

Review

Breeding for Abiotic Stress Adaptation in Chickpea (*Cicer arietinum* L.): A Comprehensive Review

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ABSTRACT

Chickpea is an important legume crop, providing a protein rich diet for humans and animal feed. Globally, chickpea is grown in over 56 countries, occupying a production area of approximately 17.8 million ha. The crop is grown mainly in arid and semi-arid regions under rainfed conditions, where it is highly vulnerable to abiotic stresses such as heat, frost and drought at various growth stages during the season. Severe yield losses due to abiotic stresses have been recorded, especially when the crop is exposed to adverse conditions during the reproductive phase, causing instability in chickpea production worldwide. Breeding for tolerant chickpea that is widely adaptable to various growth conditions and diverse growing regions is the best strategic approach but requires a fine-tuned combination of advanced phenotyping and genotyping methods. However, breeding and selection of suitable chickpea genotypes is complicated by its narrow genetic base which limits the sources of novel alleles, and its indeterminate growth habit that at times allows it to recover, flower, set pods and yield following stressful events if subsequent conditions are favorable. This manuscript provides an insight into common abiotic stresses affecting chickpea production worldwide with an emphasis on heat, frost and drought. We will elaborate on breeding approaches and application of advanced genotyping and phenotyping tools commonly used to develop tolerant chickpea varieties. Finally, key crop tolerance traits that can be easily screened for by using genotypic and phenotypic technologies will be discussed.

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KEYWORDS: adaptation; genomic selection; high-throughput phenotyping; phenomic selection; genebank phenomics; quantitative trait locus

ABBREVIATIONS

QTL, quantitative trait locus; HTP, high-throughput phenotyping; SPS, sucrose phosphate synthase; SS, sucrose synthase; TE, transpiration efficiency; ABA, abscisic acid; GS, genomic selection; GEBV, genomic estimated breeding values; MAS, marker assisted selection; LOD, logarithm of the odds; GBS, genotyping by sequencing; IRGA, infrared gas analyzer; CT, canopy temperature; RGB, red green blue; MRI, magnetic resonance imaging; X-ray CT, X-ray computed tomography; NDVI, normalized difference vegetation index; HI, harvest index; PVC, polyvinyl chloride cylinder

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important cool season food legume crop after common bean (*Phaseolus vulgaris* L.) and is mainly grown in the arid and semi-arid regions of the world. It belongs to genus *Cicer*, tribe Cicereae, family Fabaceae, and subfamily Papilionaceae, and has nine annual and 34 perennial species, has hypogeal germination, a growth habit that can be erect, semi-erect, spreading, semi-spreading or prostrate, with branches emanating from the stem [1,2]. Chickpea is a highly self-pollinated diploid crop with an outcrossing rate of less than 1%, $2n = 16$ chromosomes, a genome size of 738.09 Mb and an estimated 28,269 genes [1,3]. Globally it is cultivated in 56 countries over an area of approximately 17.8 million hectares (Mha) with an annual production of 17.2 million tons, but yield has only increased from 0.6 t/ha in 1960 to 0.9 t/ha in 2014 representing a 0.006% increase per annum [4]. This increase is most likely not enough, to feed the increasing world population, though adoption of modern technologies and breeding approaches can play an important role in increasing the rate of genetic gain. The Indian subcontinent (India, Pakistan, Myanmar, Bangladesh and Nepal) is a major producer of chickpea, with India being by far the largest producer in the world, accounting for 68% of the production, and about 69% of the cultivated area [4,5]. In Australia it is grown across an area of 107,514 ha and producing 998,231 tons, thus yielding 0.929 t/ha on average [4]. Two distinct types of chickpea are cultivated, the small seeded *desi* and the larger seeded *kabuli*. They differ in seed coat morphology and flower color, and also vary in their geographic adaptation and tolerance to abiotic stresses [6] and the seed characteristics such as color and size determine its market value.

Chickpea is widely used both as human and animal feed as it contains significant amounts of carbohydrates, all the essential amino acids except sulfur containing types, nutritionally important unsaturated fatty acids such as linoleic and oleic acids, vitamins such as riboflavin, niacin, thiamine, folate and the vitamin A precursor b-carotene, calcium, magnesium, phosphorus and potassium [7]. It has been suggested that chickpea may have potential nutritional and health benefits and might

reduce human diseases such as cardio vascular disease, type 2 diabetes, digestive diseases and some cancers [7]. Chickpea is important for farming system rotations as a disease break crop for cereal cropping and enriches soil fertility as it fixes atmospheric nitrogen. Nitrogen fixing ability also has environmental benefits due to reduced emissions of nitrous oxide greenhouse gas. On average the mean shoot nitrogen fixed by chickpea is 40 kg/ha but can range from 0 to 124 kg/ha depending on agronomy, precipitation, soil management and inoculation [8]. It thus decreases inoculum build up and subsequent disease outbreaks and reduces the need for the application of exogenous fertilizers.

Chickpea producing regions are largely affected by extreme temperatures such as heat, chilling and/or frost and drought at different time points in the growing season [9]. Furthermore, production is expanding beyond the Indian subcontinent into areas previously deemed unsuitable for production including various regions of Australia. In these new production areas, a range of biotic and abiotic factors are encountered that hinder adaptation and overall productivity. Globally, abiotic stresses, individually or in combination, cause annual chickpea yield losses that translates into severe financial penalties. This necessitates identification and/or development of suitable genotypes tolerant to these abiotic stresses. Future food and nutritional security would require increasing current crop yields through a combination of breeding approaches, to counteract the impact of a range of stressful environmental factors and overall climate change. This would be accompanied by intensified research in pulse crops including chickpea which forms a large component of the diet, especially in many developing countries. Like most pulse crops, research on chickpea improvement has lagged behind compared to major cereal crops. Agronomic practices such as management of sowing time, in combination with the crop's phenology, can be effectively used to select varieties suitable for different agroecological zones. The rationale of varying sowing time is to identify varieties that are able to reach the highly sensitive reproductive growth phase when the risks of major abiotic stresses are low. The genes controlling the timing and duration of key growth phases are largely characterized in chickpea [5] and can be effectively deployed in developing new varieties using modern breeding approaches.

Genetic approaches such as quantitative trait locus (QTL) mapping, marker assisted breeding, and genomic selection are of value in understanding genes controlling important adaptation traits and thus enabling rapid selection of superior genotypes and acceleration of the breeding cycle. Genes and QTLs (with both main and epistatic effect), associated with important chickpea traits, both under normal and stressful production environments have been reported [10–15]. These identified genes and QTLs can be incorporated into breeding programs and used as molecular markers to facilitate faster germplasm selection and reduce the costs associated with multi-environmental phenotyping.

However, more often the usefulness of the genetic approaches is limited by the fact that many key traits are polygenic and under the control of many alleles, some with minor but additive effects. Also, the reliability, reproducibility and overall usefulness of the QTLs and/or markers depends on the availability of high quality phenotypic data. As such, the value of genetic approaches is closely intertwined with the adoption of modern high throughput and precision phenotyping approaches. Modern phenotyping technologies are increasing the speed and reliability of germplasm selection and overall development of suitable genotypes.

Genetic gains and breeding progress in chickpea are slowed by the relatively narrow genetic diversity available from the cultivated elite gene pool and the current phenotyping approaches that are often slow and laborious. The narrow genetic diversity is largely due to loss of genes during domestication. Wild relatives and landraces can be valuable sources of new genes and alleles, and are an important resource for breeders to further exploit allelic richness for germplasm improvement and overall broadening and enrichment of the domesticated gene pool [16–19]. However, wild relatives have been largely underutilized because of interspecific hybridization barriers, limited data on specific agronomic traits, linkage drag of negative flanking regions, and targeting short-term outputs by most breeding programs [16]. The dominant interspecific hybridization barriers include difficulties with crossability and non-synchronised growth rate of stigma and anthers, but these can be minimized through careful choice of the female parent. The limited compatibility leads to impaired meiosis and formation of unbalanced gametes and ultimately production of infertile hybrids. That said, there have been successful crosses involving wild relatives such as introgression of genes for resistance/tolerance to phytophthora root rot, cyst nematode, root-lesion nematode, pod borer, ascochyta blight, botrytis grey mould and low temperatures [17,20].

Furthermore, the adoption of a range of complementary approaches such as conventional, molecular, and physiological breeding will aid in the release of varieties that can yield productively even under abiotic stresses. Post-green revolution challenges will be addressed through assembling appropriate gene combinations in elite varieties and developing ideal genotypes using a combination of modern genomics and phenomics approaches [21]. Description of traits that can easily be screened for tolerance using genotypic and phenotypic markers will aid breeding programs in releasing highly adapted varieties. Breeding for crop adaptation requires knowledge of the germplasm and type of environmental conditions as large genotype by environment ($G \times E$) interactions are often observed [22–25].

This review aims to provide an insight into common abiotic stresses affecting chickpea production worldwide with an emphasis on heat, frost and drought. It elaborates and provides an overview on conventional and advanced breeding approaches currently being applied to develop more

tolerant chickpea varieties. It further discusses key crop tolerance traits that can be easily screened for by using high-throughput phenotyping (HTP). Increase in genetic gains and continuous genetic improvement through the implementation and integration of phenomics, genomics-assisted breeding approaches and rapid generation advancement will significantly reduce variety development time.

COMMON ABIOTIC CONSTRAINTS

Plants utilize a variety of strategies to cope under stressful growing conditions, with escaping stress being the most effective, avoidance being the second-best strategy, and tolerance being the last resort as it results in severe yield penalties [26,27]. Abiotic stresses are major constraints to chickpea production in most regions with the most encountered ones being extreme temperatures (high and low), and moisture stress (drought). These stresses affect different traits, metabolic and physiological processes, and elicit different responses from plants (Table 1). Plant responses to abiotic stresses differ depending on the growth/developmental stage, the severity, frequency and length of exposure to the stress. While it is mostly sensitive during the reproductive phase, chickpea does display to some extent, sensitivity to abiotic stress at early vegetative stages which can reduce seed number [28]. These abiotic stresses reduce yield largely through their effect on flower set, pollen viability, pod set/abortion and retention, all being traits that are key determinants of seed number.

Table 1. The main chickpea traits and processes affected by common abiotic stresses.

Abiotic stress	Key processes affected	References
Heat	Crop growth rate and duration	[29–31]
	Reproductive organs	[31–33]
	Enzymatic activity	[31]
Cold	Reproductive organs	[34–36]
	Membrane integrity and enzymatic activity	[37–39]
	Crop growth rate and duration	[36,37,40]
	Germination and/or establishment	[24,36,39,41]
	Photosynthesis	[37]
Drought	Plant growth duration	[42,43]
	Reproductive growth	[44,45]
	Abscisic acid (ABA) accumulation in the seed or pod	[44]

High Temperature Stress

Temperatures above 35 °C (heat stress) especially during the reproductive phase can adversely affect chickpea growth and development and cause significant yield losses [29,46]. Heat stress can be subdivided into: (1) chronic, which is fatal and can lead to total crop failure; and (2) acute, which is of shorter duration but still leads to yield reduction [33]. Plants with prior exposure to a moderately elevated, non-lethal

temperature can acquire thermo-tolerance to a subsequent potentially lethal heat shock, a phenomenon known as heat acclimation. In addition to acclimation, the response of the crop to high temperatures can also depend on prevailing conditions such as the crop's recovery ability, resource availability and interactions with other stresses.

Heat stress progressively hastens the onset of flowering, podding and maturity, leaf senescence and affects a range of yield components and/or related traits such as harvest index (HI) [29–31]. Chickpea copes with heat stress through escape, avoidance and tolerance, with early maturing genotypes able to escape late season heat stress, while those with late maturity get exposed during the flowering and podding stages and potentially suffer yield penalties. Days to flowering show negative correlation with yield, with pod number per plant and HI most strongly related to grain yield under heat stress [29,31,46].

High temperatures affect both the male and female reproductive organs and thus inhibits the potential to set viable seed. It reduces pod set through the effect on pollen viability, pollen load, pollen germination (in vivo and in vitro), ovule viability and stigma receptivity [32,47]. As such, reproductive efficiency expressed as pod to flower ratio is reduced indicating that under high temperatures yield is reduced through impairment of reproductive development [30,31]. Assimilate accumulation prior to or during the reproductive phase is critical and also contributes towards overall reproductive organ viability.

In other crops such as wheat, seed weight is less plastic than seed number [48], and in chickpea it is also less affected by heat stress, with seed number and pod set being the most sensitive traits and major determinants of yield [46]. However, there are instances where a reduction in seed weight has been reported [30]. Often, in most crops, an increase in seed size leads to a reduction in number of seeds per plant as well as grain yield, and this negative correlation between seed size and number is also observed in chickpea [49]. The selection for both these favourable traits is a breeding challenge even with the adoption of modern breeding approaches such as marker assisted selection and QTL mapping. The critical period to determine chickpea yield is extended due to its indeterminate nature but is centred around flowering, with seed number which is related to both pod number and seeds per pod accounting for most of the variation [28]. Heat stress decreases grain yield through increasing the proportion of unfilled pods and decreasing the duration of the reproductive growth stage [50].

Heat stress during the seed filling stage impacts seed growth by affecting the physiological and biochemical processes. At the physiological level, it reduces stomatal conductance, leaf water content, chlorophyll, membrane integrity and photochemical efficiency. At the biochemical level, it decreases the enzymatic activities of carbon-fixing enzyme Rubisco, sucrose-cleaving enzyme invertase, and sucrose-synthesising enzymes sucrose phosphate synthase (SPS) and sucrose synthase (SS) with

the consequent impact of reduced sucrose content in the leaves and anthers [31,51]. Tolerance to heat stress is closely related to the rate of transpiration and evaporation. Genotypes with higher transpiration rates tend to maintain cooler canopies and functional physiological processes than those with hotter canopies. Canopy temperature is widely used in crop physiology as a selection trait for favourable genotypes and correlates highly with yield. Furthermore, heat stress reduces ground cover through impairing seedling vigour and biomass accumulation thus increasing water loss from the soil through evaporation [29]. In cereals, early vigour is selected as a desirable trait to conserve water and outcompete weeds [52] and this can be equally true in chickpea. The most commonly used screening approach for heat tolerance is the use of heat chambers in controlled conditions/glasshouses and delayed sowing in the field. Screening under controlled conditions is more accurate, but under field conditions there is always interaction with other environmental factors. To avoid the confounding effect of drought under field conditions, the screening experiments tend to be well watered. However, this approach does not account for radiation, humidity and day length which increases in spring going into summer compared to wintertime in Mediterranean environments. To this effect, this is not an entirely accurate screening technique for heat stress tolerance in the field, more so for a day length (photoperiod) sensitive crop such as chickpea.

Low Temperature Stress

Low temperature can be subdivided into a chilling range ($-1.5\text{ }^{\circ}\text{C}$ to $15\text{ }^{\circ}\text{C}$) and a freezing/frost range (below $-1.5\text{ }^{\circ}\text{C}$), which have overlapping effects on chickpea growth and production [39,41]. Tolerance to low temperatures can be acquired by prior exposure to reduced temperature, a physiological process referred to as cold acclimation [39]. The effect of chilling temperature on delaying pod set in chickpea is well documented. Frost damage suppress pollen viability, stigma receptivity, *in vivo* pollen germination and pollen tube growth, ultimately leading to ovule fertilization failure and reduced seed production [34-36]. *Kabuli* types, have a thinner testa which allows rapid imbibition of water and greater imbibitional damage, and hence tend to be more susceptible to low temperature damage than *desi* types [41]. This has also been demonstrated by their greater reduction in flower numbers, lower pollen viability and germination [34]. Due to high susceptibility, they also experience an increase in electrolyte leakage, loss of chlorophyll, decrease in sucrose content, reduction in the accumulation of starch, proteins, fats, protein fractions (albumins, globulins, prolamins and glutelins), crude fibre and water status in leaves [38].

Sowing chickpea at low temperatures delays emergence due to the longer time to accumulate the required minimum threshold of approximately 115 growing degree days, and subsequent low temperature or frost events decrease the rate of plant growth eventually lengthening

the duration of the vegetative growth stage and delaying flowering, podding and maturity [36,37,40,50]. Depending on the production region, this resultant long season can expose the plants to later season rainfall, thus potentially increasing yield. However, the moist conditions due to the rainfall also increases the incidence of fungal diseases especially after canopy closure. Importantly, in Mediterranean environments the longer growth season would expose plants to detrimental conditions such as heat and terminal drought stresses and associated yield losses. The reproductive stage is more susceptible to the freezing range temperatures than the seedling stage, with frost damage following pod set resulting in the abortion of pods and large yield reductions [38,41]. Low temperature especially in susceptible genotypes results in repeated cycles of flowering and flower abortion and thus delay pod set [34].

Freezing and/or chilling range temperatures cause poor establishment, reduced vigor resulting in stunted seedlings, reduced leaf expansion thus retarding plant growth and dry matter production, causes leaf wilting, flower, pod or seed abortion and increases susceptibility to soil-borne pathogens and, in extreme cases, may lead to plant death [24,36,37,39,41]. This affects the source-sink balance by markedly decreasing the source of assimilates for grain filling which, in turn, reduces potential yield. Also, reduced establishment and seedling vigor increase water loss through soil evaporation. Seed priming prior to sowing can mitigate the adverse effects of chilling stress by improving stand establishment, growth, water relations, photosynthesis, α -amylase activity, sugar metabolism, antioxidant enzyme activity, membrane stability, and leaf accumulation of proline, nitrogen, potassium and soluble phenolics [53].

Frost damage negatively affects days to pod set, number of pod nodes, number of aborted flowers, total number of pods per plant, seed number, size and shape, rate and duration of seed filling, and yield and also causes accumulation of anthocyanins in the basal part of the stem, branches and leaves [36,41]. It can also discolor the seed coat, probably through affecting the remobilization of plant assimilates and pigments [41]. Using flower color as a morphological marker, Clarke et al. [35] showed that chilling tolerant pollen fertilizes significantly more ovules at low temperature than its intolerant counterpart. Selection of chilling tolerant pollen also allows earlier podding at lower temperatures [54].

At the cellular level, frost damage destroys the integrity of membranes and intracellular organelles, leading to solute and electrolyte leakage thus disrupting metabolic processes [37,39]. Physiologically, it results in a decrease in chlorophyll content and relative leaf water content, especially in sensitive genotypes [37]. The reduction in chlorophyll content might reduce photosynthesis and photosynthetic products as evidenced by a decrease in total sugars and starch in sensitive genotypes, which is accompanied by decreased activity of key enzymes such as β -amylase, invertase and sucrose synthase in the leaves [37]. By and large, there is little genetic variation for chilling tolerance within cultivated *C. arietinum*

germplasm in either the *desi* or *kabuli* types [34]. However, some wild species of chickpea, such as *C. echinospermum* L. which is inter-fertile with the cultivated species, are more tolerant and low temperatures do not impact their pollen germination, viability, frequency on the stigma surface and subsequent pod set [34]. Therefore, there is breeding scope to use wild relatives as donors of genes for chilling tolerance but also to understand the underlying genetic and/or physiological basis of chilling tolerance.

Drought

Drought stress arises if precipitation is significantly less than evaporation during the growing season, with water use efficiency and transpiration efficiency (TE) being important traits for drought tolerance in pulse crops [27]. Continuous drought from the onset of the season has significant effects by either not allowing planting or impacting proper establishment, ultimately reducing productivity. Drought shortens the plant growth duration by reducing the days to flowering and maturity [42,43], however, the impact largely depends on the growth stage of the crop and the overall soil water status. *Kabulis* generally have a shorter vegetative and longer reproductive duration than *desis*, and as a result, accumulate less dry matter which translates to lower HI and overall yield [6]. Water uptake and requirement depends on the crop's development stage and other environmental conditions and increases as plants grow and accumulate more biomass. Drought can be terminal, allowing early maturing genotypes to escape, or cyclic which are periods of drought interspersed by water availability as is often experienced in Mediterranean regions such as the southern parts of Australia. Therefore, sustainable productivity under drought conditions can be achieved through escape due to early phenology, avoidance through deep and expansive root traits which allows longer duration genotypes to extract water and maintain the plant-water balance, or tolerance through osmotic adjustment and TE [24,27,55].

Roots play an important role in drought adaptation, with the root distribution at various depths differing during the crop cycle. Roots behave physiologically different under optimal conditions compared to drought stress. Under optimal conditions roots might be concentrated in the upper layers, but under diminishing water levels they might grow into deeper layers in search of water. Root traits show varying responses to drought, with terminal drought stress increasing root length density, depth, deep root dry weight and root to shoot ratio but decreasing the root diameter [42,43]. Generally, tolerant genotypes have high root growth vigor and deeper soil root proliferation under drought stress, allowing them to extract water from all soil depths and maintain yield and HI. *Kabulis* quickly lose root cortical layers and have a greater number of wider xylem vessels which allows them to use more water, and are therefore generally more susceptible to drought stress than the *desis* [6]. However, root traits are complex and time consuming to measure in the

field, more so for large numbers of genotypes. As a result, surrogate measurements of root traits such as carbon isotope discrimination and rate of partitioning (sink activity) which permit high throughput assessment and are cost effective as selection tools have been effectively used in chickpea [56], and wheat [57]. Canopy temperature is also considered a surrogate measure of rooting depth, with genotypes capable of extracting water from depth able to continue transpiring and maintain cooler canopies [58,59].

Early establishment and high vigor increase ground cover and reduce direct evaporation from soil which can represent a substantial loss of water. Early maturing genotypes with high vigor are preferred in environments where terminal drought occurs frequently. However, highly vigorous late maturing genotypes might use most of the available soil water early in the season and suffer moisture stress during grain filling period and result in “haying off”. It is widely accepted that deeper and denser rooting offers competitiveness under drought stress, and is a strategy adopted by drought tolerant genotypes [55].

Drought reduces above-ground biomass, reproductive growth, HI and seed yield, with yield penalties up to 33% having been reported [55]. Ability to maintain high flower number, filled pods and seed number under water stress will lead to high seed yield. Moisture limitation induces flower and pod abortion either through reduced assimilate supply to the developing pod due to stomatal closure and the decrease in leaf photosynthesis, or by ABA accumulation in the seed or pod, or possibly even by both mechanisms [44]. Generally, but to a lesser extent, drought reduces overall yield in chickpea and other crops by reducing the less plastic seed size trait [45]. Under terminal drought, differences in shoot characteristics become more noticeable in later developmental phases than during the vegetative phase [42].

BREEDING APPROACHES

The objective of breeding programs is to shorten the breeding cycle and release more resilient high yielding chickpea varieties for targeted environments through the application of a range of technologies in controlled and field conditions. More often breeding programs aim to breed for early maturing varieties adapted to short season environments that can escape late season stresses. Generally, in Mediterranean environments such as Australia, these varieties if sown at the optimum time tend to flower late enough to avoid early season frost events, but early enough to avoid the onset of late season heat and terminal drought. Equally, a longer season would expose chickpea to frost risk in temperate environments such as Canada [60]. The current improvement rate of chickpea is probably inadequate to meet current and future demands because of long breeding generation times which runs into years. This is largely because about 2–3 generations under normal glasshouse conditions and 1–2 generations under field conditions per year can be

generated. Some of the commonly adopted approaches that shorten the breeding cycle and are amenable to both conventional and modern breeding techniques are shuttle and speed breeding. Shuttle breeding allows for off season testing at different localities and under different environmental conditions. However, while it is broadly applied in wheat breeding and was key to the green revolution [61], it is not used widely in chickpea breeding, and thus would provide opportunities to reduce the breeding cycle in chickpea. The advantages of shuttle breeding are that the breeding material gets exposed two contrasting locations all with different abiotic stresses, disease types and incidents, and soil types. If the environments are at different altitudes and latitudes, photoperiod responses can also be detected at early stages. Therefore shuttle breeding can be a form of early generation multi-environment testing (MET), allowing early identification of superior genotypes, as MET is usually conducted at late stages.

Speed breeding, accommodating up to seven generations per year is now widely applied [62] and involves inducing early flowering through 24 hours of photoperiod from emergence till flowering. To speed the process, fully developed but immature green seeds are harvested and planted for the next cycle, and together with embryo rescue, this approach is applicable to a wide range of crops [63]. The process requires fully-enclosed controlled-environment growth chambers with supplemental lighting process. For chickpea speed breeding, a temperature-controlled glasshouse that allows for careful control of temperature, humidity and lighting fitted with functional high-pressure lamps is required to extend the photoperiod to 22 hours [62–64]. Extended photoperiod hastens crop growth and optimization of photosynthesis, and in chickpea it has been observed that time to anthesis can decrease on average by 33 days compared to normal glasshouse conditions, but with no penalties on seed production (g/plant) [63,64]. Importantly time to anthesis was shown to be more uniform than in a normal glasshouse.

Explore Wild Relatives and Landraces Available at Grain Genebanks

Diversity in the *desi* type is slightly higher than in the *kabuli* type which is defined by post domestication traits such as large and light colored seeds [3]. However, the overall narrow genetic base within the elite chickpea germplasm due to the domestication bottleneck necessitates the need to intensify the use of wild relatives and landraces as sources of adaptive traits/genes to confer resistance to abiotic stresses (Table 2) and improve genetic gains [18,51,65,66]. Wild relatives are native and adapted, through evolution, to the environmental conditions experienced at the crop's centers of origin. This makes them potential genetic sources of abiotic stress tolerance through exploiting the mechanisms and strategies they use to survive adverse conditions at the areas of origin. Their use also offers opportunities to recover genes lost during domestication or those that evolved independently post domestication [21]. This broadening of

the genetic base through facilitating recombination of genes at many loci can be key to increasing the genetic gains and development of high yielding varieties that are tolerant to a range of abiotic stresses.

Although there are up to eight annual wild *Cicer* species, only *C. reticulatum*, *C. judaicum* and to some extent *C. echinospermum* seem to be readily crossable with the cultivated chickpea, with the others often producing infertile hybrids [66,67] (personal observations by the authors). The wild chickpea relative, *C. judaicum* has been used to develop a pre-breeding line with a high number of primary branches per plant, more pods per plant and green seeds and this line is now routinely used as a donor of these traits [68]. It is possible to introgress the favorable genes into adapted varieties and still retain the basic seed quality traits important for commercialization and consumption [17]. The importance of wild relatives have also been observed in other pulse crops for example in lentils, where incorporation of favorable traits such as reduced transpiration rates and deeper rooting systems into modern varieties, enables lentil to escape, avoid, or tolerate drought conditions [69].

Therefore, pre-breeding research can underpin breeding programs by screening genetic resources including wild relatives and landraces available from genebanks for identification of adaptive and tolerance alleles to abiotic stresses [34,51,70–72]. A large reservoir of wild species germplasm is held in genebanks across the world, and proper characterization and evaluation of these genetic resources through phenomic and genomic approaches is imperative to enable the selection of the best crossing parents for breeding [65,67,73–76].

Table 2. Abiotic stress response of the eight annual wild relatives of domesticated chickpea.

Species	Gene pool	Abiotic stress response	References
<i>C. reticulatum</i>	Primary	Tolerant to low temperature, heat and drought	[77–79]
<i>C. echinospermum</i>	Primary	Tolerant to low temperature	[34,77,78]
<i>C. pinnatifidum</i>	Secondary	Tolerant to low temperature and drought	[78,79]
<i>C. judaicum</i>	Secondary	Sensitive to low temperature	[78]
<i>C. bijugum</i>	Secondary	Tolerant to low temperature	[78]
<i>C. yamashitae</i>	Tertiary	Sensitive to low temperature	[78]
<i>C. cuneatum</i>	Tertiary	Sensitive to low temperature	[1]
<i>C. chorassanicum</i>	Tertiary	Sensitive to low temperature	[1]

Conventional Breeding

Conventional breeding involving simple backcrosses to a recurrent parent forms the backbone of breeding and has been widely used to introduce novel traits within breeding programs and produce plant varieties suitable for targeted environments and cropping systems. Through conventional breeding, lines of varying maturity can be selected that are suitable for production in different agroecological zones. Over the past five decades, significant improvement has been achieved in crop yield

and productivity through conventional breeding, which has contributed to the development of more than 200 high yielding chickpea varieties tolerant to major biotic and abiotic stresses [80]. The main limitation of relying solely on conventional breeding is that it is largely successful for highly heritable and easy to score and/or visualize adaptive traits such as phenological development, growth habit, plant vigor, height, architecture, leaf characteristics and final yield [18,51]. However, these traits are often visually scored based on pre-determined scales and are therefore prone to human error and/or individual scoring biases. Furthermore, adaptive traits that confer resistance/tolerance to abiotic stresses are multigenic, have low heritability, display epistatic and large $G \times E$ interactions, further limiting the success of the conventional breeding approach.

Leaf characteristics such as rolling, size, area, weight, growth rate and stomatal density have been used during conventional breeding to understand plant responses to drought stress [44,81]. Plant height, bottom pod height and resistance to lodging are selected to enable efficient mechanical harvesting in chickpea and are often easily introgressed within conventional breeding approaches. To increase overall chickpea adaptation and sustainable production there is a need to complement conventional breeding with modern approaches and accurately measure other adaptive morphological, physiological or biochemical traits that explain complex responses such as abiotic stress tolerance [18]. Due to its labourious nature, slow speed and high probabilities of error/bias, conventional breeding is now routinely used in conjunction with other breeding approaches.

Molecular Breeding

Molecular breeding strategies can be deployed to target less heritable abiotic stress tolerance traits, as we now have a better understanding of the linkage between molecular markers and morphological and physiological traits [82]. It offers the opportunity to dissect the complex traits into component traits and study their underlying genetic basis in chickpea. Genetic approaches such as marker assisted selection (MAS) and marker assisted backcrossing can aid in the introgression of hard to phenotype traits such as root characteristics, which can be time consuming and require sophisticated equipment and analytical methods. These techniques further allow the improvement of one or two traits in the targeted elite variety without interfering or diluting the impact of favorable traits already present by reducing linkage drag of genes with deleterious effects from wild donor parents [83]. QTL analysis, genomics research and genotyping platforms are used to speed up the breeding process through exploiting variation at the genome level [13]. There is often co-location of QTLs associated with correlated traits, for example shoot weight and root traits, thus suggesting either a pleiotropic effect of one gene controlling both traits or presence of closely linked genes. Co-

location or QTL clusters offer the opportunity to select for multiple traits simultaneously.

There is scope to use genetic regions and genes associated with phenology to breed and fine tune varieties suited to different agroecological zones. Up to four genes of differing effect, *efl-1* [84], *efl-2* [85], *efl-3* [86], and *efl-4* [87], and numerous QTLs have been shown to control flowering in chickpea [88-90], with lateness largely dominant over earliness [49,87]. As molecular breeding is dependent mostly on molecular markers of major effect, in chickpea it would most likely rely on flowering time markers such as *efl-1*, *efl-2*, *efl-3* and *efl-4*. Additionally, numerous genes and QTLs associated with important chickpea traits have been widely reported under a range of conditions with some of the studies using either the same mapping population or common parents (Table 3). These genetic regions can be effectively selected to breed for early maturing varieties that can escape late season abiotic stresses and to match the sowing date with potential favorable conditions and increase chickpea productivity. One such genetic region on chromosome/linkage group 4 harboring several stable and consistent QTLs for drought tolerance-related traits, and associated with up to 12 other traits, and explaining up to 58.2% of the observed variation, has subsequently been referred to as the “*QTL-hotspot*” region [11,55,91]. However, QTLs for heat and frost tolerance in chickpea have been sparsely reported with Thudi et al. [92] and Mugabe et al. [93], respectively claiming theirs were the first reports. There seem to be no research reporting QTLs under heat and cold stresses published thereafter.

Some of the reported QTLs are photoperiod and vernalization responsive thus necessitating understanding the role and influence of these requirements in chickpea. As observed in cereals (wheat and barley) [94,95], vernalization and photoperiod genes are also associated with flowering in chickpea. A major vernalization response QTL, with logarithm of the odds (LOD) score of 27 and explaining 55% of the phenotypic variation was identified on chromosome/linkage group 3 [10]. Vernalization induced early flowering when plants were exposed to low temperatures with the wild relative parent showing a response while the elite cultivated parent did not respond, although a negative effect on yield in the elite cultivated parent was observed [10,96].

Table 3. List of some genes and QTLs conferring adaptation to various abiotic stresses in chickpea.

Treatment	Traits	Gene/Robust QTL number	Variation explained (%) **	References
Vernalization and non-vernalization	Vernalization response	1	47.90–54.90	[10]
Vernalization and non-vernalization	Flowering time	2	8.70–13.00	[10]
Short and long days	Flowering time	8	3.60–58.60	[14]
Post rainy season	Flowering time	10	4.04–88.19	[89]
Rainout shelter	Root traits	1	66.49	[13]
Rainfed and irrigated	Morphological, phenological, yield-related and drought indices traits	16	10.60–34.82	[13]
Rainfed and irrigated	Morphological, phenological, yield-related and drought indices traits	46	10.08–39.32	[13] *
Rainout shelter	Root traits	3	10.65–13.56	[15]
Rainfed and irrigated	Morphological, phenological, yield-related and drought indices traits	36	10.05–67.71	[15]
Rainfed and irrigated	Morphological, phenological, yield-related and drought indices traits	20	10.06–31.32	[15]*
Rainout shelter	Root traits	3	10.26–16.67	[11]
Rainfed and irrigated	Morphological, phenological, yield-related and drought indices traits	22	10.00–58.20	[11]
Drought	Harvest index, flowering time, physiological maturity, stomatal conductance, canopy temperature, air temperature, grain yield, days from flowering to maturity, plant height, drought tolerance score	15	7.00–52.10	[106]
Heat stress (early and late sown)	pod set, filled pods, seed number, grain yield	13	3.92–16.56	[12]
Cold stress	Cold tolerance	3	5.16–48.40	[93]

Table 3. Cont.

Treatment	Traits	Gene/Robust QTL number	Variation explained (%) **	References
Drought	Biotic and abiotic stresses	Aquaporins gene family (CaAQPs)	-	[107]
Drought	Abiotic stress responsive	<i>CarERF116</i>	-	[108]
Drought	Drought stress response	Differentially expressed genes	-	[109]
Drought, heat and cold	Plant developmental processes	<i>CarLEA4</i>	-	[110]
Drought and heat stresses	Root, morphological, phenological, transpiration efficiency related traits, yield and yield components	Marker-trait associations	4.14–96.55	[92]

* Used a second mapping population under the same experimental conditions.

** Only applied for QTL analysis

Genomic selection (GS) as a breeding tool increases the selection accuracy and thus enhances the rate of genetic gain, thereby reducing the length of the breeding cycle and associated costs through minimizing multi-year evaluation trials for each generation [97–99]. The varieties or lines to be used as parents in crossing blocks are selected based on their individual genomic estimated breeding values (GEBV). Genomic selection can effectively account for $G \times E$ interactions without compromising selection accuracy and ensures that alleles or QTLs with both low heritable and small-effect are effectively captured [100–102]. High heritability is key for selection of stable varieties across diverse environments. MAS is successful with highly heritable traits, and some traits of interests such as yield display large $G \times E$ interactions and have low heritability, making them not amenable to MAS. Using genome-wide high-density molecular markers, provided by genotyping by sequencing (GBS) or other genotyping platforms, GS can overcome some of the limitations of MAS and accurately predict the genetic value of traits of interest, such as yield and abiotic stress tolerance [103–105].

Physiological Breeding

Generally, crop breeding for complex traits comprises three steps: generating genetic variations through crossing, selection of the best progenies from the crosses and synthesizing the best progenies into a newly improved variety. However, most of the past research has focused mainly on the use of molecular markers for direct selection of the best progenies from the crosses, rather than choosing crossing parents [111]. Physiological breeding, on the other hand, offers the most promising approach to increase genetic gains in plant breeding as it relies heavily on advances in phenomics and genomics to create favorable allele combinations and has already demonstrated potential to significantly increase genetic gains [58]. It involves characterizing genetic resources for a large number of complementary traits, some of which are genetically complex, prior to designing precise crossing strategies. Most traits of importance such as yield and abiotic stress tolerance are genetically complex, polygenic and involve many genes of small effects, making it hard for conventional and molecular breeding approaches to effectively improve them. However, advances in phenomics and genomics are making it possible to dissect complex traits into component traits and facilitate various beneficial trait/allele combinations.

Distinct physiological traits, as constituents of yield formation and abiotic stress tolerance, hold promise to speed the breeding process compared to directly targeting the complex and polygenic final yield [100]. Physiologically traits such as photosynthetic rate, chlorophyll content, cell membrane stability, canopy temperature, root characteristics, water soluble carbohydrates have been shown to be associated with crop adaptation to abiotic stresses such as drought and/or heat stress [58,59,112,113]. Other physiological traits such as stay green, leaf rolling

and senescence generally reduce active leaf area and transpiration rate and thus also contribute to drought avoidance and yield improvement in crops [100,114] and these approaches can be of value to chickpea breeding.

COMMON ADAPTIVE TRAITS AND HIGH THROUGHPUT PHENOTYPING APPROACHES

High throughput phenotyping technology involves application of sensor or image-based tools, which, in contrast to manual and destructive methods, is able to non-invasively measure crop traits across time and space [115,116]. Sensor and imagery tools are fundamentally designed to capture the characteristic signature of the reflectances returning from the interaction between natural electromagnetic spectrum and plant cellular components. These reflectances can be analyzed and used as proxies of the crop's important morphological, agronomical and physiological properties e.g. phenology, early vigor, crop growth status, water content, biomass, and yield potential [117,118]. A plethora of optical devices such as passive (FieldSpec spectroradiometer; [119]) and active sensors (Crop Circle; [120]); red, green and blue (RGB) [121,122], multispectral [123], hyperspectral camera [124] and thermal camera [125] are available. The Light Detection and Ranging (LiDAR; [126]) and LeasScan PlantEye® [127] scanning systems emit laser pulses that capture the timing and intensity of the pulse bouncing back from the crop canopy to reconstruct 3D properties of crop canopies. The HTP technology has been extensively used in agriculture and plant science research [128] and is a promising tool for breeding chickpea against abiotic stresses [129,130].

HTP play a critical role in physiological breeding and genomic selection, enabling scientists to establish and verify key quantitative adaptive traits to strategically design crossing parents and training populations [131]. In this section, we will elaborate and discuss important traits that confer adaptation and tolerance to chickpea in response to abiotic stresses with emphasis on heat, cold and drought [132]. To avoid skepticism and reluctance to adopt HTP methods due to their complexity, cost and sometimes unproven reliability, we only recommend 'breeder friendly' HTP approaches that can quantitatively measure these traits on a large number of genetic resources or progeny experimental units [133], which are detailed in Table 4.

Phenology

For decades chickpea breeders have been focusing on selecting lines whose growth duration suits targeted specific environments. However, adaptation is dependent on the season, sowing date and water regime combinations, and these combinations affect phenological development (thermal time to flowering, pod set and end of flowering and the duration of flowering) with accelerated development under late sowing and dry conditions [134]. Super early chickpea lines that mature in less than 85 days have been developed but these are generally lower yielding

compared to the longer duration lines, and generally early flowering plants display biomass accumulation, height and yield penalties and have fewer pods and seeds per plant than the late maturing plants [87]. This might be because they do not have sufficient growth time to accumulate assimilates for subsequent remobilization to the developing grain. The shorter vegetative growth phase can potentially limit biomass accumulation and formation of additional branches and podding nodes, while the shorter podding phase might be insufficient for grain filling unless the rate of grain filling is significantly accelerated.

In chickpea, flowering and podding (reproductive and grain filling stages respectively) are generally the most critical stages affected by adverse conditions. Conventionally, flowering is assessed visually as percentage of plants per plot [89], which is subjective and can be prone to human error. An image-based phenotyping method can be used to measure these qualitative traits effectively as a replacement of the conventionally visual method. For instance, HTP technology has been used to phenotype heading and flowering [135,136] of various crop species (Table 4). This suggests that assessment of flowering time in chickpea by HTP is very feasible and should be vigorously explored to avoid subjective variation between scorers and/or days.

Early Vigor

Early vigor is a beneficial trait in chickpea, and it contributes to weed competitiveness, water use efficiency and grain yield under certain growing environments. In semi-arid environments such as India, early vigor is not a favorable trait because crops will quickly exhaust stored water causing terminal drought at the reproductive phase [154]. However, in Mediterranean climates such as Australia where cropping systems mainly depend on winter rainfall, early vigor traits can facilitate crop growth by enhancing ground cover, reducing water run-off and evaporation by preserving moisture in the soil profile for later use in the season [155,156]. Early vigor is an adaptive trait for drought and chilling stress in chickpea [41,72].

To assess early vigor, several conventional methods such as visual scores based on a pre-determined scale [127,157] or vegetative biomass harvest are usually used [23]. Although effective, these methods are highly subjective and/or labor intensive and thus, not suitable on large-scale field trials. HTP technology using sensors or multispectral imagery offers a robust and rapid assessment of early vigor in various grain crop species such as wheat [138], barley [122] and field pea [121]. This suggests that early vigor can also be evaluated by HTP methods to boost genetic gains in chickpea.

Table 4. HTP approaches for key adaptive traits to abiotic stresses.

Crop traits	Adaptation	Environment	Description and HTP approach	Species	References
Phenology	Drought/heat	Field	Detecting flowering and heading date by a gantry mounted RGB camera	<i>Triticum aestivum</i>	[136]
		Field	Predicting flowering time by aerial multispectral imagery	<i>Zea mays</i>	[135]
		Field	Flowering dynamics by time-series RGB imagery	<i>Oryza sativa</i>	[137]
Early vigor	Drought/chilling	Field/greenhouse	Early vigor assessment by handheld multispectral sensor and RGB imagery platform	<i>Pisum sativum</i>	[121]
		Field	Airborne RGB and multispectral imagery to evaluate early vigor	<i>Hordeum vulgare</i>	[122]
		Field	Early vigor evaluation by handheld multispectral sensors and RGB imagery	<i>Triticum aestivum</i>	[138]
Canopy properties	Drought/heat	Field	LeasyScan PlantEye® 3D scanners to measure canopy related traits	<i>Cicer arietinum</i>	[127]
	Drought/heat	Field	Canopy temperature measurement by airborne thermography and infrared thermometers	<i>Triticum aestivum</i>	[139]
		Field	Canopy temperature assessment by aerial thermal and RGB imagery	<i>Zea mays</i>	[125]
Root properties	Drought/heat	Greenhouse	A growth and automated imaging unit, GROWSCREEN-Rhizo, to phenotype shoot and root traits simultaneously	<i>Brassica napus</i> , <i>Hordeum vulgare</i> , <i>Oryza sativa</i> , <i>Zea mays</i>	[140,141]
			MRI and X-ray CT technologies for 3D imaging of root systems in soil	<i>Phaseolus vulgaris</i>	[142]

Table 4. Cont.

Crop traits	Adaptation	Environment	Description and HTP approach	Species	References
Stay-green	Drought/heat	Field	Stay-green assessment by handheld multispectral sensor	<i>Avena sativa</i>	[143]
		Field	Monitoring green leaf area dynamics by aerial multispectral imagery	<i>Zea mays</i>	[123]
		Field	Evaluating senescence rate	<i>Triticum aestivum</i>	[144]
		Greenhouse	Stay-green evaluation by RGB imagery platform	<i>Triticum aestivum</i> , <i>Cicer arietinum</i>	[145]
Pollen fertility		Laboratory	Counting stained viable pollens from digital microscopy RGB imagery	<i>Carduus acanthoides</i>	[146]
		Laboratory	PollenCounter to count stained pollens from digital microscopy RGB images	<i>Vitis vinifera</i>	[147]
Photosynthesis	All stresses	Field	Measuring photosynthetic capacities by handheld hyperspectral sensor	<i>Nicotiana tabacum</i>	[148]
		Field	Leaf photosynthesis evaluated by handheld hyperspectral sensor	<i>Zea mays</i>	[149]
		Greenhouse	Fluorescence imagery to early evaluate drought stress	<i>Lycopersicon esculentum</i>	[150]
		Greenhouse	Chlorophyll fluorescence imaging to screen genotypes for drought	<i>Zea mays</i>	[151]
		Field	Airborne multispectral imagery to detect chlorophyll fluorescence of various crop species under different irrigation regimes	<i>Olea europaea</i> , <i>Prunus persica</i> , <i>Citrus sinensis</i>	[152]
Biomass and grain yield	All stresses	Field	Biomass, ground cover and canopy height estimates by LiDAR	<i>Triticum aestivum</i>	[126]
		Field	Grain yield prediction by canopy airborne hyperspectral imagery	<i>Triticum aestivum</i>	[124]
		Greenhouse	Estimation of shoot biomass and yield by RGB imagery platform	<i>Triticum aestivum</i>	[153]

Root Traits

Root traits such as root length density, volume, root depth and root mass play a critical role in drought and heat adaptation in chickpea [43,55] and several QTLs controlling root traits have been reported [11,158–160]. Accurate phenotyping of root traits is challenging because roots grow underground, and they are difficult to fully recover from soil. Common methods for characterizing root traits in chickpea and food legumes are using polyvinyl chloride cylinder (PVC) growth systems [159], soil cores [161], semi-hydroponic systems [162], shovelomics [163] with subsequent WinRhizo imagery analysis. These methods could yield good results, but they are time consuming and highly laborious. Advanced image-based root phenotyping methods such as X-ray computer tomography; magnetic resonance imaging, positron emission tomography, GROWSCREEN-Rhizo are promising for chickpea germplasm improvement against drought and heat stresses since they combine phenotyping of shoot and root simultaneously (reviewed in: [164]).

Stomatal Conductance, Canopy Temperature and Stay-Green

Stomatal conductance and canopy temperature (CT) are well-known adaptive traits for terminal drought and heat tolerance in chickpea, with several QTLs associated with these traits reported [106,127]. Canopy temperature can be measured by handheld [157,165] or airborne [166,167] thermal and hyperspectral imagery to screen crop genotypes for drought and heat adaptation. Stay-green is the plant's ability to retain their green leaves and photosynthetic activities for an extended period post-anthesis and is associated with enhanced drought and heat tolerance in various crop species [168]. Functional stay-green has been shown to link with deeper roots and cooler CT, which are adaptive traits for heat and drought adaptation, and higher yielding [169]. Thus stay-green traits have been extensively used by various crop breeding programs, including chickpea, for drought and heat adaptation improvement [132,170,171]. Conventionally, the stay-green trait is assessed by visual scoring, which is subjective, labor intensive and prone to human errors and bias. Proximal and remote sensing technology using sensors and cameras can be a method of choice for HTP screening of stay-green phenotypes of different crop species [123,143] and chickpea [145].

Pollen Viability

The reproductive growth stage is the most sensitive to heat, cold and drought stresses in grain crops. The stresses delay anther dehiscence; reduce flower numbers and pollen viability; decrease pollen germination rate and pollen tube growth; cause fertilization failure and pod abortion in chickpea [35,41,172,173]. Thus, pollen viability is a key adaptive trait for heat, cold and drought stresses and pollen quality traits have been used as

selection criteria in breeding chickpea [54], other food legumes [174], canola [175] and tomato [176].

Screening pollen quality traits for adaptation using standard microscopy methods is useful, but it is a tedious and a labor-intensive process, and results are sometimes cumbersome, especially when it is used for screening a large number of genotypes [27]. Advances in image-based phenotyping methods have enabled automated quantitative analysis of pollen fertility (reviewed in: [177]). For example, Costa and Yang [146] developed an image processing pipeline to effectively count the number of stained viable pollens from digital microscopy RGB images. Similarly, Tello et al. [147] introduced a novel method using PollenCounter software to successfully quantify fertile pollen grains within stained aliquots of pollen suspension under a microscope.

Photosynthesis Related Traits

Enhancing functional photosynthetic components is a strategic approach to increase photosynthetic efficiency and seed yield in chickpea, especially under abiotic stress conditions [178]. Water stress decreases net photosynthetic rate, chlorophyll content and photosystem efficiency in chickpea [179,180], and thus, sustaining photosynthetic activities under abiotic stresses is a desirable adaptive trait. Photosynthetic related traits are usually measured by a gas exchange system such as infrared gas analyzer (IRGA) or handheld chlorophyll fluorescence devices such a fluorometer. These are excellent tools to assess photosynthetic efficiency, to study plant-water relations [180] and frost damage [181]. However, given their relatively slow speed of data acquisition and mode of operation, they are unsuitable for automation and large-scale trials, especially under field conditions. In this context, chlorophyll fluorescence imagery can be an excellent alternative to automatically and rapidly capture photosynthetic activities of crops [182,183] and such platforms have been widely used for data capture under abiotic stresses in controlled [184] and field conditions [152].

Biomass, HI and Grain Yield

Grain yield potential or sink strength, a function of biomass and HI, is the most valuable and targeted trait for phenotyping in any breeding program because it is the final outcome of the $G \times E$ interactions under optimum crop management practices. However, yield itself is a complex trait, and direct selection of yield from early breeding lines does not always result in desirable outcome. Instead, selection of relevant secondary traits contributing to yield, e.g., biomass, can be a feasible approach in physiological breeding. Moreover, it is imperative to optimize the balance between biomass (the source) and HI (the sink) to achieve yield potential in crops [185].

Improving biomass and HI is a critical metric to increase genetic gains in chickpea and other grain crops under controlled and abiotic stress

conditions. Multiple QTLs related to HI have been reported under water stress condition [11,15,106,160,186]. High-throughput estimation for biomass is a typical approach in various crop species and can be conducted fairly straightforward by proximal and remote sensing tools [128]. Normalized difference vegetation index (NDVI) is an inexpensive screening tool to capture physiological characteristics such as yield and crop growth rate in chickpea [130]. Recently, airborne multispectral imagery has been deployed to evaluate yield potential in chickpea, where the mean NDVI was found to be consistently correlated to dry seed yield [187].

CHALLENGES AND FUTURE PERSPECTIVES

Abiotic stresses such as heat, frost and drought cause significant chickpea yield losses, especially if the crops are exposed during the mainly grown in risk prone marginal areas under rainfed conditions and residual soil moisture. They affect a range of metabolic and reproductive phase, with huge financial implications to growers. The abiotic stresses cause year on year fluctuations in chickpea yield as it is physiological plant processes, and the yield reduction is primarily due to the effect of stress on flower set, pollen viability, pod set/abortion and retention, all key determinants of grain number. Therefore, integration of various breeding approaches and coordination of phenotyping and genotyping platforms will improve selection efficiency, effectiveness, shorten the breeding cycle and ensure rapid attainment of genetic gains even under stressful conditions.

The immediate challenge, especially for smallholder farmers, is availability of widely adapted and tolerant varieties, and to overcome this would involve pyramiding many genes, some with minor effects into a desirable genotype. The major genetic sources of tolerance to abiotic stresses can come from crop's centers of origin—the geographical regions where plants normally grow under such stressful conditions and can involve using wild relatives as donors of favorable genes. Genebanks around the world hold large reservoirs of genetic material including wild relatives and landraces that needs to be characterized [76], and they should continue to strive to conserve these valuable resources and make them readily available to breeding programs.

While growth chambers and glasshouses create an artificial environment that allows the study of one experimental factor at a time without the confounding influence of others, as happens in the field, results need to be treated with care as pot-grown plants behave completely differently from plants growing as a community in the field. In the field the environmental factors are continuously changing throughout the growing season and there can be inter and intra plot variations. High throughput precision phenotyping platforms that can be easily deployed to field studies, will enable faster, accurate and unbiased screening of large numbers of genotypes, which will increase genetic gains. The

concept of “envirotyping” [188], complements the phenotyping platforms and improves environmental characterization and generation of high quality phenotypic data in a range of diverse environments. It allows for better control of experimental errors, understanding and management of environmental factors that affect crop development and productivity, and identification of environments suitable for specific genotypes.

Despite concerted genetic research, there is still a disconnection between the identified markers and/or genes published in journals, and their translation, adoption and implementation by breeding programs [21,189]. This is largely due to lack of fine mapping or validation of the reported markers and/or QTLs across large populations to ensure their consistency and applicability for use in routine screening applications. Even though dense genetic maps are available, the lack of common markers in the diverse maps makes the interpretation of the exact map positions of the identified QTLs ambiguous, rendering comparison of QTLs located in the same chromosome/linkage group difficult. This can be minimised through availability of an up to date germplasm database containing key phenotypic and genotypic information, and that is easily accessible to breeding programs. A user-friendly, efficient and interactive QTLBase database that catalogues identified QTLs from human genome research is available and data can be readily searched, queried, visualized, retrieved and compared across multiple tissues [190]. Such an approach can certainly be applied for chickpea and other grain crops and will ensure that QTLs reported in numerous studies and information regarding their respective map positions, alleles with positive effects and variance explained, are readily available to researchers. Importantly, it will minimize duplication of reported QTLs detected in different populations/studies and facilitate their active uptake by breeding programs.

AUTHOR CONTRIBUTIONS

LM and GNN conceived the topic and wrote the manuscript. MFR and SLN provided critical comments, edits and acquired the funding. All authors contributed to editing, revision and approval of the manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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