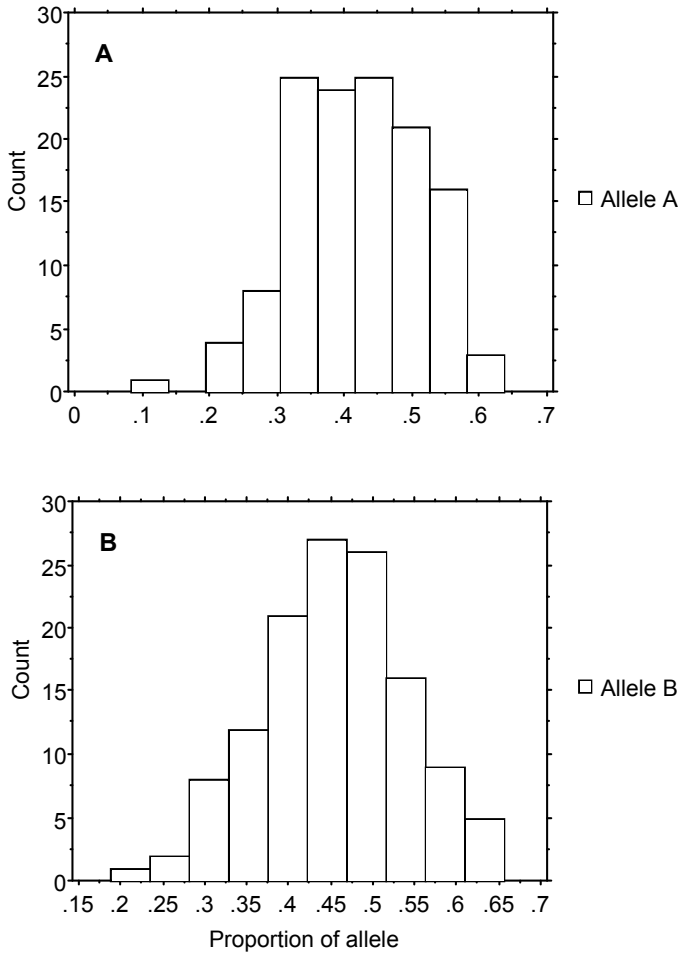


Figure 19. Histogram of parental allele proportions in the *Babax/Seri* RILs subset: A, Proportion of A (Seri) allele; B, Proportion of B (Babax allele).

Table 16. Markers utilised in BSA analyses at CSIRO-Plant Industry and CIMMYT, INT

Marker	Chromosome	Laboratory
GWM135#1 *	1A-1	CSIRO
wmc097#1	1A-1	CSIRO
barc065#1	1B-1	CSIRO
GWM131#1	1B-1	CSIRO
GWM273#1	1B-1	CSIRO
GWM301b#2	1B-1	CSIRO
GWM413#1	1B-1	CSIRO
GWM582#1	1B-1	CSIRO
barc169#1	1D-1	CSIRO
GWM337#1 *	1D-1	CSIRO
GWM301d#4	1D-2	CSIRO
gdm093#1	2A-2	CSIRO
GWM526#1	2A-2	CSIRO
GWM636#1	2A-3	CSIRO
GWM191a#1	2B-1	CSIRO
GWM388#1	2B-1	CSIRO
GWM301a#1	2B-2	CSIRO
GWM301c#3	2B-2	CSIRO
GWM102#1	2D-1	CSIRO
GWM369#1 *	3A-1	CSIRO
gdm008#1	3B-1	CSIRO
GWM301e#5	3B-1	CSIRO
GWM644#1	3B-1	CSIRO
barc087#1	3B-2	CSIRO
barc147#1	3B-2	CSIRO
GWM389#1 *	3B-2	CSIRO
barc070#1	4A-1	CSIRO
GWM350#1	4A-1	CSIRO
GWM397#1	4A-2	CSIRO
wmc048c#3	4A-2	CSIRO
wmc048d#4	4A-2	CSIRO
barc020#1	4B-1	CSIRO
GWM006a#1 *	4B-1	CSIRO
GWM375#1 *	4B-1	CSIRO
wmc048a#1	4B-1	CSIRO
cfd023#1	4D-1	CSIRO
cfd071#1	4D-1	CSIRO
wmc048b#2	4D-1	CSIRO
barc040#1	5A-1	CSIRO
barc100#1	5A-1	CSIRO
barc186#1	5A-1	CSIRO
GWM304#1	5A-1	CSIRO
GWM617a#1 *	5A-1	CSIRO
GWM132c#3	5A-2	CSIRO
GWM112#1	5B-2	CSIRO
GWM133#1	5B-2	CSIRO
GWM191b#2	5B-2	CSIRO
GWM213#1	5B-2	CSIRO
GWM274#1	5B-2	CSIRO
GWM371#1	5B-2	CSIRO
gdm063#1	5D-1	CSIRO
gdm133#1	5D-1	CSIRO

NOTE:
*, common marker in the two molecular databases
UA, Unassigned chromosomic position

Table 16 (*continued*). Markers utilised in BSA analyses at CSIRO-Plant Industry and CIMMYT, INT

Marker	Chromosome	Laboratory
GWM334#1	6A-1	CSIRO
GWM459#1	6A-1	CSIRO
GWM518b#2 *	6A-1	CSIRO
GWM617b#2 *	6A-2	CSIRO
GWM132b#2	6B-1	CSIRO
GWM518a#1 *	6B-1	CSIRO
gdm098#1	6D-1	CSIRO
gdm132#1	6D-2	CSIRO
GWM325#1	6D-2	CSIRO
GWM469#1	6D-2	CSIRO
barc121#1	7A-1	CSIRO
GWM282#1	7A-1	CSIRO
GWM635a#1	7A-1	CSIRO
gdm086#1	7B-1	CSIRO
GWM635b#2	7B-1	CSIRO
GWM130#1	7D-2	CSIRO
GWM437#1	7D-2	CSIRO
GWM473#1	7D-2	CSIRO
GWM006b#2 *	4B, 4D	CSIRO
GWM190#1 *	5D	CSIRO
gdm035#1	UA-1	CSIRO
GWM132a#1	6B	CSIRO
L-GWM6#1	4B, 4D	CIMMYT
L-GWM113#1	4B	CIMMYT
L-GWM120#1	2B	CIMMYT
L-GWM135#1 *	1A	CIMMYT
L-GWM149v1#1	4B, 4D	CIMMYT
L-GWM149v2#1	4B, 4D	CIMMYT
L-GWM190#1 *	5D	CIMMYT
L-GWM251#1	4B, 4D	CIMMYT
L-GWM261#1	2D	CIMMYT
L-GWM311#1	2A, 2D, 6B	CIMMYT
L-GWM337#1 *	1B, 1D	CIMMYT
L-GWM340#1	3B	CIMMYT
L-GWM369#1 *	3A, 4B	CIMMYT
L-GWM375#1 *	4B	CIMMYT
L-GWM382#1	2A, 2B, 2D	CIMMYT
L-GWM389#1 *	3B	CIMMYT
L-GWM427#1	3B	CIMMYT
L-GWM480#1	3A	CIMMYT
L-GWM499#1	5B	CIMMYT
L-GWM508#1	6B	CIMMYT
L-GWM518#1 *	6B	CIMMYT
L-GWM566#1	3B	CIMMYT
L-GWM617#1	5A, 6A	CIMMYT
PstACGMseCGG1#1	unknown	CIMMYT
PstACGMseCGG2#2	unknown	CIMMYT
PstACCMseiCTA1#1	unknown	CIMMYT
PstACCMseiCTA2#2	unknown	CIMMYT
PstAAGMseCGT1#1	unknown	CIMMYT
PstAAGMseCGT2#2	unknown	CIMMYT
PstAAGMseCTA1#1	unknown	CIMMYT
PstAAGMseCTA2#2	unknown	CIMMYT
PstAAGMseCTA3#3	unknown	CIMMYT
PstACCMseCGC1#1	unknown	CIMMYT

NOTE:
*, common marker in the two molecular databases
UA, Unassigned chromosomic position

4.6.2.3.2.2 Linkage

Linkage analyses in all the marker/ genotypic combinations resulting from the association parameters (LOD score=3.0, θ =0.40) were congruous with published chromosomic locations. Some chimerical associations (*i.e.*, loci closely or moderately associated in a linkage group with reported or published chromosomic locations in distantly-related chromosomic groups) were observed. Where applicable, no chimerical linkage groups were considered for the association analyses between genotypic and phenotypic datasets. Rather, the loci found in the chimerical associations (spurious linkage) were handled as separate linkage groups, as there are indications (Burr & Burr 1991) in that it is quite likely to observe association of unlinked loci because of their similar allele distributions in a small, restricted population or genotypic subset –such as the 20 genotype subset utilised in the INTEGRAL BSA. One-way analysis of variance at every locus identified by individual markers rendered numerous associations, restricting their reporting and analysis to the threshold of $F \geq 1.00$. For the purposes of this scientific report, the threshold was declared at $p < 0.05$ and $F \geq 5.00$.

4.6.2.3.2.3 Bulk classes and target loci associations

4.6.2.3.2.4 Genomic phenomena

A detailed description for each locus in every bulk class (*i.e.*, trait) is shown in APPENDIX 3. When analysing the various genotypic combinations for all classes, a large number of marker/trait associations (387) were verified across all categories (*i.e.*, treatments: DRT, RED and IRR; or latitudes: AUS). From published resources, there were 335 assigned chromosomic positions of the marker alleles or known loci utilised for the analysis of the BSA, whereas only 52 were not. Loci in the three hexaploid wheat genomes (A, B, D) and across all seven chromosomic groups per genome were detected to be varingly contributing to explain the phenotypic variation of the diverse bulk classes, except for chromosomic group 3D. In total, the number of markers explaining the phenotypic variations via the INTEGRAL BSA strategy were, per categories: for DRT, 93 (67 assigned, 26 unassigned); for RED, 12-4 (116 assigned, 8 unassigned); for IRR, 93 (81 assigned, 12 unassigned); and, for AUS, 77 (71 assigned, 6 unassigned).

The most significant (p<0.05) chromosomic groups that encompass the scattered markers were, respective to their abundance across the various classes and categories, as follows: group 1 for DRT (21) and RED (32); group 4 for IRR (20); and, group 5 for AUS (22). Overall, as for the least significant (p<0.05) chromosomic regions, relative to the abundance of interesting loci, groups 6 and 7 were of note.

Overall, the various contributions of the assessed loci for each bulk class (Table 18), when considering all classes, in the four categories: in DRT, 19-69%; in RED, 19-62%; in IRR, 19-74%; and in AUS, 19-44%.

Table 18. INTEGRAL BSA– Ranges of the relative contribution of the various target loci per trait bulk under different treatments in Mexico (DRT, RED & IRR) and Australia (AUS)

TRAIT	DRT (%)	RED (%)	IRR (%)	AUS (%)
CHLO-boot (SPAD)	22-67	23-44	21-48	20-44
CHLO-gf (SPAD)	26-66	20-54	31	19-36
CTBOOTAM	19-55	.	.	.
CTBOOTPM	19-49	.	.	19-43
CTAM-gf (^{o}C)	21-63	19-35	19-35	20-31
CTPM-gf (^{o}C)	19-51	25-39	26-74	19-32
OP-gf (MPa)	24-32	20-30	22-58	20-37
RNDVI-boot	28-30	.	.	.
RNDVI-gf	23-25	.	.	.
LROLL-gf	23-33	20-39	20-46	20-43
HEIGHT-gf (cm)	20-33	23-44	19-53	20-34
KNO	19-47	24-59	20-50	.
ATKW (g)	20-56	19-53	20-48	.
YIELD (gm^{-2})	19-69	20-62	21-45	20-44

NOTE: CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised difference in vegetative index at booting (red spectrum); RNDVI-gf, Normalised difference in vegetative index at grain filling (red spectrum); LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

4.6.2.3.2.5 Cross-dissection dynamics description

A cross-comparison of the findings in the different classes (traits) and across categories (treatments/latitudes), with a description of the significant (p<0.05) marker/trait associations explaining the phenotypic variation at specific loci are presented in Tables 19A-D. A description, on a class (trait) basis, follows:

Chlorophyll at booting

One locus in 3B was found to be explaining the phenotypic variation in the trait under DRT, RED and IRR, while not in AUS. Two distinct, single locus were located in 1D, while there was only one in 7A under DRT. Only under DRT, three loci were associated in 4B.

Under RED, a number of loci were of significance: Six in 1B, two in 7D, one in 5A and a 5A, 6A ubiquitous. Under IRR, there were significant loci in 2A (two), 3B (one), 4A (one), 5D (two), a ubiquitous (5A, 6A) and in 6A (one). In AUS, significant loci were located in 1B (three), 1D (one), 2A (two) 6A (two) and two ubiquitous (a 3A, 4B, and a 5A, 6A).

Chlorophyll at grain filling

Under DRT, loci were located in same groups as in the previous class (chlorophyll at booting), in 1D (one) and in 4B (two).

Under RED, single locus were located in 2B, 3A, 4B, 5D and a ubiquitous one (3A, 4B).

Under IRR, one single locus was explaining the variation (in 4A).

In AUS, single locus were found in 3B, 6B and 7B, plus a ubiquitous (5A, 6A).

CT at booting, in the morning and in the afternoon

Under DRT, the two bulk classes differed in the three 3B loci that are of significance only for the morning bulk. For the rest, they share the same significant loci: four loci in 1B and two single locus in 5D in 6A.

When comparing the DRT class at booting in the afternoon with that in AUS, they only share loci in the chromosome region 5. In AUS, single locus were found in 3A, 4A, 5A and 5D, whereas two loci were of significance in 7A.

CT at grain filling, in the morning and in the afternoon

Under DRT, these bulk classes (in the morning and in the afternoon) differ from their counterparts at booting in all loci, but share 3 relevant chromosomic groups (one locus in 3 (B), three loci in 5 (A, B and D), and one in 6 (B). A ubiquitous locus (2A, 2B, 2D), not found at booting, is also of significance.

Under DRT, the two classes share markers in the 5th chromosomic region (5B in the morning; 5A and 5D in the afternoon), differing in four single significant locus (ubiquitous in 2A, 2B, 2D; 3B; 4A; and, 6B) that were only detected for the morning class, and in a single locus (7A) for the afternoon one.

Under RED, the morning and afternoon classes share only one locus (3B) and scattered loci in the 6th chromosomic region (divergent loci in both 6A and 6B for in the morning and in the afternoon classes). While two 4D loci, and two single locus in 5A and 7B were uniquely found in the morning class, there was only one additional 3B loci for the afternoon class.

Under IRR, the two classes (morning and afternoon) share loci in the 3 (3B in the morning, and an ubiquitous in 3A, 4B in the afternoon) and 6A groups (one locus in the morning, while a pair in the afternoon). However, they differ in a single 7A locus (morning) and in two 4B loci and one 5B locus (afternoon).

In AUS, they share scattered markers in chromosomic regions 3 (different pairs of 3B loci) and 5 (an 5A, 6A ubiquitous and a 5D in the morning; five 5A loci and a single 5D locus in the afternoon). The classes differed on the single locus in 1A, 2D, 6B and a ubiquitous 4B, 4D (all present in the morning).

When comparing significant loci across the different categories, chromosome group 1 was the least contributing to the expression of the trait in any class (only a 1A locus was detected in AUS). With respect of chromosome 2, only DRT and AUS possessed a significant single locus. Also, while the analyses revealed one single locus in chromosome groups 4 and 6 in DRT and AUS, there was a pair of loci in RED and IRR instead. While there seems to be more loci significantly associated to the expression of the trait in chromosome group 5 under DRT and in AUS, such differ from those observed in chromosomes 3, 4 and, more importantly, 6 for the RED and IRR categories.

In all treatments in Mexico (DRT, RED and IRR), a single locus in chromosome group 7 was verified, which was coincidentally absent in the AUS category.

Osmotic potential at grain filling

Under DRT, three chromosomic regions (1A; 2A, 2B, 2D; and, 3B) were harbouring significantly associated loci. Under RED, loci were located in a pair of chromosomic regions (four 1B and two 5A loci). Under IRR and AUS, significant loci were detected in four chromosomic regions (IRR: a single locus in 1A, four 5A loci, and two single locus in 6A and 7A; AUS: single locus in 1A, 2A, 4B, 5A and 5B, two 4A loci, and a ubiquitous 4B, 4D).

When cross-comparing categories, DRT, RED and IRR showed clustered loci in different chromosomic regions. Whereas in AUS, the significant loci were more evenly distributed.

Normalised difference in vegetative index at booting and grain filling (red spectrum)

The only category in where the traits were assessed was under DRT. Different loci were identified for the two stages. At booting, loci were located at 2A and 4A, while at grain filling a pair of loci were identified amplifying sequences in 2A.

Leaf rolling at grain filling

Under DRT, a single locus was located in 1B, and an ubiquitous (3A, 4B).

Under RED, chromosomic regions 2 and 3 were the only ones not associated to the expression of the trait. Single locus were at 1D, 5B, 5D, 6B, 6D and 7D, while there were two ubiquitous (4B, 4D).

Under IRR, only three single locus were found associated with the phenotypic expression in 4A, 4B and 5B.

In AUS, chromosomic regions 3 and 6 were not involved in the trait expression. Scattered loci were identified at 1D, 2D, 5B, 7A, 7B and 7D, one was ubiquitous (4B, 4D), and two were located along 4B.

Overall, DRT was the category with the least interacting regions, closely followed by IRR. AUS showed the most regions associated with the expression of the trait.

Height at grain filling

Under DRT, two loci (4B and 5D) were solely explaining the phenotypic expression in height.

Under RED, chromosomic regions 3 and 6 showed no contributing loci. Those were found in 1A (one),1B (four), 2B (two), 4B (one), 5D (two) and 7D (two).

Under IRR, chromosomic regions without relevance were 6 and 7. Loci were detected in 1B (five), 2B (two), 3B (one), 4B (four) and 5A (one).

In AUS, 3, 5 and 6 regions did not harbour any significant loci. However, there were loci in chromosomic regions 1 (single locus in 1A, two in 1D), 2 (two 2B loci), 4 (one 4A locus) and 7 (two 7D loci).

Overall, RED and IRR, were the categories wherein the classes shared the most of the associated chromosomic regions, only differing in regions 3, 6 and 7.

Kernel number

Under DRT, all but chromosomic regions 3 and 4 harboured associated loci for the expression of the trait. Loci were located in 1B (three), 2B, 5A, 6B, 7B and 7D.

Under RED, region 7 did not have any associated loci. The two regions with more contributing loci were 1 (six in 1B, one in 1D) and 4 (three in 4A; five in 4B; two ubiquitous 4B, 4D), followed by region 2 (two 2A loci and couple in 2B) and 6 (a single 6B locus and a pair in 6D). Two single locus were located in 3B and 5B.

Under IRR, two major regions harboured the most significant loci: 1 (four in 1B, one in 1D) and 4 (three in 4A and three in 4B). As for the rest, three loci were located in 5 (two in 5A) and 6 (6D).

Overall, the latter three categories shared associated regions 1, 5 and 6. DRT and RED showed loci in region 2, but not IRR. RED and IRR shared a significantly important set of loci in region 4 that were absent in DRT. However, DRT was the only category with associations found in region 7, while RED in 3.

A thousand kernels weight

Under DRT, the majority of the significant loci were located in 1B (six). Single locus were found in 3B and 4A, while there was a 3A, 3B ubiquitous one.

Under RED, four regions were associated to the expression, namely: 1 (a pair of loci in 1A), 2 (a pair in 2B and a single locus in 2D), 3 (a pair of 3B loci) and 7 (two 7B and one 7D).

Under IRR, two loci were found in chromosomic region 7 (7A and 7D), while scattered single locus were found in 1B, 2D, 4A and 5B.

Overall, the category with the most associated regions was IRR, being evenly distributed across the genome (except for contribution of regions 3 and 6). RED and IRR seemed to shared the same associated chromosomic regions, except for regions 3, 4 and 5.

Yield

Under DRT, in four chromosomic regions a number of loci were identified: two ubiquitous 4B, 4D loci, and three single locus in 2B, 6A and 7B.

Under RED, all regions harboured significantly associated loci, except for region 7. Associated loci was found in 1B (five), 1D (two), 2A (one), 3B (five), 4A (three), 4B (five), 5B (one), 6A (two), 6D (two) and a ubiquitous 4B, 4D locus.

Under IRR, all regions contributed except 5. Significant loci were found in 1A (one), 1B (five), 2B (three), 3B (three), 4A (three), 6A (two), 6B (one) and 7D (one).

In AUS, regions with no significant loci were 2, 6 and 7. Significant loci were located in 1D (one), 3B (one), 4A (one) and 5B (seven).

Overall, DRT and AUS were the categories with the least significantly associated regions (four), but DRT had the lesser a number of associated loci. RED and IRR had six contributing regions, but RED had a larger number of loci significantly explaining the phenotypic variation.

All traits

In a close inspection of the classes within the categories, different levels of phenotypic variation resulted from the INTEGRAL BSA. Under DRT, the class that resulted in the one with most loci (12) was CT at booting in the morning, while the least (2) were the normalised difference in vegetative index assessments.

Under RED, yield had the most associated loci (28), while CT at grain filling in the afternoon had the least (4).

Under IRR, yield was the class with the largest number of significant loci (19), while chlorophyll at booting was the one with the meagrest (1).

In AUS, chlorophyll at booting and leaf rolling at grain filling were the traits with the largest number of associated loci, while chlorophyll at grain filling and CT at booting in the afternoon had the most modest one (6).

Table 19-A. INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under drought stress (average of data from cycles 1999/2000, 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -boot	CTPM -boot	CTAM -gf	CTPM -gf	OP -gf	RNDVI -boot	RNDVI -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
L-GWM135#1	1A	L78							0.31							
GWM135#1	1A-1	L1														
wmc097#1	1A-1	L2														
barc065#1	1B-1	L3			0.19	0.19									0.36	
GWM131#1	1B-1	L4												0.19	0.32	
GWM273#1	1B-1	L5			0.19	0.19								0.19	0.22	
GWM301b#2	1B-1	L6			0.19	0.19									0.36	
GWM413#1	1B-1	L7													0.21	
GWM582#1	1B-1	L8			0.19	0.19						0.23		0.19	0.35	
barc169#1	1D-1	L9														
GWM337#1	1D-1	L10														
GWM301d#4	1D-2	L11	0.25	0.40												
gdm093#1	2A-2	L12									0.25					
GWM526#1	2A-2	L13							0.26		0.23					
GWM636#1	2A-3	L14														
L-GWM120#1	2B	L77							0.27	0.30						0.24
GWM191a#1	2B-1	L15														
GWM388#1	2B-1	L16														
GWM301a#1	2B-2	L17												0.24		
GWM301c#3	2B-2	L18														
L-GWM261#1	2D	L83							0.32							
GWM102#1	2D-1	L19														
L-GWM382#1	2A, 2B, 2D	L89					0.22									

NOTE:

k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised difference in vegetative index at booting (red spectrum); RNDVI-gf, Normalised difference in vegetative index at grain filling (red spectrum); LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-A (*continued*). INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under drought stress (average of data from cycles 1999/2000, 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -boot	CTPM -boot	CTAM -gf	CTPM -gf	OP -gf	RNDVI -boot	RNDVI -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
L-GWM480#1	3A	L92														
GWM369#1	3A-1	L20														
L-GWM340#1	3B	L86														
L-GWM566#1	3B	L96					0.23									
gdm008#1	3B-1	L21	0.34		0.28											
GWM301e#5	3B-1	L22														
GWM644#1	3B-1	L23														
barc087#1	3B-2	L24			0.22				0.24							
barc147#1	3B-2	L25			0.23				0.30						0.20	
GWM389#1	3B-2	L26														
L-GWM369#1	3A, 4B	L87										0.32			0.26	
barc070#1	4A-1	L27													0.29	
GWM350#1	4A-1	L28														
GWM397#1	4A-2	L29					0.21									
wmc048c#3	4A-2	L30								0.28						
wmc048d#4	4A-2	L31														
L-GWM113#1	4B	L76														
L-GWM375#1	4B	L88														
barc020#1	4B-1	L32	0.22													
GWM006a#1	4B-1	L33	0.67	0.66									0.22			
GWM375#1	4B-1	L34	0.38	0.26												
wmc048a#1	4B-1	L35														
cfd023#1	4D-1	L36														
cfd071#1	4D-1	L37														
L-GWM6#1	4B, 4D	L75														
L-GWM149v1#1	4B, 4D	L79														0.27
L-GWM149v2#1	4B, 4D	L80														0.25
L-GWM251#1	4B, 4D	L82														
barc040#1	5A-1	L39														
barc100#1	5A-1	L40														
barc186#1	5A-1	L41														
GWM304#1	5A-1	L42														
GWM617a#1	5A-1	L43												0.19		
GWM132c#3	5A-2	L44						0.23								
L-GWM499#1	5B	L93														
GWM112#1	5B-2	L45														
GWM133#1	5B-2	L46														
GWM191b#2	5B-2	L47														
GWM213#1	5B-2	L48														
GWM274#1	5B-2	L49														
GWM371#1	5B-2	L50					0.26									
L-GWM190#1	5D	L81											0.20			
gdm063#1	5D-1	L51						0.19								
gdm133#1	5D-1	L52			0.32	0.36										
L-GWM617#1	5A, 6A	L97														

NOTE:
k, chromosome.
CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised difference in vegetative index at booting (red spectrum); RNDVI-gf, Normalised difference in vegetative index at grain filling (red spectrum); LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-A (*continued, bis*). INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under drought stress (average of data from cycles 1999/2000, 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -boot	CTPM -boot	CTAM -gf	CTPM -gf	OP -gf	RNDVI -boot	RNDVI -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
L-GWM427#1	6A	L91														
GWM334#1	6A-1	L53														
GWM459#1	6A-1	L54														
GWM518b#2	6A-1	L55														
GWM617b#2	6A-2	L56			0.26	0.26										0.19
L-GWM508#1	6B	L94												0.22		
L-GWM518#1	6B	L95														
GWM132b#2	6B-1	L57					0.22									
GWM518a#1	6B-1	L58														
gdm098#1	6D-1	L59														
GWM325#1	6D-2	L61														
barc121#1	7A-1	L63	0.25													
GWM282#1	7A-1	L64						0.30								
GWM635a#1	7A-1	L65														
gdm086#1	7B-1	L66												0.27		0.26
GWM635b#2	7B-1	L67														
GWM130#1	7D-2	L68												0.20		
GWM437#1	7D-2	L69														
GWM473#1	7D-2	L70														
GWM006b#2	unknown	L71														
GWM190#1	unknown	L72														
gdm035#1	unknown	L73	0.31	0.33												
GWM132a#1	unknown	L74	0.57	0.62								0.29				
PstACGMseCGG2#2	unknown	L99														
PstACCMseiCTA1#1	unknown	L100			0.42	0.41	0.55	0.48				0.33		0.47	0.56	0.69
PstACCMseiCTA2#2	unknown	L101			0.46	0.49	0.63	0.51				0.33	0.32	0.47		0.69
PstAAGMseCGT1#1	unknown	L102														
PstAAGMseCGT2#2	unknown	L103			0.55	0.42		0.30								
PstAAGMseCTA1#1	unknown	L104														0.41
PstAAGMseCTA3#3	unknown	L106											0.33			
PstACCMseCGC1#1	unknown	L107														

NOTE:

k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised difference in vegetative index at booting (red spectrum); RNDVI-gf, Normalised difference in vegetative index at grain filling (red spectrum); LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-B. INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under reduced irrigation (average of data from cycles 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -gf	CTPM -gf	OP -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
L-GWM135#1	1A	L78							0.32			
GWM135#1	1A-1	L1									0.20	
wmc097#1	1A-1	L2									0.19	
barc065#1	1B-1	L3	0.36				0.22			0.52		0.31
GWM131#1	1B-1	L4	0.44						0.33	0.32		
GWM273#1	1B-1	L5	0.44						0.23	0.51		0.20
GWM301b#2	1B-1	L6	0.36				0.22		0.30	0.52		0.20
GWM413#1	1B-1	L7	0.33				0.30			0.49		0.30
GWM582#1	1B-1	L8	0.44				0.28		0.38	0.52		0.20
barc169#1	1D-1	L9						0.29				0.26
GWM337#1	1D-1	L10										
GWM301d#4	1D-2	L11								0.31		0.35
gdm093#1	2A-2	L12								0.42		
GWM526#1	2A-2	L13								0.49		0.23
GWM636#1	2A-3	L14										
L-GWM120#1	2B	L77										
GWM191a#1	2B-1	L15		0.21					0.37	0.37	0.28	
GWM388#1	2B-1	L16							0.43	0.29	0.28	
GWM301a#1	2B-2	L17										
GWM301c#3	2B-2	L18										
L-GWM261#1	2D	L83									0.35	
GWM102#1	2D-1	L19										
L-GWM382#1	2A, 2B, 2D	L89										
L-GWM480#1	3A	L92		0.25								
GWM369#1	3A-1	L20										
L-GWM340#1	3B	L86			0.34	0.39						0.25
L-GWM566#1	3B	L96										
gdm008#1	3B-1	L21	0.33									0.33
GWM301e#5	3B-1	L22				0.39						0.26
GWM644#1	3B-1	L23								0.26		0.51
barc087#1	3B-2	L24										0.26
barc147#1	3B-2	L25									0.50	
GWM389#1	3B-2	L26									0.45	
L-GWM369#1	3A, 4B	L87		0.31								
barc070#1	4A-1	L27										
GWM350#1	4A-1	L28										
GWM397#1	4A-2	L29								0.47		0.24
wmc048c#3	4A-2	L30								0.31		0.39
wmc048d#4	4A-2	L31								0.59		0.49
L-GWM113#1	4B	L76								0.35		
L-GWM375#1	4B	L88								0.50		0.23
barc020#1	4B-1	L32								0.42		0.62
GWM006a#1	4B-1	L33		0.20					0.25			0.27
GWM375#1	4B-1	L34								0.39		0.56
wmc048a#1	4B-1	L35								0.42		0.62
cfd023#1	4D-1	L36			0.19							
cfd071#1	4D-1	L37			0.19							
L-GWM6#1	4B, 4D	L75								0.53		
L-GWM149v1#1	4B, 4D	L79						0.20				
L-GWM149v2#1	4B, 4D	L80						0.35				
L-GWM251#1	4B, 4D	L82								0.52		0.23

NOTE:

k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling; KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-B (*continued*). INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under reduced irrigation (average of data from cycles 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -gf	CTPM -gf	OP -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
barc040#1	5A-1	L39					0.20					
barc100#1	5A-1	L40					0.23					
barc186#1	5A-1	L41			0.20							
GWM304#1	5A-1	L42										
GWM617a#1	5A-1	L43	0.23									
GWM132c#3	5A-2	L44										
L-GWM499#1	5B	L93						0.23		0.24		0.25
GWM112#1	5B-2	L45										
GWM133#1	5B-2	L46										
GWM191b#2	5B-2	L47										
GWM213#1	5B-2	L48										
GWM274#1	5B-2	L49										
GWM371#1	5B-2	L50										
L-GWM190#1	5D	L81						0.36				
gdm063#1	5D-1	L51		0.29					0.23			
gdm133#1	5D-1	L52							0.36			
L-GWM617#1	5A, 6A	L97	0.28									
L-GWM427#1	6A	L91			0.19							0.30
GWM334#1	6A-1	L53										
GWM459#1	6A-1	L54										
GWM518b#2	6A-1	L55				0.25						
GWM617b#2	6A-2	L56										0.21
L-GWM508#1	6B	L94								0.34		
L-GWM518#1	6B	L95			0.20			0.28				
GWM132b#2	6B-1	L57										
GWM518a#1	6B-1	L58										
gdm098#1	6D-1	L59				0.29		0.39		0.27		0.24
GWM325#1	6D-2	L61								0.25		0.40
barc121#1	7A-1	L63										
GWM282#1	7A-1	L64										
GWM635a#1	7A-1	L65										
gdm086#1	7B-1	L66			0.20						0.53	
GWM635b#2	7B-1	L67									0.53	
GWM130#1	7D-2	L68						0.20			0.38	
GWM437#1	7D-2	L69	0.26						0.44			
GWM473#1	7D-2	L70	0.23						0.44			
GWM006b#2	unknown	L71										
GWM190#1	unknown	L72		0.23								
gdm035#1	unknown	L73										
GWM132a#1	unknown	L74		0.24								
PstACGMseCGG2#2	unknown	L99		0.54								
PstACCMseiCTA1#1	unknown	L100										
PstACCMseiCTA2#2	unknown	L101										
PstAAGMseCGT1#1	unknown	L102										
PstAAGMseCGT2#2	unknown	L103			0.41							
PstAAGMseCTA1#1	unknown	L104			0.35							
PstAAGMseCTA3#3	unknown	L106						0.63				
PstACCMseCGC1#1	unknown	L107		0.24								0.35

NOTE:

k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling; KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-C. INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under full irrigation (average of data from cycles 1999/2000, 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -gf	CTPM -gf	OP -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
L-GWM135#1	1A	L78										
GWM135#1	1A-1	L1										0.21
wmc097#1	1A-1	L2					0.25					
barc065#1	1B-1	L3							0.23	0.22	0.26	0.23
GWM131#1	1B-1	L4							0.29			
GWM273#1	1B-1	L5								0.21		0.23
GWM301b#2	1B-1	L6							0.29	0.30		0.30
GWM413#1	1B-1	L7							0.23			0.32
GWM582#1	1B-1	L8							0.29	0.43		0.43
barc169#1	1D-1	L9										
GWM337#1	1D-1	L10										
GWM301d#4	1D-2	L11								0.30		
gdm093#1	2A-2	L12	0.22									
GWM526#1	2A-2	L13										
GWM636#1	2A-3	L14	0.29									
L-GWM120#1	2B	L77										0.22
GWM191a#1	2B-1	L15							0.27			
GWM388#1	2B-1	L16							0.21			
GWM301a#1	2B-2	L17										0.26
GWM301c#3	2B-2	L18										0.26
L-GWM261#1	2D	L83									0.21	
GWM102#1	2D-1	L19										
L-GWM382#1	2A, 2B, 2D	L89										
L-GWM480#1	3A	L92										
GWM369#1	3A-1	L20										
L-GWM340#1	3B	L86							0.19			
L-GWM566#1	3B	L96	0.36									
gdm008#1	3B-1	L21	0.36									
GWM301e#5	3B-1	L22										
GWM644#1	3B-1	L23			0.19							
barc087#1	3B-2	L24										0.24
barc147#1	3B-2	L25										0.26
GWM389#1	3B-2	L26										0.31
L-GWM369#1	3A, 4B	L87				0.27						
barc070#1	4A-1	L27		0.31								
GWM350#1	4A-1	L28						0.23			0.20	0.28
GWM397#1	4A-2	L29	0.23							0.30		
wmc048c#3	4A-2	L30								0.22		0.26
wmc048d#4	4A-2	L31								0.50		0.25
L-GWM113#1	4B	L76										
L-GWM375#1	4B	L88										
barc020#1	4B-1	L32				0.45			0.53	0.35		
GWM006a#1	4B-1	L33						0.20	0.47			
GWM375#1	4B-1	L34							0.39	0.25		
wmc048a#1	4B-1	L35				0.46			0.42	0.33		
cfd023#1	4D-1	L36										
cfd071#1	4D-1	L37										
L-GWM6#1	4B, 4D	L75										
L-GWM149v1#1	4B, 4D	L79										
L-GWM149v2#1	4B, 4D	L80										
L-GWM251#1	4B, 4D	L82										

NOTE: k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling; KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-C (*continued*). INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Mexico under full irrigation (average of data from cycles 1999/2000, 2000/2001 &2001/2002)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTAM -gf	CTPM -gf	OP -gf	LROLL -gf	HEIGHT -gf	KNO	ATKW	YIELD
barc040#1	5A-1	L39					0.34					
barc100#1	5A-1	L40					0.32					
barc186#1	5A-1	L41					0.30			0.21		
GWM304#1	5A-1	L42					0.27			0.26		
GWM617a#1	5A-1	L43							0.30			
GWM132c#3	5A-2	L44										
L-GWM499#1	5B	L93						0.22				
GWM112#1	5B-2	L45										
GWM133#1	5B-2	L46										
GWM191b#2	5B-2	L47										
GWM213#1	5B-2	L48										
GWM274#1	5B-2	L49										
GWM371#1	5B-2	L50				0.26					0.27	
L-GWM190#1	5D	L81										
gdm063#1	5D-1	L51	0.39									
gdm133#1	5D-1	L52	0.21									
L-GWM617#1	5A, 6A	L97	0.27									
L-GWM427#1	6A	L91	0.43									
GWM334#1	6A-1	L53				0.35						0.40
GWM459#1	6A-1	L54				0.32						0.45
GWM518b#2	6A-1	L55										
GWM617b#2	6A-2	L56			0.35							
L-GWM508#1	6B	L94					0.22					
L-GWM518#1	6B	L95										
GWM132b#2	6B-1	L57										0.21
GWM518a#1	6B-1	L58										
gdm098#1	6D-1	L59								0.22		
GWM325#1	6D-2	L61										
barc121#1	7A-1	L63									0.28	
GWM282#1	7A-1	L64			0.35							
GWM635a#1	7A-1	L65					0.39					
gdm086#1	7B-1	L66										
GWM635b#2	7B-1	L67										
GWM130#1	7D-2	L68									0.30	0.25
GWM437#1	7D-2	L69										
GWM473#1	7D-2	L70										
GWM006b#2	unknown	L71	0.29									
GWM190#1	unknown	L72						0.31				
gdm035#1	unknown	L73	0.21									
GWM132a#1	unknown	L74	0.37				0.29					
PstACGMseCGG2#2	unknown	L99	0.39									
PstACCMseiCTA1#1	unknown	L100					0.58	0.46				
PstACCMseiCTA2#2	unknown	L101					0.47	0.46				
PstAAGMseCGT1#1	unknown	L102										
PstAAGMseCGT2#2	unknown	L103										
PstAAGMseCTA1#1	unknown	L104										
PstAAGMseCTA3#3	unknown	L106	0.48									
PstACCMseCGC1#1	unknown	L107				0.74						

NOTE: k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling; KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Table 19-D. INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Australia (averaged phenotypic data from Charlick and Roseworthy in 2001)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTPM -boot	CTAM -gf	CTPM -gf	OP -gf	LROLL -gf	HEIGHT -gf	YIELD
L-GWM135#1	1A	L78									
GWM135#1	1A-1	L1				0.29		0.20			
wmc097#1	1A-1	L2								0.20	
barc065#1	1B-1	L3	0.20								
GWM131#1	1B-1	L4									
GWM273#1	1B-1	L5	0.20								
GWM301b#2	1B-1	L6									
GWM413#1	1B-1	L7	0.20								
GWM582#1	1B-1	L8									
barc169#1	1D-1	L9	0.36								0.44
GWM337#1	1D-1	L10								0.23	
GWM301d#4	1D-2	L11							0.36	0.27	
gdm093#1	2A-2	L12	0.44					0.24			
GWM526#1	2A-2	L13	0.41								
GWM636#1	2A-3	L14									
L-GWM120#1	2B	L77									
GWM191a#1	2B-1	L15								0.24	
GWM388#1	2B-1	L16								0.24	
GWM301a#1	2B-2	L17									
GWM301c#3	2B-2	L18									
L-GWM261#1	2D	L83									
GWM102#1	2D-1	L19				0.26			0.20		
L-GWM382#1	2A, 2B, 2D	L89									
L-GWM480#1	3A	L92									
GWM369#1	3A-1	L20			0.19						
L-GWM340#1	3B	L86									
L-GWM566#1	3B	L96									
gdm008#1	3B-1	L21				0.23					
GWM301e#5	3B-1	L22		0.20							0.24
GWM644#1	3B-1	L23				0.20					
barc087#1	3B-2	L24									
barc147#1	3B-2	L25					0.31				
GWM389#1	3B-2	L26					0.28				
L-GWM369#1	3A, 4B	L87	0.26								
barc070#1	4A-1	L27						0.21			
GWM350#1	4A-1	L28									
GWM397#1	4A-2	L29									
wmc048c#3	4A-2	L30			0.36						
wmc048d#4	4A-2	L31								0.34	0.20
L-GWM113#1	4B	L76									
L-GWM375#1	4B	L88									
barc020#1	4B-1	L32						0.22			
GWM006a#1	4B-1	L33									
GWM375#1	4B-1	L34							0.34		
wmc048a#1	4B-1	L35							0.22		
cfd023#1	4D-1	L36									
cfd071#1	4D-1	L37									
L-GWM6#1	4B, 4D	L75				0.28		0.22			
L-GWM149v1#1	4B, 4D	L79							0.29		
L-GWM149v2#1	4B, 4D	L80									
L-GWM251#1	4B, 4D	L82									

NOTE:

k, chromosome.

CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling; YIELD, yield.

Table 19-D (*continued*). INTEGRAL BSA- One-way analysis of variance for determining marker/ trait associations at the identified loci that explain the phenotypic variation for the traits in Australia (averaged phenotypic data from Charlick and Roseworthy in 2001)

MARKER	K	Marker / Locus Code	CHLO -boot	CHLO -gf	CTPM -boot	CTAM -gf	CTPM -gf	OP -gf	LROLL -gf	HEIGHT -gf	YIELD
barc040#1	5A-1	L39					0.28				
barc100#1	5A-1	L40					0.28				
barc186#1	5A-1	L41					0.28	0.22			
GWM304#1	5A-1	L42					0.32				
GWM617a#1	5A-1	L43			0.23		0.22				
GWM132c#3	5A-2	L44									
L-GWM499#1	5B	L93						0.37			0.33
GWM112#1	5B-2	L45									0.33
GWM133#1	5B-2	L46									0.23
GWM191b#2	5B-2	L47									0.33
GWM213#1	5B-2	L48									0.30
GWM274#1	5B-2	L49									0.31
GWM371#1	5B-2	L50			0.22				0.38		0.30
L-GWM190#1	5D	L81				0.31					
gdm063#1	5D-1	L51					0.19				
gdm133#1	5D-1	L52									
L-GWM617#1	5A, 6A	L97	0.32	0.36		0.26					
L-GWM427#1	6A	L91									
GWM334#1	6A-1	L53	0.25								
GWM459#1	6A-1	L54	0.25								
GWM518b#2	6A-1	L55									
GWM617b#2	6A-2	L56									
L-GWM508#1	6B	L94									
L-GWM518#1	6B	L95				0.27					
GWM132b#2	6B-1	L57									
GWM518a#1	6B-1	L58		0.23							
gdm098#1	6D-1	L59									
GWM325#1	6D-2	L61									
barc121#1	7A-1	L63			0.43						
GWM282#1	7A-1	L64			0.28						
GWM635a#1	7A-1	L65							0.21		
gdm086#1	7B-1	L66		0.19					0.20		
GWM635b#2	7B-1	L67									
GWM130#1	7D-2	L68							0.35		
GWM437#1	7D-2	L69								0.23	
GWM473#1	7D-2	L70								0.23	
GWM006b#2	unknown	L71									
GWM190#1	unknown	L72									0.28
gdm035#1	unknown	L73									
GWM132a#1	unknown	L74									
PstACGMseCGG2#2	unknown	L99									
PstACCMseiCTA1#1	unknown	L100									
PstACCMseiCTA2#2	unknown	L101									
PstAAGMseCGT1#1	unknown	L102		0.31			0.26				
PstAAGMseCGT2#2	unknown	L103									
PstAAGMseCTA1#1	unknown	L104									
PstAAGMseCTA3#3	unknown	L106									
PstACCMseCGC1#1	unknown	L107		0.36		0.31			0.43		

NOTE:
k, chromosome.
CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling; YIELD, yield.

4.6.2.3.2.6 INTEGRAL BSA modification- Increasing the number of genotypes per bulk

An increase in the number of genotypes used for preparing bulks (up to 30 per bulk class) was sufficient for estimating a significant (p<0.05) decrease in the sensitivity of the methodology, when such modification was evaluated for a selection of traits. The percentage in variation explained by the significant (p<0.05 and $F \geq 5.00$) loci was considerably reduced when outlining only four bulks for the DRT treatment with 127 *Babax/Seri* RILs in the four selected traits: CT at booting, in the morning (CTBOOTAM); CT at booting, in the afternoon (CTBOOTPM); CT at grain filling, in the morning (CTGFAM); CT at grain filling, in the afternoon (CTGFPM). Results (Table 20) showed that with the bulks corresponding to the booting stage, CTBOOTAM and CTBOOTPM, target loci only explained 6-19% and 6-10%, respectively; whereas with the grain filling bulks, CTGFAM and CTGFPM, the variation was slightly increased, resulting in 7-24% and 6-13%, respectively. In summary, when comparing these results with those obtained for the same traits via INTEGRAL BSA with 10 genotypes per bulk class (Table 18), there was an overall decrease in the phenotypic variance explained by the target markers. The reductions per trait were, as follows: CTBOOTAM, 32-35%; CTBOOTPM, 20-32%; CTGFAM, 33-38%; and, CTGFPM, 26-32%. The complete results details of the methodology modification is described, in full, in APPENDIX 4.

Table 20. INTEGRAL BSA- Increasing the number of genotypes in the CT bulks and its relevance to explaining variation through marker/trait associations at the identified loci

MARKER	CHROMOSOME	CTPM -boot	CTAM -boot	CTAM -gf	CTPM -gf
PstACCMseiCTA2#2	unknown	0.19	0.11	0.20	0.13
L-GWM499#1	5B	0.11	0.06		
PstACCMseiCTA1#1	unknown	0.17		0.24	
L-GWM311#1	2A, 2D, 6B	0.06			
L-GWM508#1	6B		0.10		
L-GWM120#1	2B		0.09		
GWM102#1	2D-1		0.07		
barc020#1	4B-1			0.10	
GWM132c#3	5A-2			0.10	
wmc048a#1	4B-1			0.09	
GWM375#1	4B-1			0.07	
GWM282#1	7A-1				0.09
L-GWM389#1	3B				0.07
barc186#1	5A-1				0.06

NOTE: p<0.05. CTPM-boot, Canopy temperature at booting; CTAM-boot, Canopy temperature at booting; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon

DISCUSSION

5.1 RESPONSE OF SERI AND BABAX TO DROUGHT

Both parental lines used in this study are high-yielding, semi-dwarf cultivars widely adapted to various environments (CIMMYT 1986), but their response to drought stress seems to be quite different, as evidenced from their agronomic performance in Mexico and Australia. When comparing parental agronomic performance under DRT, Babax's kernel weight is reduced (21%) almost as much as Seri's (23%), but its kernel number is reduced by 40% in contrast to Seri's 50% reduction. Such 10% difference in kernel set suggests an early reproductive response under DRT (Table 7A).

Babax showed higher yield performance than Seri's (Table 7A, Fig.5) –particularly under DRT– and its consistently low canopy temperature (Table 8A, Figs. 8 &.9) –irrespective of time of collection, phenological stage or latitude (Tables 8A & B)–, indicates it avoids drought stress via evapotranspiration across the cycle (Jackson 1982) thereby sustaining turgor and physiological activity. The latter suggests that Babax possesses a greater difference, when compared to Seri, in plant water potential than that of the soil under an increased evaporative demand (Turner & Begg 1981). Babax´s ability to access water across the crop cycle (Fig.9) is more evident as stress increased (RED to DRT) (Fig.8), when a greater difference in CT response between the parental genotypes was observed (Table 8A, Fig.9). This was supported by measurements of stomatal conductance with a viscous-flow porometer (as that described by Rawson 1996) on both parents at booting, which showed a significant (p<0.001) higher conductance in Babax than in Seri both in the morning (*ca*.60%) and afternoon (*ca*.50%).

Not only was the canopy of Babax cooler than that of Seri, suggesting a higher photosynthetic activity, but flag leaf chlorophyll content in Babax was also greater both in Mexico (in DRT) and Australia; in contrast, Seri's chlorophyll content diminished between booting and grain filling as stress increased (Table 8A). The occurrence of leaf rolling in Babax (Table 8A), especially under DRT, may reduce direct leaf area exposure to solar radiation, while Seri's open leaves would tend to expose them to more radiation, which could be associated with photo-inhibition and premature senescence. Such decrease in leaf area exposure might account for the observed reduction in Babax's RNDVI signal in Mexico (Table 8A), even though Babax consistently maintained a cool environment relative to air temperature and to Seri (Table 8A). The fact that Babax did not show considerable height reduction in any

latitude (Tables 7A & B) is indicative of 1the effects of a cool CT and a lesser impact by drought stress during early phenological stages.

A low OP and null osmotic adjustment in Babax when subjected to DRT, as well as at Charlick in 2001, might indicate that it maintains sufficient hydration such that osmotic adjustment is not cued (Fig.10).

As it was found that the metabolic apparatus of Babax does not generate high levels of putrescine, in contrast with previous reports on high-yielding drought-tolerant wheat (Ye *et al.* 1997), it suggests that either the antioxidative polyamine protection is not exerted in this genotype –the drought avoidance mechanism might not be effecting the synthesis of antioxidants.

5.2 RILs ADAPTATION AND PERFORMANCE

While it has been argued that some traits determining adaptation to drought stress may be mutually exclusive to high yield potential (Blum 1996), it was observed in the Seri/Babax RILs under DRT that yield potential *per se* contributes significantly to drought adaptation (r^2=0.49, p<0.01) and that the highest yielding lines under DRT included some that possessed the highest yield potential (Fig.5). The RILs performance in the Australian experiments at Charlick seem to be comparable to the Mexican experiments, as in 2001 yield potential contributed to drought adaptation as much as in DRT (r^2=0.47, p<0.01), but at a less extent in 2002 (r^2=0.38, p<0.01). Nonetheless, even though direct selection for yield is recognised as necessary for high-yielding environments (Turner & Begg 1981; Rajaram 1995; Slafer *et al.* 1996) or for environments where intermittent drought occurs (Pfeiffer 1988; Rajaram *et al.* 1996; Kirigwi *et al.* 2004), there is increased evidence that selecting for specific drought-adaptive traits can significantly increase performance when soil moisture is the environmental constraint (Ceccarelli 1989; Loss & Siddique 1994; Araus 1996; Richards 1996; van Ginkel *et al.* 1998; Araus *et al.* 2002). The latter appears to be case in the Seri/Babax RILs. A closer inspection of the apparent association between yield under DRT and IRR (Fig.5), reveals that when considering only the top 50% performing lines under DRT, the association falls from an r^2= 0.50 to r^2= 0.10. In fact, the top 12 ranking lines under DRT showed a range from the 7th to 98th place when considering their ranking under IRR, encompassing only 2 of the top 12 ranking lines. However, a comparison of trait expression under IRR of the highest yield potential

lines that contrasted sharply in drought adaptation, revealed no obvious characteristics associated with their divergent response to moisture stress.

Under DRT, CT was the trait most strongly associated with yield performance (Tables 9 & 12), as previously reported for fixed lines (Blum *et al.* 1982, 1989) and other families of RILs, including the ITMI population (Reynolds *et al.* 2000). In the current study, the association of yield with CT was examined for the effect of phenological stage (booting vs. grain filling) and time of day (morning vs. afternoon). However, the association with yield remained high in all situations (Fig.9, Table 9), considering both phenotypic and genotypic correlations (Tables 9, 12 & 21). When CT data measured in different situations in Mexico were regressed on yield using multiple regression, it was clear that CT could explain over 60% of phenotypic variation irrespective of when it was measured (Table 22), despite factors influencing GxE interactions. While CT measured in the afternoon during booting obtained the highest association (r^2=0.64, p<0.0001), adding the CTs measured in the other environments increased the r^2 by no more than 10 percentage points in total. Hence, CT is a robust trait that explains the most of the genetic variation in yield under DRT (Table 9), regardless of phenological stage or time of day (Table 21); a similar conclusion as that established under hot, irrigated environments (Reynolds *et al.* 1994; Amani *et al.* 1996). Thus, the evident evapotranspiration in Seri/Babax RILs, as a component within a drought avoidance strategy, is a decisive mechanism in their genotypic performance under DRT.

A dehydration avoidance strategy (Blum 1988), involving a constantly cool CT in the high yielding genotypes, suggests a high-risk tactic initiated early at the cycle in pursuit of additional water when facing early signs of soil moisture stress and an increased water extraction capability, which might partially explain yield potential and the association of yield under DRT and IRR (r=0.7, p<0.0001).

Table 21. Phenotypic and genetic correlations between selected traits and CT under drought stress (Yaqui valley, Mexico)

TRAIT	CTAM-boot (°C)		CTPM-boot (°C)		CTAM-gf (°C)		CTPM-gf (°C)	
	r	r(g)	r	r(g)	r	r(g)	r	r(g)
YIELD (g/m²)	-0.75	-0.99	-0.80	-0.98	-0.77	-0.92	-0.78	-0.90
CHLO-boot (SPAD)	-0.23	-0.61	-0.21	-0.53	-0.19	-0.55	-0.15	-0.41
CHLO-gf (SPAD)	-0.41	-0.65	-0.41	-0.88	-0.34	-0.54	-0.36	-0.41
OP-gf (MPa) [A]	-0.03	0.83	0.07	0.39	0.09	0.86	0.11	0.83
RNDVI-boot (nm)[A]	-0.62	-0.97	-0.67	-0.99	-0.54	-0.97	-0.58	-0.90
RNDVI-gf (nm)[A]	0.13	0.29	0.18	0.26	0.19	0.24	0.17	0.23
CTAM-boot (°C)	.	.	0.77	0.98	0.69	0.87	0.73	0.96
CTPM-boot (°C)	.	.	.	.	0.75	0.95	0.79	0.99
CTAM-gf (°C)	.	.	.	.	.	.	0.82	0.99

NOTE:
n=169; *p*<0.0001.
All data calculated from the mean of data from cycles 1999/2000, 2000/2001 & 2001/2002, except: [A], Mean calculated using datasets corresponding to cycles 2000/2001 & 2001/2002.
CHLO-boot, Chlorophyll SPAD measurements at booting; CHLO-gf, Chlorophyll SPAD measurements at grain filling; CTAM-boot, Canopy temperature at booting, in the morning (9:00-11:00h); CTPM-boot, Canopy temperature at booting, in the afternoon (14:00-16:00h); CTAM-gf, Canopy temperature at grain filling, in the morning (9:00-11:00h); CTPM-gf, Canopy temperature at grain filling, in the afternoon (14:00-16:00h); OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised Difference in Vegetative Index (red spectrum), at booting; RNDVI-gf, Normalised Difference in Vegetative Index (red spectrum), at grain filling; YIELD, yield.

Table 22. Stepwise regression per phenological stage for yield under drought stress (Yaqui valley, Mexico)

Booting stage

TRAIT	PARTIAL R^2	MODEL R^2	*F*
CTPM-boot	0.64	0.64	301.6***
HEIGHT	0.08	0.73	49.7***
CTAM-boot	0.02	0.74	11.1**
RNDVI-boot	0.01	0.75	4.80*
CHLO-boot	0.00	0.75	2.11^{ns}

Grain filling stage

TRAIT	PARTIAL R^2	MODEL R^2	*F*
CTPM-gf	0.61	0.61	258.0***
CTAM-gf	0.05	0.66	25.9***
RNDVI-gf	0.04	0.70	20.3***
CHLO-gf	0.01	0.70	3.03*

NOTE:
***, *p*<0.0001; **, *p*<0.001; *, *p*<0.05
All variables are significant at the 0.15 level.
No other variable met the 0.15 significance level for entry into the model.

Even though drought escape is a documented crop response (Loss & Siddique 1994; Slafer & Rawson 1994), the Seri/Babax RILs seem not to be exerting such strategy in Obregon. The observed shift to a reproductive stage by *ca.*10 days (Fig.7), as a response to a warmer plant temperature under DRT (Table 8A), is not associated with the dehydration avoidance response: there is a lack of associations between CT and anthesis date or with the reduction in days to anthesis under DRT. The latter is in consonance with the trait relationships in Australia. A combined difference in the latitude-dependent solar radiation with lower temperatures (maximum: at Charlick 9^{o}C, at Roseworthy, 8^{o}C; minimum: Charlick 0.6^{o}C, at Roseworthy, 1.44^{o}C) than those in Mexico (Tables 6A & B), might have effected the observed alterations in phasic development, as reported in other hexaploid wheat plant materials (Piratesh & Welsh 1980; Fischer 1985; Slafer & Rawson 1994; Slafer *et al.* 1996; Reynolds *et al.* 2002).

Dehydration avoidance was correlated with increased kernel set, as evidenced by the association of CT at booting stage with: (*i*) kernel number under DRT (averaged, r= –0.73, p<0.0001); and, (*ii*) the reduction in kernel number between IRR and DRT conditions (r=0.47, p<0.0001); the inevitable consequence being a strong association between kernel number and yield. As kernel weight also shows a significant but weaker association with CT during grain filling (r= –0.40, p<0.0001), it suggests that the dehydration mechanism still operates throughout grain filling (Tables 5 & 7).

A number of physiological and agronomic parameters support the overall conclusion that drought adaptation in the Seri/Babax RILs was largely driven by the ability to access water (Table 11), an strategy that seems to be consistent with a suggested (Reynolds *et al.* 2005) concomitant association between a low CT and a decreased residual soil moisture resulting from a high water extraction capacity of the root system. For instance, there was a significant association (averaged, r= –0.64, p<0.0001) of height with CT measured during booting in DRT, indicating that those RILs able to access more water were less susceptible to environmentally-determined effects on the height of genotypes. Similarly, there were significant associations between CT and flag leaf chlorophyll (Tables 12 & 21) under DRT, suggesting that chlorophyll loss might have been a symptom of inability to access water. In DRT, the association of CT and NDVI during booting (Table 21) suggest that rapid early growth

–that would require a higher assimilation rate– may have been due to increased access to water. The fact that CT was not well associated with NDVI –in contrast with other studies (Gutiérrez-Rodríguez *et al.* 2004)– during grain filling in DRT is not surprising, as NDVI values were reduced by leaf rolling as stress intensified after heading. Leaf rolling itself was associated with warmer canopies and negatively with NDVI during grain filling (Table 21).

OP association with yield was small (Table 9), in contrast with other reports (Morgan 1983; Morgan & Condon 1986; Blum & Pnuel 1990). However, unlike previous reports, these data were collected in field grown plots where plant water status was influenced by the ability of individual genotypes to access water from different soil depths. Thus, osmotic adjustment was presumably confounded by water availability --reflected in the null heritability reported in Table 9-- and may have reflected the ability to access water, or perceived moisture stress, rather than potential for osmotic adjustment *per se.* This hypothesis is supported by the strong positive genetic correlations observed between OP and CT under DRT (Table 21).

5.3 TRANSGRESSIVE SEGREGATION OF RILs

RILs' transgressively segregated for agronomic performance under contrasting conditions (Fig.5) suggesting a complex genetic background controlling yield performance. The large yield reduction range in the RILs subjected to DRT indicated a significantly ample response to moisture deficit (Fig.5), not solely explained by yield potential *per se* (*ca.*50%). It suggests that performance under DRT is contributed to by the expression of adaptive physiological traits (CT, NDVI, chlorophyll) that, *per se*, show transgressive segregation in all soil moisture environments. The transgressive segregation is revealed when observing the distribution in physiological traits across the RILs genetic background under DRT (Figs. 8, 9, 10 & 11). Such segregation is observed in the Seri/Babax RILs because even though both parents resemble each other in the expression of specific traits (Tables 8A & B), they presumably do not possess the same allelic or genetic composition controlling such traits (Lynch & Walsh 1998).

While selection for a physiological trait associated to yield performance *per se* has proven to increase yield (Reynolds *et al.* 1994; Richards *et al.* 2001; Rebetzke *et al.* 2002), there have been difficulties in analysing the genetic phenomena (epistasis,

non-additive gene action or overdominance) that govern the inheritance and expression of such traits, even more when the allelic composition in transgressive populations (as in the Seri/Babax RILs) are regulating complex traits as CT. For discerning those genetic factors responsible for the positive transgressive phenotypes for CT in an eventual selection scheme, one methodology is the quantitative trait loci (QTL) mapping analysis (Sax 1923; Paterson *et al.* 1988), with which it is possible to determine the chromosomal regions that contribute to the superior performance and the identification of specific parental allelic compositions that contribute at each QTL for its transfer from one genetic background to another using marker-assisted selection (Tanksley & Nelson 1996).

5.4 IMPLICATIONS FOR PHYSIOLOGICAL BREEDING

By assessing the response of RILs in a close-to-farm scenario under various moisture stresses, traits that contribute specifically to yield under water-limited environments can be identified, and the concept of the cereal ideotype model for high yield first expounded by Donald (1968) for wheat breeding can be extrapolated to dry environments (Reynolds *et al.* 2005). Identifying the sources of genetic gains via such physiological adaptations to drought and transferring them conventionally or transgenetically to well-adapted materials, can significantly augment yield productivity. Not only are the current findings in an ample genetic background confirmatory of the latter, but they concurrently permit a direct estimate of genetic gains associated with indirect selection for CT (Fig.9). Moreover, they ratify the originally proposed idea, by Blum *et al.* (1989), on utilising CT as a selection tool for drought-adapted genotypes in dryland conditions.

Although CT expression can be affected by environmental variations (e.g., soil moisture access, air humidity and temperature, eolic dynamics, solar radiation) and agronomic factors (soil toxicities or deficiencies, soil-borne parasitic diseases) or plant characteristics (phenology, height, canopy architecture), when used correctly and in appropriate conditions, it consistently shows highly-significant phenotypic and genetic associations with yield (Tables 9, 10 & 21). The latter is true, even across different environments and latitudes (Table 10). CT's large contribution to yield under DRT (r^2=0.74, p<0.0001), its high broad sense heritability (Table 9) and high genetic correlations (Tables 9 & 21), support its importance as an effective, consistent,

indirect methodology for predicting yield performance under DRT (Reynolds *et al,* 2000), or as a selection index for drought tolerance in hexaploid wheat (Blum *et al.* 1982, 1989). The methodology, deemed cost-effective (Brennan 2006) and facile in its utilisation when compared to other indirect tools (Condon *et al.* 2006), has already been examined to be used by breeders for genotype selection for its further implementation in breeding programmes (van Ginkel *et al.* 2006). Results validate the concept implemented in developing a population (Seri/Babax RILs) with similar height and phenological characteristics thereby avoiding these confounding factors during measurement of complex physiological traits.

Breeding for the complex, polygenic trait that CT is, either for its contribution to drought adaptation or as a high yield performance predictor under DRT, will be easier to genetically modify through improved understanding of the genetic control and its various avenues of expression and regulation.

5.5 CIMMYT-CSIRO COMPOSITE MOLECULAR ASSESSMENTS

The molecular characterisation via INTEGRAL BSA of the drought-adaptive trait that CT is, and its associated physiological traits in the dehrydration avoidance mechanism, required the assessment of the Seri/Babax RILs utilising different markers in two research facilities in Mexico (CIMMYT) and Australia (CSIRO). The efforts rendered molecular data that, when integrated into a composite, comprehensive database, proved to be an effective strategy that increased the understanding of the drought-adaptive traits when associated with specific markers identifying loci in the hexaploid wheat genome.

Different alleles were assessed at the two CIMMYT and CSIRO laboratories in a small subset of common microsatellite loci. The multiallelic nature (Tautz 1989; Pejic *et al.* 1998; Sandhu & Gill 2002) and hypervariable length (Röder *et al.* 1995) of microsatellites explain the observation, as the DNA substrate was of comparable quality and purity, having been extracted (in both premises) following the same standardised protocol. Hence, the common designated alleles used for the assessments in the INTEGRAL BSA were considered different, yet complementary.

Overall, the missing datapoint information –including non-amplified alleles– across the subsets of all the debulked RILs (used to prepare the bulks) using both marker systems comprised *ca.*10% of the database, mainly due to the AFLP dominant

marker nature. The codominant nature of microsatellites (Rafalski & Tingey 1993; Senior *et al.* 1996), explained that it was in this class of markers in where the heterozygote detection occurred (only 3% of the total alleles), revealing the phenotypic effects of both alleles equally (Griffiths *et al.* 2000).

The high level of polymorphism revealed by the microsatellite and other PCR-based marker systems is congruous with previous reports (Wu & Tanksley 1993; Morgante *et al.* 1994; Röder *et al.* 1995; Pejic *et al.* 1998). Microsatellites and PCR-based markers, as useful markers in genotypic characterisation, fingerprinting and mapping, vary in their usefulness (Röder *et al.* 1995; Smith *et al.* 1997), depending on particular species and application combinations/ intersections (Lee 1995*a*, *b*; Smith *et al.* 1997; Devos & Gale 1998; Pejic *et al.* 1998). In wheat, microsatellites (Röder *et al.* 1995, 1998; Pestsova *et al.* 2000) have proven to be crucial genetic tools for the construction of genetic maps and on the elucidation of the species' genomic architecture (Salina *et al.* 1998; Somers *et al.* 2004). The abundance of alleles revealed by the microsatellite system (Plashke *et al.* 1995; Röder *et al.* 1995; Gupta & Varshney 2000) contrasts with the generalised –and, depending on the genetic pool assessed, justified– conclusions (González de León *et al.* 1995) on that the AFLP methodology provide solutions to restricted polymorphism detection due to its multiloci nature (Vos *et al.* 1995; Pejic *et al.* 1998). When comparing amplification efficiency and productivity –in terms of polymorphic alleles rendered per laboratory assay– in all the assays, PCR-amplified markers were more efficient, faster and less a laborious methodology (Weining & Langridge 1991; Röder *et al.* 1995; Ribaut *et al.* 1997; Gupta & Varshney 2000) than the time-consuming, multistep AFLP procedure –even though the 10% selection efficiency obtained with the AFLP assortments was more than twice that of the referential source (William *et al.* 2003) and ten times that of reports using random primers (William *et al.* 1997). In addition, the availability of published resources for locating the microsatellite markers make them ideal for linkage endeavours, as the AFLP products have unknown chromosomic position *per se*.

5.6 PARENTAL GENOTYPIC PURITY AND POLYMORPHISM

Parental intergenotypic assessment

As the parental intravarietal analysis with three AFLP assortments –a 75% of the used in prospective analysis– rendered some doubts on the purity of the Seri parental line used in the original crosses (*Seri/Babax* and *Babax/Seri*) from which the population was derived, it suggested that the genotypes were not genetically identical (Fig.14). The diagrammatic analysis of the Seri allele distribution (Fig.19A) confirmed the latter, as a transgressive segregation pattern was observed across the 127 RILs subset histogram. However, the occurrence of more than one Seri plant did not render any segregation distortion in the population (Table 17), as the allelic proportion was of a 1 : 1 ratio. Thus, it confirms the well-understood notions (Sinnott *et al.* 1958) on that members of a pure line do not necessarily have identical genotypes and that the genotypic uniformity among them is likely to be greater than in a progeny obtained by cross-fertilisation of different individuals.

Parental polymorphism level

Although a low genetic polymorphism has been intrinsically encountered in certain plant species (Bonierbale *et al.* 1988; Ganal *et al.* 1988; Morgante & Olivieri 1993), it is with the genetically-narrowed, bred crops (Welsh 1981; Lee 1995) that the circumstance is more evident. However, the degree of sequence variation differs amongst the domesticated plant species, being higher in the out-crossed (Helentjaris *et al.* 1985; Shattuck-Eidens *et al.* 1990; Senior & Heun 1993; Smith *et al.* 1997; Bennetzen & Ramakrishna 2002) than in the self-pollinated species (Saghai-Maroof *et al.* 1984; Akkaya *et al.* 1992; Wu & Tanskley 1993; Bryan *et al.* 1999; Sandhu & Gill 2002).

Hexaploid wheat is a self-pollinated, cultivated species with a large-sized genome (*ca.*16000 Mbp[15]/1C[16], Arumuganathan & Earle 1991) characterised by a very low rate of nucleotide polymorphism (*ca.*1 polymorphic nucleotide per 1000 basepairs) (Bryan *et al.* 1999) and a reduced percentage (*ca.*10%) of low gene-containing regions (Sandhu & Gill 2002). The majority of the genome (*ca.*90%) of the polyploid species (7 homoeologous chromosomic groups in AA, BB, DD genomes)

[15] Mbp, Million base pairs.

comprises highly repetitive sequences (Britten and Kohne 1995; Röder *et al.* 1998) of genome-specific (Devos & Gale 2000), non-coding DNA (Sandhu & Gill 2002). Thus, an intrinsic high monomorphism was expected *a priori* in the parental polymorphism screening for the two parental genotypes (Seri and Babax), even more as both are Veery'S-derived genotypes and possess a closely related pedigree history –Seri and Babax have 25% of a common genome, with a realised coefficient of parentage of 33% (C.L. McIntyre, *pers.comm.*).

The low level of polymorphism observed between the parents, when assessed with the CIMMYT marker collection (28%) and that from CSIRO (25%), did not prevent to observe sufficient phenotypic differences between them and within the population, particularly under drought stress conditions [*see* **4.3.1.1 Agronomic traits**; Olivares-Villegas *et al.*]. It would be highly probable that if the selection of parents for the Seri/Babax population was performed utilising methods for polymorphism prospecting –such as polymorphism information content (Anderson *et al.* 1993)– or considering the conventional notion for selecting from a restricted pool for significant polymorphism (Bernatzky and Tanksley 1986; Bonierbale *et al.* 1988; Tanksley *et al.* 1989; Helentjaris *et al.* 1986), it would have been difficult to have them being chosen for generating a genetic pool with sufficient genetic diversity so as to measure complex traits interactions. Novel methodologies developed for the selection of parents with polygenically inherited traits have either integrated trait and marker information to simulate predictions and provide a decision support tool for genotypic selection (Eagles *et al.* 2001), or estimated classifications of genetic resources by combining marker information and phenotypic attributes (Franco *et al.* 1998, 2001). Any physiological breeding strategy, not only for selecting parental genotypes for genetic mapping or micro-, macro-molecular expression, but for specific pre-breeding purposes, would have to follow the latter approaches.

[16] 1C, the unreplicated haploid genome of the species (1C=17.8 pg).

5.7 POPULATION HOMOZYGOSITY AND SEGREGATION

Homozygosity

RILs are produced by continually selfing or sib-mating the progeny of individual members of an F_2 population until homozygosity is achieved (Haldane 1930; Haldane & Waddington 1931; Burr & Burr 1991). Thus, the genotypic uniformity is greater than it was in the initial crossbred population (Sinnot *et al.* 1958). RILs are genetic stocks considered homogeneous (Blake *et al.* 1991), facile in their production (Knott & Kumar 1975; Burr *et al.* 1988) and maintenance (in terms of homozygosity), with *fixed* linkage blocks –almost no recombination–, and essentially immortal (Soller & Beckmann 1990) –can be propagated indefinitely without further segregation, especially for polyploids, in where genomic redundancy results in absence of genomic coalescence (James 1992)–, which make them amenable of being easily shared between projects (Blake *et al.* 1991; McIntyre *et al.* 2006). The present collaborative research is proof of the latter, as the Seri/Babax population, bearing the aforementioned genetic attributes, has been readily evaluated in the field for a large number of traits in different environments in Mexico and South Australia over different cycles (Olivares-Villegas *et al.*), as well as in New South Wales and Queensland (S.C. Chapman, *pers.comm.*; McIntyre *et al.* 2006), while its partial –this report– and full molecular characterisation (McIntyre *et al.* 2006) have been performed at two different research facilities without the concern of further genetic recombination in the genetic pool.

While a major objective when originally developing the Seri/Babax RILs (*Seri/Babax*, *Babax/Seri*) was the study of drought-adaptive attributes in a hexaploid wheat genetic pool without the confounding factors of phenology and height, it was also decisive to conduct such studies in a genetically-stable population within the *Triticum aestivum* species. The development of the Seri/Babax RILs [*see* **3.1.1.2 Population development**] involved two particular methods for reducing the inherent heterozygosity after having initially followed a pedigree breeding scheme (Hayes & Immer 1942): a) at one generation (F_2) a single seed descent method (Goulden 1939) was utilised to advance them; and, b) single plants were taken from selected F_7 plots (generated via mass-pedigree, Harrington 1937) to generate seed for the 194 $F_{7:8}$ lines, as the calculated level of heterozygosity (*ca.*12.5%) for such

generation was considered high for their genetic analysis via genomics. The series of heterozygosis expansion/reduction procedures in the generation advancement for this population aimed, primarily, at expanding the allelic base (information content) for its ultimate fixation with restricted recombination.

An analysis of the debulked *Babax/Seri* genotypes used for the INTEGRAL BSA assessment (CIMMYT-CSIRO composite database) revealed a low level of heterozygosity (*ca.*3.7%), in consonance with the expected one (3.13%). However, it was suspected that in the original crosses more than one Seri parental plant was used and, potentially, different parental alleles were sampled. The implications of such a genetic condition are exclusively observed on the genetic drift for either of the parental alleles in the subsequent generations, for either parental allele will be eventually segregated and fixed, altering the expected segregation ratio not visible yet –*ca.*50 generations are required (Haldane & Waddington 1931). Effects of the existence of more than one Seri parental plant in the allele segregation are the observations of a transgressive segregation of the Seri allele (Figure 8) and the heterozygous pattern in 40 RILs when analysed with a set of markers, which were excluded of any further marker/trait analyses.

Segregation

For avoiding the heterozygous pattern in the BSA, a reduced number of genotypes (127) was selected, a decision justified by the ample phenotypic response and the significant transgressive segregation observed across all traits under every irrigation regime and latitude [*see* **5.3 TRANSGRESSIVE SEGREGATION OF RILs**]. Although it is considered that large population sizes are as important a factor as phenotypic heritability or multi-environmental sampling (Beavis 1998), in this study it was observed that the reduction on the utilised population size was not of a significant genetic effect, given that in the RILs genetic stocks type, the transgressive segregation allows for identifying sufficient allelic variation with little recombination in the variance for the traits of interest (Blake *et al.* 1991). Concomitantly, the combination of different methods for producing the Seri/Babax population at various steps in the generations advancement, effected positively in enhancing genetic diversity within the genetic pool.

Polymorphism

Whereas the population segregates with ample continuous categories at the phenotypic level, reduced polymorphic genomic regions were found. The insufficient number of polymorphic PCR-based markers prevented from developing a complete map, which in the case of wheat should minimally comprise a total of well-distributed 250 molecular markers (Röder *et al.* 1998) (*ca.*90 randomly distributed per 1000 centiMorgans, Kashi *et al.* 1986). Alternatively, a suitable strategy for the detection of marker/trait interactions was to be sought, considering the population type the Seri/Babax is.

Detecting effects of a certain magnitude via markers in a RIL population, requires a number of individuals to be scored similar to that in a double haploid population (Cowen 1988), but smaller than that with F_2 or F_3 population types (Soller & Beckmann 1990; Beckmann 1991). Direct scoring of RILs with minimal sampling is useful in detecting significant linked main effects (Cowen 1988), as in RILs the absolute size of the genetic effect detected as significant depends to a great extent on *r*, the map distance (in centiMorgans) –or percentage of recombination frequency– between the marker and the genetic loci underlying a quantitative trait. With RILs, the ratio of non-recombinant to recombinant is (1-R):R, for any marker class tends to $R= 2r/(1+2r)$ in the limit (Haldane & Waddington 1931). As RILs have twice the amount of observed recombination between closely linked markers as compared with a population derived from a single meiosis (Haldane & Waddington 1931), the linkage is difficult to detect in the initial steps of map construction, especially when the number of polymorphic markers is limited. However, the possibility of replicating the RILs in different environments or experimental treatments for assessing and quantifying the fluctuations (variance) under varying conditions (Sorrell & Beckmann 1990; Lorieux 1994; Paterson 2002), makes them ideal for discriminating genotypes upon their response (Quarrie *et al.* 1999). The fact that the population was assessed replicatedly in different environments and under differing water regimes not only enhanced the quality in the analyses of the phenotypic assessments, but also the power of determining significant marker/trait interactions (Knapp & Bridges 1990).

Hence, the genetic pool the Seri/Babax RILs was amply suitable for a genetic analysis through an interaction of markers with a quantitative trait. Even though the circumstance of insufficient markers across the genome was evident, the

considerable trait phenotyping of the total population genotypes performed was a strong basis upon which the genome analyses were to be performed (Lebowitz *et al.* 1987). In order to pursue an alternative to complete genome mapping, it was necessary: a) to rely on the consistently ample response within a limited number of RILs, recorded from replicated trials [*see* **4.3.1.2 Physiological traits**]; b) to focuss in traits that were of relevance to the adaptation to drought and that, while assessed in diverse environments, their heritabilities or genetic correlations were high (Soller & Genizi 1978; Knapp & Bridges 1990; Liu 1998) [*see* **5.2 RILs ADAPTATION AND PERFORMANCE**]; c) to define specific markers for selected groups of genotypes differing in their response (Stuber *et al.* 1982; Soller & Beckmann 1983; Michelmore *et al.* 1991; Darvasi & Soller 1992); and, d) to evaluate the pertinence of applying the strategy for any expressed trait –segregating in continuous categories (Lander & Botstein 1989)– to various soil-moisture environments in different latitudes.

5.8 INTEGRAL BSA STRATEGY- IMPLEMENTING A STRATEGY FOR THE MOLECULAR CHARACTERISATION OF DROUGHT-ADAPTIVE QUANTITATIVE TRAITS

Justification

The genetic analysis of the continuous variation in the Seri/Babax RILs followed the proposal of Soller & Beckmann (1983), similar to that suggested by Stuber *et al.* (1982), analysed by Lebowitz *et al.* (1987), Lander & Botstein (1989) and Darvasi & Soller (1992), while being applied and termed by Michelmore *et al.* (1991): Bulked Segregant Analysis (BSA). Scoring genetic markers in only those individuals that show extreme phenotypes for a particular trait, as the two allelic pools (bulks) will differ in the frequency of alleles affecting, in this case, the quantitative trait. While such pools integrate a composite of alleles genotypically-identical only in a particular locus (target locus or region), they will vary at the loci unlinked to the selected region. Consequently, the resulting two bulks will differ at the selected region, being heterozygous and monomorphic for all other regions. A significant deviation of *observed* from *expected* marker frequencies or a significant difference in marker allele frequencies between the two allelic pools, would be an indication of linkage between the locus and the QTL affecting (controlling or regulating) the trait of interest

(Lebowitz *et al.* 1987). Thus, marker alleles affecting the trait of interest in the vicinity of a quantitative trait loci (QTL) can be identified without the necessity of scoring all offspring with respect to the marker alleles.

Implementation

The application of the BSA methodology was originally intended for qualitative (mono- or oligogenically-controlled, quantitatively-expressed) traits involved in biotic stress, such as fungal (Michelmore *et al.* 1991; Yang *et al.* 1994; Eastwood *et al.* 1994; William *et al.* 1997, 2003), viral (Salava *et al.* 2002) or parasitic (Williams *et al.* 2001) diseases. However, the basic conceptual principles were applied in this study for a large number of quantitative (polygenically-controlled) traits, as an alternative to a complete QTL mapping. While there are reports on the utilisation of the methodology to dissect quantitative (polygenically) traits involved in abiotic stresses –silicon uptake (Ma *et al.* 2004), manganese efficiency (Khabaz-Saberi *et al.* 2002), paraquat (Altinkut *et al.* 2003*a*, *b*) or Aluminum tolerance (Ma *et al.* 2005)– or resulting of –yield, Quarrie *et al.* 1999–, there has been no reports on the utilisation of BSA for the thorough identification of genomic factors strongly associated with the drought-adaptive trait expression under varying water regimes, over different cycles and in different latitudes. This is the first successful study in where an assessment via BSA is performed on the highly heritable, single-most drought-adaptive attribute (canopy temperature) that explains a higher performance under drought stress in Mexico and Australia within a large hexaploid wheat RIL population **[5.2 RILs ADAPTATION AND PERFORMANCE]**. In addition, this study not only reports on the utilisation of the BSA assessment of the correlated quantitative traits to the drought-adaptive strategy (Table 9), with high genetic correlation with yield but moderate heritabilities (Table 9: chlorophyll and NDVI), but it also reports on the BSA of those traits with zero or low heritabilities (<4%) and a high genetic correlation with yield (Table 9: osmotic potential, leaf rolling); the latter type of traits (with low or null heritabilities) were concurrently assessed to provide information on the possibility of finding false positives through this methodology and, thus, providing information on potential misleading marker-trait associations. Furthermore, the concurrent molecular characterisation of the large hexaploid wheat RIL population was performed for their expression under reduced irrigation and under optimum

conditions to serve as a complete platform for expanding the understanding of the genomic associations and divergences within this genetic pool.

Genomic phenomena

Whereas a total of 387 marker/trait associations were located in all four categories (DRT, RED, IRR and AUS), their abundance and intensity were not homogeneous across the categories. When dissecting the contributions of the various hexaploid wheat genomes to the interactions in all categories, the B genome was the one with the largest contribution (*ca.*46%), followed by the A (*ca.*23%), while the D genome was the least contributing (*ca.*13%). Thus, the assigned significant loci totalled a *ca.*81%, while the unassigned (unknown chromosomic position) loci a *ca.*13%, and the ubiquitous loci only a *ca.*5%.

The different magnitude and disparate occurrence of marker/trait associations in every category reflects the divergent response of physiological and agronomic traits at various stages of crop development, at both the different latitudes in where the population was evaluated. Such a divergence can be explained by the impact of the environment [*see* **4.5 INFLUENCE OF ENVIRONMENT IN RESPONSE**] and revealed by the poor, if not null, correlations between traits measured at the two drought stressed environments –namely, DTR and AUS. Results indicate that QTL associations are largerly site-specific, as reported by other workers (Peccoud *et al.* 2004; Quarrie *et al.* 2005).

Evidently, the low level of polymorphism but ample response found within the Seri/Babax RILs suggest that the genotypic response is not strictly governed at the genetic level, but that there is a major influence of the transcriptomic systems in trait expression when subjected to drought (De Vienne 1991; Prioul *et al.* 1997; Cushman & Bohnert 2000). While QTL analysis aims at detecting relationships between genomic regions with the continuous distribution of traits, the INTEGRAL BSA approach followed in this scientific approach is reflecting the control of any such trait (as in the methodology described for this BSA study) following the mode of action of oligogenes (Mather 1943): large phenotypic variations explained by few regions (Tables 19A-D).

The latter assumption is supported by the fact that of the 107 markers, a total of 99 (*ca.*93%) were of significance for explaining the phenotypic variation in the bulks classes. Of such a figure, the markers of relevance were 68 GWM microsatellites,

23 non-GWM PCR-based markers and 8 AFLPs, indicating the efficiency of PCR-based markers versus the AFLPs in genotypic discrimination. Such markers either permit to identify variability in a highly monomorphic species in families of repetitive sequences (with probable regulatory roles in macromolecules syntheses or structural roles) or to detect unique, tagged sequences (that effect gene expression mechanisms) (Britten & Kohne 1968; Voet & Voet 1990).

5.9 DEVELOPMENT OF MARKERS FOR DROUGHT-ADAPTIVE TRAITS VIA INTEGRAL BSA

Marker loci associated with canopy temperature (CT)

Whilst there has been ample reporting on the association of CT to performance under stress (Blum *et al.* 1982; Blum & Gozlan 1989; Reynolds *et al.* 2000) and on their implications to breeding for stressed environments, there are no published accounts of the development of markers for such a complex trait. The present contribution aimed at investigating the possibility of developing significantly (p<0.05) associated markers to the expression of CT not only under different soil water regimes, but in different agroecological localities (Tables 19A-D). Results allow to speculate on the relevance of the INTEGRAL BSA strategy. As the significant genomic loci differ not only within treatments and across latitudes, but also in their magnitude, there were indications of significant environmental influences in the expression of the trait [in consonance with **4.5.1 GxE interactions**] as well as on the adaptation of the RILs studied [as described in **5.2 RILs ADAPTATION AND PERFORMANCE**]. Thus, logic implications are inferred on the feasibility of tailoring markers for specific agroecological niches or sites. Focussing on the significant (p<0.05) marker/trait associations explaining the phenotypic variation of CT, it is evident that the portfolio of site-specific markers for the trait is ample for any stage, as there were found at least twelve loci of relevance under DRT and thirteen for AUS (Table 23: summary of previous data, reported in **RESULTS** section). While the BSA methodology has some limitations *per se* (low number of individuals assessed and unavailability of linkage associations with other traits aside from the bulked ones), these markers (or other in close proximity in linkage maps) are to be considered to be utilised in furthering the molecular characterisation for the assessed trait. Feasibility of utilising single or

multiple markers, either in tandem or multi-trait selection, is a matter of consideration. Howewer, for some traits (like OP or leaf rolling), it should be a matter of caution, as a great a number of markers were identified of significance, even though those traits had a low or null heritability indicating the possibility of *noise*.

While the relevance of the marker/trait interactions differed across phenological stages (booting or grain filling) and latitude (AUS from DRT), there were remarkable consistencies in 20% of the chromosomic loci between the two drought-stressed sites. Further characterisation of either the Seri/Babax population can aim at dissecting the chromosomic regions of interest (chromosomes: 3B; 4A; and, 6A; but notably, the 5A; 5B; 5D; and, 6B).

Meaningful recent evidence, in process of being published by CSIRO-Plant Industry (G.J.Rebetzke, *pers.comm.*), on significant (p<0.05) chromosomic loci for CT has confirmed the DRT and AUS chromosomic positions herein reported. The fact that the loci, identified in Australian germplasm using QTL mapping, is in consonance with the loci characterised in 75 and 85% with the DRT and in AUS, respectively, is important evidence of latitude-consistency in the expression of the trait. The reasoning that the mapping confirms the significance and chromosomic position of the loci, permits to infer a certain extent of robustness for the INTEGRAL BSA strategy.

Table 23. Summary of significant (p<0.05) loci in the *Babax/Seri* RILs identified for explaining complex traits variation via marker/trait association in Mexico and Australia

	DRT	AUS
CHROMOSOME	TRAIT (%)[1]	TRAIT (%)
1A	OP-gf (31)	CT-gf (29), OP-gf (20)
1B	CT-boot (19), CT-gf (19), LROLL-gf (23), KNO (19), ATKW (30)	CHLO-boot (20)
1D	CHLO-boot (25), CHLO-gf (40)	CHLO-boot (36), LROLL-gf (36), YLD (44)
2A	NDVI-gf (24), OP-gf (25)	CHLO-boot (43), OP-gf (24)
2B	NDVI-boot (30), OP-gf (30), KNO (24), ATKW (30), YLD (24)	.
2D	OP-gf (32)	CT-gf (26), CT-gf (20)
2A, 2B, 2D	CT-gf (22)	.
3A	.	CT-boot (19)
3B	CT-boot (23), CT-gf (19), OP-gf (27), ATKW (20), YLD (20)	CT-gf (22), CHLO-gf (20), YLD (24)
3D	.	.
3A,4B	LROLL-gf (32), ATKW (26), YLD (26)	CHLO-boot (26)
4A	CT-gf (21), NDVI-boot (28), ATKW (29)	CT-boot (36), OP-gf (21), YLD (20)
4B	CHLO-gf (46)	OP-gf (22), LROLL-gf (34)
4D	.	.
4B,4D	.	CT-gf (28), OP-gf (22), LROLL-gf (28)
5A	CT-gf (23), KNO (19)	CT-boot (23), OP-gf (22)
5B	CT-gf (26)	CT-boot (22), OP-gf (37), LROLL-gf (38), YLD (30)
5D	CT-boot (32), CT-gf (19)	CT-gf (31)
5A,6A	.	CT-gf (26), CHLO-boot (32), CHLO-gf (36)
6A	CT-boot (25), YLD (19)	CHLO-boot (25)
6B	CT-gf (19), KNO (22)	CT-gf (27), CHLO-boot (23)
6D	.	.
7A	CHLO-boot (25)	CT-boot (36), CHLO-gf (19), LROLL-gf (21)
7B	KNO (27), YLD (26)	LROLL-gf (20)
7D	KNO (20)	LROLL-gf (35)

NOTE:
DTR, averaged data of cycles 1999/2000, 2000/2001 & 2001/2002.
AUS, averaged data of South Australian trials at Charlick and Roseworthy in 2001.
[1], %, Percentage of variation explained by the marker/trait association at the identified loci.
Trait codes are, as follows: CHLO-boot, Chlorophyll at booting; CHLO-gf, Chlorophyll at grain filling; CTAM-boot, Canopy temperature at booting in the morning; CTPM-boot, Canopy temperature at booting in the afternoon; CTAM-gf, Canopy temperature at grain filling in the morning; CTPM-gf, Canopy temperature at grain filling in the afternoon; OP-gf, Osmotic potential at grain filling; RNDVI-boot, Normalised difference in vegetative index at booting (red spectrum); RNDVI-gf, Normalised difference in vegetative index at grain filling (red spectrum); LROLL-gf, Leaf rolling at grain filling; HEIGHT-gf, Plant height at grain filling, KNO, kernel number; ATKW, a thousand kernels weight; YIELD, yield.

Marker loci associated with traits related to CT

The molecular characterisation of traits associated (positively or negatively) to CT is shown in (Tables 19A-D), and summarised (for DRT and AUS) in Table 23**.** A close inspection of relevant chromosomic regions (in terms of the magnitude of association in the identified loci) suggest that in both DRT and AUS there were chromosomic regions harbouring significant (p<0.05) loci involved in the dehydration avoidance mechanism [**5.2 RILs ADAPTATION AND PERFORMANCE**], although their occurrence was divergent. Under DRT, there were more loci focalised in specific chromosomic regions (5th and 6th), where there was relevance to the expression of CT and yield (or its components) (namely, 5A; 5B; 5D; 6A; and, 6B). In contrast, there were alternate, dispersed regions in where a wider array of traits were identified with the dehydration avoidance mechanism in either direction (at 1B; 3B; and, 4A). In AUS, it was the reverse. While there are indications of loci identified for the dehydration avoidance mechanism, they seemed to be dispersed across the genome (2D; 5A,6A; 6B; and, 7A). This was true for the coincident loci –yet genomically dispersed–, with relevance for CT and associated traits, either positively or negatively, and yield (3B; 4A; 5B).

In terms of marker development for specific traits related to CT, there was loci concentrated in chromosomes in homoeologous groups 1, 2, 3, 4 and 7 under DRT, whereas for AUS they are dispersed. To infer different trait interactions or to suggest a basis for the divergent response under the different soil water profiles at the different latitudes is not yet to be attained. Whether these results provide indications of relevant loci with the expression of CT, they remain to be analysed in depth. To infer pleiotropic and interallelic interactions in the governance of one or more traits and their relatedness to the influence of GxE interactions (Aastveit & Aastveit 1993), is unfeasible given the nature of the assessment; yet, it is evident that the GxE interactions under DRT are subject of consideration when deciding the study of one or more traits for that particular soil water profile in Mexico. For as the INTEGRAL BSA evidences to be a robust methodology, it is still to be considered a platform for advanced studies of molecular characterisation. Nonetheless, there was a relevant number of loci identified for the marker/trait association explaining the variance of NDVI (an estimate of biomass) –for which there are no previous reports of associated markers, at least in the public realm–, chlorophyll, leaf rolling, osmotic potential,

yield components and yield *per se*. Further, such relevance is consistent with the Seri/Babax QTL map (McIntyre *et al.* 2006) for height (2B and 4A in AUS) and kernel size (a thousand kernels weight in 1B under DRT), while there is coincidence (in AUS: 1D; 2B; 3B; 4B, 4D; 4A; and, 5B; under DRT: 2B, 4B, 4D; 6A; 7A; and, 7B) with a high-density QTL map of hexaploid wheat for yield across a range of environments (Quarrie *et al.* 2005).

Modification of BSA

As Michelmore *et al.* (1991) predicted when applying the BSA methodology for the dissection of qualitative traits, the detection of a decreased linkage disequilibrium would be found between the target markers and the polymorphic loci when unequal amounts of alleles were increasingly combined in the bulks as more recombinants were selected for the bulks. The latter was evidently corroborated, as when 10 genotypes per bulk were utilised in the BSA, a mere 8% of the population was represented; in contrast, with the 24% of the total population alleles represented when increasing using 30 genotypes per bulk (Table 20), a significant (p<0.05) reduction of both the resolution and effectiveness of the assessment was observed (23-34% range in reduction). This is in close terms with the stated excluding threshold (25%) in allelic species per subset established by Darvasi & Soller (1992). It was evident that even when characterising polygenic, quantitative traits involved in drought-adaptation of hexaploid wheat, as little as ten genotypes per bulk is sufficient a figure for estimating the association of genomic factors involved in explaining a significant proportion of the phenotypic expression under varying water regimes and in agroecological localities in distant latitudes.

5.10 INTEGRAL BSA- IMPLICATIONS FOR MOLECULAR BREEDING AND FOR OTHER GENOMIC APPROACHES FOR THE DISSECTION OF DROUGHT RESPONSE

Cost-effectiveness of the INTEGRAL BSA molecular dissection strategy

Map saturation has been a significantly expensive endeavour (Ragot & Hoisington 1993) that has been considerably reduced (Beckmann & Soller 1995) over the past years due to the development of PCR and of PCR-based markers, automatisation,

relative cost decrease in reagents and laboratory disposables, and availability (Gelfand 1989; Mullis 1989; Newton and Graham 1995; Röder *et al.* 1998; Gupta & Varshney 2000). As the changes in the financial climate within the global economic network are forcing to adapt research programmes to increased economic restrictions not only at the national level but also at the international one (Heisey *et al.* 1999; CIMMYT 2004, Borlaug 2006), for policy-makers and, consequently for agribiotechnological researchers, the financial effectiveness is a matter of utmost concern. The allocation of restricted resources, thus, prevents the establishment of long-term research programmes, but estimulate creativity. In the case of the development of a saturated map for wheat or the characterisation of expressed factors, the inherent complexities of genomically studying the species have prompted researchers to establish global collaborative efforts (ITEC, ITMI, *grain genes*[17]) or specific alliances (Seri/Babax: CRCMPB and CIMMYT, INT; and, CIMMYT, INT and CSIRO-Plant Industry) and share the investments and returns.

While the saturated mapping of factors is a limitation for many programmes, the utilisation of single or multiple markers for selection of genes associated with traits is not. Economic analyses have been conducted for establishing the cost-effectiveness of utilising molecular markers at one or various stages of the plant breeding process, in the same view of assessing other elements of the plant industry (Brennan 1997; Brennan & Morris 2001; Brennan 2006). This, not only with the specific purposes of project management within a commercial strategy (private sector: Eathington 2006), but for efficaciously incorporating them in national breeding programmes (Australia: Spielmeyer *et al.* 2001; Langridge 2003; CRCMPB 2003, 2004) and international public research (CIMMYT: William *et al.* 2005; William 2006). Whereas it has been reported (Dreher *et al.* 2002) that the financial investment for producing one data point (at CIMMYT) would involve USD$2.13, only a year later (Dreher *et al.* 2003) the figure decreased to USD$1.35 when involving 10 or more markers and over 100 samples, but that figure was calculated to be abated to USD$1.26 should the analysis involved 50 chemiluminiscently-labelled markers on 200 samples. A competitive, similar figure (A$1.00) is estimated (C.L. McIntyre, *pers. comm.*) at the CSIRO-Plant Industry facilities for radiolabelled markers. Collaborative efforts, technological trends (automatisation) combined with outsourced molecular

cxxxiv

[17] wheat.pw.usda.gov

datapoint production are likely mechanisms by which budget-restricted research programmes can develop markers should the infrastructure capabilities are limited.

The ample number of significant (p<0.05) marker/trait associations for an ample repertoire of drought-adaptive traits for drought-stressed and reduced irrigation conditions (Northwestern Mexico) and for a rainfed agroenvironment (South Australia), INTEGRAL BSA allow to establish that they are useful strategies for developing simple, cost-efficient methodologies that can reduce the cost (Darvasi & Soller 1992) and complexity of marker-assisted selection (MAS) for selected traits under an abiotic stress. The strategy aforementioned can meet expectations from national agricultural scientists from budget-restricted breeding programmes, as it is cost-efficient for inbred line conversion if studied on a case by case basis (Dreher *et al.* 2002; Morris *et al.* 2003), or for pyramiding other traits associated with adaptation or tolerance to drought stress; with the two essential elements in applying the strategy being: a) a large phenotypic data collection of heritable traits, and b) a suitable genetic pool. Already an independent economic analysis have showed (Brennan 2006) that the physiological trait that has been the most significantly-associated to yield under drought in Mexico and Australia (CT) is a low-cost option that can lead to significant cost savings in selecting for yield. The efficient selection tool –already in use by breeders (van Ginkel *et al.* 2006)–, can be coupled with the INTEGRAL BSA strategy for developing a portfolio of powerful physiological and molecular marker tools for assisting breeding programmes in the selection of drought-adaptive hexaploid wheat germplasm with a positive high investment-return ratio.

Impacts for Molecular Breeding

Provided sufficient funding and evidence of its efficiency in any given agribiotechnology programme, the development of markers via INTEGRAL BSA can serve as an ideal platform not only for the further characterisation of the regions significantly associated to the traits of importance, but also for its expansion into a complete full QTL genomic map for the analysis of polygenic, complex traits. When this circumstance is not encountered, the impacts of the aforementioned INTEGRAL BSA for the development of markers for abiotic stresses can be summarised in four major categories:

I. Efficient development of markers for abiotic stresses in a marker-assisted selection (MAS) scheme for polygenic, quantitative traits (Lee 1995) evaluated in different agroenvironments. This, either for exclusive (phenophasic stages, latitudes, experimental conditions) or generalised conditions (overall averaged response). The former, related to the MAS scheme for specific environmental niches, depending on soil water profile within genotypes and pools of *Triticum aestivum*. The latter, for amply heritable traits across regions or sites with significant (p<0.0001) associations with yield.

II. Potential combination of five strategies for the development of markers for distinct, varying soil water conditions:

1) Development of a BSA strategy (Soller & Beckmann 1983; Michelmore *et al.* 1991), tailoring marker sets for divergent allelic pools upon continuous variation of traits. This would lead to elucidate the specific genetic architecture of quantitative traits and allow for selection of favourable allelic pools, allelic combinations and individual alleles or loci (Stuber *et al.* 1982; Beckmann & Soller 1988) that lye in the vicinity or reveal the presence of a loci associated to the phenotypical *favourable* response.

2) Characterisation of genotypes with favourable attributes either for the associated loci or for a larger marker set aiming at genomic coverage of the region of significance.

3) Selective genotyping of appropriate genotypes within a genetic pool, as rationalised by Darvasi & Soller (1992) and reviewed by Prioul *et al.* (1997) based in the conclusions of Lander & Botstein (1989) –for continuous traits, linkage of markers and traits is markedly increased when the analysis is restricted to individuals in the high and low phenotypic tails of the population.

4) Introgression of dominant or overdominant QTL alleles or of those loci with large additive effects (Wehrhahn & Allard 1965) from the adapted genotypes in a population –in the case of this study, the Babax parent or the drought-adapted RILs– to other less-adapted genotypes, sister lines or F_2s, F_3s segregants (as proposed by Stuber *et al.* 1982) of commercial and production value, either for global cultivation or for specific environmental and market niches. This, with the express purpose of improve the efficiency of hexaploid wheat quantitative trait breeding.

5) Generation of expressed sequence tags (ESTs) via complementary DNA clones (cDNA) (Adams *et al.* 1991) from selected individuals or from pools of contrasting genotypes (*à la* INTEGRAL BSA), as a strategy for identifying novel coding regions in genomic sequences, discovering genes associated to specific traits and for mapping of the Seri/Babax genome. Conversion of ESTs into PCR-based markers would ensue (Scott *et al.* 2000).

III..Evaluation of the transfer and convertibility of PCR-based markers (Guyomarc'h *et al.* 2002; Kuleung *et al.* 2004; Nicot *et al.* 2004) for their utilisation in the molecular characterisation of species within the *Triticeae* and *Poaceae*. Based on identifying the site in the linear genomic organisation (Moore *et al.* 1995; Gale & Devos 1998) of the significantly associated loci to the traits of interest, an exploration of the syntenic relationships and colinearities of genes or chromosomes (Devos & Gale 1997) can permit to establish relevance of the genetic factors across species. Ultimately, the approach can have important implications in the structural estimates (Bennetzen & Freeling 1993; Fulton *et al.* 2002), domestication tempo (Paterson 2002), evolutionary or phylogenetic studies (Devos & Gale 1997, 2000; Gale & Devos 1998; Paterson *et al.* 2000) of stress response processes (Langridge *et al.* 2006).

Employ of strategy in functional genomics of abiotic stresses

Upon the effectiveness of the BSA methodology for identifying genetic and genomic factors associated to the phenotypic variation of a trait, there have been proposals of its utilisation in functional genomic studies for the targeted dissection of candidate genes (Quarrie *et al.* 1999; Dietrich *et al.* 2006; compgenomics.ucdavis.edu[18], 2006), induced expression molecules (Habben *et al.* 1999; Bohnert *et al.* 2000; Bohnert *et al.* 2002) and expressed metabolites (de Vienne & Zivy 2000). The basic requirements that the functional analyses in those proposals remain the same as for the INTEGRAL BSA with the Seri/Babax RILs: high quality, phenotypic data on suitable, large populations and accessions to efficiently correlate with candidate genes.

Whilst the molecular characterisation rendered a clear perspective for further genomic studies on drought-adaptation and a precise establishment of an effective marker development platform, there is relevance for a prospective functional genomics approach. The molecular characterisation of the Seri/Babax RILs via

[18] *Compositae* Genome Project Whitepaper proposal.

INTEGRAL BSA suggested that there are different A, B or D *Triticum aestivum* chromosomes (some in common in both latitudes) of relevance for the variation in the expression of molecules involved in drought tolerance or adaptation where there are significant indications of the polygenic governance of CT. Although there is evidence on the clustering of a class of dehydrins[19] in chromosomes 5 and 6 (Campbell & Close 1997; Close *et al.* 2000) of both *Hordeum vulgare* and *Triticum aestivum*, and on the localisation of ATPases[20] in chromosome 7A of *Triticum monococcum* (Dubcovsky *et al.* 1995), it is a matter of further investigation if they are clustering in the presently outlined loci (for the marker/trait association) for CT. The intrincatedly complex multilevel networking of the functional operation of enzymes, transcription factors, polypeptides, hormones and secondary metabolites is expressed integratively as secondary traits in which category the polygenic CT is. however, this remains to be investigated. Already, regions of the *Triticeae* genome have been identified (Cativelli *et al.* 2002) to be governing the tolerance or adaptation to drought stress, which are, incidentally, the regions (major: 5, 6 and 7; minor: 1 and 2) for which the most significant and abundant loci were identified for being associated with the interaction of the markers developed via INTEGRAL BSA and the expression of CT in both Mexico and South Australia.

Further genomic and transcriptomic expression analyses of CT is paramount in order to understand the interaction and control of the complex physiological networking in the Seri/Babax RILs, exerted early in the crop cycle. Such understanding will further help to unravel the complex relationship between yield performance and drought adaptation.

cxxxviii———————————

[19] Polypeptides involved in drought stress.

[20] Adenosine triphosphatases, *i.e.*, enzymes involved in the catalysis (hydrolysis) of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), releasing usable energy within the cell (Voet & Voet 1990).

CONCLUSIONS

6.1 PHENOTYPIC EVALUATION

In the population studied under various drought-stressed wheat-growing environments in Mexico and South Australia, tolerant genotypes response encompassed a mechanism of drought escape via dehydration avoidance expressed at early stages of the crop cycle and maintained until physiological maturity. Regardless of the environments in which the population was studied, CT was the single-most important drought-adaptive trait contributing to yield performance.

Breeding for tolerance via indirect methodologies describing integrative physiological traits is an approach which the present study examined, and confirming CT's utility as a simple and practical tool. CT is significantly and consistently associated with yield under drought stress across cycles in Mexico and to a lesser extent at the South Australian locations where it was examined, despite the difficulties of collecting the data because of cloudy and windy conditions. Hence, the robustness of the physiological methodology supports its use in breeding as an important predictor of yield performance or as a part of a selection index under drought stress.

As the complex trait response is inextricably bound to yield performance and appears to penalise the highest yielding lines under full irrigation, additional genomic investigations were conducted on the Seri-Babax population leading to understand the genetic governance and physiological adaptations of the sister lines under drought stress and their contributions to higher productivity. The large genetic diversity observed was sufficient to further the understanding of the genetics of drought adaptation.

6.2 GENOTYPIC ASSESSMENT

A molecular characterisation for the complex drought-adaptive trait, CT, and for associated quantitatively-expressed physiological and agronomic traits was performed via BSA on a subset of the Seri/Babax RILs population. The examined approach resulted in the efficient development of molecular markers for specific traits at specific stages of crop development. A synergistic research endeavour for accomplishing the marker development was established between an international agriculture centre, CIMMYT INT, and a world-class Australian research organisation,

CSIRO-Plant Industry, aiming at advancing research resulting from previous collaborations of the former within the Australian Cooperative Research Centre Molecular Plant Breeding with relevance for the Mediterranean-type environment of the South Australian wheat-growing region. The purposes of such a synergy was to further the understanding of the hexaploid wheat adaptation to drought and to develop technology pertinent to Australian environments, particularly for the water-limited northern wheat-growing region.

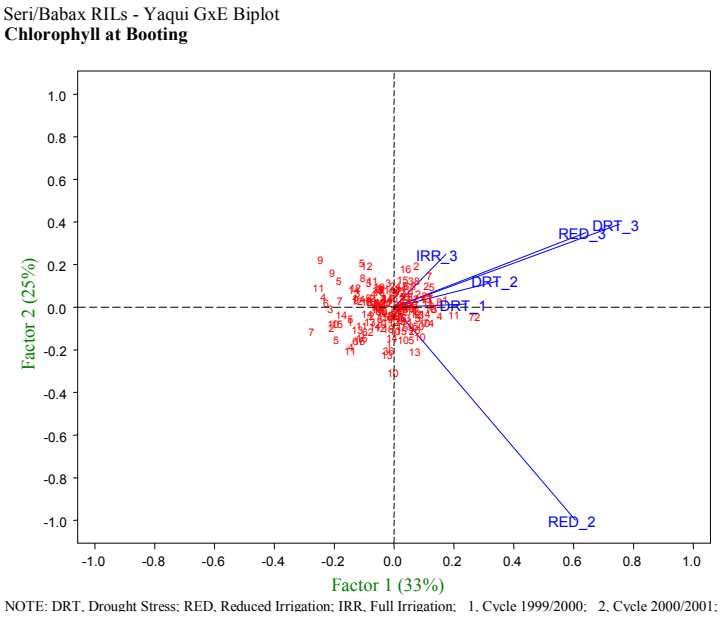
Abundant genomic loci were identified with markers whose significant association with the complex traits explained, in various orders of magnitude, the phenotypic variation, regardless of soil-water regimes or latitudes. Specific marker development for CT and for other complex traits associated, positively or negatively, with the dehydration avoidance mechanism, was particularly tailored for two different drought-stressed and rainfed agroecological sites in Mexico and South Australia in where the genotypic response was studied at different phenological stages. Loci identified via this methodology were consistent with other, more descriptive, genomic studies conducted in Queensland and Canberra, Australia. Hence, the robustness and cost-efficiency of the BSA strategy for complex traits involved in drought stress adaptation or tolerance, supports its utilisation in molecular breeding not only for budget-restricted breeding programmes, but also for medium- and long-term funded research plans. While it was developed as an alternative to full QTL mapping in response to financial constraints at the host institution during the development of the Ph.D. research, the robust methodology did not preclude the furthering of the molecular characterisation of the population. On the contrary, as in the case of the Seri/Babax RILs, its physiological attributes and significant prospective genomic studies, allowed researchers at CSIRO-Plant Industry to pursue its full QTL mapping to provide information and molecular tools to enable indirect selection and pyramiding of genomic regions with relevance to the Australian wheat industry. Genomic dissection for gene identification, QTL cloning and genome modification via engineering are avenues of research for the genetic design of genotypic attributes specific for Australian wheat environments. Comparative mapping and syntenic relationships for an enhanced understanding of the genomic control in the tolerance and adaptation of drought within the *Triticeae* and *Poaceae* remains an area of research and technology development that can be pursued from the further study of genomic loci relevant to drought adaptation.

Molecular breeding of complex traits, as examined and verified in the present study, can be utilised as an effective stategy for the development of drought-adapted genotypes for specific agroecological Australian and world regions. A validation of the herein reported marker-trait associations in other segregating populations (subjected to drought stress in different latitudes), can allow to confirm its potential use as molecular tools which, alone or in combination with the robustness of the physiological indirect selection tool, could be utilised for performance prediction or genotypic assessment under varying levels of drought stress.

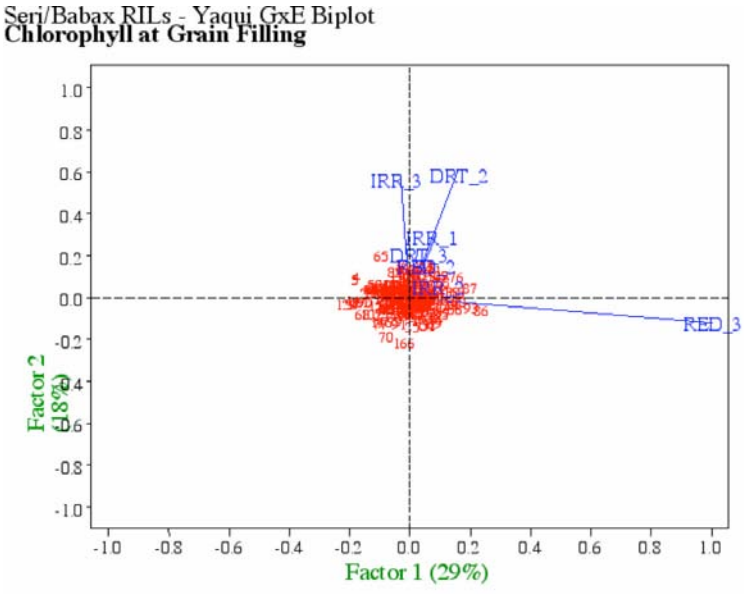
A large genetic diversity and significant transgressive segregation within the Seri/Babax RILs enabled the efficient characterisation of the complex trait involved in the dehydration avoidance mechanism. This, in spite of the low levels of polymorphism between the parental genotypes, suggesting that the diverse genotypic response is not strictly governed at the genetic level. Relevant information and regulation systems, as the transcriptome and proteome, remain to be investigated in depth for broadening the understanding of the cascade of biological phenomena combined with environmental characterisation resulting in the expression of CT under drought stress and its implications on yield potential in wheat for Australian environments.

APPENDICES

APPENDIX 1. Genotype-by-environment interactions for a number of selected traits under various hydric conditions (DRT, drought stress; RED, reduced irrigation; and, IRR, full irrigation). Seri/Babax population in Yaqui valley, Mexico (cycles: 1, 1999/2000; 2, 2000/2001; 3, 2001/02002).

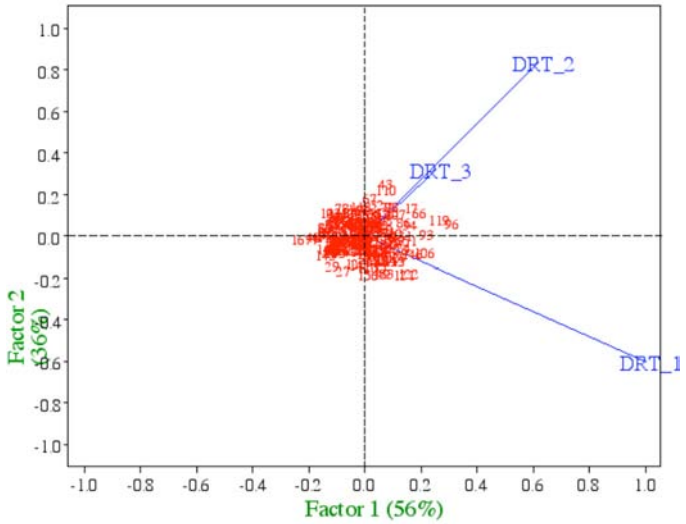


NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001;



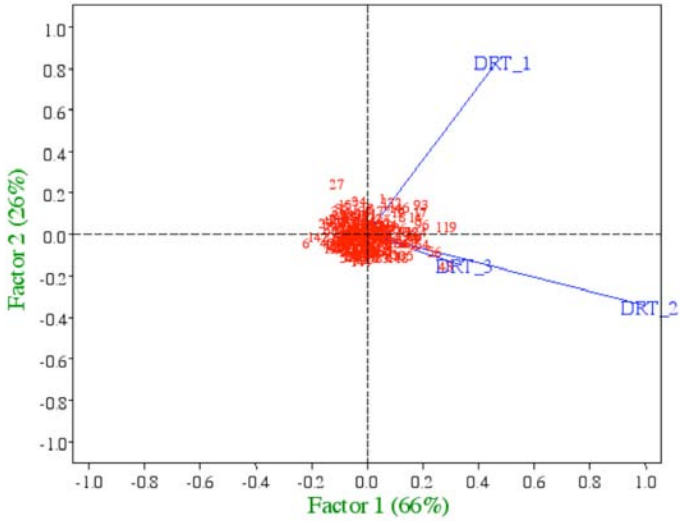
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Canopy Temperature at Booting, in the Morning



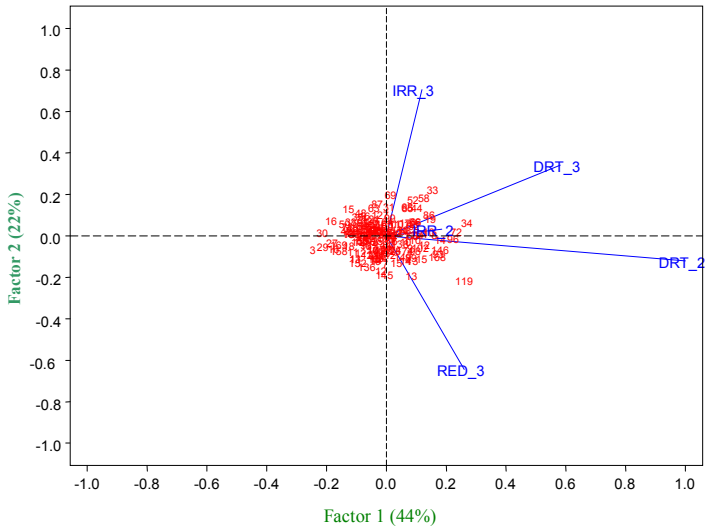
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Canopy Temperature at Booting, in the Afternoon



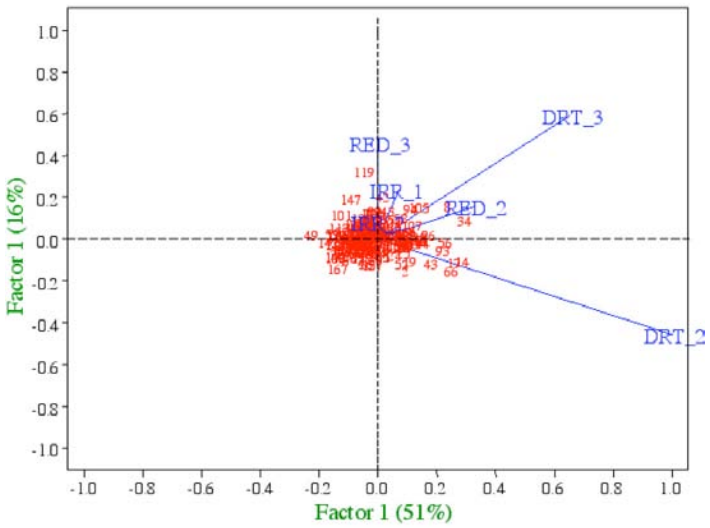
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Canopy Temperature at Grain Filling, in the Morning



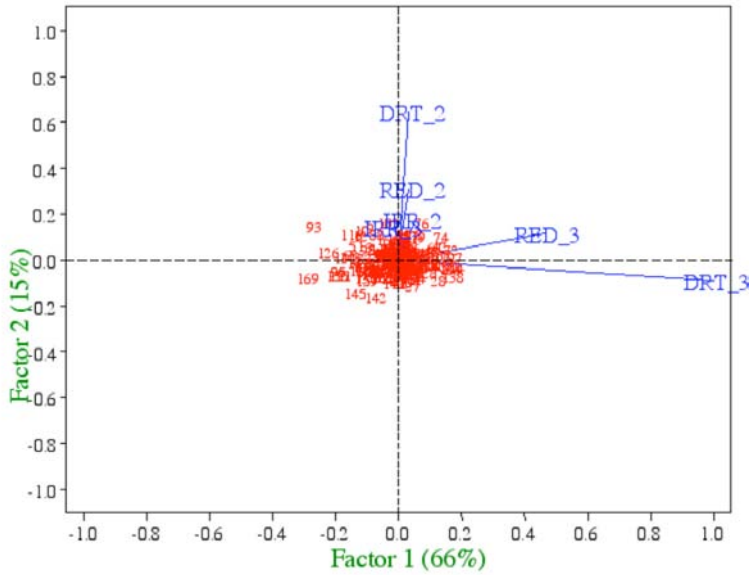
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Canopy Temperature at Grain Filling, in the Afternoon



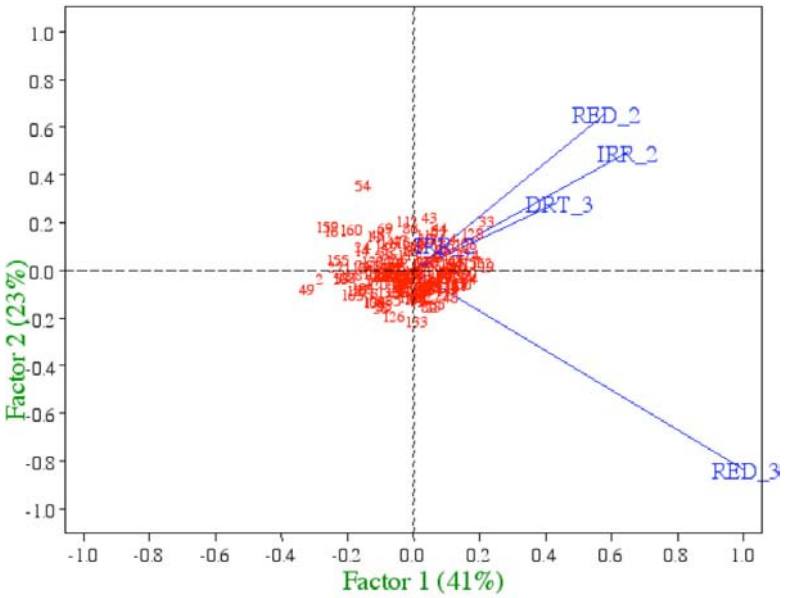
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Osmotic Potential at Grain Filling



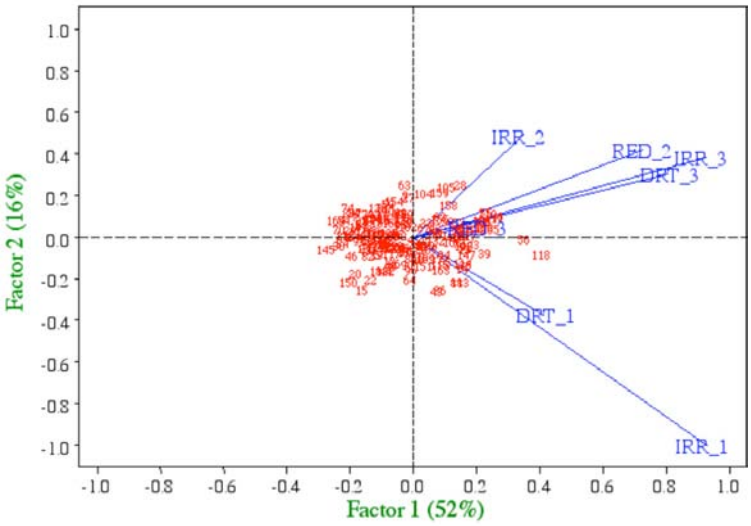
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Leaf Rolling at Grain Filling



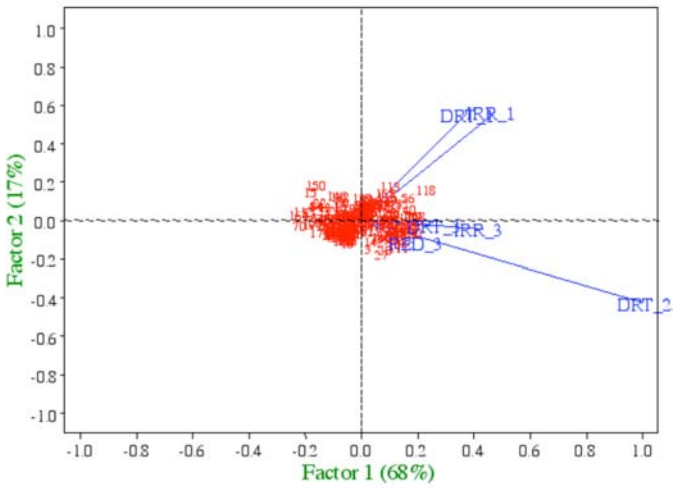
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Days to Anthesis



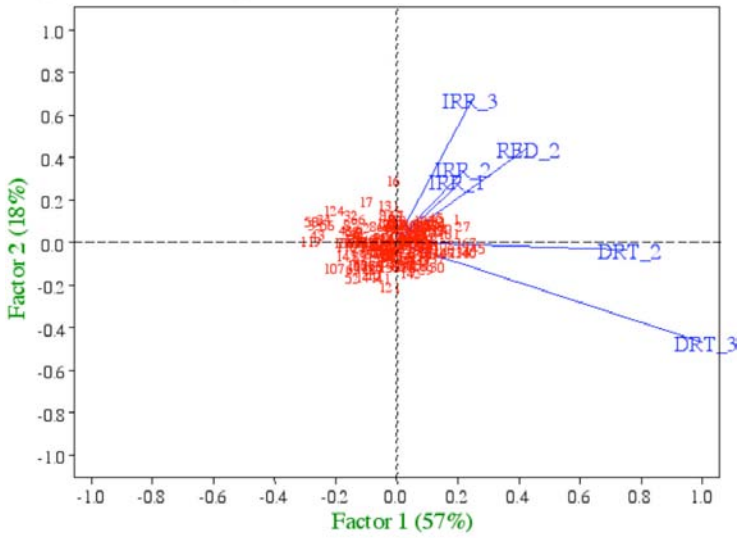
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Days to Physiological Maturity



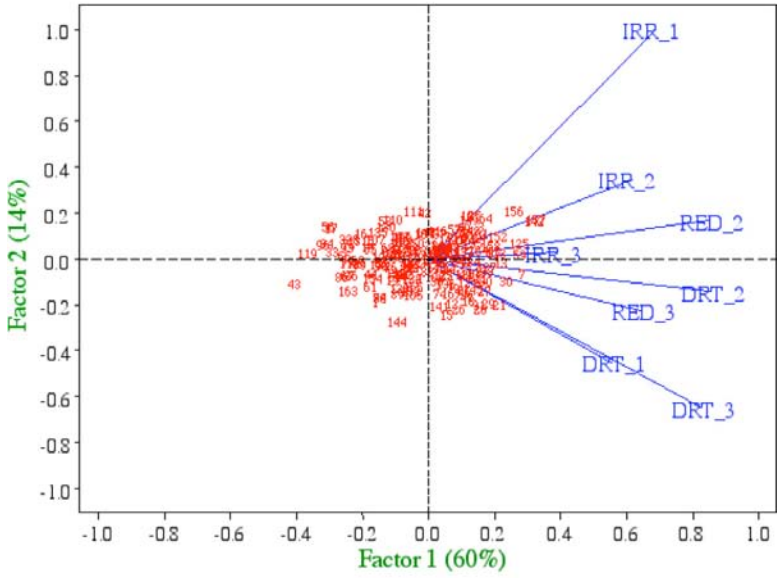
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Height at Grain Filling



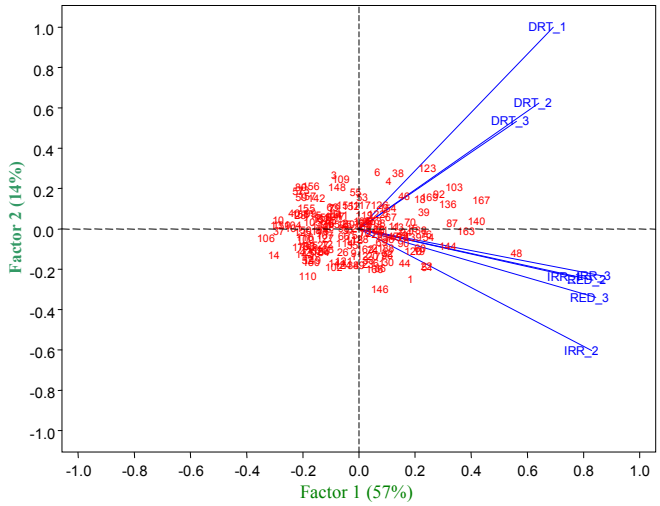
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Kernel Number



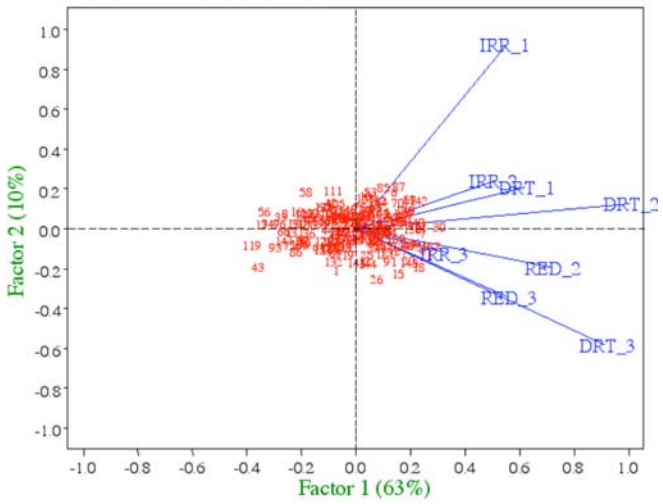
NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
A Thousand Kernel Weight



NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

Seri/Babax RILs - Yaqui GxE Biplot
Yield



NOTE: DRT, Drought Stress; RED, Reduced Irrigation; IRR, Full Irrigation; _1, Cycle 1999/2000; _2, Cycle 2000/2001; _3, Cycle 2001/2002.

APPENDIX 2A. Bulked segregant analysis from a 127 *Babax/Seri* RILs subset. Drought stress in Yaqui valley, Northwestern Mexico

RIL	CHLO-BOOT	RIL	CHLO-GF	RIL	CTAM-BOOT	RIL	CTPM-BOOT	RIL	CTAM-GF	RIL	CTPM-GF	RIL	OP-GF
126	50,7	126	49,7	55	21,5	6	26,2	3	20,9	11	24,7	BABAX	-1,53
24	50,2	78	48,7	7	21,5	55	26,6	29	21,2	123	24,9	110	-1,68
85	50,3	BABAX	48,8	11	21,6	7	26,7	20	21,5	70	24,9	72	-1,85
64	50,0	30	48,1	3	21,7	70	26,8	BABAX	21,5	55	25,1	81	-1,81
98	50,2	124	48,8	47	21,7	BABAX	26,7	123	21,6	29	25,2	114	-1,96
89	50,0	84	47,8	12	21,7	11	26,9	86	21,6	3	25,3	23	-2,03
84	49,7	12	47,9	BABAX	21,5	28	26,8	45	21,7	20	25,3	6	-1,90
23	49,8	123	47,8	6	21,6	84	26,9	11	21,6	27	25,3	66	-1,99
87	50,4	70	47,7	92	21,7	78	27,0	52	21,7	6	25,3	123	-2,05
10	50,3	65	48,0	110	21,7	23	27,0	126	21,7	88	25,2	64	-2,06
SERI	47,9	98	48,1	70	21,8	112	26,8	94	21,8	BABAX	25,2	7	-2,01
BABAX	47,9	SERI	44,0	SERI	22,2	SERI	27,7	81	23,0	SERI	26,3	SERI	-2,20
72	46,6	127	43,7	113	22,8	121	28,3	89	23,1	122	26,9	43	-2,33
83	45,7	81	43,5	81	22,7	14	28,6	115	23,3	125	27,1	80	-2,42
81	46,1	43	44,0	8	22,7	108	28,8	108	23,3	108	27,7	68	-2,43
109	46,3	110	43,9	122	22,8	79	28,5	14	23,4	82	27,0	14	-2,53
27	44,7	122	43,9	108	23,1	109	28,2	38	23,3	79	27,8	112	-2,49
119	45,7	85	42,4	121	23,1	111	28,7	100	23,3	85	27,3	2	-2,36
50	45,4	27	43,3	100	22,9	38	28,5	SERI	23,4	14	28,1	8	-2,41
43	45,4	1	43,3	18	23,2	8	28,5	8	23,5	111	27,4	85	-2,50
68	45,4	52	42,2	79	23,2	100	28,4	111	23,7	18	28,2	89	-2,50
125	44,8	83	40,5	111	23,6	18	28,9	85	23,8	8	28,5	87	-2,51

NOTE:
Drought-adapted genotypes selected for "Tolerant" bulk are on the top, while those less drought-adapted genotypes selected for "Susceptible" bulk are at the bottom.

APPENDIX 2A (*continued*). Bulked segregant analysis from a 127 *Babax/Seri* RILs subset. Drought stress in Yaqui valley, Northwestern Mexico

RIL	RNDVI-BOOT	RIL	RNDVI-GF	RIL	LROLL-GF	RIL	HEIGHT-GF	RIL	KNO	RIL	ATKW	RIL	YIELD
12	0,827	116	0,640	38	5,2	29	80,9	29	20779,0	118	43,6	7	768,0
11	0,805	47	0,643	50	5,2	123	78,2	70	20204,6	BABAX	42,4	70	747,9
20	0,777	124	0,628	21	5,0	BABAX	78,8	7	20340,1	107	42,2	11	759,8
28	0,796	SERI	0,623	8	4,9	1	77,4	22	19634,9	101	41,7	55	728,9
27	0,791	94	0,622	109	4,9	55	78,8	23	20309,3	20	41,5	23	754,9
BABAX	0,791	6	0,616	111	5,1	3	73,7	105	18824,8	45	41,9	22	723,6
23	0,789	43	0,613	100	4,9	88	75,4	11	19351,9	6	41,2	20	748,9
78	0,786	30	0,609	22	4,9	11	75,4	55	18511,9	83	40,2	29	769,5
48	0,779	29	0,622	107	4,8	52	74,0	92	17163,2	3	39,8	105	676,3
29	0,779	126	0,619	11	4,7	20	74,6	110	16999,4	124	39,8	6	661,8
76	0,777	96	0,629	BABAX	4,2	94	73,6	BABAX	15054,4	89	39,5	BABAX	642,8
SERI	0,761	BABAX	0,580	6	3,5	SERI	60,7	SERI	12375,6	SERI	34,4	SERI	432,9
119	0,713	38	0,467	87	3,6	109	56,3	121	9712,8	10	34,3	122	346,3
9	0,710	100	0,465	2	3,5	79	52,6	125	9646,1	108	34,2	121	325,6
85	0,708	50	0,438	66	3,4	18	55,9	14	8685,0	115	34,0	100	305,4
50	0,707	79	0,441	117	3,5	81	55,9	100	8904,6	116	34,2	38	269,3
74	0,705	1	0,465	SERI	3,4	122	55,3	38	7633,5	43	33,8	50	222,1
125	0,700	65	0,457	68	3,4	125	54,5	108	7298,1	126	33,7	111	257,1
38	0,687	9	0,436	123	3,1	85	54,1	111	6998,2	63	33,2	125	308,4
115	0,686	83	0,454	124	3,3	8	53,5	50	5749,3	121	32,9	108	250,0
122	0,686	24	0,419	29	3,2	50	44,9	85	6422,3	117	33,3	85	231,0
100	0,681	21	0,396	64	3,1	38	45,5	18	5403,6	125	31,7	18	181,2

NOTE:
Drought-adapted genotypes selected for "Tolerant" bulk are on the top, while those less drought-adapted genotypes selected for "Susceptible" bulk are at the bottom.

APPENDIX 2B. Bulked segregant analysis from a 127 *Babax/Seri* RILs subset. Reduced irrigation in Yaqui valley, Northwestern Mexico

RIL	CHLO -BOOT	RIL	CHLO -GF	RIL	CTAM -GF	RIL	CTPM -GF	RIL	OP-GF	RIL	LROLL -GF	RIL	HEIGHT -GF	RIL	KNO	RIL	ATKW	RIL	YIELD
72	50,1	86	55,6	39	16,9	71	25,6	BABAX	-1,49	106	4,6	1	112,4	7	19568,1	48	41,0	30	648,5
111	48,2	87	54,5	52	16,9	125	25,6	126	-1,64	19	4,4	120	106,3	21	18698,8	22	37,9	21	644,7
108	48,2	76	54,4	71	17,2	39	25,7	125	-1,68	84	4,3	92	105,8	3	18633,8	76	37,9	18	640,7
48	48,1	71	53,9	38	17,3	BABAX	25,7	123	-1,72	90	4,3	3	104,8	20	18502,3	19	37,2	23	620,7
112	48,1	SERI	53,8	16	17,4	6	25,7	116	-1,72	122	4,2	53	104,6	125	18497,8	90	37,1	29	617,4
50	47,9	89	53,6	36	17,4	108	25,8	6	-1,73	113	4,1	48	104,0	113	18476,7	1	37,0	20	611,1
81	47,9	81	52,8	66	17,4	30	25,9	113	-1,74	121	4,1	80	103,3	121	18416,9	87	36,8	22	609,7
101	47,7	28	52,5	76	17,4	52	25,9	99	-1,75	39	4,1	27	102,5	30	18371,7	70	36,4	121	601,2
36	47,7	103	52,5	50	17,4	50	25,9	5	-1,77	124	4,1	91	102,4	26	18339,8	92	36,4	7	597,0
84	47,6	110	52,4	BABAX	17,5	99	25,9	82	-1,79	22	4,1	BABAX	102,4	91	18198,2	39	36,2	65	593,8
SERI	46,1	119	52,3	108	17,5	27	25,9	98	-1,79	BABAX	3,7	90	102,1	BABAX	15972,2	BABAX	35,4	BABAX	562,4
BABAX	45,9	BABAX	49,7	113	18,7	122	26,9	SERI	-1,93	SERI	3,3	SERI	90,1	SERI	13938,4	SERI	31,8	SERI	444,9
77	43,6	14	47,1	124	18,8	118	27,0	50	-2,00	74	2,8	10	88,3	44	13096,9	119	29,3	118	416,3
86	43,6	30	46,8	103	18,8	106	27,0	70	-2,01	108	2,7	101	88,2	48	13024,5	99	29,1	16	405,9
123	43,0	126	46,8	112	18,8	43	27,0	48	-2,02	3	2,7	14	88,2	16	12945,5	100	29,1	105	403,4
5	42,9	78	46,8	SERI	18,8	SERI	27,1	80	-2,02	27	2,7	65	87,3	36	12551,1	80	29,0	94	402,4
43	42,5	90	46,5	107	19,0	12	27,1	71	-2,02	5	2,6	52	86,9	66	12516,7	62	28,1	8	391,5
68	41,8	29	45,8	117	19,1	121	27,1	76	-2,03	118	2,6	119	86,5	39	12463,0	10	28,1	14	383,0
70	41,6	80	45,5	89	19,1	105	27,2	119	-2,05	24	2,3	106	85,7	94	11955,6	105	28,1	43	375,2
7	41,4	44	45,4	121	19,2	14	27,2	74	-2,06	11	2,3	121	84,8	118	11945,2	101	28,0	96	374,6
1	41,4	7	45,2	12	19,3	107	27,4	72	-2,06	14	2,2	100	79,6	43	11502,8	106	27,7	119	368,0
116	41,1	5	44,3	119	20,0	119	27,9	22	-2,07	2	2,0	107	79,0	96	11449,6	14	27,2	66	365,2

NOTE:
Genotypes selected for "Tolerant" bulk are on the top, while those less drought-adapted genotypes selected for "Susceptible" bulk are at the bottom.

APPENDIX 2C. Bulked segregant analysis from a 127 *Babax/Seri* RILs subset. Full irrigation in Yaqui valley, Northwestern Mexico

RIL	CHLO -BOOT	RIL	CHLO -GF	RIL	CTAM -GF	RIL	CTPM -GF	RIL	OP-GF	RIL	LROLL -GF	RIL	HEIGHT -GF	RIL	KNO	RIL	ATKW	RIL	YIELD
77	54,6	SERI	53,6	BABAX	20,5	88	24,6	14	-1,37	41	3,1	16	104,0	64	19754,7	48	50,7	70	805,7
99	53,7	53	53,5	3	20,7	113	24,7	125	-1,37	122	3,0	45	102,3	125	19736,6	87	46,1	112	786,9
117	53,1	74	53,0	10	20,8	79	24,7	6	-1,40	30	3,0	27	102,2	113	19426,5	103	45,9	87	773,9
48	52,6	76	53,0	83	20,8	21	24,7	108	-1,42	10	3,0	92	102,0	3	19259,6	1	45,4	64	762,1
41	52,5	19	52,9	7	20,8	81	24,8	78	-1,43	68	3,0	103	101,3	10	19214,4	22	45,4	6	761,5
118	52,3	41	52,7	27	20,9	26	24,8	81	-1,44	11	3,0	1	100,7	85	19075,2	44	44,9	30	761,0
111	52,3	65	52,6	123	20,9	41	24,9	5	-1,44	61	3,0	50	99,2	6	19067,2	94	44,8	120	753,4
86	52,3	71	52,6	98	21,0	65	24,9	83	-1,45	26	3,0	87	99,2	7	19020,4	19	44,6	113	751,1
121	52,2	46	52,4	89	21,0	63	24,9	109	-1,46	BABAX	3,0	123	98,7	99	19014,1	120	44,6	45	750,6
20	52,1	109	52,3	22	21,0	80	24,9	113	-1,46	113	3,0	83	98,3	70	18939,9	92	44,4	11	745,5
SERI	49,5	5	52,3	88	21,0	BABAX	25,2	BABAX	-1,47	126	3,0	BABAX	93,6	BABAX	18735,8	BABAX	43,6	BABAX	732,5
BABAX	49,0	BABAX	51,8	SERI	21,0	SERI	25,6	46	-1,65	SERI	2,5	SERI	92,5	SERI	16709,8	SERI	39,6	SERI	633,5
24	47,5	98	48,4	43	21,9	97	25,8	8	-1,65	1	2,0	72	89,7	96	14067,2	84	38,1	61	605,2
115	47,5	78	48,3	86	21,9	27	25,8	70	-1,66	103	2,0	99	88,8	36	14000,1	125	38,0	8	605,2
91	47,3	105	48,2	47	21,9	118	25,8	88	-1,67	66	2,0	100	88,1	61	13744,3	3	38,0	72	602,7
63	47,2	79	48,2	127	22,0	103	25,8	97	-1,67	46	1,9	65	87,9	48	13725,3	116	37,8	78	599,1
61	47,2	116	48,1	85	22,0	99	25,9	9	-1,69	16	1,9	127	87,4	44	13677,6	50	37,7	100	594,5
27	46,9	89	47,9	21	22,0	96	26,0	SERI	-1,71	24	1,5	107	87,1	1	13614,1	115	37,6	55	589,9
62	46,4	8	47,8	46	22,1	5	26,0	48	-1,72	76	1,5	10	86,9	78	13603,5	28	37,3	86	586,3
36	46,3	16	47,7	52	22,1	68	26,0	29	-1,72	23	1,5	121	86,9	86	13504,7	10	36,6	52	585,3
16	46,2	7	46,5	106	22,2	124	26,1	19	-1,73	62	1,5	41	86,3	119	12596,5	80	36,5	119	540,4
108	44,3	91	46,2	44	22,4	119	26,5	44	-1,77	22	1,5	52	84,8	43	12049,7	106	36,5	43	533,2

NOTE:
Genotypes selected for "Tolerant" bulk are on the top, while those less drought-adapted genotypes selected for "Susceptible" bulk are at the bottom.

APPENDIX 2D. Bulked segregant analysis from a 127 *Babax/Seri* RILs subset. Rainfed conditions in South Australia (average of data from Charlick and Roseworthy)

RIL	CHLO -BOOT	RIL	CHLO -GF	RIL	CTPM -BOOT	RIL	CTAM -GF	RIL	CTPM -GF	RIL	OP-GF	RIL	LROLL -GF	RIL	HEIGHT -GF	RIL	YIELD
111	46,2	83	43,5	6	16,8	62	16,6	82	19,9	23	-1,08	24	5,0	1	98,2	23	365,1
118	44,5	22	46,9	92	16,9	2	16,7	53	19,8	1	-1,15	22	5,0	BABAX	102,0	125	501,9
89	44,2	61	45,9	98	16,9	98	16,5	97	19,8	88	-1,08	84	5,0	45	101,2	112	485,8
88	44,1	118	45,4	BABAX	16,9	53	16,7	117	19,9	30	-1,16	1	4,0	24	90,3	79	487,5
53	44,0	1	38,4	53	17,0	BABAX	16,7	98	20,0	81	-1,28	21	5,0	92	101,1	126	474,9
41	43,6	97	43,9	9	17,2	11	16,8	25	20,1	89	-1,16	29	4,0	48	100,3	109	508,8
20	43,4	109	44,0	19	17,2	92	16,9	16	20,1	18	-1,31	30	4,0	123	100,7	121	471,6
11	43,2	92	44,5	44	17,2	38	16,9	20	20,1	22	-1,61	9	3,9	9	89,3	9	339,5
108	43,1	41	43,7	48	17,2	77	16,9	62	19,8	36	-1,20	25	4,8	87	91,4	18	500,9
109	43,1	89	39,5	79	17,2	96	16,8	111	19,8	85	-1,19	36	4,0	83	90,9	24	336,6
BABAX	42,3	SERI	41,8	81	17,2	81	16,9	BABAX	19,3	BABAX	-1,47	BABAX	4,3	117	98,5	SERI	430,6
SERI	39,0	BABAX	39,8	SERI	17,5	44	17,4	SERI	21,1	103	-1,54	116	3,0	SERI	89,8	BABAX	403,9
43	37,6	119	39,0	125	18,2	84	17,7	91	21,3	26	-1,54	27	2,8	76	87,6	98	383,8
47	37,5	99	38,9	10	18,3	23	17,9	22	20,8	116	-1,55	109	3,0	21	87,2	61	382,4
98	37,3	120	38,6	41	18,3	114	17,5	30	21,0	21	-1,56	97	2,8	107	87,2	122	363,1
105	37,2	43	38,5	87	18,3	20	17,5	76	21,5	94	-1,70	62	2,7	80	86,5	127	369,5
77	36,9	91	35,7	108	18,3	SERI	17,7	121	21,4	38	-1,57	14	2,5	10	82,8	120	365,3
78	36,7	106	38,2	105	18,4	108	17,6	1	21,3	118	-1,66	96	2,6	125	85,5	12	361,9
106	36,1	124	38,3	96	18,5	36	18,0	36	21,0	48	-1,61	50	2,5	28	84,5	48	357,3
127	35,6	44	37,5	127	18,6	88	17,9	87	20,7	50	-1,64	110	2,5	2	81,8	76	336,5
91	35,3	98	36,8	110	18,7	9	18,3	107	21,6	SERI	-1,63	119	2,5	100	81,0	94	347,4
10	33,9	127	35,1	80	18,9	87	18,1	83	20,8	92	-1,65	SERI	2,5	121	75,3	43	290,3

NOTE:
Drought-adapted genotypes selected for "Tolerant" bulk are on the top, while those less drought-adapted genotypes selected for "Susceptible" bulk are at the bottom.

APPENDIX 3A. Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Chlorophyll at Booting

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
GWM006a#1	4B-1	L33	23	CSIRO	35,8	0,67	4,75	0,0000
GWM132a#1	UA-1	L74	49	CSIRO	23,5	0,57	3,62	0,0001
GWM375#1	4B-1	L34	22	CSIRO	11,0	0,38	2,07	0,004
gdm008#1	3B-1	L21	12	CSIRO	9,22	0,34	1,80	0,01
gdm035#1	UA-1	L73	48	CSIRO	8,03	0,31	1,60	0,01
GWM301d#4	1D-2	L11	5	CSIRO	5,66	0,25	1,19	0,03
barc121#1	7A-1	L63	39	CSIRO	5,59	0,25	1,17	0,03
barc020#1	4B-1	L32	22	CSIRO	5,09	0,22	1,08	0,04

Chlorophyll at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
GWM006a#1	4B-1	L33	22	CSIRO	35,1	0,66	4,70	0,0000
GWM132a#1	UA-1	L74	46	CSIRO	29,1	0,62	4,18	0,0000
GWM301d#4	1D-2	L11	6	CSIRO	11,2	0,40	2,09	0,004
gdm035#1	UA-1	L73	46	CSIRO	8,68	0,33	1,71	0,01
GWM375#1	4B-1	L34	22	CSIRO	6,37	0,26	1,32	0,02

APPENDIX 3A (*continued*). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Booting, in the Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstAAGMseCGT2#2	unknown	L103	76	CIMMYT	15,0	0,55	2,46	0,002
gdm133#1	5D-1	L52	30	CSIRO	8,38	0,32	1,66	0,01
PstACCMseiCTA2#2	unknown	L101	74	CIMMYT	6,74	0,46	1,33	0,03
GWM617b#2	6A-2	L56	33	CSIRO	6,19	0,26	1,28	0,02
gdm008#1	3B-1	L21	13	CSIRO	5,46	0,28	1,14	0,03
barc147#1	3B-2	L25	17	CSIRO	5,45	0,23	1,15	0,03
PstACCMseiCTA1#1	unknown	L100	73	CIMMYT	5,16	0,42	1,08	0,06
barc087#1	3B-2	L24	16	CSIRO	4,97	0,22	1,06	0,04
GWM582#1	1B-1	L8	3	CSIRO	4,23	0,19	0,92	0,05
GWM301b#2	1B-1	L6	3	CSIRO	4,23	0,19	0,92	0,05
GWM273#1	1B-1	L5	3	CSIRO	4,23	0,19	0,92	0,05
barc065#1	1B-1	L3	3	CSIRO	4,23	0,19	0,92	0,05

Canopy Temperature at Booting, in the Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
gdm133#1	5D-1	L52	33	CSIRO	10,0	0,36	1,93	0,01
PstAAGMseCGT2#2	unknown	L103	78	CIMMYT	8,76	0,42	1,67	0,01
PstACCMseiCTA2#2	unknown	L101	76	CIMMYT	7,76	0,49	1,47	0,02
GWM617b#2	6A-2	L56	36	CSIRO	6,22	0,26	1,29	0,02
PstACCMseiCTA1#1	unknown	L100	76	CIMMYT	4,77	0,41	1,02	0,07
GWM582#1	1B-1	L8	3	CSIRO	4,22	0,19	0,91	0,05
GWM301b#2	1B-1	L6	3	CSIRO	4,22	0,19	0,91	0,05
GWM273#1	1B-1	L5	3	CSIRO	4,22	0,19	0,91	0,05
barc065#1	1B-1	L3	3	CSIRO	4,22	0,19	0,91	0,05

APPENDIX 3A (*continued*). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Grain Filling, in the Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseiCTA2#2	unknown	L101	73	CIMMYT	18,5	0,63	2,78	0,001
PstACCMseiCTA1#1	unknown	L100	73	CIMMYT	12,0	0,55	2,06	0,01
GWM371#1	5B-2	L50	28	CSIRO	6,39	0,26	1,32	0,02
L-GWM566#1	3B	L96	69	CIMMYT	5,41	0,23	1,14	0,03
GWM132b#2	6B-1	L57	34	CSIRO	5,14	0,22	1,09	0,04
L-GWM382#1	2A, 2B, 2D	L89	59	CIMMYT	5,09	0,22	1,08	0,04
GWM397#1	4A-2	L29	19	CSIRO	4,68	0,21	1,00	0,04

Canopy Temperature at Grain Filling, in the Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseiCTA2#2	unknown	L101	72	CIMMYT	9,23	0,51	1,69	0,01
PstACCMseiCTA1#1	unknown	L100	72	CIMMYT	7,37	0,48	1,42	0,03
GWM282#1	7A-1	L64	40	CSIRO	6,75	0,30	1,38	0,02
GWM132c#3	5A-2	L44	28	CSIRO	5,19	0,23	1,10	0,04
PstAAGMseCGT2#2	unknown	L103	74	CIMMYT	5,09	0,30	1,07	0,04
gdm063#1	5D-1	L51	31	CSIRO	4,27	0,19	0,92	0,05

APPENDIX 3A (*continued*). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Osmotic Potential at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM261#1	2D	L83	58	CIMMYT	8,63	0,32	1,70	0,01
L-GWM135#1	1A	L78	53	CIMMYT	8,15	0,31	1,62	0,01
barc147#1	3B-2	L25	15	CSIRO	7,65	0,30	1,54	0,01
L-GWM120#1	2B	L77	52	CIMMYT	6,59	0,27	1,35	0,02
GWM526#1	2A-2	L13	7	CSIRO	6,28	0,26	1,30	0,02
barc087#1	3B-2	L24	15	CSIRO	5,61	0,24	1,18	0,03

Normalised Difference in Vegetative Index at Booting (Red Spectrum)

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM120#1	2B	L77	52	CIMMYT	7,31	0,30	1,48	0,02
wmc048c#3	4A-2	L30	20	CSIRO	7,00	0,28	1,43	0,02

Normalised Difference in Vegetative Index at Grain Filling (Red Spectrum)

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
gdm093#1	2A-2	L12	7	CSIRO	5,86	0,25	1,22	0,03
GWM526#1	2A-2	L13	7	CSIRO	4,96	0,23	1,06	0,04

APPENDIX 3A (*continued*). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Leaf Rolling at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM369#1	3A, 4B	L87	62	CIMMYT	8,46	0,32	1,67	0,01
GWM132a#1	UA-1	L74	49	CSIRO	7,28	0,29	1,48	0,01
PstACCMseiCTA2#2	unknown	L101	73	CIMMYT	5,81	0,33	1,20	0,03
PstACCMseiCTA1#1	unknown	L100	73	CIMMYT	5,81	0,33	1,20	0,03
GWM582#1	1B-1	L8	3	CSIRO	5,15	0,23	1,09	0,04

Height at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM006a#1	4B-1	L33	24	CSIRO	4,85	0,22	1,04	0,04
PstACCMseiCTA2#2	unknown	L101	78	CIMMYT	5,07	0,32	1,07	0,05
L-GWM190#1	5D	L81	61	CIMMYT	4,47	0,20	0,96	0,05
PstAAGMseCTA3#3	unknown	L106	82	CIMMYT	4,99	0,33	1,05	0,05

Yield

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseiCTA2#2	unknown	L101	76	CIMMYT	19,6	0,69	2,76	0,002
PstACCMseiCTA1#1	unknown	L100	76	CIMMYT	19,6	0,69	2,76	0,002
L-GWM149v1#1	4B, 4D	L79	58	CIMMYT	6,66	0,27	1,37	0,02
gdm086#1	7B-1	L66	46	CSIRO	6,48	0,26	1,34	0,02
L-GWM149v2#1	4B, 4D	L80	59	CIMMYT	6,02	0,25	1,25	0,02
L-GWM120#1	2B	L77	56	CIMMYT	5,79	0,24	1,21	0,03
PstAAGMseCTA1#1	unknown	L104	79	CIMMYT	5,46	0,41	1,13	0,05
GWM617b#2	6A-2	L56	36	CSIRO	4,34	0,19	0,94	0,05

APPENDIX 3A (*continued*). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Kernel Number

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseiCTA2#2	unknown	L101	74	CIMMYT	10,7	0,47	1,94	0,01
PstACCMseiCTA1#1	unknown	L100	74	CIMMYT	10,7	0,47	1,94	0,01
gdm086#1	7B-1	L66	44	CSIRO	6,54	0,27	1,35	0,02
GWM301a#1	2B-2	L17	10	CSIRO	5,48	0,24	1,15	0,03
L-GWM508#1	6B	L94	68	CIMMYT	4,96	0,22	1,06	0,04
GWM130#1	7D-2	L68	46	CSIRO	4,41	0,20	0,95	0,05
GWM617a#1	5A-1	L43	27	CSIRO	4,34	0,19	0,94	0,05
GWM582#1	1B-1	L8	2	CSIRO	4,29	0,19	0,93	0,05
GWM273#1	1B-1	L5	2	CSIRO	4,29	0,19	0,93	0,05
GWM131#1	1B-1	L4	2	CSIRO	4,29	0,19	0,93	0,05

A Thousand Kernels Weight

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM301b#2	1B-1	L6	2	CSIRO	10,2	0,36	1,95	0,01
barc065#1	1B-1	L3	2	CSIRO	10,2	0,36	1,95	0,01
GWM582#1	1B-1	L8	2	CSIRO	9,19	0,35	1,78	0,01
GWM131#1	1B-1	L4	2	CSIRO	8,57	0,32	1,69	0,01
barc070#1	4A-1	L27	17	CSIRO	7,34	0,29	1,49	0,01
L-GWM369#1	3A, 4B	L87	58	CIMMYT	6,18	0,26	1,28	0,02
GWM273#1	1B-1	L5	2	CSIRO	5,03	0,22	1,07	0,04
GWM413#1	1B-1	L7	2	CSIRO	4,66	0,21	1,00	0,04
barc147#1	3B-2	L25	16	CSIRO	4,53	0,20	0,97	0,05
PstACCMseiCTA1#1	unknown	L100	69	CIMMYT	6,41	0,56	1,25	0,05

APPENDIX 3B. Marker/trait associations under reduced irrigation (cycles 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Chlorophyll at Booting

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM582#1	1B-1	L8	3	CSIRO	13,9	0,44	2,49	0,002
GWM273#1	1B-1	L5	3	CSIRO	13,9	0,44	2,49	0,002
GWM131#1	1B-1	L4	3	CSIRO	13,9	0,44	2,49	0,002
GWM301b#2	1B-1	L6	3	CSIRO	9,97	0,36	1,91	0,01
barc065#1	1B-1	L3	3	CSIRO	9,97	0,36	1,91	0,01
GWM413#1	1B-1	L7	3	CSIRO	7,81	0,33	1,55	0,01
L-GWM617#1	5A, 6A	L97	69	CIMMYT	6,90	0,28	1,41	0,02
gdm008#1	3B-1	L21	15	CSIRO	7,01	0,33	1,41	0,02
GWM437#1	7D-2	L69	45	CSIRO	5,95	0,26	1,24	0,03
GWM617a#1	5A-1	L43	26	CSIRO	5,05	0,23	1,07	0,04
GWM473#1	7D-2	L70	45	CSIRO	4,68	0,23	1,00	0,05

Chlorophyll at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACGMseCGG2#2	unknown	L99	70	CIMMYT	17,5	0,54	2,85	0,001
L-GWM369#1	3A, 4B	L87	60	CIMMYT	8,26	0,31	1,64	0,01
gdm063#1	5D-1	L51	27	CSIRO	7,41	0,29	1,50	0,01
L-GWM480#1	3A	L92	63	CIMMYT	6,08	0,25	1,26	0,02
GWM132a#1	UA-1	L74	47	CSIRO	5,54	0,24	1,17	0,03
GWM190#1	u-9	L72	45	CSIRO	5,49	0,23	1,16	0,03
GWM191a#1	2B-1	L15	8	CSIRO	4,77	0,21	1,02	0,04
GWM006a#1	4B-1	L33	20	CSIRO	4,56	0,20	0,98	0,05
PstACCMseCGC1#1	unknown	L107	77	CIMMYT	4,42	0,24	0,95	0,05

APPENDIX 3B (*continued*). Marker/trait associations under reduced irrigation (cycles 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Grain Filling, in the Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM340#1	3B	L86	60	CIMMYT	9,33	0,34	1,81	0,01
PstAAGMseCGT2#2	unknown	L103	74	CIMMYT	7,67	0,41	1,49	0,02
PstAAGMseCTA1#1	unknown	L104	75	CIMMYT	5,36	0,35	1,12	0,04
gdm086#1	7B-1	L66	42	CSIRO	4,55	0,20	0,98	0,05
barc186#1	5A-1	L41	24	CSIRO	4,43	0,20	0,96	0,05
L-GWM518#1	6B	L95	67	CIMMYT	4,41	0,20	0,95	0,05
cfd071#1	4D-1	L37	23	CSIRO	4,30	0,19	0,93	0,05
cfd023#1	4D-1	L36	23	CSIRO	4,30	0,19	0,93	0,05
L-GWM427#1	6A	L91	63	CIMMYT	4,25	0,19	0,92	0,05

Canopy Temperature at Grain Filling, in the Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM340#1	3B	L86	58	CIMMYT	11,5	0,39	2,15	0,003
GWM301e#5	3B-1	L22	13	CSIRO	10,0	0,39	1,90	0,01
gdm098#1	6D-1	L59	35	CSIRO	6,64	0,29	1,36	0,02
GWM518b#2	6A-1	L55	31	CSIRO	6,13	0,25	1,27	0,02

APPENDIX 3B (*continued*). Marker/trait associations under reduced irrigation (cycles 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Osmotic Potential at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
GWM413#1	1B-1	L7	2	CSIRO	7,64	0,30	1,54	0,01
GWM582#1	1B-1	L8	2	CSIRO	7,12	0,28	1,45	0,02
barc100#1	5A-1	L40	25	CSIRO	5,25	0,23	1,11	0,03
GWM301b#2	1B-1	L6	2	CSIRO	5,16	0,22	1,09	0,04
barc065#1	1B-1	L3	2	CSIRO	5,16	0,22	1,09	0,04
barc040#1	5A-1	L39	25	CSIRO	4,50	0,20	0,97	0,05

A Thousand Kernels Weight

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
gdm086#1	7B-1	L66	18	CSIRO	20,4	0,53	3,29	0,0003
GWM635b#2	7B-1	L67	43	CSIRO	20,4	0,53	3,29	0,0003
barc147#1	3B-2	L25	17	CSIRO	18,0	0,50	3,01	0,001
GWM389#1	3B-2	L26	17	CSIRO	14,0	0,45	2,47	0,002
GWM130#1	7D-2	L68	44	CSIRO	11,0	0,38	2,07	0,004
L-GWM261#1	2D	L83	56	CIMMYT	9,76	0,35	1,88	0,01
GWM388#1	2B-1	L16	9	CSIRO	6,87	0,28	1,40	0,02
GWM191a#1	2B-1	L15	9	CSIRO	6,87	0,28	1,40	0,02
GWM135#1	1A-1	L1	1	CSIRO	4,54	0,20	0,98	0,05
wmc097#1	1A-1	L2	2	CSIRO	4,25	0,19	0,92	0,05

APPENDIX 3B (*continued*). Marker/trait associations under reduced irrigation (cycles 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Leaf Rolling at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
gdm098#1	6D-1	L59	34	CSIRO	10,1	0,39	1,91	0,01
L-GWM190#1	5D	L81	53	CIMMYT	9,76	0,36	1,87	0,01
L-GWM149v2#1	4B, 4D	L80	52	CIMMYT	9,50	0,35	1,84	0,01
L-GWM518#1	6B	L95	65	CIMMYT	7,09	0,28	1,44	0,02
barc169#1	1D-1	L9	3	CSIRO	6,60	0,29	1,35	0,02
L-GWM499#1	5B	L93	63	CIMMYT	5,38	0,23	1,14	0,03
PstAAGMseCTA3#3	unknown	L106	75	CIMMYT	8,58	0,63	1,52	0,03
GWM130#1	7D-2	L68	42	CSIRO	4,64	0,20	1,00	0,05
L-GWM149v1#1	4B, 4D	L79	51	CIMMYT	4,48	0,20	0,97	0,05

Height at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM473#1	7D-2	L70	46	CSIRO	14,0	0,44	2,49	0,002
GWM437#1	7D-2	L69	46	CSIRO	14,0	0,44	2,49	0,002
GWM388#1	2B-1	L16	9	CSIRO	13,3	0,43	2,40	0,002
GWM582#1	1B-1	L8	3	CSIRO	10,4	0,38	1,96	0,01
gdm133#1	5D-1	L52	31	CSIRO	10,1	0,36	1,93	0,01
GWM191a#1	2B-1	L15	9	CSIRO	10,2	0,37	1,93	0,01
GWM131#1	1B-1	L4	3	CSIRO	8,89	0,33	1,74	0,01
L-GWM135#1	1A	L78	53	CIMMYT	7,95	0,32	1,58	0,01
GWM301b#2	1B-1	L6	3	CSIRO	7,29	0,30	1,47	0,02
GWM006a#1	4B-1	L33	24	CSIRO	6,04	0,25	1,26	0,02
GWM273#1	1B-1	L5	3	CSIRO	5,30	0,23	1,12	0,03
gdm063#1	5D-1	L51	30	CSIRO	5,23	0,23	1,11	0,03

APPENDIX 3B (*continued*). Marker/trait associations under reduced irrigation (cycles 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Kernel Number

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
wmc048d#4	4A-2	L31	21	CSIRO	25,5	0,59	3,83	0,0001
GWM582#1	1B-1	L8	2	CSIRO	19,7	0,52	3,21	0,0003
GWM301b#2	1B-1	L6	2	CSIRO	19,7	0,52	3,21	0,0003
barc065#1	1B-1	L3	2	CSIRO	19,7	0,52	3,21	0,0003
L-GWM251#1	4B, 4D	L82	22	CIMMYT	19,3	0,52	3,17	0,0003
GWM273#1	1B-1	L5	2	CSIRO	17,6	0,51	2,93	0,001
L-GWM375#1	4B	L88	22	CIMMYT	17,2	0,50	2,89	0,001
GWM397#1	4A-2	L29	21	CSIRO	16,2	0,47	2,79	0,001
GWM526#1	2A-2	L13	6	CSIRO	16,0	0,49	2,74	0,001
GWM413#1	1B-1	L7	2	CSIRO	15,4	0,49	2,63	0,001
L-GWM6#1	4B, 4D	L75	22	CIMMYT	15,7	0,53	2,61	0,001
wmc048a#1	4B-1	L35	22	CSIRO	13,1	0,42	2,37	0,002
barc020#1	4B-1	L32	22	CSIRO	13,1	0,42	2,37	0,002
gdm093#1	2A-2	L12	6	CSIRO	13,0	0,42	2,36	0,002
GWM375#1	4B-1	L34	22	CSIRO	11,6	0,39	2,16	0,003
GWM191a#1	2B-1	L15	9	CSIRO	10,5	0,37	2,00	0,005
L-GWM113#1	4B	L76	10	CIMMYT	9,79	0,35	1,89	0,01
L-GWM508#1	6B	L94	62	CIMMYT	9,18	0,34	1,79	0,01
GWM131#1	1B-1	L4	2	CSIRO	8,34	0,32	1,65	0,01
wmc048c#3	4A-2	L30	21	CSIRO	8,11	0,31	1,62	0,01
GWM388#1	2B-1	L16	9	CSIRO	7,28	0,29	1,48	0,01
GWM301d#4	1D-2	L11	5	CSIRO	7,23	0,31	1,46	0,02
GWM644#1	3B-1	L23	16	CSIRO	6,35	0,26	1,31	0,02
GWM325#1	6D-2	L61	38	CSIRO	6,01	0,25	1,25	0,02
gdm098#1	6D-1	L59	36	CSIRO	5,99	0,27	1,24	0,03
L-GWM499#1	5B	L93	61	CIMMYT	5,79	0,24	1,21	0,03

APPENDIX 3B (*continued*). Marker/trait associations under reduced irrigation (cycles 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Yield

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
wmc048a#1	4B-1	L35	22	CSIRO	29,9	0,62	4,25	0,0000
barc020#1	4B-1	L32	22	CSIRO	29,9	0,62	4,25	0,0000
GWM375#1	4B-1	L34	22	CSIRO	23,0	0,56	3,58	0,0001
GWM644#1	3B-1	L23	15	CSIRO	19,0	0,51	3,13	0,0004
wmc048d#4	4A-2	L31	21	CSIRO	17,5	0,49	2,95	0,001
GWM325#1	6D-2	L61	39	CSIRO	12,1	0,40	2,23	0,003
wmc048c#3	4A-2	L30	21	CSIRO	11,3	0,39	2,12	0,004
GWM301d#4	1D-2	L11	5	CSIRO	9,71	0,35	1,87	0,01
barc065#1	1B-1	L3	2	CSIRO	8,24	0,31	1,64	0,01
gdm008#1	3B-1	L21	13	CSIRO	7,81	0,33	1,55	0,01
GWM413#1	1B-1	L7	2	CSIRO	7,38	0,30	1,49	0,01
L-GWM427#1	6A	L91	62	CIMMYT	7,26	0,30	1,47	0,02
GWM006a#1	4B-1	L33	23	CSIRO	6,79	0,27	1,39	0,02
GWM301e#5	3B-1	L22	14	CSIRO	6,47	0,26	1,33	0,02
barc087#1	3B-2	L24	16	CSIRO	6,17	0,26	1,28	0,02
L-GWM340#1	3B	L86	60	CIMMYT	5,99	0,25	1,25	0,02
L-GWM499#1	5B	L93	64	CIMMYT	5,88	0,25	1,23	0,03
PstACCMseCGC1#1	unknown	L107	76	CIMMYT	6,33	0,35	1,29	0,03
GWM397#1	4A-2	L29	21	CSIRO	5,57	0,24	1,17	0,03
L-GWM375#1	4B	L88	51	CIMMYT	5,51	0,23	1,16	0,03
L-GWM251#1	4B, 4D	L82	51	CIMMYT	5,51	0,23	1,16	0,03
barc169#1	1D-1	L9	3	CSIRO	5,52	0,26	1,16	0,03
gdm098#1	6D-1	L59	37	CSIRO	5,05	0,24	1,07	0,04
GWM526#1	2A-2	L13	6	CSIRO	4,99	0,23	1,06	0,04
GWM582#1	1B-1	L8	2	CSIRO	4,44	0,20	0,96	0,05
GWM301b#2	1B-1	L6	2	CSIRO	4,44	0,20	0,96	0,05
GWM273#1	1B-1	L5	2	CSIRO	4,44	0,20	0,96	0,05
GWM617b#2	6A-2	L56	34	CSIRO	4,39	0,21	0,95	0,05

APPENDIX 3C. Marker/trait associations under full irrigation (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Chlorophyll at Booting

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM427#1	6A	L91	59	CIMMYT	12,9	0,43	2,33	0,00
gdm063#1	5D-1	L51	28	CSIRO	11,3	0,39	2,12	0,00
GWM132a#1	UA-1	L74	10	CSIRO	10,6	0,37	2,01	0,00
L-GWM566#1	3B	L96	64	CIMMYT	9,98	0,36	1,92	0,01
gdm008#1	3B-1	L21	15	CSIRO	9,34	0,35	1,81	0,01
PstACGMseCGG2#2	unknown	L99	67	CIMMYT	8,33	0,39	1,61	0,01
GWM006b#2	u-8	L71	44	CSIRO	7,46	0,29	1,51	0,01
GWM636#1	2A-3	L14	10	CSIRO	7,22	0,29	1,46	0,02
PstAAGMseCTA3#3	unknown	L106	73	CIMMYT	8,29	0,48	1,56	0,02
L-GWM617#1	5A, 6A	L97	65	CIMMYT	6,37	0,27	1,31	0,02
GWM397#1	4A-2	L29	22	CSIRO	5,11	0,23	1,08	0,04
gdm093#1	2A-2	L12	9	CSIRO	5,01	0,22	1,07	0,04
gdm133#1	5D-1	L52	29	CSIRO	4,88	0,21	1,04	0,04
gdm035#1	UA-1	L73	10	CSIRO	4,71	0,21	1,01	0,04

Chlorophyll at Grain FIlling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
barc070#1	4A-1	L27	18	CSIRO	8,19	0,31	1,63	0,01

APPENDIX 3C (*continued*). Marker/trait associations under full irrigation (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Grain Filling, in the Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM282#1	7A-1	L64	40	CSIRO	9,56	0,35	1,85	0,01
GWM617b#2	6A-2	L56	33	CSIRO	9,14	0,35	1,78	0,01
GWM644#1	3B-1	L23	14	CSIRO	4,22	0,19	0,91	0,05

Canopy Temperature at Grain Filling, in the Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseCGC1#1	unknown	L107	82	CIMMYT	36,9	0,74	4,38	0,00
wmc048a#1	4B-1	L35	23	CSIRO	15,3	0,46	2,67	0,00
barc020#1	4B-1	L32	23	CSIRO	13,7	0,45	2,44	0,00
GWM334#1	6A-1	L53	34	CSIRO	9,52	0,35	1,84	0,01
GWM459#1	6A-1	L54	34	CSIRO	7,88	0,32	1,57	0,01
L-GWM369#1	3A, 4B	L87	64	CIMMYT	6,29	0,27	1,30	0,02
GWM371#1	5B-2	L50	31	CSIRO	6,21	0,26	1,29	0,02

APPENDIX 3C (*continued*). Marker/trait associations under full irrigation (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Osmotic Potential at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM635a#1	7A-1	L65	39	CSIRO	11,4	0,39	2,13	0,003
barc040#1	5A-1	L39	24	CSIRO	9,42	0,34	1,83	0,01
barc100#1	5A-1	L40	24	CSIRO	8,11	0,32	1,61	0,01
GWM132a#1	UA-1	L74	47	CSIRO	7,21	0,29	1,46	0,02
barc186#1	5A-1	L41	24	CSIRO	7,16	0,30	1,45	0,02
PstACCMseiCTA1#1	unknown	L100	70	CIMMYT	9,48	0,58	1,67	0,02
GWM304#1	5A-1	L42	24	CSIRO	6,59	0,27	1,35	0,02
wmc097#1	1A-1	L2	2	CSIRO	5,93	0,25	1,24	0,03
PstACCMseiCTA2#2	unknown	L101	71	CIMMYT	7,07	0,47	1,38	0,03
L-GWM508#1	6B	L94	65	CIMMYT	4,78	0,22	1,02	0,04

Leaf Rolling at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseiCTA2#2	unknown	L101	76	CIMMYT	8,38	0,46	1,59	0,02
PstACCMseiCTA1#1	unknown	L100	76	CIMMYT	8,38	0,46	1,59	0,02
GWM190#1	u-9	L72	51	CSIRO	6,63	0,31	1,35	0,02
GWM350#1	4A-1	L28	20	CSIRO	5,44	0,23	1,15	0,03
L-GWM499#1	5B	L93	69	CIMMYT	4,93	0,22	1,05	0,04
GWM006a#1	4B-1	L33	24	CSIRO	4,47	0,20	0,96	0,05

APPENDIX 3C (*continued*). Marker/trait associations under full irrigation (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Height at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
barc020#1	4B-1	L32	21	CSIRO	19,1	0,53	3,10	0,00
GWM006a#1	4B-1	L33	21	CSIRO	16,1	0,47	2,78	0,00
wmc048a#1	4B-1	L35	21	CSIRO	12,5	0,42	2,27	0,00
GWM375#1	4B-1	L34	21	CSIRO	11,5	0,39	2,15	0,00
GWM131#1	1B-1	L4	3	CSIRO	7,20	0,29	1,46	0,02
GWM301b#2	1B-1	L6	3	CSIRO	6,89	0,29	1,40	0,02
GWM582#1	1B-1	L8	3	CSIRO	6,79	0,29	1,39	0,02
GWM617a#1	5A-1	L43	24	CSIRO	6,76	0,30	1,38	0,02
GWM191a#1	2B-1	L15	9	CSIRO	6,36	0,27	1,31	0,02
GWM413#1	1B-1	L7	3	CSIRO	5,38	0,23	1,14	0,03
barc065#1	1B-1	L3	3	CSIRO	5,38	0,23	1,14	0,03
GWM388#1	2B-1	L16	9	CSIRO	4,83	0,21	1,03	0,04
L-GWM340#1	3B	L86	59	CIMMYT	4,28	0,19	0,93	0,05

A Thousand Kernels Weight

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM130#1	7D-2	L68	44	CSIRO	7,85	0,30	1,57	0,01
GWM371#1	5B-2	L50	28	CSIRO	6,49	0,27	1,34	0,02
barc121#1	7A-1	L63	39	CSIRO	6,50	0,28	1,34	0,02
barc065#1	1B-1	L3	2	CSIRO	6,28	0,26	1,30	0,02
GWM350#1	4A-1	L28	19	CSIRO	4,40	0,20	0,95	0,05
L-GWM261#1	2D	L83	58	CIMMYT	4,43	0,21	0,96	0,05

APPENDIX 3C (*continued*). Marker/trait associations under full irrigation (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Kernel Number

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
wmc048d#4	4A-2	L31	19	CSIRO	17,9	0,50	3,00	0,00
GWM582#1	1B-1	L8	2	CSIRO	12,1	0,43	2,20	0,00
barc020#1	4B-1	L32	21	CSIRO	9,83	0,35	1,89	0,01
wmc048a#1	4B-1	L35	21	CSIRO	8,28	0,33	1,64	0,01
GWM301b#2	1B-1	L6	2	CSIRO	7,60	0,30	1,53	0,01
GWM397#1	4A-2	L29	19	CSIRO	7,43	0,30	1,50	0,01
GWM301d#4	1D-2	L11	6	CSIRO	6,99	0,30	1,42	0,02
GWM304#1	5A-1	L42	23	CSIRO	6,46	0,26	1,33	0,02
GWM375#1	4B-1	L34	21	CSIRO	6,11	0,25	1,27	0,02
wmc048c#3	4A-2	L30	20	CSIRO	4,87	0,21	1,04	0,04
GWM273#1	1B-1	L5	2	CSIRO	4,86	0,21	1,04	0,04
barc065#1	1B-1	L3	2	CSIRO	4,86	0,21	1,04	0,04
barc186#1	5A-1	L41	23	CSIRO	4,64	0,20	1,00	0,05
gdm098#1	6D-1	L59	34	CSIRO	4,55	0,22	0,98	0,05

APPENDIX 3C (*continued*). Marker/trait associations under full irrigation (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Yield

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
GWM582#1	1B-1	L8	2	CSIRO	12,7	0,43	2,30	0,002
GWM334#1	6A-1	L53	30	CSIRO	11,8	0,40	2,18	0,003
GWM459#1	6A-1	L54	30	CSIRO	12,1	0,45	2,18	0,003
GWM413#1	1B-1	L7	2	CSIRO	8,30	0,32	1,65	0,01
GWM301b#2	1B-1	L6	2	CSIRO	7,87	0,30	1,58	0,01
GWM389#1	3B-2	L26	15	CSIRO	7,33	0,31	1,47	0,02
GWM350#1	4A-1	L28	17	CSIRO	7,01	0,28	1,43	0,02
barc147#1	3B-2	L25	15	CSIRO	6,42	0,26	1,32	0,02
wmc048c#3	4A-2	L30	19	CSIRO	6,35	0,26	1,31	0,02
GWM301c#3	2B-2	L18	9	CSIRO	6,25	0,26	1,29	0,02
GWM301a#1	2B-2	L17	9	CSIRO	6,25	0,26	1,29	0,02
GWM130#1	7D-2	L68	43	CSIRO	6,03	0,25	1,25	0,02
wmc048d#4	4A-2	L31	20	CSIRO	5,89	0,25	1,23	0,03
barc087#1	3B-2	L24	15	CSIRO	5,66	0,24	1,19	0,03
GWM273#1	1B-1	L5	2	CSIRO	5,43	0,23	1,14	0,03
barc065#1	1B-1	L3	2	CSIRO	5,43	0,23	1,14	0,03
L-GWM120#1	2B	L77	51	CIMMYT	5,03	0,22	1,07	0,04
GWM132b#2	6B-1	L57	33	CSIRO	4,92	0,21	1,05	0,04
GWM135#1	1A-1	L1	1	CSIRO	4,84	0,21	1,03	0,04

APPENDIX 3D. Marker/trait associations in Australia, 2001 (average of Charlick and Roseworthy sites). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Chlorophyll at Booting

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
gdm093#1	2A-2	L12	6	CSIRO	14,3	0,44	2,53	0,001
GWM526#1	2A-2	L13	6	CSIRO	12,3	0,41	2,26	0,003
barc169#1	1D-1	L9	3	CSIRO	9,46	0,36	1,83	0,01
L-GWM617#1	5A, 6A	L97	69	CIMMYT	8,02	0,32	1,59	0,01
GWM459#1	6A-1	L54	30	CSIRO	5,93	0,25	1,24	0,03
GWM334#1	6A-1	L53	30	CSIRO	5,93	0,25	1,24	0,03
L-GWM369#1	3A, 4B	L87	61	CIMMYT	5,69	0,26	1,19	0,03
GWM413#1	1B-1	L7	2	CSIRO	4,37	0,20	0,94	0,05
GWM273#1	1B-1	L5	2	CSIRO	4,37	0,20	0,94	0,05
barc065#1	1B-1	L3	2	CSIRO	4,37	0,20	0,94	0,05

Chlorophyll at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM617#1	5A, 6A	L97	69	CIMMYT	8,82	0,36	1,72	0,01
PstACCMseCGC1#1	unknown	L107	77	CIMMYT	7,39	0,36	1,47	0,02
PstAAGMseCGT1#1	unknown	L102	73	CIMMYT	5,94	0,31	1,23	0,03
GWM518a#1	6B-1	L58	35	CSIRO	5,17	0,23	1,10	0,04
GWM301e#5	3B-1	L22	14	CSIRO	4,44	0,20	0,96	0,05
gdm086#1	7B-1	L66	42	CSIRO	4,29	0,19	0,93	0,05

APPENDIX 3D (*continued*). Marker/trait associations in Australia, 2001 (average of Charlick and Roseworthy sites). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Booting, in the Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
barc121#1	7A-1	L63	40	CSIRO	12,9	0,43	2,33	0,002
wmc048c#3	4A-2	L30	22	CSIRO	10,2	0,36	1,95	0,01
GWM282#1	7A-1	L64	41	CSIRO	6,64	0,28	1,36	0,02
GWM371#1	5B-2	L50	30	CSIRO	4,97	0,22	1,06	0,04
GWM617a#1	5A-1	L43	28	CSIRO	4,96	0,23	1,06	0,04
GWM369#1	3A-1	L20	12	CSIRO	4,25	0,19	0,92	0,05

Canopy Temperature at Grain Filling, in the Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
L-GWM190#1	5D	L81	58	CIMMYT	7,50	0,31	1,51	0,01
GWM135#1	1A-1	L1	1	CSIRO	7,41	0,29	1,50	0,01
PstACCMseCGC1#1	unknown	L107	79	CIMMYT	6,85	0,31	1,39	0,02
L-GWM518#1	6B	L95	69	CIMMYT	6,40	0,27	1,32	0,02
GWM102#1	2D-1	L19	10	CSIRO	6,32	0,26	1,31	0,02
L-GWM617#1	5A, 6A	L97	71	CIMMYT	5,76	0,26	1,20	0,03
L-GWM6#1	4B, 4D	L75	53	CIMMYT	5,50	0,28	1,15	0,03
gdm008#1	3B-1	L21	12	CSIRO	4,95	0,23	1,05	0,04
GWM644#1	3B-1	L23	14	CSIRO	4,53	0,20	0,97	0,05

APPENDIX 3D (*continued*). Marker/trait associations in Australia, 2001 (average of Charlick and Roseworthy sites). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Grain Filling, in the Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
GWM304#1	5A-1	L42	24	CSIRO	8,46	0,32	1,67	0,01
barc147#1	3B-2	L25	17	CSIRO	8,10	0,31	1,61	0,01
barc186#1	5A-1	L41	24	CSIRO	6,85	0,28	1,40	0,02
barc100#1	5A-1	L40	24	CSIRO	6,85	0,28	1,40	0,02
barc040#1	5A-1	L39	24	CSIRO	6,85	0,28	1,40	0,02
GWM389#1	3B-2	L26	17	CSIRO	6,77	0,28	1,38	0,02
PstAAGMseCGT1#1	unknown	L102	73	CIMMYT	5,27	0,26	1,11	0,04
GWM617a#1	5A-1	L43	25	CSIRO	4,87	0,22	1,04	0,04
gdm063#1	5D-1	L51	28	CSIRO	4,34	0,19	0,94	0,05

Osmotic Potential at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM499#1	5B	L93	67	CIMMYT	10,6	0,37	2	0,005
gdm093#1	2A-2	L12	7	CSIRO	5,71	0,24	1,2	0,03
barc020#1	4B-1	L32	24	CSIRO	4,80	0,22	1,03	0,04
barc186#1	5A-1	L41	26	CSIRO	4,73	0,22	1,01	0,04
L-GWM6#1	4B, 4D	L75	51	CIMMYT	4,69	0,22	1,01	0,04
barc070#1	4A-1	L27	19	CSIRO	4,65	0,21	1	0,04
GWM135#1	1A-1	L1	1	CSIRO	4,47	0,20	0,96	0,05

APPENDIX 3D (*continued*). Marker/trait associations in Australia, 2001 (average of Charlick and Roseworthy sites). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Leaf Rolling at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
GWM371#1	5B-2	L50	27	CSIRO	11,1	0,38	2,09	0,004
PstACCMseCGC1#1	unknown	L107	77	CIMMYT	10,4	0,43	1,92	0,01
GWM130#1	7D-2	L68	46	CSIRO	9,57	0,35	1,85	0,01
GWM375#1	4B-1	L34	21	CSIRO	9,39	0,34	1,82	0,01
GWM301d#4	1D-2	L11	5	CSIRO	8,04	0,36	1,58	0,01
L-GWM149v1#1	4B, 4D	L79	55	CIMMYT	7,34	0,29	1,49	0,01
wmc048a#1	4B-1	L35	21	CSIRO	5,13	0,22	1,09	0,04
GWM102#1	2D-1	L19	10	CSIRO	4,52	0,20	0,97	0,05
GWM635a#1	7A-1	L65	43	CSIRO	4,51	0,21	0,97	0,05
gdm086#1	7B-1	L66	44	CSIRO	4,45	0,20	0,96	0,05

Height at Grain Filling

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
wmc048d#4	4A-2	L31	20	CSIRO	9,45	0,34	1,83	0,01
GWM301d#4	1D-2	L11	6	CSIRO	6,70	0,27	1,37	0,02
GWM388#1	2B-1	L16	9	CSIRO	5,77	0,24	1,21	0,03
GWM191a#1	2B-1	L15	9	CSIRO	5,77	0,24	1,21	0,03
GWM337#1	1D-1	L10	5	CSIRO	5,34	0,23	1,13	0,03
GWM473#1	7D-2	L70	45	CSIRO	5,16	0,23	1,09	0,04
GWM437#1	7D-2	L69	45	CSIRO	5,16	0,23	1,09	0,04
wmc097#1	1A-1	L2	2	CSIRO	4,39	0,20	0,95	0,05

APPENDIX 3D (*continued*). Marker/trait associations in Australia, 2001 (average of Charlick and Roseworthy sites). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Yield

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
barc169#1	1D-1	L9	4	CSIRO	12,6	0,44	2,27	0,003
GWM191b#2	5B-2	L47	29	CSIRO	8,98	0,33	1,76	0,01
GWM112#1	5B-2	L45	29	CSIRO	8,98	0,33	1,76	0,01
L-GWM499#1	5B	L93	68	CIMMYT	8,70	0,33	1,71	0,01
GWM274#1	5B-2	L49	29	CSIRO	8,00	0,31	1,6	0,01
GWM371#1	5B-2	L50	29	CSIRO	7,64	0,30	1,54	0,01
GWM213#1	5B-2	L48	29	CSIRO	7,64	0,30	1,54	0,01
GWM190#1	u-9	L72	49	CSIRO	6,50	0,28	1,34	0,02
GWM301e#5	3B-1	L22	14	CSIRO	5,58	0,24	1,17	0,03
GWM133#1	5B-2	L46	29	CSIRO	5,48	0,23	1,15	0,03
wmc048d#4	4A-2	L31	22	CSIRO	4,55	0,20	0,98	0,05

APPENDIX 4. Modification of the Integral BSA strategy. Increase in the number of genotypes per bulk (30). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Booting, Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
PstACCMseiCTA2#2	unknown	L101	61	CIMMYT	7,37	0,19	1,53	0,01
L-GWM499#1	5B	L93	54	CIMMYT	6,90	0,11	1,46	0,01
PstACCMseiCTA1#1	unknown	L100	61	CIMMYT	6,16	0,17	1,3	0,02
L-GWM311#1	2A, 2D, 6B	L84	47	CIMMYT	3,94	0,06	0,86	0,05

Canopy Temperature at Booting, Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	F	RSq	LOD	P
L-GWM508#1	6B	L94	57	CIMMYT	6,2	0,10	1,32	0,02
L-GWM120#1	2B	L77	44	CIMMYT	5,41	0,09	1,16	0,02
PstACCMseiCTA2#2	unknown	L101	61	CIMMYT	4,39	0,11	0,95	0,04
GWM102#1	2D-1	L19	9	CSIRO	4,08	0,07	0,89	0,05
L-GWM499#1	5B	L93	56	CIMMYT	3,83	0,06	0,83	0,06

APPENDIX 4 (*continued*). Modification of the Integral BSA strategy. Increase in the number of genotypes per bulk (30). Marker/trait associations under drought stress (cycles 1999/2000, 2000/2001 & 2001/2002). Debulking with a 127 *Babax/Seri* RILs subset. CSIRO-CIMMYT Molecular Databases

Canopy Temperature at Grain Filling, Morning

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
PstACCMseiCTA1#1	unknown	L101	63	CIMMYT	11,4	0,24	2,26	0,002
PstACCMseiCTA2#2	unknown	L100	63	CIMMYT	8,58	0,20	1,76	0,006
barc020#1	4B-1	L32	17	CSIRO	6,65	0,10	1,41	0,01
GWM132c#3	5A-2	L44	21	CSIRO	6,58	0,10	1,4	0,01
wmc048a#1	4B-1	L35	17	CSIRO	5,78	0,09	1,24	0,02
GWM375#1	4B-1	L34	17	CSIRO	4,33	0,07	0,94	0,04

Canopy Temperature at Grain Filling, Afternoon

Marker	Chromosome	Marker/ Locus Code	Linkage Group	Marker Dataset	**F**	**RSq**	**LOD**	**P**
GWM282#1	7A-1	L64	33	CSIRO	5,06	0,09	1,09	0,03
PstACCMseiCTA2#2	unknown	L101	62	CIMMYT	4,61	0,13	0,99	0,04
L-GWM389#1	3B	L90	53	CIMMYT	4,41	0,07	0,95	0,04
barc186#1	5A-1	L41	19	CSIRO	3,81	0,06	0,83	0,06

BIBLIOGRAPHY

Aastveit AH, Aasveit K (1993) Effects of genotype-environment interactions on genetic correlations. *Theoretical and Applied Genetics* (86): 1007-1013.

Abeles FB (1973) Ethylene in plant biology. Academic Press. New York.

Adams MD, Kelley JM, Gocayne JD, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, Kerlavage AR, McCombie WR, Venter JC (1991) Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* 252(5013): 1651-1656.

Ahn S, Anderson JE, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. *Mol Gen Genet* 241: 483-490.

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. *Proc Natl Acad Sci* 90: 7980-7984.

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics* 132:1131-1139.

Altinkut A, Kaza K, Gozukirmizi N (2003*a*) AFLP marker linked to water-stress-tolerant bulks in barley (*Hordeum vulgare* L.)*Genetics and Molecular Biology* 26: 77-82.

Altinkut A, Gozukirmizi N (2003*b*) Search for microsatellite markers associated with water-stress tolerance in wheat through bulked segregant analysis. *Molecular Biotechnology* 23(2):97-106.

Altieri MA (1995) The agroecosystem: Determinants, resources, processes and sustainability. *In:* Agroecology- The science of sustainable agriculture. Cambridge University Press. 2nd Edition. USA. p41-68.

Amani I, Fischer RA, Reynolds MP (1996) Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. *Journal of Agronomy and Crop Science* 176: 119-129.

Appels R, Dvorak J (1982) The wheat ribosomal DNA spacer region: Its structure and variation in populations and among species. *Theoretical and Applied Genetics* 83 : 715-719.

Appels R, Reddy P, McIntyre CL, Moran LB, Frankel OH, Clarke BC (1989) The molecular cytogenetic analysis of grasses and its application to studying relationships among species of the Triticeae. *Genome*. 31: 122-133.

Araus JL, Reynolds MP, Acevedo E (1993) Leaf posture grain yield, growth, leaf structure and carbon isotope discrimination in wheat. *Crop Science* 33: 1273-1279.

Araus JL (1996) Integrative physiological criteria associated with yield potential. *In*: Reynolds MP, Rajaram S, McNab A (1996) Increasing Yield Potential in Wheat: Breaking the Barriers. Mexico D.F.: CIMMYT. *p* 150-166.

Araus JL, Amaro T, Casadesus J, Asbati A, Nachit MM (1998) Relationships between ash content, carbon isotope discrimination and yield in durum wheat. *Australian Journal of Plant Physiology* 25: 835-842.

Araus JL, Casadesus J, Bort J (2001) Recent tools for the screening of physiological traits determining yield. *In*: Reynolds MP, Ortiz-Monasterio JI, McNab A (Eds.) (2001) Application of physiology in wheat breeding. Mexico D.F.: CIMMYT. *p* 59-77.

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C_3 cereals: What should we breed for? *Annals of Botany* 89: 925-940.

Arumuganathan K, Earle ED (1991) Nuclear content of some important plant species. *Plant Molecular Biology Reporter* 9(3): 208-218.

Ayala L, Henry M, van Ginkel M, Singh R, Keller B, Khairallah M (2002) Identification of QTLs for BYDV tolerance in bread wheat. *Euphytica* 128: 249-259.

Baldwin D, Crane V, Rice D (1999) A comparison of gel-based, nylon filter and microarray techniques to detect differential RNA expression in plants. *Curr Opin Plant Biol* 2: 96-103.

Bálint A (1984) Physiological Genetics of Agricultural Crops. Akadémiai Kiadó, Budapest.

Barlow EW, Munns R, Scott NS, Reisner AH (1971) Water potential, growth and polyribosome content of the stressed wheat apex. J Expt Bot 28: 909-916.

Bartels D, Schneider K, Terstappen G, Piatkowsky D, Salamini F (1990) Molecular cloning of abscisic acid-modulated genes which are induced during dessication of the resurrection plant *Craterostigma plantagineum*. *Planta* 181: 27-34.

Beavis WD (1998) *In*: Paterson AH (Ed.) Molecular Dissection of Complex Traits. p 145-162. CRC Press. Boca Raton, USA.

Beckmann JS (1991) Genomic genetics and plant genetic improvement. *In*: Gene-Mapping Techniques and Applications. Schook LB, Lewin HA, McLaren DG (1991) Marcel Dekker Inc. Basel, Switzerland. p201-229.

Beckmann JS, Soller M (1983) Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. *Theoretical and Applied Genetics* 67: 35-43.

Beckmann JS, Soller M (1986) Restriction fragment length polymorphisms in plant genetic improvement. *Oxford Surveys of Plant Molecular & Cell Biology* 3:196-250.

Beckmann JS, Soller M (1990) Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. *Bio/Technology*. 8: 939-932.

Bennett MD (1972) Nuclear DNA content and minimum generation time in herbaceous plants. *Proc.R.Soc.LondonSer.* B: 181:109-135.

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, collinearity and compatibility. *Trends in Genetics* 9(8)

Bennetzen JL (2000) Comparative genomics approaches to the study of drought tolerance. *In:* Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. p41-44.

Bennetzen JL, Ramakrishna W (2002) Exceptional haplotype variation in maize. *Proceedings of the National Academy of Sciences* USA 99 (14): 9093-9095.

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics* 112: 887-898.

Bevan M, Bancroft I, Mewes HW, Martienssen R, McCombie R (1999) Clearing a path through the jungle: progress in *Arabidopsis* genomics. *Bioessays* 21: 110-120.

Bianchi G, Gamba A, Murelli C, Salamini F, Bartels D (1991) Novel carbohydrate metabolism in the resurrection plant *Craterostigma plantagineum*. Plant J 1: 355-359.

Bingham J (1961) Varietal response in wheat to water supply in the field and male sterility caused by a period of drought in a glasshouse experiment. *Ann Appl Biol 5*7: 365-377.

Blake T, Lybeck N, Hayes P (1991) Good, bad and untested ideas in RFLP and QTL analysis. *Plant Breeding Abstracts*, C.A.B. International. Vol 61. No.1 p1-7.

Blum A (1988) Plant breeding for stress environments. CRC press USA.

Blum A (1996) Yield potential and drought resistance: Are they mutually exclusive? *p* 90-100. *In*: Reynolds MP, Rajaram S, McNab A (1996) Increasing Yield Potential in Wheat: Breaking the Barriers. Mexico D.F.: CIMMYT.

Blum A, Mayer J, Gozlan G (1982) Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. *Field Crops Research* 5: 137-146.

Blum A, Mayer J, Gozland G (1983) Association between plant production and some physiological components of drought resistance in wheat. *Plant Cell Environ* 6: 219-225.

Blum A, Shpiler L, Gozlan G, Mayer J (1989) Yield stability and canopy temperature of wheat genotypes under drought-stress. *Field Crops Research* 22: 289-296.

Blum A, Pnuel Y (1990) Physiological attributes associated with drought resistance of wheat cultivars in a mediterranean environment. *Australian Journal of Agricultural Research* 41: 799-810.

Blum A, Zhang J, Nguyen HT (1999) Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. *Field Crops Res* 64: 287-291.

Bohnert H, Fischer R, Kawasaki S, Michlaowski C, Wang H, Yale J, Zepeda G (2000) *In*: Ribaut JM, Poland D (Eds.) (2000) Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. p156-161.

Bohnert HJ, Bressan RA, Burnap R, Cushman JC, Galbraith DW, Hasegawa PM, Prade R, Zhu JK (2002) Transcript profiling of environmental stress responses through expression categories and microarrays. *In*: Plant, Animal & Microbe Genomes X Conference. 12th-16th January. San Diego, California.

Bonierbale MW, Plaisted RL, Tanskley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* 120: 1095-1103.

Borlaug NE, Dowswell CR (2005) Feeding a world of ten billion people: A 21st century challenge. *In*: In the wake of the double helix: From the green revolution to the gene revolution. Tuberosa R, Phillips RL, Gale M (Eds.). Proceedings of an International Congress. 27th-31st May. Univeristy of Bologna, Italy. Avenue media. 3-23.

Borlaug NE (2006) International collaborative wheat improvement: Future challenges. *In:* Reynolds MP, Godínez D (Ed.) Challenges to International Wheat Breeding- International Symposium on Wheat Yield Potential. 20th-24th March. Ciudad Obregón, Sonora, Mexico. Extended Abstracts. p1.

Bray EA (1993) Molecular responses to water deficit. *Plant Physiol* 103: 1035-1040.

Brennan PS, Byth DE, Drake DW, DeLacy IH, Butler DG (1981) Determination of the location and number of test environments for a wheat cultivar evaluation program. *Australian Journal of Agricultural Research* 32:189-201.

Brennan JP, Morris ML (1997) Economic aspects of quality issues in wheat variety improvement. *In*: Steele JL, Chung OK (Eds.) International Wheat Conference Proceedings. p363-376.

Brennan JP, Morris ML (2001) Economic issues in assessing the role of physiology in wheat breeding programs. *In*: Reynolds MP, Ortiz-Monasterio JI, McNab A (Eds.) Application of physiology in wheat breeding. Mexico D.F.: CIMMYT. p78-86.

Brennan JP (2006) Returns to investment in new breeding technologies. *In:* Reynolds MP, Godínez D (Ed.) Challenges to International Wheat Breeding- International Symposium on Wheat Yield Potential. 20th-24th March. Ciudad Obregón, Sonora, Mexico. Extended Abstracts. p33.

Briggle LW, Curtis BC (1987) Wheat worldwide. *In*: Wheat and wheat improvement. Heyne EG (Ed.) (Agronomy; No. 13) Second Edition. USA. 1-32.

Britten RJ, Kohne DE (1968) Repeated sequences in DNA. *Science* 161 (3841): 529-540.

Bureau of Meteorology. Drought statement (on Drought for the 3, 6 and 10-month periods ending 31st October 2006) issued 3rd November 2006 by the National Climate Centre. Australian Government.

Burbank RT, Taylor WC (1995) Regulation of photosynthesis in C3 and C4 plants: A molecular approach. *Plant Cell* 7: 797-807.

Burr B, Burr FA, Thompson KH, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. *Genetics* 118: 519-526.

Burr BJ, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: Theoretical and practical considerations. Trends in Genetics 7 (2): 55-60.

Byerlee D, Moya P (1993) Impacts of international wheat breeding research in the developing world, 1966-1990. Mexico D.F.: CIMMYT.

Caetano-Anollés, Bassam BJ, Gresshoff PM (1991) DNA amplification fingerprinting: A strategy for genome analysis. *Plant Molecular Biology Reporter* 9 (4): 294307.

Campbell SA, Close TJ (1997) Dehydrins: genes, proteins, and associations with phenotypic traits. *The New Phytologist* 137 (1): 61-74.

Campbell TA, Rathjen AJ, Paull JG, Islam AKMR (1998) Method for screnning bread wheat for tolerance to boron. *Euphytica* 100: 131-135.

Carpenter JF, Crowe JH (1988) The mechanism of cryoprotection of proteins by solutes. *Cryobiol* 25: 244-255.

Cattivelli L, Baldi P, Crosatti C, Di Fonzo N, Faccioli P, Grossi M, Mastrangelo AM, Pecchioni N, Michele Stanca A (2002) Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*. *Plant Molecular Biology* 48: 649-665.

Ceccarelli S (1984) Plant responses to water stress—A review. *Genetica-Agraria* 38(1): 43-73.

Ceccarelli S (1989) Wide adaptation: How wide? *Euphytica* 40:197-205.

Ceccarelli S, Nachit MM, Ferrara GO, Mekni MS, Tahir M, van Leur J, Srivastava JP (1987) Breeding strategies for improving cereal yield and stability under drought. *In*: Drought tolerance in winter cereals. Srivastava JP, Porceddu E, Acevedo E, Varma S (Eds.) Proceedings of an International Workshop 27-31 October 1985, Capri, Italy. ICARDA: John Wiley & Sons. Great Britain. p101-114.

Chapin FS (1991) Integrated responses of plants to stress. A centralised system for physiological responses. *Bioscience* 40: 29-31.

Chapman SC, Mathews KL, Cooper M, Jensen N, Wang E, Butler D, Sheppard J, Sahama T (2001) Using environment characterisation to interpret wheat yield in water-limited environments. *In*: Eastwood R, Hollamby G, Rathjen AJ, Gororo N (Eds.) 10th Assembly Proceedings of the Wheat Breeding Society of Australia, Inc. 16th-21st September, Mildura, Australia. p136-139.

CIMMYT Wheat Personnel (1986) Veery 'S': Bread wheats for many environments. El Batan, Mexico: CIMMYT. 23 p.

CIMMYT (2004) Perspectives on CIMMYT's future: A summary of external stakeholder consultations conducted for strategic planning at CIMMYT. Mexico DF: CIMMYT.

Close TJ, Choi D-W, Campbell SA, Koag M-C, Zhu B (2000) The dehydrin multigene family in the Triticeae and maize. Bennetzen JL (2000) Comparative genomics approaches to the study of drought tolerance. *In:* Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. p167-170.

Close TJ, Kortt AA, Chandler PM (1989) A cDNA based comparison of dehydration-induced proteins (dehydrins) in barley and corn. *Plant Mol Biol* 13:95-108.

Cohen A, Bray EA (1990) Characterization of three mRNAs that accumulate in wilted tomato leaves in response to elevated levels of endogenous abscisic acid. *Planta* 182: 27-33.

Cooke R, Raynal M, Laudie M, Grellet F, Delseny M, Morris PC, Guerrier D, Giraudet J, Quigley F, Clabaut G, Li YF, Mache R, Krivitzky M, Fy IJJ, Kreis M, Lecharney A, Parmentier Y, Marbach J, Fleck J, Clement B, Philips G, Herve C, Bardet C, Tremousaye D, Lescure B, Lacomme C, Roby D, Jourjon MF, Chabrier P, Charpenteau JL, deprez T, Amselem J, Chiapello H, Hofte H (1996) *Plant J* 9: 101-124.

Cooper M, Woodruff DR, Eisemann RL, Brennan PS, DeLacy IH (1995) A selection strategy to accomodate genotype-by-environment interaction for grain yield in wheat: Managed environments for selection among genotypes. *Theoretical and Applied Genetics* 90: 493-502.

Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. *Crop Science* 27: 996-1001.

Condon AG, Richards RA, Farquhar GD (1992) The effect of variation in soil water availability, vapour pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. *Australian Journal of Agricultural Research* 43: 935-947. 27: 996-1001.

Condon AG, Reynolds MP, Rebetzke GJ, van Ginkel M, Trethowan R, Brennan J, Bonnett DG (2006) Stomatal aperture relate traits and yield potential in bread wheat. *In:* Reynolds MP, Godínez D (Ed.) Challenges to International Wheat Breeding- International Symposium on Wheat Yield Potential. 20th-24th March. Ciudad Obregón, Sonora, Mexico. Extended Abstracts. p29.

Cowen NM (1988) The use of replicated progenies in marker-based mapping of QTL's. *Theoretical and Applied Genetics* 75: 857-862.

CRCMPB (2003) Cooperative Research Molecular Plant Breeding. Adelaide, Australia. Annual Report. 104 pp.

CRCMPB (2004) Cooperative Research Molecular Plant Breeding. Adelaide, Australia. Annual Report. 112pp.

Crossa J, Cornelius PL (1997) Sites regression and shifted multiplicative model clustering of cultivar trial sites under heterogeneity of error variance. *Crop Science* 37: 406-415.

Crowe JH, Crowe LM, Carpenter JF, Rudolph AS, Winstrom CA, Spargo BJ, Anchordoguy TJ (1988) Interactions of sugars with membranes. *Archive of Biochemistry and Biophysics* 947: 367-384.

Csonnka LN, Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. *Annu Rev Microbiol* 45: 569-606.

Cullis BR, Gleeson AC (1991) Spatial analysis of field experiments—An extension to two dimensions. *Biometrics* 47: 1449-1460.

Curtis H (1986) Biología. Editorial Médica Panamericana. 4a Edición. México. 1255 pp.

Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. *In:* Current Opinion in Plant Biology. Roberts K & Somerville C (Eds.). 3: 117-124.

Çakir M, Karakousis A (2000) A guide to molecular genetic mapping and analysis of quantitative trait loci. University of Western Australia. Cooperative Research Centre Molecular Plant Breeding. Grains Research & Development Corporation: National Barley Molecular Marker Program (NBMMP). 50pp.

Darvasi A, Soller M (1992) Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theoretical and Applied Genetics* 85: 353-359.

Davis WJ, Mansfield TA (1983) The role of abscisic acid in drought avoidance. *In*: Addicott FT (Ed) Abscisic Acid. New York.

Desprez T, Anselem J, Caboche M, Höfte H (1998) Differential gene expression in *Arabidopsis* monitored using cDNA arrays. *Plant J* 14: 643-652.

de Vienne D (1991) Molecular markers in quantitative genetics. Plant Sciences Today. La Colle-sur-Loup (France), 16-18 Octobre. Ed. Inra, Paris (Les Colloques n°59). p 283-284.

de Vienne D, Zivy M (2000) Proteomic and genetical approach of physiological and molecular resposnes to drought in maize. *In*: Ribaut JM, Poland D (Eds.) (2000) Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. p151-153.

Devos KM, Gale MD (1997) Comparative genetics in the grasses. *Plant Molecular Biology* 35: 3-15.

Devos KM, Gale MD (2000) Genome Relationships: The Grass Model in Current Research. *The Plant Cell*å 12: 637-646.

Devos KM, Chao S, Li QY, Simonetti MC, Gale MD (1994) Relationship between chromosome 9 of maize and wheat homeologous group 7 chromosomes. *Genetics* 138: 1287-1292.

Devos KM, Dubcovsky J, Dvorák J, Chinoy CN, Gale MD (1995) Structural evolution of wheat chromosomes 4A, 5A and 7B and its impact on recombination. *Theoretical & Applied Genetics* 91: 282-288.

Dhindsa RS, Cleland RE (1975) Water stress and protein synthesis. I. Differential inhibition of protein synthesis. *Plant Physiol* 55: 778-781.

Dietrich R, Grivet L, Zhu T, Chilcott C, Dunn M, Xia Y, Zhou N, Long N, Puddepaht I, Bontems S, Bonnet G (2006) Affymetrix microarrays as tools for generating maps and markers in tomato. PAA Solanaceae 2006 Conference: Genomics meets Biodiversity. 23th-27th July. Madison, Michigan. Botanical Society of America.

Donald CM (1968) The breeding of crop ideotypes. *Euphytica* 17: 385-403.

Dreher K, Morris M, Khairallah M, Ribaut JM, Pandey S, Srinivasan G (2002) Is marker-assisted selection cost-effective compared with conventional plant breeding methods? The case of quality protein maize. *In:* Economic and Social Issues in Agricultural Biotechnology. Evenson RE, Santaniello V, Zilberman D (Eds.) CAB International. 204-236.

Dreher K, Khairallah M, Ribaut JM, Morris M (2003) Money matters (I): costs of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. *Molecular Breeding* 11: 221-234.

Dreisigacker S, Zhang P, Warburton ML, Skovmand B, Hoisington D, Melchinger AE (2005) Genetic diversity among and within CIMMYT wheat landrace accessions investigated with SSRs and implications for plant genetic resources management. *Crop Science* 45: 653-661.

Dubcovsky J, Luo MC, Dvorak J (1995) Linkage relationships among stress-induced in wheat. *Theoretical and Applied Genetics* 91(5): 795-801.

Dunford RP, Kurata N, Laurie DA, Money TA, Minobe Y, Moore G (1995) Conservation of fine-scale DNA marker order in the genomes of rice and the *Triticeae.* Nucl Acids Res 23: 2724-2728.

D'Ovidio R, Anderson OD (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread-making quality. *Theoretical and Applied Genetics* 88: 759-763.

Dure L III, Crouch M, Harada J, Ho TH, Quatrano R, Thomas T, Sung ZR (1989) Common amino acid sequence domains among the LEA proteins of higher plants. *Plant Mol Biol* 12: 475-486.

Eagles HA, Hollamby GJ, Eastwood RF, Podlich DW (2001) Use of markers for monogenically and polygenically inherited traits. *In:* Eastwood R, Hollamby G, Rathjen T, Gororo N (Eds.) Wheat Breeding society of Australia, Inc. 10th Assembly Proceedings, Mildura, Australia. p 35-37.

Eastwood RF, Lagudah ES, Appels R, 1994. A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticium tauschii*. *Genome*. 37:311-319

Eathington S (2006) Practical uses of molecular markers in a commercial breeding program (Monsanto). *In*: Higman S (Ed.) International Plant Breeding Symposium – Honouring John Dudley. 20-25 August. Proceedings (*in press*).

Ehdaie B, Hall AE, Farquhar GD, Nguyen HT, Waines JG (1991) Water-use efficiency and carbon isotope discrimination in wheat. *Crop Science* 31: 1282-1288.

Ellis MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theoretical and Applied Genetics* 105:1038-1042.

Engledow FL, Wadham SM (1923) Investigations on yield in the cereals. Part 1. *J.Agric.Sci.* 13: 390-439.

Eujayl L, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellites markers for genotyping the A & B genomes of wheat. *Theoretical and Applied Genetics* 104: 399-407.

Evans LT, Wardlaw IF, Fischer RA (1975) Wheat. *In*: Crop Physiology- Some case histories. Evans LT (Ed.) Cambridge University Press. Great Britain. 101-149.

Evans LT, Fischer RA (1999) Yield potential: Its definition, measurement and significance. *Crop Science* 39:1544-1551.

Falconer DS (1989) Introduction to Quantitative Genetics. John Wiley & Sons. New York. USA.

Finlay KW, Wilkinson GN (1963) The analysis of adaptation in a plant breeding programme. *Australian Journal of Agricultural Research* 14(6): 742-754.

Fischer RA (1985) Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science* 100: 447-461.

Fischer RA (1999) Wheat cropping in Australia *In*: Wheat: Ecology and Physiology of Yield Determination. Satorre EH, Slafer GA (Eds.) The Haworth Press. Chapter 13. 277-294.

Flores HE, Galston AW (1984) Osmotic stress-induced polyamine accumulation in cereal leaves. Physiological parameters of the response. *Plant Physiology* 75: 102-109.

Food and Agriculture Organisation (2006) Economics and Statistics (www.fao.org).

Fox P, López C, Skovmand B, Sánchez H, Herrera R, White JW, Duveiller E, van Ginkel M (1996) International Wheat Information System (IWIS), v.1. Mexico D.F.: CIMMYT. Compact Disc.

Franco J, Crossa J, Villaseñor J, Castillo A, Taba S, Eberhart SA (1998) Classifying genetic resources by categorical and continuous variables. *Crop Science* 38: 1688-1696.

Franco J, Crossa J, Ribaut JM, Betran J, Warburton ML, Khairallah M (2001) A method of combining molecular markers and phenotypic attributes for classifying plant genotypes *Theorethical and Applied Genetics* 103: 944-952.

Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis and utilization of conserved ortholog set markers for comparative genomics in higher plants. *The Plant Cell* 14: 1457-1467.

Gaff DF (1980) Protoplasmic tolerance of extreme water stress. *In:* Turner NC, Kramer PJ (Eds). Adaptation of plants to water and high temperature stress. John Wiley and Sons. New York.

Gale MD, Devos KM (1998) Comparative genetics in the grasses. *Proc. Natl. Acad. Sci.* USA 95:1971-1974.

Galston AW, Sawhney RK (1990) Polyamines in plant physiology. *Plant Physiology* 94: 406-410.

Ganal MW, Lapitan NLV, Tanksley SD (1988) A molecular and cytogenetic survey of major repeated DNA sequences in tomato (*Lycopersicon esculentum*) *Molecular and General Genetics* 213: 262-268.

Gehlot HS, Mali S, Agarwal S (1998) Stress Proteins in Plants. *Agro Ann Rev Pl Physiol* 249-289.

Geldermann H (1975) Investigations of inheritance of quantitative characters in animals by gene markers. I. Methods. *Theoretical and Applied Genetics* 46: 319.

Gelfand DH (1989) *Taq* DNA polymerase. *In*: PCR Technology—Principles and Applications for DNA amplification. Erlich HA (Ed.). Stockton Press. USA. 1st Edition. p 17-22.

Goday A, Jensen AB, Culianez-Macia FA, Mar Alba M, Figueras M, Serratosa J, Torrent M, Pages M (1994) The maize abscisic acid-responsive protein Rab17 is located in the nucleus and interacts with nuclear localization signals. *Plant Cell* 6: 351-360.

Goff SA (1999) Rice as a model for cereal genomics. *Curr Opi Plant Biol* 2: 86-89.

Goggin DE, Setter TL (2004) Fructosyltransferase activity and fructan accumulation during development in wheat exposed to terminal drought. *Functional Plant Biology* 31: 11-21.

González-de-León D, Hoisington DA, Hu X, Jiang C, Khairallah M (1995) Molecular Marker Applications to Plant Breeding- A Workshop offered by the Applied Molecular Genetic Laboratory CIMMYT, INT. Mexico. 27 November-8 December, El Batán, México.

Goulden CH (1939) Problems in plant selection. *In:* Burnett RC (Ed.) Proceedings of the 7th International Genetics Congress, Edinburgh, Scotland. Cambridge University Press. p 132-133.

Goodwin SB, Hu X, Shaner G (1998) Proc 9th Intl Wheat Genet Symp 3: 108-111.

Grains Research and Development Corporation (2006) Annual Report 2004-2005. Australian Government (www.grdc.com.au)

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM (2000) An introduction to genetic analysis. WH Freeman & Co. USA. 860pp.

Gutiérrez-Rodríguez M, Reynolds MP, Escalante-Estrada JA, Rodríguez-González MT (2004) Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. *Australian Journal of Agricultural Research* 55: 1139-1147.

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 163-185.

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier MF, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp PJ, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theoretical and Applied Genetics* 105: 413-422.

Guyomarc'hH, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. *Theoretical and Applied Genetics* 104: 1164-1172.

Habben J, Helentjaris T, Sun Y, Zinselmeier C (2000) *In*: Ribaut JM, Poland D (Eds.) (2000) Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. p153-154.

Haldane JBS (1919) The combination of linkage values and the calculation of distance between the loci of linked factors. *Journal of Genetics* 8: 299-309.

Haldane JBS (1930) Theoretical genetics of autopolyploids. *Journal of Genetics* 22: 359-372.

Haldane JBS, Waddington CH (1931) Inbreeding and linkage. *Genetics* 16:357-374.

Hanson H, Borlaug NE, Anderson RG (1982) Wheat in the Third World. International Maize and Wheat Improvement Center. Westview Press, Inc. USA. 174pp.

Hare PD, Cress WA, van Staden J (1999) Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *Journal of Experimental Botany* 50 (333): 413-434.

Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. *Science* 153: 1074-1080.

Harlan JR (1975) Crops and man. *Am Soc Agron Crop Sci Soc Am.* Madison, Wisconsin. USA.

Harrington JB (1937) The mass-pedigree method in the hybridization improvement of cereals. *Journal of the American Society of Agronomy* 29: 379-384.

Hartl L, Weiss H, Stephan U, Zeller FJ, Jahoor A (1995) *Theoretical & Applied Genetics* 90: 601-606.

Hartl L, Mori S, Schweizer G (1998) Proc 9th Intl Wheat Genet Symp 3: 111-113.

Hayes HH, Immer FR (1942) Methods of plant breeding. McGraw-Hill Co. Inc. New York, USA. 432 p.

Heikkila JJ, Papp JET, Schultz GA, Bewley D (1984) Induction of heat shock protein messenger RNA in maize mesocotyls by water stress, abscisic acid and wounding. *Plant Physiology* 76: 270-274.

Heisey PW, Lantican MA, Dubin HJ (1999) Assessing the benefits of international wheat breeding research: An Overview of the global wheat impacts study. *In*: Pinglai PL (Ed.) Global Wheat Research in a Changing World: Challenges and Achievements. Mexico DF: CIMMYT. p19-26.

Helentjaris T, King G, Slocum M, Sidenstrang C, Wegman S (1985) Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. *Plant Molecular Biology* 5: 109-118.

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of gentic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Theoretical and Applied Genetics* 72: 761-769.

Hieter P, Boguski M (1997) Functional Genomics: It's all how you read it. *Science* 278:601-604

Higuchi R (1989) Simple and rapid preparation of samples for PCR. *In*: PCR Technology—Principles and Applications for DNA Amplification. Erlich HA (Ed.). Stockton Press. USA. 1st Edition. p 31-38.

Hobbs PR, Sayre KD (2001) Managing experimental breeding trials. *In*: Reynolds MP, Ortiz-Monasterio JI, McNab A (Eds.) (2001) Application of physiology in wheat breeding. Mexico D.F.: CIMMYT. *p* 48-58.

Hoisington DA, González-de-León D (1993) HyperMapData —A HyperCard stack for entry and analysis of molecular genetic segregation data. *Agronomy abstracts*. Annual meetings, Cincinnati, Ohio, Nov. 7-12, 1993.

Hoisington DA, Khairallah M, González-de-León D (1994) Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. 2nd Edition. Mexico City, Mexico.

Hollamby G, Bayraktar A (1996) Breeding objectives, philosophies and methods in South Australia. *In*: Reynolds MP, Rajaram S, McNab A (Eds.) Increasing yield potential in wheat: Breaking the barriers. Mexico, DF: CIMMYT. p52-65.

Holappa LD, Walker-Simmons MK (1995) The wheat abscisic acid-responsive protein kinase mRNA, PKABAI, is up-regulated by dehydration, cold temperature, and osmotic stress. *Plant Physiol* 108: 1203-1210.

Hsiao (1973) Plant responses to water stress. *Annual Review of Plant Physiology* 24: 519-570.

Hu XY, Ohm HW, Dweikat I (1997) *Theoretical and Applied Genetics* 94: 832-840.

Hulbert SH, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proc Natl Acad Sci* 87: 4251-4255.

Hurd EA (1968) Growth of roots of seven varieties of spring wheat at high and low moisture levels. *Agron. J.* 60:201-205.

International Soil Fertility Manual (1995) Potash & Phosphate Institute. First printing. USA. 124pp.

Innes P, Blackwell RD, Quarrie SA (1984) Some effects of genetic variation in drought-induced abscisic acid accumulation on the yield and water-use of spring wheat. *J. Agric. Sci. Camb.* 102:341.

Isbell RF (2002) The Australian Soil Classification. CSIRO Publishing: Melbourne, Australia.

Jackson RD (1982) Canopy temperature and crop water stress. *Advances in Irrigation* 1: 43-55.

James SH (1992) Inbreeding, self-fertilization, lethal genes and genomic coalescence. *Heredity* 68: 449-456.

Kashi Y, Soller M, Hallerman E, Beckmann JS (1986) Restriction fragment length polymorphisms in dairy cattle genetic improvement. *In:* Proceedings of the Third World Congress on Genetics Applied to Livestock Production. Nebraska. 12: 57-63.

Kameli A, Losel DM (1995) Contribution of carbohydrates and other solutes to osmotic adjustment in wheat leaves under water stress. *J Plant Physiol* 145: 363-366.

Kehoe DM, Villand P, Sommerville S (1999) Microarrays for studies of higher plants and other photosynthetic organisms. *Trends Plant Sci* 4: 38-41.

Keim DL, Kronstad WE (1981) Drought response of winter wheat cultivars grown under field stress conditions. *Crop Science* 21: 11-15.

Khabaz-Saberi H, Graham RD, Pallotta MA, Rathjen AJ, Williams KJ (2002) Genetic markers for manganese efficiency in durum wheat. *Plant Breeding* 121: 224-227.

Kihara H (1944) Discovery of the DD-analyser, one of the ancestors of vulgare wheats. *Agri Hort* 19: 889-890.

Killian A, Kudrna DA, Kleinhofs A, Yano M, Kurata N, Steffenson B, Sasaki T (1995) *Nucl Acids Res* 23: 2729-2733.

Kirigwi FM, van Ginkel M, Trethowan R, Sears RG, Rajaram S, Paulsen GM (2004) Evaluation of selection strategies for wheat adaptation across water regimes. *Euphytica* 135: 361-371.

Knapp SJ, Bridges WC (1990) Using molecular markers to estimate quantitative trait locus parameters: Power and genetic variuances for unreplicated and replicated progeny. Genetics 126: 769-777.

Kramer PJ (1969) Plant and soil water relationships: A modern synthesis. McGraw-Hill, New York.

Kramer PJ (1983) Water Relations of Plants. Academic Press. New York.

Kramer PJ (1980) Drought, stress and the origin of adaptations. *In*: Turner NC, Kramer PJ (Eds). Adaptation of plants to water and high temperature stress. John Wiley and Sons. New York.

Kuleung C, Baenzinger PS, Dweikat I (2004) Transferability of SSR markers among wheat, rye and triticale. *Theoretical and Applied Genetics* 108: 1147-1150.

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimaon T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma Y, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) *Nature Gen* 8: 365-372.

Lacerenza NG, Cattivelli L, Fonzo ND (1995) Expression of drought induced genes in durum wheat under water stress in field conditions. *J Plant Physiol* 146: 169-172.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174-181.

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121: 186-199.

Langridge (2003) Molecular breeding of wheat and barley. *In*: Tuberosa R, Phillips RL, Gale M (Eds.) In the Wake of the Double Helix- From the Green Revolution to the Gene Revolution. Proceedings of an International Congress. 27-31 May. Bologna, Italy. p 279-286.

Langridge P, Paltridge N, Fincher G (2006) Functional genomics of abiotic stress tolerance in cereals. *Briefings in functional genomics and proteomics* 4(4): 343-354.

Lee M (1995) DNA markers and plant breeding programs. *Advances in Agronomy* 55: 265-344.

Lee SJ, Penner GA, Devos KM (1995) Characterization of *loci* containing microsatellite sequences among Canadian wheat cultivars. *Genome* 38: 1037-1040.

Lemieux B, Aharoni A, Schena M (1998) Overview of DNA chip technology. *Mol Breed* 4: 277-289.

Leonard WH, Martin JH (1963) Cereal crops. The Macmillan Co. New York.

Levitt J (1980) Responses of Plants to Environmental Stress. Academic Press. New York.

Limón-Ortega A, Sayre KD, Francis CA (2000) Wheat and maize yields in response to straw management and nitrogen under a bed-planting system. *Agronomy Journal* 92: 295-302.

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Gen.* 44: 397-401.

Liu BH (1998) Statistical genomics- Linkage, mapping and QTL analysis. CRC Press. USA. 611p

Liu CY, Paull JG, Rathjen AJ (2000) Shoot mineral composition and yield of wheat genotyps grown on a sodic and a non-sodic soil. *Australian Journal of Experimental Agriculture*. 40: 69-78.

Liu H-C, Sofer L, Burnside J, Cheng HH (2000) Identification of positional candidate genes for QTL through DNA microarrays and genetic mapping. *In*: Proceedings & Abstracts. Plant & Animal Genome VIII—The International Conference on the Status of Plant & Animal Genome Research. Jan 9-12, 2000. San Diego, USA. 227pp.

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Anioxidative defense system, pigment composition, and photo efficiency in two wheat cultivars subjected to drought. *Plant Physiol* 119: 1091-1100.

Lorieux M (1994) Aspects statistiques de la cartographie des marquers moléculaires. Documents de travail de la mission biométrie, Unité de recherche BIOTROP, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD). No 1. France. 49pp.

Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in mediterranean environments. *Advances in Agronomy* 52: 229-276.

Lucas P (2000) Corrélation entre l'accumulation de polyamines et la résistance à la sécheresse chez le mais. M.Sc.Thesis. Université D'Orsay (Paris XI). DESS Economie Agricole Internationale. 29 p.

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. *Advances in Agronomy* 43: 107-153.

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Inc. Canada.

Ma JF, Mitani N, Nagao S, Konishi S, Tamai K, Iwashita T, Yano M (2004) Characterization of the silicon uptake system and molecular mapping of the silicon transporter gene in rice. *Plant Physiology* 136: 3284-3289.

Ma H, Bai G, Zhou L, Carver B (2005) Molecular mapping of a quantitative trait locus for aluminum tolerance in wheat cultivar Atlas 66. *Theoretical and Applied Genetics* 112: 51-57.

Mason HS, Mullet JE, Boyer JS (1988) Polysomes, messenger RNA, and growth in soybean stems during development and water deficit. *Plant Physiol* 86: 725-733.

Mather K (1943) Polygenic balance in the canalization of development. *Nature* 151: 68-71.

Mather K (1951) The measurement of linkage in heredity. John Wiley & Sons. New York, USA.

Mayoral ML, Atsmon D, Shimshi D, Gromet-Elhanan Z (1981) Effect of water stress on enzyme activities in wheat and related wild species: Carboxylase activity, electron transport and photophosphorylation in isolated chloroplasts. *Aus J Plant Physiol* 8: 385-392.

Macindoe SL (1975) History of production of wheat in Australia. *In*: Australian Field Crops, Vol.1: What and other temperate cereals. Lazenby A, Matheson EM (Eds.) Angus & Robertson, Sydney, AUS 99-121. *In*: Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environment. *Adv. Agron.* 52:229-276.

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. *J Hered* 37: 81-89.

McIntyre CL, Chapman SC, Mathews KL, van Herwaarden A, Reynolds MP, Shorter R (2006) Identification of germplasm and genomic regions for traits relevant to wheat production in

drought environments. Proceedings of the 13th Australasian Plant Breeding Conference. Christchurch, New Zealand, April. CD.

McWilliam (1986) The national and international importance of drought and salinity effects on agricultural production. *Australian Journal of Plant Physiology* 13:1-13.

McWilliam JR (1989) The dimensions of drought. *In*: Drought resistance in cereals. Baker FWG (Ed.) International Council of Scientific Unions (ICSU): CAB International. United Kingdom. p 1-11.

McCouch S (1998) Toward a plant genomics initiative: thoughts on the value of cross-species and cross-genera comparisons in the grasses. *Proc. Natl. Acad. Sci. USA.* 95:1983-1985.

Miralles DJ, Slafer GA (1999) Wheat development. *In*: Wheat: Ecology and Physiology of Yield Determination. Satorre EH, Slafer GA (Eds.) The Haworth Press. Chapter 2. 13-43.

Molnár I, Gáspár L, Sárvári E, Dulai S, Hoffmann B, Molnaár-Láng M, Galiba G (2004) Physiological and morphological responses to water stress in *Aegilops biuncialis* and *Triticum aestivum* genotypes with differing tolerance to drought. *Functional Plant Biology* 31: 1149-1159.

Moore G, Devos KM, Wang Z, Gale MD (1995) *Curr Opn Biol* 5: 737-739.

Morgan JM (1980a) Possible role of abscisic acid in reducing seed set in water-stressed wheat plants. *Nature*. 285: 655-657.

Morgan (1980b) Osmotic adjustment in the spikelets and leaves of wheat. *J Exp Bot* 31: 655-665.

Morgan JM (1983) Osmoregulation as a selection criterion for drought tolerance in wheat. *Aust Journal of Agricultural Research* 34: 607-614.

Morgan JM, Condon AG (1986) Water-use, grain yield and osmoregulation in wheat. *Australian Journal of Plant Physiology* 13:523-532.

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. *Plant J.* 3: 175-182.

Morgante M, Rafalski A, Biddle P, Tingey S, Olivieri AM (1994) Genetic mapping and variability of seven soyabean simple sequence repeat loci. *Genome* 37: 763-769.

Morris ML, Belaid A, Byerlee D (1991) Wheat and barley production in rainfed marginal environments of the developing world. *In:* CIMMYT World Wheat Facts and Trends, 1990-1991. CIMMYT, Mexico.

Morris M, Dreher K, Ribaut JM, Khairallah M (2003) Money matters (II): costs of inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. *Molecular Breeding* 11: 235-247.

Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked o disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc.Nat.Acad.Sci.USA*. 88:9828-9832.

Mullis KB (1989) The Polymerase Chain Reaction: Why it works. *In*: Polymerase Chain Reaction. Erlich HA, Gibbs R, Kazazian Jr HH (eds.) Current Communications in Molecular Biology. Cold Spring Harbor Laboratory Press. p 237-243.

Munns R, Weir R (1981) Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits at two light levels. *Aust J Plant Physiol* 8: 93-105.

Naidu R, Rengasamy P (1993) Ion interactions and constraints to plant nutrition in Australian sodic soils. *Australian Journal of Soil Research* 31: 801-819.

Naidu R, Rengasamy P, de Lacy NJ, Zarcinas BA (1995) Soil solution composition of some sodic soils. *In:* "Australian sodic soils: distribution, properties and management" (Eds. Naidu R, Sumner ME, Rengasamy P). CSIRO Melbourne, Australia. *p*155-161.

Nakamura Y, Leppert M, O'Connell P, Wolf R, Holm T, Culver M, Martin C, Fugimoto E, Hoff M, Kumlin E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* 235: 1616-1622.

Nelson JC (1997) QGENE: Software for marker-based genomic analysis and breeding. *Molecular Breeding* 3: 239-245.

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995*a*) Molecular mapping of wheat: Major genes and rearrangements in homoeologous groups 4, 5 and 7. *Genetics* 141: 721-731.

Nelson JC, Van Deynze AE, Autrique AE, Sorrells ME, Lu, YH, Merlino M, Atkinson M, Leroy P, (1995*b*) *Genome* 38: 516-524.

Neuhaus G, Neuhaus G (1993) The use of the nonradioactive digoxigenin chemiluminescent technology for plant genomic Southern blot hybridization: A comparison with radioactivity. *Transgenic Research* 2: 115-120

Newman T, de Bruijn FJ, Green P, Keegstra K, Kende H, McIntosh L, Ohlrogge J, Raikhel N, Somerville S, Thomashow M, Retzel E, Somerville C (1994) *Plant Physiol* 106: 1241-1255.
Parker GD, Chalmers KJ, Rathjen AJ, Langridge P (1998) Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.) *Theor Appl Genet* 97: 238-245.

Newton CR, Graham A (1995) PCR. Bios-Scientific Publishers. United Kingdom. 1st Edition. 162 pp.

Nicol JM (2000) Important nematode pests of cereals. *In*: FAO (2000) Curtis BC (Ed.) Wheat Production and Improvement. FAO Plant Production and Protection Series.

Nicol JM, Ortiz-Monasterio I (2004) Effects of the root-lesion nematode, *Pratylenchus thornei*, on wheat yields in Mexico. *Nematology* 6(4): 485.

Nicol JM, Rivoal R, Trethowan RM, van Ginkel M, Mergoum M, Singh RP (2001). CIMMYT's approach to iedntify and use resistance to nematodes and soil-borne fungi, in developing superior wheat germplasm. *In*: Wheat in a global environment. Bedö Z, Láng L (Eds.) Proceedings of the 6th International Wheat Conference, 5-9 June 2000, Budapest, Hungary. Kluwer Academic Publishers. *p*381-389.

Nicot N, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P, Bernard M, Sourdille P (2004) Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags. *Theoretical and Applied Genetics* 104: 800-805.

Northcote KH, Skene JKM (1972) Australian soils with saline and sodic properties. Australian soils with saline and sodic properties. Australian Soil Publication No. 27. CSIRO: Melbourne, Australia.

Olivares-Villegas JJ, Reynolds MP, McDonald GK (2007) Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. *Functional Plant Biology* 34: 189-203.

Orion D, Amir J, Krikun J (1984) Field observations on *Pratylenchus thornei* and its effects on wheat under arid conditions. *Revue Nématologie* 7(4): 341-345.

O'Toole JC, Cruz RT (1980) Genotypic variation in epicuticular wax of rice. *Plant Physiology* 64: 428-430.

Paillaird S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). *Theorethical and Applied Genetics* 107:1235-1242.

Passioura JB (1986) Resistance to Drought and Salinity: Avenues for improvement. *Aust J Plant Physiol* 13: 191-201.

Paterson A (2002) What has QTL mapping taught us about plant domestication? Tansley review no.137 *New Phytologist* 154 : 591-608.

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete map of restriction fragment length polymorphisms. *Nature* 335: 721-726.

Paterson AH, LinYR, Li S, Schertz KF, Doebley JF, Pinson SRM, Liu SC, Stansel JW Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci *Science* 269: 1714-1717.

Paterson AH, Bowers JE, Burow MD, Draye X, Elsik CG, Jiang CX, Katsar CS, Lan TH, Lin YR, Ming R, Wright RJ (2000) Comparative genomics of plant chromosomes. *The Plant Cell* 12: 1523-1539.

Paterson AH (2002) What has QTL mapping taught us about plant domestication? *New Phytologist* 154: 591-608.

Peccoud J, Velden KV, Podlich D, Winkler C, Arthur L, Cooper M (2004) The selective values of alleles in a molecular network model are context dependent. *Genetics* 166: 1715-1725.

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs.*Theorethical and Applied Genetics* 97: 1248-1255.

Pelah D (1999) Abiotic stress response in plants: basic and applied studies. Agro´s Ann Rev Pl Physiol (B&A) IV. Purohit SS (Ed). Agrobios. India. pp 226-248.

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* 43: 689-697.

Pfeiffer WH (1988) Drought tolerance in bread wheat –Analysis of yield improvement over the years in CIMMYT germplasm. *In*: Wheat production constraints in tropical environments.

Proceedings of the International Conference. Klatt AR (Ed.) Chiang Mai, Thailand, 19-23 January, 1987 *p*274-284.

Pfeiffer W, Trethowan R (1999) Challenges and future strategies in breeding wheat for adaptation to drought stressed environments: A CIMMYT Wheat Program perspective. *In*: Ribaut JM, Poland D (Eds.) Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. *p*49-53.

Piratesh B, Welsh JR (1980) Effect of temperature on the heading date of wheat cultivars under a lengthening photoperiod. *Crop Science* 20: 453-456.

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theorethical and Applied Genetics* 91: 1001-1007.

Powell DBB (1978) Regulation of plant water potential by membranes of the endodermis in young roots. *Plant Cell Environ* 28: 192-203.

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.* 1: 215-221.

Quarrie SA, Jones HG (1977) Effects of abscisic acid and water stress on development and morphology of wheat. *J Exp Bot* 28: 192-203.

Quarrie SA (1981) Genetic variability and heritability of drought-induced abscisic acid accumulation in spring wheat. *Plant Cell Environ* 4 : 147-151.

Quarrie S (1996) New molecular tools to improve the efficiency of breeding for increased drought resistance. *Plant Growth Regulation*20: 167-178.

Quarrie SA, Steed A, Lebreton C, Gulli M, Calestani C, Marmiroli N (1994) QTL analysis of ABA production in wheat and maize and associated physiological traits. *Russ J Plant Physiol* 41: 565-571.

Quarrie SA, Lazic-Jancic V, Kovacevic D, Steed A, Pekic S (1999) Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. *Journal of Experimental Botany*. 50, 337: 1299-1306.

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva A, Tuberosa R, Sanguinetti MC, Hollington PA, Aragués R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum*, L.) from the cross Chinese Spring x SQ1 and its use to compare QTLs for grain yield across a range of environments.*Theoretical and Applied Genetic*s 110: 865-880.

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. *Trends Genet.* 9: 275-280.

Ragot M, Hoisington DA (1993) Molecular markers for plant breeding: comparisons of RFLP and RAPD genotyping costs. *Theoretical and Applied Genetics* 86: 975-984.

Rajaram S (1995) Wheat Germplasm Improvement: Historical Perspectives, Philosophy, Objectives and Missions. *In*: Wheat breeding at CIMMYT: Commemorating 50 years of research

in Mexico for global wheat improvement. Rajaram S and Hettel GP (Eds.) Ciudad Obregón, Sonora, Mexico, 21-25 March, 1994. *p*1-10.

Rajaram S, Nelson WL (1982) Wheat production system in arid and semi-arid regions. Presented to the International Summer School on Agriculture, Royal Society of Dublin. p 305-324. *In*: Osmanzai M, Rajaram S, Knapp EB (1987) Breeding for moisture-stressed areas. *In*: Drought tolerance in winter cereals. Srivastava JP, Porceddu E, Acevedo E, Varma S (Eds.) Proceedings of an International Workshop 27-31 October 1985, Capri, Italy. ICARDA: John Wiley & Sons. Great Britain. p151-161.

Rajaram S, Mann CE, Ortiz-Ferrara G, Mujeeb-Kazi A (1983) Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats. *In*: Proceedings of the 6th International Wheat Genetics Symposium. Plant Germplasm Institute, Kyoto. *p*613-621.

Rajaram S, Skovmand B, Curtis BC (1984) Philosophy and methodology of an international wheat breeding program. *In*: Gene manipulation in plant improvement. Gustafson JP (Ed.) 33-60.

Rajaram S, van Ginkel M, Fischer RA (1994) CIMMYT's wheat breeding mega-environments (ME). *In*: Proceedings of the 8thInternational Wheat Genetic Symposium. 19-24 July, 1993. Beijing, China.

Rajaram S, Braun HJ, van Ginkel M (1996) CIMMYT's approach to breed for drought tolerance. *Euphytica* 92: 147-153.

Rathjen AJ, Brand JD, Liu C-Y, Paull JG, Cooper D (1999) Breeding for soil toxicities. 11th Australian Plant Breeding Conference Proceedings, Adelaide, South Australia, 19-23 April. Vol 1. p34-40.

Rawson HM (1996) An inexpensive pocket-sized instrument for rapid ranking of wheat genotypes for leaf resistance. *In*: Proceedings of the 8th Assembly of the Wheat Breeding Society of Australia. Richards *et al.* (Eds.) Canberra, ACT. *p*127-128.

Rebetzke GJ, Condon AG, Richards RA, Read JJ (2001) Phenotypic variation and sampling for leaf conductance in wheat (*Triticum aestivum* L.) breeding populations. *Euphytica* 100: 1-7.

Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop Science* 42: 739-745.

Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA (1994) Physiological and morphological traits associated with spring wheat yield under hot, dry irrigated conditions. *Australian Journal of Plant Physiology* 21: 717-730.

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larqué-Saavedra A, Quick JS (1998) Evaluating physiologial traits to complement empirical selection for wheat in warm environments. *Euphytica* 100:85-94.

Reynolds MP, Skovmand B, Trethowan R, Pfeiffer W (2000) Evaluating a conceptual model for drought tolerance. *In*: Ribaut JM, Poland D (Eds.) Molecular approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop. CIMMYT: El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. *p*49-53.

Reynolds MP, Nagarajan S, Razzaque MA, Ageeb OAAA (2001) Heat tolerance. *In*: Reynolds MP, Ortiz-Monasterio JI, McNab A (Eds.) (2001) Application of physiology in wheat breeding. Mexico D.F.: CIMMYT. *p*124-135.

Reynolds MP, Trethowan R, Crossa J, Vargas M, Sayre KD (2002) Physiological factors associated with genotype by environment interaction in wheat. *Field Crops Research* (75): 139-160.

Reynolds MP, Mujeeb-Kazi A, Sawkins M (2005) Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environments. *Annals of Applied Biology* 146: 239-259.

Ribaut J-M, Jiang C, González-de-León D, Edmeades GO, Hoisington DA (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics* 94: 887-896.

Ribaut JM, Hu X, Hoisington D, González-de-León D (1997) Use of STSs and SSRs as rapid and reliable preselection tools in a marker-assisted selection scheme. *Plant Molecular Biology Reporter* 15(2): 154-162.

Ribaut JM, William HM, Khairallah M, Worland AJ, Hoisington DA (2001) *In*: Reynolds MP, Ortiz-Monasterio JI, McNab A (Eds.) (2001) Application of physiology in wheat breeding. Mexico D.F.: CIMMYT. *p*29-47.

Ried JL, Walker-Simmons MK (1993) Group 3 late embryongenesis abundant proteins in dessication-tolerant seedlings of wheat (*Triticum aestivum* L.). *Plant Physiol* 102: 125-131.

Richards RA (1991) Crop improvement for temperate Australia: Future opportunities. *Field Crops Research* 26: 141-169.

Richards RA (1996) Defining selection criteria to improve yield under drought. *Plant Grwth Reg*. 20:157-166.

Richards RA (1996) Increasing the yield potential of wheat: Manipulating sources and sinks. *In*: Reynolds MP, Rajaram S, McNab A (1996) Increasing Yield Potential in Wheat: Breaking the Barriers. Mexico D.F.: CIMMYT. *p* 134-149.

Richards RA Rebetzke GF, Condon AG, van Herwaarden AF, Duggan BL, Munns R (1999) Targeting physiological traits to overcome environmental limitations and raise the yield potential of dryland crops. *In*: 11th Australian Plant Breeding Conference: Proceedings. Langridge P, Barr A, Auricht, Collins G, Granger a, Handford D, Paull J (Eds) Adelaide, Australia. April 19-23. (1): 27-33.

Richards RA, Condon AG, Rebetzke GJ (2001) Traits to improve yield in dry environments. *In*: Reynolds MP, Ortiz-Monasterio JI, McNab A (Eds.) (2001) Application of physiology in wheat breeding. Mexico D.F.: CIMMYT. *p*88-100.

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal SW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. *Molecular and General Genetics* 246: 327-333.

Röder MS, Korzun V, Wendehake K, Plaschke, Tixier M-H, Leroy P, Ganal M (1998) A microsatellite map of wheat. *Genetics* 149: 2007-2023.

Rotchild MF, Soller M (1997) *Probe* 8:13-20.

Rounsley SD, Glodek A, Sutton G, Adams MD, Somerville CR, Venter JC, Kerlavage AR (1996) The Construction of Arabidopsis Expressed Sequence Tag Assemblies (A New Resource to Facilitate Gene Identification) *Plant Physiol* 112: 1177-1183.

Ruan Y, Gilmore J, Conner T (1998) Towards Arabidopsis genome analysis: monitoring expression profiles of 1400 genes using cDNA microarrays. *Plant J* 15: 821-833.

Saghai-Maroof, M.A., K. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc Natl. Acad. Sci. USA* 81:8014–8018.

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mulllis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487-491.

Saiki RK (1989a) Optimization of the Polymerase Chain Reaction. *In:* Polymerase Chain Reaction. Erlich HA, Gibbs R, Kazazian Jr HH (Eds.) Current Communications in Molecular Biology. Cold Spring Harbor Laboratory Press. USA. p 25-30.

Saiki RK (1989b) The Design and Optimization of the PCR. *In:* PCR Technology-Principles and Applications for DNA Amplification. Erlich HA (Ed.). Stockton Press. USA. 1a Edición. p 7-16.

Saini HS, Aspinall D (1981) Effect of water deficit on sporogenesis in wheat (*Triticum aestivum* L.) *Ann Bot* 48: 623-644.

Salava J, Krska B, Wang E, Polak J, Kominek P, Reighard GL, Miller W, Dowler, Abbott AG (2002) Bulked segregant analysis and construction of a linkage map in apricot (*Prunus armeniaca* L.) crosses segregating for ersistance to plum pox virus. Plant, Animal & Microbes Genomes X Conference. 12-16 January, San Diego, California, USA.

Salina EA, Pestsova EG, Adonina IG, Vershinin AV (1998) Identification of a new family of tandem repeats in *Triticeae* genomes. *Euphytica* 100: 231-237.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning —A Laboratory Manual. Cold Spring Harbor Laboratory Press. Tomo 1. 2a Edición. Nueva York, E.U.A. p 6.3-6.62.

Sandhu D, Gill KS (2002) Gene-containing regions of wheat and the other grass genomes. *Plant Physiology* 128: 803-811.

Sanguinetti CJ, Dias E, Simpson AJG (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 915-921.

Sankoff D, Ferretti V, Roby D (1986) Allele and locus classification in electrophoretic population studies. *Journal of Theoretical Biology* 119: 397-407.

SAS User's Guide: Basics (1985) Version 5. Cary, N.C. USA: SAS Institute, Inc. 1290 pp.

Sax K (1923) The association of size differences with seedcoat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* 8:552.

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270: 467-470.

Schobert B (1977) Is there an osmotic regulatory mechanism in algae and higher plants? *J Theor Biol* 68: 17-26.

Schwab KB, Gaff DF (1986) Sugar and ion content in leaf tissues of several drought tolerant plants under water stress. *J Plant Physiol* 125: 257-266.

Schwab KB, Gaff DF (1990) Influence of compatible solutes on soluble enzymes from dessication-tolerant *Sporobolus stapfianus* and dessication-sensitive *Sporobolus pyramidalis*. *J Plant Physiol* 137(2): 208-215.

Scott KD, Eggler P, Seaton G, Rossetto, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. *Theoretical and Applied Genetics* 100:723-726.

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. *Genome* 36: 884-889.

Shalon D, Smith SJ, Brown PO (1996) A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Meth* 6: 639-645.

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of amylase sequences in wheat and its relatives. *Theor Appl Genet* 75: 286-290.

Shattuck-Eidens DM, Bell RN, Neuhausen SL, Helentjaris T (1990) DNA sequence variation within maize and melon: observations from polymerase chain reaction amplification and direct sequencing. *Genetics* 126: 207-217.

Sherman JD, Fenwick AL, Namuth DM, Lapitan NLV (1995) A barley RFLP map: Alignment of three barley maps and comparisons to Gramineae species. *Theor Appl Genet* 91: 681-690.

Shimshi D, Mayoral ML, Atsmon D (1982) Responses to water stress in wheat and related wild species. *Crop Science* 22: 123-128.

Shinozaki K, Tamaguchi-Shinozaki K, Seki (2003) Regulatory network of gene expression in the drought and cold stress responses. *In:* Current Opinion in Plant Biology. Roberts K & Somerville C (Eds) 6: 410-417.

Sinnott EW, Dunn LC, Dobzhansky T (1958) Principles of Genetics. Mc Graw-Hill Book Company, Inc. USA. p 22.

Sirault XRR, Fettell N, Condon AG, Rebetzke GJ (2004) Does leaf rolling slow water use and maintain wheat leaf area in a terminal drought? *In:* Cereals 2004. Black CK, Panozzo JF, Rebetzke GF (Eds.) Proceedings 54 Australian Cereal Chemistry Conference and 11th Wheat Breeders Assembly. 21-24 September, Canberra. p52-55.

Skovmand B, Villareal R, van Ginkel M, Rajaram S, Ortiz-Ferrara (1997) Semidwarf bread wheats: Names, parentages, pedigrees and origins. Mexico, D.F.: CIMMYT.

Slafer GA, Calderini DF, Miralles DJ (1996) Yield components and compensation in Wheat: opportunities for further increasing yield potential. *In*: Reynolds MP, Rajaram S, McNab A (Eds.) (1996) Increasing Yield Potential in Wheat: Breaking the Barriers. Mexico D.F.: CIMMYT. *p* 101-133.

Slafer GA, Rawson HM (1994) Sensitivity of wheat phasic development to major environmental factors: a reexamination of some assumptions made by physiologists and modellers. *Australian Journal of Plant Physiology* 21: 393-426.

Slafer GA, Satorre EH (1999) An introduction to the physiological-ecological analysis of wheat yield. *In*: Wheat: Ecology and Physiology of Yield Determination. Satorre EH, Slafer GA (Eds.) The Haworth Press. Chapter 1. 3-12.

Smirnoff N, Cumbes QJ (1989) Hydroxyl radical scavenging activity of compatible solutes. *Phytochem* 28: 1057-1060.

Smith DB, Flavell RB (1975) Characterisation of the wheat genome by renaturation kinetics. *Chromosoma* 50: 223-242.

Soanes C, Hawker S (2005) Compact Oxford English Dictionary of Current English. Oxford University Press. Third Edition.

Soller M, Genizi A (1978) The efficiency of experimental designs for the detection of linkage between a marker locus and a locus affecting a quantitative trait in segregating populations. *Biometrics* 34: 47-55.

Soller M Beckmann JS (1983) Genetic polymorphism in varietal identification and genetic improvement. Theoretical and Applied Genetics 67: 25-33.

Soller M, Beckmann JS (1990) Marker-based mapping of quantitative trait loci using replicated progenies. *Theoretical and Applied Genetics* 80: 205-208.

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. *Genome* 46: 555-564.

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.).*Theorethical and Applied Genetics* 109:1105-1114.

Sorrells M (1998) The application of comparative genetics to wheat improvement. *In*: Application of Biotechnologies to wheat breeding. Kohli MM, Francis M (Eds.) Proceedings of a Conference at La Estanzuela, Uruguay. 19-20 November. Montevideo, Uruguay. p17-32.

Sorrells M (1999) Comparative genomics in crop improvement. 11th Australian Plant Breeding Conference. Conference Proceedings. Adelaide (April 19-23, 1999) Vol.1: Invited Papers. p 56-63.

Sorrells ME (2000) Marker Assisted Selection: Is it practical? *In*: Kohli MM Francis M. Application of Biotechnologies to Wheat Breeding. Proceedings of a Conference at La Estanzuela, Uruguay. 19-20 November, Montevideo, Uruguay: CIMMYT. p103-110.

Spielmeyer W, Bonnett D, Ellis M, Rebetzke G, Richards R (2001) Implementation of molecular markers to improve selection efficiency in CSIRO wheat breeding program. Wheat Breeding Assembly of Australia, Inc. 10th Assembly Proceedings. 16th-21th Mildura, Australia. p 88-91.

Stack S, Campbell L, Henderson K, Eujayl I, Hannafey M, Powell W, Wolters P (2000) Development of EST-derived microsatellite markers for mapping and germplasm analysis in wheat. *In*: Proceedings & Abstracts. Plant & Animal Genome VIII—The International Conference on the Status of Plant & Animal Genome Research. Jan 9-12, 2000. San Diego, USA. 227pp.

Steel RGD, Torrie JH (1980) Principles and Procedures of Statistics. 2nd Edition. McGraw-Hill Book Co. New York.

Steudle E (2000) Water uptake by plant roots: An integration of views. *Plant and soil* 226: 45-56.

Stewart CR, Hanson AD (1980) Proline accumulation as a metabolic response to water stress. *In*: Turner NC, Kramer PJ (Eds). Adaptation of plants to water and high temperature stress. John Wiley and Sons. New York.

Stuber CW, Goodman MM, Moll RH (1982) Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. *Crop Science* 22:737-740.

Subbarao GV, Nam NH, Chauhan YS, Johansen C (2000) Osmotic adjustment, water relations and carbohydrate remobilization in pigeonpea under water deficits. *J Plant Physiol* 157: 651-659.

Tanskley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: New tools for an old science. *Bio/Technology* 7: 257-264.

Tanksley SD (1983) Molecular markers in plant breeding. *Plant Molecular and Biology Reporter* 1: 3-8.

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: New tools for an old science. *Bio/Technology* 7: 257-264.

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics* 92: 191.

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* 39: 277-287.

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucl. Acids Res.* 12: 4127-4138.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucl. Acids Res.* 17: 6463-6471.

Taylor C, Sheperd KW, Langridge P (1998) A molecular genetic map of the long arm of chromosome 6R or rye incorporating the cereal cyst nematode resistance gene, Cre R. *Theor Appl Genet* 97: 1000-1012.

This D, Teulat-Merah B (2000) Towards comparative genomics of drought tolerance in cereals: lessons from a QTL analysis in barley. *In*: Molecular approaches for the genetic improvement of cereals for stable production in water-limited environmentsó*An International Workshop* (June 21-25, 1999). CIMMYT, Mexico. .

Trethowan RM, Pfeiffer WH (2000) Challenges and future strategies in breeding wheat for adaptation to drought stressed environments. *In*: Ribaut JM, Poland D (Eds.) (2000) Molecular

approaches for the improvement of cereals for stable production in water-limited environments. A strategic planning workshop held at CIMMYT, El Batan, Mexico, 21-25 June 1999. Mexico D.F.: CIMMYT. *p*49-53.

Tucker CJ, Holben BN, Elgin Jr. JH, McMutrey III JE (1980) Relationship of spectral data to grain yield variation*Photogrammetric Engineering and Remote Sensing* 46(5): 657-666.

Turner NC (1979) Drought resistance and adaptation to water deficits in crop plants. *In*: Stress physiology in crop plants. Mussell H, Staples RC (Eds.) John Wiley & Sons. USA p343-372.

Turner NC (1981) Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil* 58: 339-366.

Turner NC (1986) Adaptation to water deficits: a changing perspective. *Aust J Plant Physiol* 13: 175-190.

Turner NC, Jones MM (1980) Turgor maintenance by osmotic adjustment: A review and evaluation. *In:* Turner NC, Kramer PJ (Eds). Adaptation of plants to water and high temperature stress. John Wiley and Sons. New York.

Turner NC, Begg JE (1981) Plant water relations and adaptation to stress. *Plant and Soil* 58: 97-131.

Turner NC, Asseng S (2005) Productivity, sustainability and rainfall-use efficiency in Australian rainfed Mediterranean agricultural systems. *Australian Journal of Agricultural Research* 56:1123-1136.

Valluri JV, Castillon J, Newton RJ, Soltes EJ (1989) Water stress-induced changes in protein synthesis of slash pine hypocotyls. *J Plant Physiol* 135: 355-360.

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorák J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995*a*) Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. *Genome* 38: 45-59.

Van Deynze AE, Nelson JC, O´Donoghue LS, Ahn SN, Sirinpoonwiwat W, Harrington SE, Yglesias ES, Braga DP, McCouch SR, Sorrells (1995*b*) Comparative mapping in grasses. Oat relationships. *Mol Gen Genet* 249: 349-356.

Van Deynze AE, Nelson JC, Yglesias ES, Harrington SE, Braga DP, McCouch SR, Sorrells ME (1995*c*) Comparative mapping in grasses. Wheat relationships. *Mol Gen Genet* 248: 744-754.

van Ginkel M, DS Calhoun, Gebeyehu G, Miranda A, Tian-you C, Pargas Lara R, Trethowan RM, Sayre K, Crossa J, Rajaram S (1998) Plant traits related to yield of wheat in early, late or continuous drought conditions. *Euphytica* 100: 109-121.

van Ginkel M, Reynolds MP, Trethown R, Hernandez E, Condon A (2006). Complimenting the breeders eye with canopy temperature measurements. *In:* Reynolds MP, Godínez D (Eds.) Challenges to International Wheat Breeding- International Symposium on Wheat Yield Potential. 20th-24th March. Ciudad Obregón, Sonora, Mexico. Extended Abstracts. p 10-11.

Vanstone VA, Taylor S (1992) Distribution of *Pratylenchus* (root lesion nematodes) in South Australia. Crop Science Society of South Australia Incorporated, Newsletter No. 115.

Varshney RK, Kumar A, Balyan HS, Roy JK, Prasad M, Gupta PK (2001) Integrated physical maps of 2DL, 6 BS and 7DL, carrying loci for grain protein content and pre-harvest sprouting tolerance in bread wheat. *Cereal Research Communications* 29: 33-40.

Villareal RL, del Toro E, Mujeeb-Kazi A, Rajaram S (1995) The 1BL/1RS chromosome translocation effect on yield characteristics in a *Triticum aestivum* L. cross. *Plant Breeding* (114) 497-500.

Voet D, Voet JG (1990) Biochemistry. John Wiley & Sons. USA 1223pp.

Vos P, Hogers R, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new concept for DNA fingerprinting. *Nucl. Acids Res.* 23:4407-4414.

Walbot V (1999) Genes, Genomes, Genomics. What can plant biologists expect from the 1998 National Science Foundation Plant Genome Research Program? *Plant Physio.* 119:1151-1155.

Walton DC (1980) Biochemistry and physiology of abscisic acid. *Ann Rev Plant Physiol* 31: 453-489.

Wardlaw IF (1971) The early stages of grain development in wheat: Response to water stress in a single variety. *Aust J Biol Sci* 24: 1047-1055.

Weining S, Langridge P (1991) Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. *Theor. Appl. Genet.* 82: 209-216.

Welsh JR (1981) Fundamentals of plant genetics and breeding. John Wiley & Sons. USA.

Wehrhahn C, Allard RW (1965) The detection and measurement of the effects of individual genes involved in the inheritance of a quantitative character in wheat. *Genetics* 51: 109-119.

William HM (2006) Wheat Molecular Breeding- Does it offer any hope? *In:* Reynolds MP, Godínez D (Ed.) Challenges to International Wheat Breeding- International Symposium on Wheat Yield Potential. 20th-24th March. Ciudad Obregón, Sonora, Mexico. Extended Abstracts. p 42.

William HM, Hoisington D, Singh RP, González-de-León D (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. *Genome* 40: 253-260.

William HM, Khairallah M, Ayala L, Singh RP, Mujeeb-Kazi A, Hoisington DA (1998) The application of molecular markers in wheat improvement at CIMMYT. *In*: Applications of Biotechnologies to Wheat Breeding: Proceedings. Kohli MM, Francis M (Eds) La Estanzuela, Uruguay. November 19-20, 1998. Montevideo, Uruguay.

William HM, Singh RP, Huerta-Espino J, Ortiz-Islas S, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93(2):153-159.

William HM, Singh RP, Trethowan R, van Ginkel M, Pellegrineschi A, Huerta-Espino J, Hoisington DA (2005) Biotechnology Applications for Wheat Improvement at CIMMYT. *Turkish Journal of Agriculture and Forages* 29: 113-119.

Williams K, Taylos S, Bogacki P, Pallota M, Wallwork H (2001) Nematode resistance and tolerance genes located in wheat by bulked-segregant analysis combined with comparative mapping. *In*: Plant & Animal Genome Conference IX – The International Conference on the Status of Plant & Animal Genome Research. San Diego, California. p 169.

Wilson WA, Harrington SE, Woodman WL, Lee M, Sorrells ME, McCouch SR (1999) Inferences on the genome structure of progenitor maize through comparative analysis of rice, maize and the domesticated panicoids. *Genetics* 153(1):453-73.

World Meteorological Organisation (1997) Comprehensive Assessment of the Freshwater Resources of the World. Geneva, Switzerland.

Wright STC (1969) An increase in the "Inhibitor-β" content of detached wheat leaves following a period of wilting. *Planta* 86: 10-20.

Wright STC, Hiron RWP (1969) (+)-Abscisic Acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. *Nature* 224: 719-720.

Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen. Genet.* 241: 225-235.

Xue GP, Bower NI, McIntyre CL, Riding GA, Kazan K, Shorter R (2006) TaNAC69 from the NAC superfamily of transcription factors is up-regulated by abiotic stresses in wheat and recognises two consensus DNA-binding sequences. *Functional Plant Biology* 33: 43-57.

Yang HY, Korban SS, Kruger J, Schmidt H (1997) The use of a modified bulk segregant analysis to identify a molecular marker linked to a scab resistance gene in apple. *Euphytica* 94:175-182.

Ye B, Müller HH, Zhang J, Gressel J (1997) Constitutively elevated levels of putrescine and putrescine-generating enzymes correlated with oxidant stress resistance in *Conyza bonariensis* and Wheat. *Plant Physiolology* 115: 1443-1451.

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* 14: 415-421.

Zhou H, Buitenhuis B, Lamont SJ (2000) Candidate genes for polygenic control of antibody response in chickens. *In*: Proceedings & Abstracts. Plant & Animal Genome VIII—The International Conference on the Status of Plant & Animal Genome Research. Jan 9-12, 2000. San Diego, USA. 227pp.