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RESEARCH PAPER

Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought

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Abstract

Terminal drought during the reproductive stage is a major constraint to yield of chickpea in many regions of the world. Termination of watering (WS) during podding in a small-seeded desi chickpea (*Cicer arietinum* L.) cultivar, Rupali, and a large-seeded kabuli chickpea cultivar, Almaz, induced a decrease in predawn leaf water potential (*LWP*), in the rate of photosynthesis, and in stomatal conductance. Compared to well-watered (WW) controls, the WS treatment reduced flower production by about two-thirds. In the WW treatment, about 15% of the flowers aborted and 42% (Rupali) and 67% (Almaz) of the pods aborted, whereas in the WS treatment 37% and 56% of the flowers aborted and 54% and 73% of the pods aborted, resulting in seed yields of 33% and 15% of the yields in WW plants in Rupali and Almaz, respectively. *In vitro* pollen viability and germination in Rupali decreased by 50% and 89% in the WS treatment, and pollen germination decreased by 80% *in vivo* when pollen from a WS plant was placed on a stigma of a WW plant. While about 37% of the germinated pollen tubes from WW plants and 22% from the WS plants reached the ovary in the WW plants, less than 3% of pollen grains reached the ovary when pollen from either WS or WW plants was placed on a stigma of a WS plant. It is concluded that, in addition to pod abortion, flower abortion is an important factor limiting yield in chickpea exposed to terminal drought and that water deficit impaired the function of the pistil/style more than the pollen.

Key words: Flower production, pistil, pod set, pollen, seed yield, stigma, style.

Introduction

There are two types of chickpea, namely 'desi' and 'kabuli', respectively. The desi type has small, angular, dark-brown seeds, while kabuli types have large, rams-head-shaped, light-brown seeds (Malhotra *et al.*, 1982). Both types are generally grown under rainfed conditions either on stored soil moisture in subtropical environments with summer-dominant rainfall or on current rainfall in winter-dominant Mediterranean-type environments. In both environments, water shortage and high temperatures as the plant enters its reproductive phase induces the end of reproductive development (Siddique *et al.*, 2000; Turner, 2003, 2004; Turner *et al.*, 2006). This end-of-season drought is termed 'terminal drought'.

While chickpea (*Cicer arietinum* L.) is considered one of the most drought-tolerant cool-season food legumes, terminal drought still limits chickpea production. With terminal

Abbreviations: DAS, days after sowing; DAW, days after water withheld; FC, field capacity; FDA, fluorescein diacetate; *LWP*, predawn leaf water potential; *SWC*, soil water content; WS, water stress; WW, well watered.

drought, seed yields can be reduced by 58–95% compared to irrigated plants and reductions in pod production and abortion are key factors impacting final seed yield (Leport *et al.*, 1999, 2006). Yields of kabuli chickpeas are less than desi chickpea under terminal drought and pod abortion by kabuli chickpea is more sensitive to water stress than that of desi chickpea (Leport *et al.*, 2006). While pod abortion in chickpea has previously been studied (Leport *et al.*, 2006), flower production and abortion under terminal drought and the role of water deficits on pollen viability and pollen tube growth in water-stressed pistils have received little attention.

In the present study, a desi and a kabuli cultivar were used to investigate the effect of terminal drought on flower production and abortion, pod set, pod abortion, and seed production. Terminal drought was imposed when both cultivars had flower buds, flowers, and developing pods. The objectives of the study were to investigate: (i) the influence of terminal drought on flower and pod abortion, (ii) whether flower abortion was due to impairment of pollen or pistil function, and (iii) the response of pods to terminal drought at different stages in the two chickpea types.

Materials and methods

Experiment 1: design and management

Two chickpea (*Cicer arietinum* L.) cultivars, a desi type, Rupali, and a kabuli type, Almaz, were grown in a controlled-temperature greenhouse, set at 22/15 °C day/night temperature, at The University of Western Australian, Perth, Western Australia (31°57′ S, 115°47′ E). Plants were grown in polyvinyl chloride pots, 15 cm in diameter and 40 cm high, closed at one end with a perforated cover to allow free drainage of water. To reduce compaction and improve drainage, sieved, fine-textured loam (Calcic Haploxeralf) from the top 10 cm of an unfertilized and uncultivated field in Merredin was mixed 4:1 v/v with yellow sand. Filter paper was placed over the holes at the bottom of each pot and filled with 9.4 kg of the soil:sand mixture, 0.12 g of Richgro™ trace elements (K, Fe, Ca, Mg, Cu, Zn, Bo, Mo), 0.90 g potassium nitrate, 0.85 g ammonium nitrate, 1.28 g calcium nitrate, and 1.89 g triple superphosphate.

Seeds of the two cultivars were inoculated with commercial Group N *Bradyrhizobium* and germinated in Petri dishes lined with wet filter paper on 26 May 2008. Two days later, four seedlings were transferred into each pot that had previously been irrigated with 2.24 kg water to bring the soil to field capacity (FC). All pots were irrigated every 2 d to maintain the soil above 80% FC by weighing 10 randomly-selected pots to measure and replace the water lost. Fifteen days after sowing (DAS), seedlings were thinned to one per pot. For each genotype, 50 pots were used and randomly designated to one of two treatments: well-watered control (WW) and water-stressed (WS). The WS treatment was imposed by cessation of watering from 78 DAS (12 August) when both genotypes had flowers and young pods (23 and 4 pods in Rupali and Almaz, respectively). WW plants were watered every 2 d to maintain the soil above 80% FC until the WS plants reached maturity (101 DAS) when water was withheld. There were 25 pots per treatment per genotype. Five pots in each treatment and genotype were used to measure predawn leaf water potential (*LWP*) and gas exchange. The remaining 20 pots in each treatment and genotype were used to measure flower production and abortion, pod production and abortion, biomass, seed yield, and seed-yield components at each of five harvests. The 100 pots were randomly arranged on five benches in the greenhouse and benches were moved weekly to minimize any variation in light and temperature.

Podding date

The start and end of flowering and podding was recorded for each plant in each treatment and cultivar except for plants used for measuring *LWP* and gas exchange. Every second day, new flowers were tagged and the flowering and podding dates recorded on the tag.

Harvest and dry matter partitioning

There were five harvests (four plants per harvest per cultivar per treatment): (i) when the WS treatment started (78 DAS); (ii) when *LWP* in the WS treatment was about –1.2 MPa (85 DAS); (iii) when *LWP* in the WS treatment was about –2.0 MPa (89 DAS); (iv) when *LWP* in the WS treatment was about –3.0 MPa (95 DAS); and (v) at maturity (WS 115 DAS; WW 141 DAS). At each harvest plants were cut at the soil surface and sorted into primary and secondary branches. Primary branches were defined as the largest stems growing near the base, which included the main stem and branches growing from the lowest three nodes of the main stem. Secondary branches grew from nodes of primary branches (Leport *et al.*, 2006). All leaves were removed, green leaves separated from the rest, and the green leaf area measured with a scanner (WinRHIZO Root Analysis, Regent Instruments, Quebec, Canada). Leaflets that had shed at the time of harvest were not included in leaf biomass. Roots were removed from the pots and rinsed with water to remove all soil and sand. Leaves, branches, and roots were dried to constant weight in a forced-draught oven at 70 °C and weighed. After drying, tags and pods from primary and secondary branches were separated, and all pods with the same date of podding were combined, and then separated into pod wall and seeds for counting and weighing. Flower abortion was calculated from tags where no podding date was recorded. Pod abortion was calculated from tags where a podding date was recorded, but no pod was present or pods were present, but had small or no seed present at maturity (Leport *et al.*, 2006).

Seed components and pollen viability

Seed components were determined for each cultivar and treatment from dry weights measured as described above. The number of flowers, pods, aborted pods, filled pods, seeds per pod, and individual seed weights for each date were determined. Seed growth rate was determined from the rate of seed dry matter accumulation during the linear seed-filling period (Schussler *et al.*, 1991).

When *LWP* reached –1.2 MPa (86 DAS) and –2.5 MPa (90 DAS), 10 hooded flowers from each cultivar and each treatment were collected at around noon for the determination of pollen viability (only pollen was collected from Almaz at 90 DAS as there were no new flowers in Rupali) as described below for Experiment 2.

Soil and leaf water status and gas exchange

LWP of the upper expanded leaves on primary branches was measured at predawn (04.30–06.00 h, Australian Western Standard Time) using a pressure chamber (PMS Instrument Company, Albany, OR, USA) and following the precautions recommended by Turner (1988). The proximal six leaflets were removed before the leaf midrib was inserted into the pressure chamber. Five leaves were measured in each treatment and cultivar every 3–5 d for the first 23 d after water was withheld from the WS treatment. Soil water content (*SWC*) was measured gravimetrically by weighing pots after measurement of *LWP*. On similar leaves to those measured for *LWP*, photosynthetic rate, stomatal conductance,

and transpiration rate were measured between 09.30–10.30 h (Australian Western Standard Time) with a portable, open gas-exchange system (Li-Cor Inc., Lincoln, NE, USA) at a photosynthetically active radiation of 1200 μmol m^{-2} s^{-1} and a CO_2 concentration of 380 μmol mol^{-1}. After measurement, the part of the leaf inserted into the cuvette was placed in a rapid-seal plastic bag and its area measured with the WinRHIZO Root Analysis scanner used previously, in order to calculate the rate of net photosynthesis, stomatal conductance, and transpiration rate per unit leaf area.

Experiment 2: design and management

In order to determine the effect of a water deficit on pollen viability and pollen tube growth, Rupali seeds were sown in 30 pots on 23 December 2008 and treated and managed as in Experiment 1. The start and end of flowering and podding was recorded for each plant in each treatment except for plants used for measuring *LWP*. As in Experiment 1, every second day new flowers were tagged and the flowering and podding dates recorded on the tag. Half the pots were kept well-watered throughout. Terminal drought was imposed on the other half by withholding water from 39 DAS when podding had commenced. When *LWP* decreased to –0.5 MPa (39 DAS), –0.8 MPa (48 DAS), and –1.2 MPa (51 DAS) in the WS plants, 20 hooded flowers from each treatment were collected at around noon; half for the determination of pollen viability and the remainder for the determination of pollen germination *in vitro*. From 17.00–18.00 h on the same days, the second flower from the top branches in the WW and WS treatments were both self-pollinated and reciprocally-pollinated by hand at the stage when petals were visible and slightly smaller than the sepals (Clarke and Siddique, 2004). Stigmas of WW plants were pollinated with pollen from WW plants (WW+WW) and WS plants (WW+WS), while stigmas of WS plants were pollinated with pollen from WW plants (WS+WW) and WS plants (WS+WS) (10 flowers per combination). The flowers were harvested 24 h later to measure pollen germination and pollen tube growth in the pistil in each treatment. In order to identify changes in anther morphology under water stress, that may account for differences in flower abortion between WW and WS plants, flowers near the tip of branches of plants in the WS and WW treatments were photographed after removal of some petals and sepals. Photographs were taken: (i) 3 d before flowers in WW plants opened, (ii) at flowering (*LWP* in WS plants was –1.2 MPa), and (iii) 3 d after flowers opened in WW plants.

Pollen viability and germination determination

Pollen was collected in an Eppendorf tube by squeezing the keel from the base upwards with forceps until most pollen exuded through the tip.

Pollen viability was assessed using the fluorochromatic reaction adapted from Heslop-Harrison and Heslop-Harrison (1970). Fluorescein diacetate (FDA, 2 mg) was dissolved in 1 ml of acetone and a drop of the acetone–FDA solution put on a microscope slide and allowed to evaporate. Pollen was mixed with a 10% sucrose solution and a drop of the solution placed on the stain of evaporated acetone–FDA and covered with a coverslip. Pollen grains with a grey colour under a fluorescence microscope (Zeiss, Oberkochen, Germany) were assessed as having lost viability. The percentage of viable and unviable pollen was measured by examining 300 grains.

Pollen was inoculated into 100 ml of freshly-prepared pollen-culture medium (Brewbaker and Kwack, 1963), preincubated at 25 °C. The contents were mixed on a vortex mixer, and incubated in the dark. After 4 h the experiment was halted by adding one drop of acetic alcohol (glacial acetic acid:ethanol, 1:3, v/v) to the sample as a fixative. The percentage of pollen germination was estimated by examining 300 pollen grains (10–15 microscopic fields of view). A pollen grain was scored as germinated when the length of the pollen tube exceeded the diameter of the pollen grain.

In vivo *pollen germination and tube growth*

Previously-pollinated pistils were excised from flowers 24 h after pollination and fixed for 24 h in the acetic alcohol described previously, then cleared with 8 N NaOH overnight, and thoroughly rinsed before being stained with decoloured aniline blue. The preparation was observed under a fluorescence microscope (Zeiss, Oberkochen, Germany) to measure pollen germination and pollen tube growth down the style. Pollen grains were scored as germinated when the tubes were apparent between the papillate cells of the stigma, and the number of germinated pollen tubes and the number that reached the ovary were recorded.

Statistical analysis

Statistical analyses were performed using SPSS 15.0 by one-way ANOVA and *t* test. Differences between mean values of treatments were evaluated using least significant difference (LSD) at a 0.05 significance level. Regressions were fitted using Origin 7.0.

Results

Phenology

In Experiment 1, flowering commenced in Rupali and Almaz at 49 DAS and 65 DAS, and ended in Rupali at 86 DAS and 96 DAS, and in Almaz at 90 DAS and 104 DAS in the WS and WW treatments, respectively. In Experiment 2, which was sown in summer, Rupali began flowering at 27 DAS and continued flowering until 51 and 65 DAS in the WS and WW treatments, respectively. Pod set commenced 8–9 d after flowering.

Soil water content (SWC) and predawn leaf water potential (LWP)

In Experiment 1, *SWC* decreased after water was withheld (DAW) from the WS treatment to reach 39% FC by 3 DAW and 24% FC by 7 DAW. Subsequently *SWC* decreased slowly to reach 14% FC by 23 DAW (Fig. 1A). *LWP* in WW plants was between –0.4 and –0.7 MPa in both cultivars, but in WS plants it decreased steadily to about –3.2 MPa by 17 DAW in both cultivars. At 23 DAW, *LWP* of Rupali was not measured, but Almaz had decreased to –5.0 MPa (Fig. 1B). In Experiment 2, *SWC* in the WS treatment decreased more slowly than in Experiment 1 to reach 27% FC by 13 DAW (Fig. 1C), while *LWP* decreased significantly below that in the WW treatment about 7–9 DAW in both experiments (Fig. 1B, D).

Gas exchange

In Experiment 1, mean photosynthetic rate, stomatal conductance and transpiration rate in WW plants of both cultivars ranged from 17–22 μmol m^{-2} s^{-1}, 0.41–0.64 mmol m^{-2} s^{-1}, and 6.5–10.3 mmol m^{-2} s^{-1}, respectively, until 17 DAW, but then decreased after water was withheld from the WW plants at 101 DAS (23 DAW) (Fig. 2). Although *LWP* (measured predawn) changed little, photosynthetic

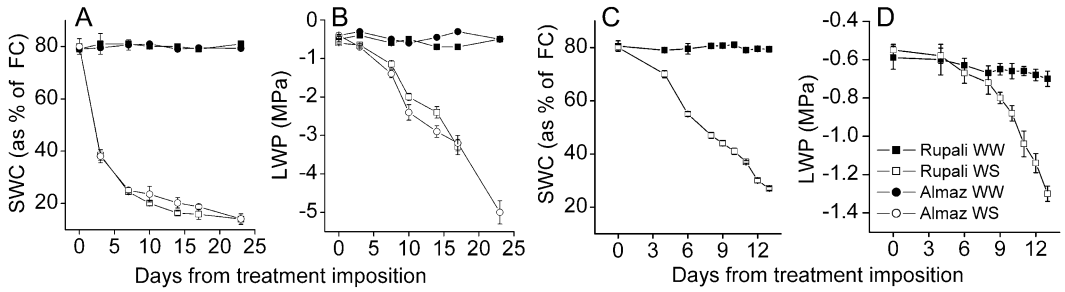

Fig. 1. Change with time after the imposition of treatments [Day 0=78 DAS (12 August) in A and B and 39 DAS (1 February) in C and D] in soil water content [*SWC*, % of field capacity (FC)] (A, C), and predawn leaf water potential (*LWP*) (B, D) of Rupali and Almaz chickpea cultivars in Experiment 1 (A, B) and in Rupali in Experiment 2 (C, D) in well-watered (WW) and water-stressed (WS) treatments. Values are means ±SE (n=5). Note change of scale of y-axis between (B) and(D).

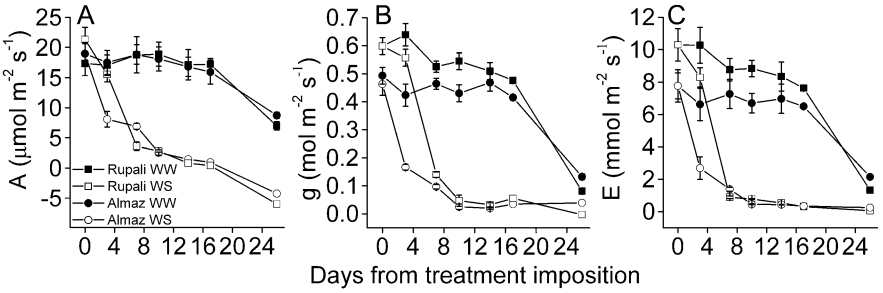

Fig. 2. Change with time after imposition of treatments (Day 0=78 DAS) in (A) photosynthetic rate, (B) stomatal conductance, and (C) transpiration rate of Rupali and Almaz chickpea cultivars in well-watered (WW) and water-stressed (WS) treatments in Experiment 1. Values are means ±SE (n=5).

rate, stomatal conductance, and transpiration rate decreased markedly in Almaz as *SWC* decreased from 80% to 39% FC in the first 3 DAW, but marked decreases in Rupali occurred from 3–7 DAW as *SWC* continued to decrease and *LWP* decreased close to –1.2 MPa. After that, all measurements decreased slowly but steadily, reaching about zero on 17 DAW (Fig. 2).

Flower and pod production and abortion

In Experiment 1, total flower production per plant in WW plants of Rupali and Almaz was similar at 105±4.5 and 98±3.6, respectively. Due to the earlier flowering in Rupali, 50±9.4 of the 105 flowers/plant were produced before the WS treatment was imposed, whereas in Almaz only 15±2.8 flowers of the 98 flowers/plant were produced before the WS treatment was imposed. In the WS treatment, flowering stopped in Rupali on 8 DAW and in Almaz on 12 DAW (Fig. 3B, D), while in the WW plants flowering continued for a further 10 d and 14 d in Rupali and Almaz, respectively, but then stopped even though water was still available and the temperature was maintained at or below 22 °C (Fig. 3A, C). In the WS treatment, only 18.2±3.9 Rupali flowers were produced after the treatment was imposed compared to 56±4.9 in the WW treatment (Fig. 3A, B). In Almaz, equivalent numbers were 23±4.1 and 82±2.7 in WS and WW treatments, respectively (Fig. 3C, D). In WW plants, pod set was 86% in Rupali and 85% in Almaz (that is flower abortion was 14–15%), while in WS plants, pod set was 63% (flower abortion 37%) in Rupali and 44% (flower abortion 56%) in Almaz (Table 1). Pod abortion ranged from 42–67% in the WW treatment and 54–73% in the WS treatment in both cultivars (Table 1). In both treatments, pod abortion was significantly higher in the kabuli cultivar (Almaz) than in the desi cultivar (Rupali) and on secondary branches than primary branches (Table 1). Pod abortion was also greater in later-produced flowers than in early-produced flowers, even in the WW treatment (Fig. 4). By maturity, in Rupali, some pods that were initiated before the WS treatment was imposed had aborted, while all of the pods formed after the WS treatment was imposed had aborted (Fig. 4A). In Almaz, 24% of the pods initiated before the WS treatment was imposed aborted, while all pods set after 93 DAS in the WS plants aborted (Fig. 4B). In Experiment 2, flower and pod abortion in Rupali was similar to those in Experiment 1 (Table 2).

Flower development, pollen viability, germination, and pollen tube growth

In Experiment 1, no more flowers developed in Rupali from 8 DAW when *LWP* decreased to –1.2±0.10 MPa, while in Almaz no further flowers developed from 12 DAW when *LWP* had decreased to –2.5±0.18 MPa. In Experiment 2, flower development in Rupali was similar to that in Experiment 1. When *LWP* decreased to –1.2 MPa, at which stage the plants still were growing, flower development was

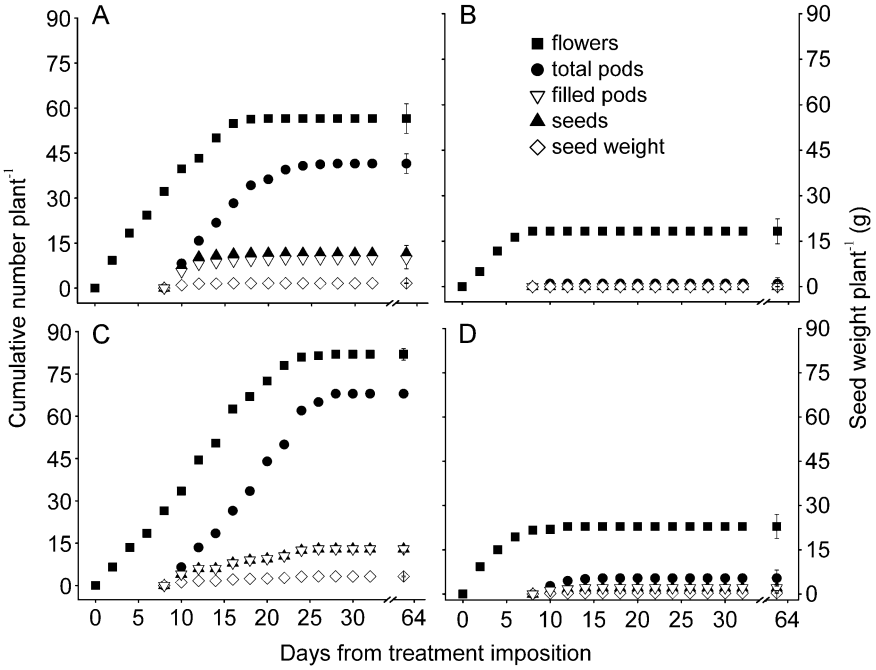

Fig. 3. Cumulative number of flowers, total pods, filled pods, and seeds, and cumulative seed weight per plant in well-watered (A, C) and water-stressed (B, D) treatments of Rupali (A, B) and Almaz (C, D) from the imposition of treatments (Day 0=78 DAS) to maturity in Experiment 1. Values are means ±SE (n=4).

Table 1. Percentage of flowers and pods that aborted and seed yield per plant at maturity on the primary and secondary branches along the whole branch, and for those produced after treatments were imposed in Experiment 1

Treatments[a]	Primary branch[b]		Secondary branch[b]		Whole plant[b]
	After drought	Whole branch	After drought	Whole branch	
Flower abortion (%)					
CR	19.1 c	10.4 c	23.3 c	18.8 b	14.2 c
SR	100.0 a	26.4 b	100.0 a	51.5 a	36.8 b
CA	12.8 c	8.9 c	20.4 c	18.3 b	14.7 c
SA	72.7 b	48.7 a	69.2 b	63.0 a	55.6 a
Pod abortion (%)					
CR	55.2 b	34.7 b	50.9 b	44.4 b	41.9 c
SR	87.5 a	50.8 ab	84.6 a	60.3 ab	54.2 bc
CA	51.7 b	51.7 ab	77.6 ab	77.6 a	67.1 a
SA	66.3 b	58.3 a	78.0 ab	78.0 a	72.8 a
Seed yield (g plant^{-1})					
CR	0.35 b	6.95 a	1.27 a	5.43 a	12.3 a
SR	0.00 c	3.04 c	0.00 b	1.01 c	4.05 c
CA	1.46 a	4.51 bc	1.76 a	2.94 b	7.45 b
SA	0.15 c	0.80 d	0.13 b	0.32 d	1.12 d

[a] C, well-watered treatment; S, water-stressed treatment; R, Rupali, A, Almaz.
[b] Values with the same letter within a column are not significantly different (P >0.05).

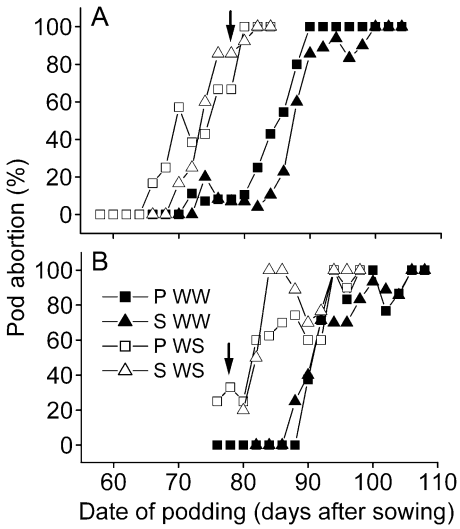

Fig. 4. Pod abortion by maturity in relation to the date of podding for pods on primary (P) and secondary (S) branches of Rupali (A) and Almaz (B) chickpeas in well-watered (WW) and water-stressed treatments (WS) in Experiment 1. The time of imposition of treatments is shown by a vertical arrow.

impaired. As a result, in the WS treatment the size of the flower near the tip of the branches was about half that of flowers formed at the same time in the WW treatment (Fig. 5). Not only was the flower size smaller, but photographs showed that the anthers in the WS plants did not burst when anthers in the WW plants, initiated at the same time, had burst (Fig. 5).

In Experiment 1, pollen viability *in vitro* was 79.4±3.1% and 82.3±5.2% in WW Rupali and WW Almaz, whereas the corresponding values were 51.3±2.7% when *LWP* was about –1.2 MPa in WS Rupali and 53.1±2.8% when *LWP* was about –2.5 MPa in WS Almaz. In Experiment 2, pollen viability *in vitro* of WW Rupali was similar to that in

Experiment 1, and decreased to 42±3.4% in the WS treatment (Figs 6, 7A) when *LWP* was about –1.2 MPa (Fig. 1D). After 4 h incubation *in vitro*, 76–80% of pollen germinated in the WW treatment, while only 8–10% germinated in the WS treatment (Fig. 7B). The number of pollen grains that germinated *in vivo* on the stigma of hand-pollinated flowers was 12.9±0.65 in WW+WW, 2.48±0.61 in WW+WS, 3.10±1.12 in WS+WW, and 0.60±0.34 in WS+WS treatments. Thirty seven per cent of pollen tubes from pollen from WW plants placed on the stigmas of WW plants reached the ovary in 24 h, but this declined to 22% when pollen from WS plants was placed on the stigmas of a WW plant (Fig. 7C). However, when pollen from either WW or WS plants was placed on the stigmas of WS plants, the number of pollen tubes that reached the ovary was less than 3% (Figs 7C, 8).

Table 2. Percentage of flowers and pods that aborted and seed yield per plant at maturity on primary and secondary branches along the whole branch, and for those produced after treatments were imposed in Experiment 2

Treatments[a]	Primary branch[b]		Secondary branch[b]		Whole plant[b]
	After drought	Whole branch	After drought	Whole branch	
Flower abortion (%)					
CR	29.1 b	19.2 b	39.8 b	17.7 b	18.4 b
SR	52.8 a	32.4 a	68.5 a	50.6 a	39.9 a
Pod abortion (%)					
CR	56.8 b	37.4 a	55.6 b	53.2 a	45.9 a
SR	78.3 a	47.8 a	73.1 a	55.9 a	49.3 a
Seed yield (g plant^{-1})					
CR	1.65 a	3.66 a	2.03 a	2.91 a	6.57 a
SR	0.40 b	2.83 a	0.32 b	0.86 b	3.70 b

[a] C, well-watered treatment; S, water-stressed treatment; R, Rupali.
[b] Values with the same letter within a column are not significantly different (P >0.05).

Seed development

In Experiment 1, the number of seeds per pod in Rupali was 1.4±0.06 in the WW treatment and decreased to 1.2±0.08 in the WS treatment. However, in the kabuli cultivar, Almaz, there was only one seed per pod in both treatments. At maturity, seed size did not differ between treatments in Rupali (Fig. 9A, B), but was significantly different in Almaz (Fig. 9C, D). Seed size tended to decrease in late-set pods compared to early set pods. There was no significant difference in seed size and seed number per pod on primary and secondary branches that podded on the same day.

The withholding of water tended to accelerate seed-filling. In the WW treatment the average seed growth rate during seed-filling was 2.6±0.08 mg seed^{-1} d^{-1} in Rupali and 6.0±0.41 mg seed^{-1} d^{-1} in Almaz; the corresponding values in WS plants were 3.9±0.21 and 7.2±0.43 mg seed^{-1} d^{-1} in Rupali and Almaz, respectively. The smaller seed size in WS Almaz compared to WW treatment was due to a shorter duration of seed growth (Fig. 9D).

Biomass allocation

Compared with Rupali, Almaz tended to allocate more resources to vegetative growth (both above- and below-ground) than to reproductive growth (Fig. 10). In Experiment 1, the seed yield in the WW treatment was 12.3±0.31 g^{-1} plant in

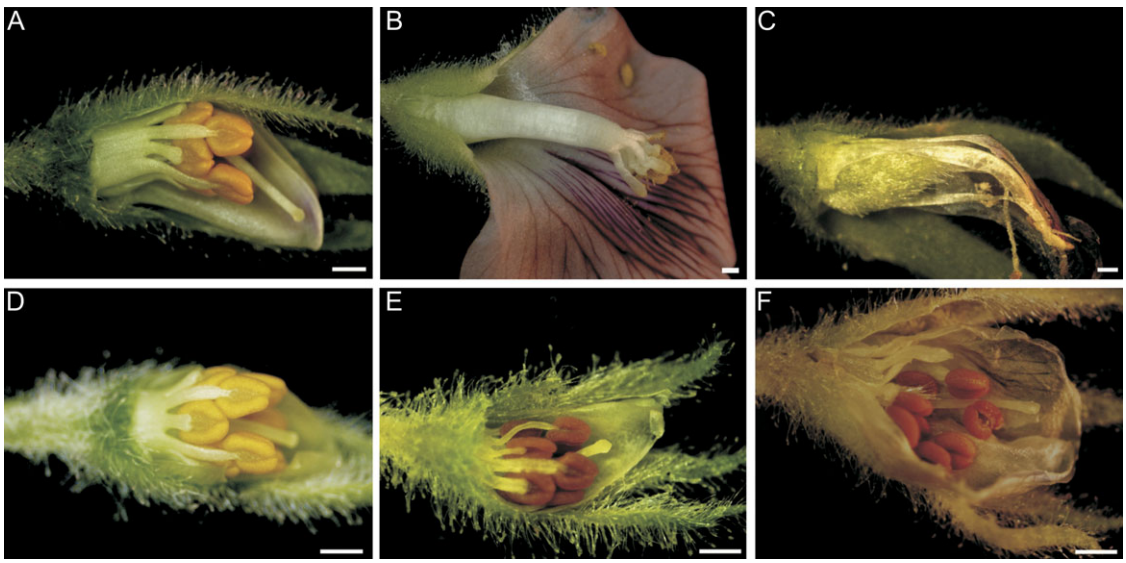

Fig. 5. Flowers of Rupali at three stages of development in well-watered (WW) (A–C) and water-stressed (WS) plants (D–F) that developed at the same time in Experiment 2: (i) 3 d before WW flowers opened (A, D); (ii) at flowering (*LWP* was –1.2 MPa in WS plants) (B, E); and (iii) 3 d after WW flowers opened (C, F). Photographs were taken after the removal of some petals and sepals and show that anthers of flowers in the WS plants did not burst when *LWP* decreased to –1.2 MPa. Scale bar=500 μm (note the smaller magnification in B and C).

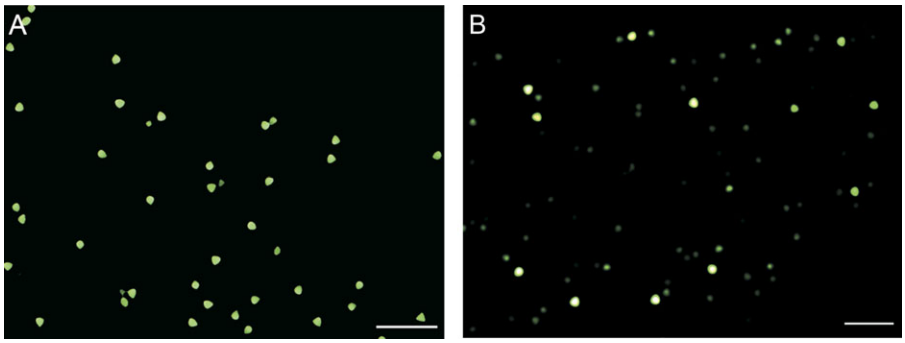

Fig. 6. Pollen viability of Rupali in well-watered (A) and water-stressed (B) treatments when predawn leaf water potential decreased to −1.2 MPa in the WS treatment in Experiment 2. Bright pollen grains are viable while grey pollen grains have lost viability. Scale bar=200 μm.

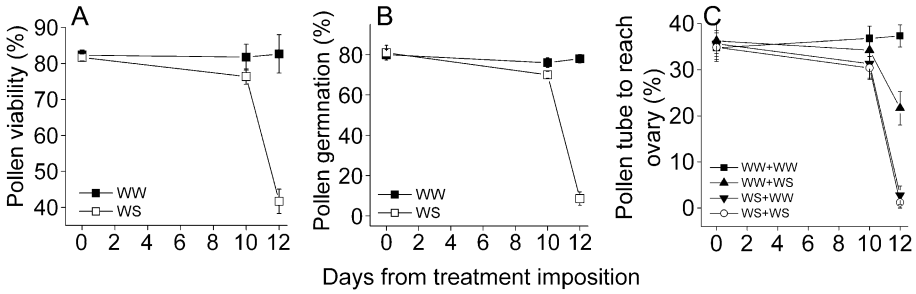

Fig. 7. Percentage pollen viability (A), percentage pollen germination during 4 h culture *in vitro* (B), and percentage of germinated pollen tubes to reach the ovary after 24 h in hand-pollinated flowers *in vivo* (C) in Rupali chickpea in well-watered (WW) and water-stressed (WS) treatments. WW+WW, stigmas of WW plants pollinated with pollen from WW plants; WW+WS, stigmas of WW plants pollinated with pollen from WS plants, WS+WW, stigmas of WS plants pollinated with pollen from WW plants; WS+WS, stigmas of WS plants pollinated with pollen from WS plants. Days are from treatment imposition (Day 0=39 DAS) in Experiment 2. Values are means ±SE (n=10).

Rupali and 7.45±0.56 g^{-1} plant in Almaz (Table 1); the corresponding values in the WS treatment were 4.05±0.51 and 1.12±0.59 g^{-1} plant, a decrease of about 67% in Rupali and 85% in Almaz (Table 1). When seeds were separated into those from flowers formed before and after WS was imposed, seed yield in the WW treatment after WS was imposed was 1.62±0.29 g^{-1} plant in Rupali and 3.22±0.59 g^{-1} plant in Almaz; corresponding values in the WS treatment were 0±0.00 and 0.28±0.08 g^{-1} plant in Rupali and Almaz (Table 1), a decrease of 100% and 91% in Rupali and Almaz, respectively (Table 1). In Experiment 2, seed yield of Rupali in the WW treatment was less than that of Experiment 1 due to the smaller plants produced in summer, but in the WS treatment seed yield was similar in both experiments (Table 2).

Discussion

A major limitation of chickpea in subtropical environments with summer-dominant rainfall or on current rainfall in winter-dominant Mediterranean-type environments is its inability to maintain yield under terminal drought (Davies *et al.*, 1999; Siddique *et al.*, 1999; Leport *et al.*, 1998, 1999). The present study has demonstrated that terminal drought reduced flower and pod production, increased flower and pod abortion and therefore reduced seed yield in both chickpea cultivars, indicating that both flower and pod abortion are important in determining seed yield. Secondly, the study showed that water deficits impaired both pollen and stigma/style function, and the impairment of pistil function was an important factor relating to flower abortion, while, thirdly, it showed that initiation date significantly affected flower and pod development with early-initiated flowers and pods less likely to abort, while late-initiated flowers and pods largely aborted.

Chickpea is generally considered to be an indeterminate annual legume with progressive development of flowers and pods. However, surprisingly in the case of the two cultivars used in this study, the maximum number of flowers produced was about 100 and further flower development ceased even though the plants had sufficient water and temperatures in the greenhouse were modest. Nevertheless, branch growth continued as the plant switched from vegetative to reproductive mode and flowering was initiated, such that the duration from time of first flower and development of water deficit played an important role in determining the number of flowers and pods that produced a seed (Turner, 2003). In this study, total flower production in the WS treatment was reduced by 30% and 62% in Rupali and Almaz, respectively, compared with WW plants.

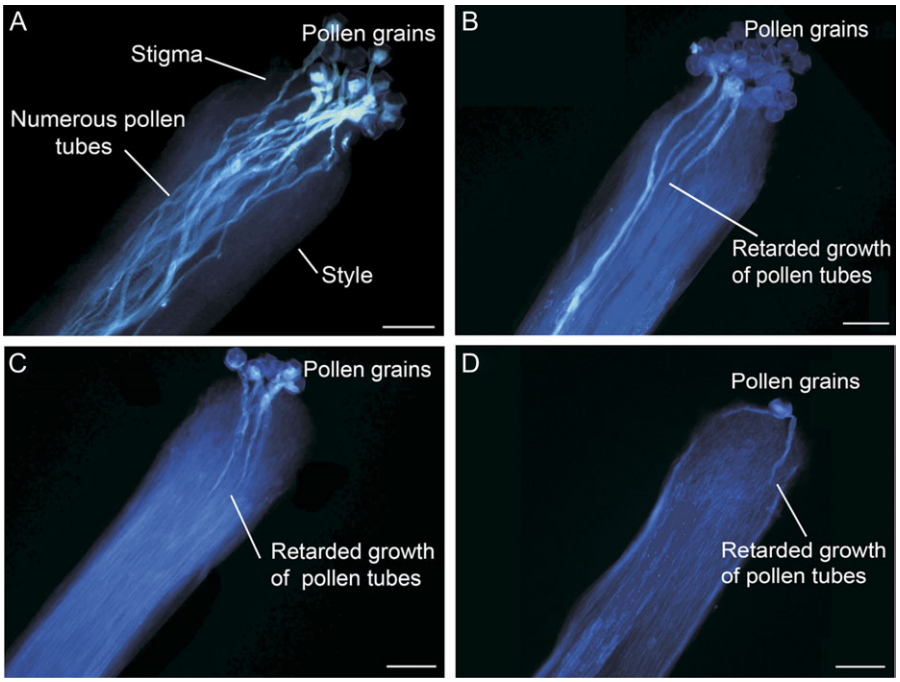

Fig. 8. Pollen tube growth down the style in pistils of (A) well-watered (WW) Rupali chickpeas pollinated with pollen from WW plants (WW+WW); (B) WW plants pollinated with pollen from water-stressed (WS) plants (WW+WS); (C) WS plants pollinated with pollen from WW (WW+WS); and (D) WS plants pollinated with pollen from WS plants (WS+WS) in Experiment 2. Styles were harvested when the predawn leaf water potential of WS plants was –1.2 MPa and then fixed 24 h later and stained with aniline blue. Scale bar=50 μm.

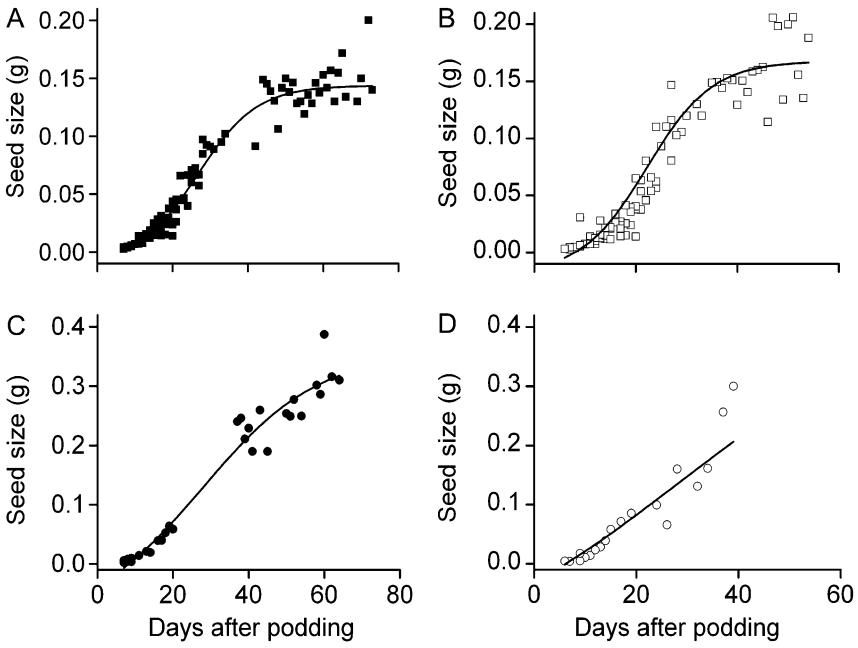

Fig. 9. Change in seed size on primary branches with time after podding in Rupali (A, B) and Almaz (C, D) chickpeas in well-watered (A, C) and water-stressed treatments (B, D) in Experiment 1. Note change of scale of *y*-axis in (C) and (D) from that in (A) and (B).

Once the WS treatment was imposed, WS plants produced one-third of the number of flowers compared with WW controls, and of these flowers, 74% and 83% produced pods under WW conditions, but only 0% and 24% produced pods in the WS treatment in Rupali and Almaz, respectively. Thus, while Leport *et al.* (2006) showed that pod abortion was a key factor influencing seed yield of chickpea when exposed to terminal drought, the present study clearly showed that flower production and abortion is an important factor reducing seed yield. Leport *et al.* (2006) also showed that pod abortion was inherently more sensitive to terminal drought in kabuli chickpeas than in desi chickpea,

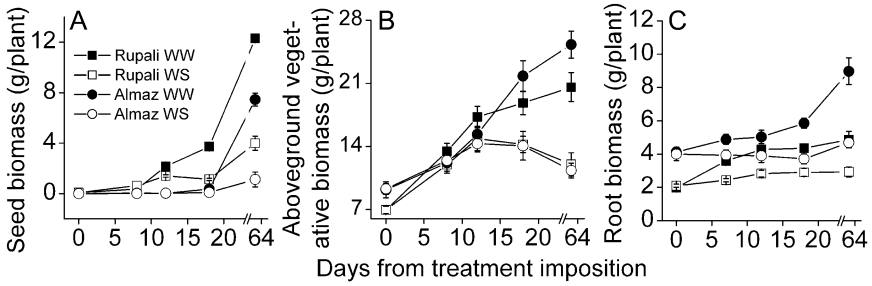

Fig. 10. Change with time after imposition of treatments (Day 0=78 DAS) in (A) seed biomass, (B) above-ground vegetative biomass (sum of leaf, pod, and stem), and (C) root biomass of Rupali and Almaz chickpeas in well-watered (WW) and water-stressed (WS) treatments in Experiment 1. Values are means ±SE (n=4).

irrespective of seed size. In the present study, the kabuli cultivar, Almaz, only produced 34% of the number of pods of the desi cultivar, Rupali, in the WS treatment, and the number of filled pods was reduced even further by pod abortion of 73% in Almaz compared to 54% in Rupali. As a result, the number of filled pods in the kabuli cultivar, Almaz, was only about 20% of that in the desi cultivar, Rupali, in the WS treatment. Further, the effect of terminal drought on pod abortion was much greater on the secondary branches compared to primary branches (Tables 1, 2). This was the result of the earlier pod set on primary branches than secondary branches, the same time of cessation of podding, and the lower likelihood of abortion in response to terminal drought in the early-set compared to late-set pods.

Drought stress induced a decrease in pollen viability and germination *in vitro*, and the pistils of WW plants pollinated with pollen from WS plants had fewer germinated pollen grains and fewer pollen tubes that reached the ovary than those of WW plants pollinated with pollen from WW plants. The decrease in germination in both media suggests that water deficits imposed during floral development had a detrimental effect on the subsequent capacity of pollen to germinate and, as the pollen tube data shows, a detrimental effect on pollen tube growth *in vivo*. Pistils in WS plants hand-pollinated with either pollen from WW or WS plants also had markedly-fewer pollen tubes that reached the ovary compared to those in WW plants crossed with WS plants, suggesting that pollen tube growth was greatly inhibited in the pistils of WS plants. Flower abortion in chickpea induced by a water deficit may be attributed not only to an impairment of pollen viability, but also to an impairment of stigma/style function. Indeed, the much greater number of pollen tubes to reach the ovary in the WW plants than the WS plants, irrespective of whether the pollen was from WS or WW plants suggests that the impairment of the function of the pistil is a more crucial factor inducing flower abortion than the viability of pollen.

A reduction in pollen viability is a common symptom in angiosperms under the stress environments (Porch and Jahn, 2001). For example, water stress during reproductive development in common bean (*Phaseolus vulgaris*) resulted in reduced pollen viability, reduced pollen germination and an abnormal exine with deeply pitted and smooth regions (Shen and Webster, 1986). However, the same phenomenon was not observed in maize as its pollen does not lose viability even at water potentials as low as –12.5 MPa (Westgate and Boyer, 1986). The water potential of flowers (excluding petals) is always lower than that of leaves. For example, the water potential in soybean flowers was nearly 0.2–0.3 MPa lower than that of leaves in a study by Westgate and Peterson (1993) and 0.5 MPa lower in a study by Kokubun *et al.* (2001). Although there are no reports showing the difference in water potential between flowers and leaves in chickpea, between 40% and 50% of the pollen grains from the stressed Rupali lost viability, as assessed by the fluorochromatic reaction, when *LWP* decreased to –1.2 MPa, and only 8–10% of pollen grains germinated *in vitro* within 4 h, showing that not every pollen grain which fluoresced in the assay was capable of germination. A considerable reduction in pollen viability might affect seed set if fewer viable pollen grains are deposited on the receptive stigma and if infertile pollen grains interfere with pollen tube growth. It is also possible that pollen from stressed plants might have a shorter lifespan and have reduced vigour; pollen tube growth may begin, but fail to reach the ovule so that no fertilization takes place (Turner, 1993). However, this study has also shown that the water deficit had a greater effect on pollen growth in the pistil than on pollen viability *in vitro* and that the effect of a water deficit is greater on the stigma/style function than on pollen viability and germination. The present study suggests that the water relations of flowers in chickpea and potential differences among genotypes in the water relations of the flower as water deficits develop is worthy of further investigation.

Although there is little agreement on the exact stage of pod development when abortion occurs, full-sized pods that contain seeds in the linear phase of growth may be relatively resistant to reproductive failure (Duthion and Pigeaire, 1991; Egli, 2005), as seen in Rupali and Almaz aborting fewer early-formed pods. Pods began to abort only under severe drought stress when leaf photosynthetic rate approached 0 μmol m^{-2} s^{-1} in both cultivars, but pod abortion occurred earlier in Rupali than in Almaz. This may be due to more severe resource competition among reproductive tissues in Rupali than in Almaz, as Rupali had

many early-set pods per plant while Almaz had fewer pods when the WS treatment was imposed.

This study has shown that seed size tends to decrease in late-set compared to early-set pods. Similar results have also been reported by Leport *et al.* (2006) in other chickpea cultivars where early water stress reduced average seed size by 28%, and late water stress had no effect compared with a WW control. However, seed growth rate in the WS treatment increased significantly compared with the WW treatment. The higher seed-filling rate may be due to drought-enhanced whole-plant senescence, leading to faster and better remobilization of carbon from vegetative tissues to the seed. Similar findings have been reported in rice (Yang *et al.*, 2001), wheat, (Yang *et al.*, 2004), and barley (Samarah, 2005). For rice and wheat, this was caused by an altered hormonal balance of grains, especially decreased gibberellic acid and increased abscisic acid, induced by soil drying during grain filling, that, in turn, enhanced mobilization of prestored carbon to the seeds and accelerated the seed-filling rate (Yang *et al.*, 2001; Yang and Zhang, 2005).

Finally, the relevance of the results of the present greenhouse study to those observed in the field needs to be considered. While the study was not designed to exactly replicate field conditions, the soil volume was similar to that for field-grown chickpea and temperatures were similar to those in the field in spring in southern Australia. In Experiment 1, the rate of development of water deficits was similar to the development of water deficits in previous greenhouse studies (Leport *et al.*, 2006) and about twice the rate of those in the field (Leport *et al.*, 1998, 1999), whereas, in Experiment 2, in which pollen viability and pistil function were evaluated, the rate of development of water deficit was similar to that in the field. The effect of the WS treatment in the greenhouse was more severe than that in the field with yields from 15% to 33% of those in the WW treatment, whereas in the field yields in the rainfed plots were from 29% to 70% those in the irrigated plots (Leport *et al.*, 1998, 1999; Davies *et al.*, 1999). Pod numbers were also reduced more in the present study, particularly in Almaz, than in the field studies using a different kabuli genotype (Leport *et al.*, 1998, 1999; Davies *et al.*, 1999). As the rate of decrease of water potential in Experiment 2 was similar to that in rainfed chickpea in the field, the resultant influence of the water deficit on pollen viability, pollen germination *in vivo* and pollen tube growth are considered likely to be similar to those in the field. It is clear that the reciprocal crosses provide a way of determining the influence of water deficits on the pistil versus pollen that should be adopted in future studies. If the importance of the water relations of the pistil on fertilization and seed set can be verified in other genotypes, variation in the water relations and pollen growth in pistils among genotypes will be worthy of investigation.

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