

Chickpea genotypes contrasting for seed yield under terminal drought stress in the field differ for traits related to the control of water use

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Abstract. Chickpea (*Cicer arietinum* L.) is often exposed to terminal drought, and deep, profuse rooting has been proposed as the main breeding target to improve terminal drought tolerance. This work tested whether plant water use at the vegetative stage and under non-limited water conditions could relate to the degree of sensitivity of chickpea to terminal drought. Transpiration response to a range of vapour pressure deficits under controlled and outdoor conditions was measured with canopy conductance using gravimetric measurements and thermal imagery in eight chickpea genotypes with comparable phenology and contrasting seed yield under terminal drought in the field. Additionally, the response of plant growth and transpiration to progressive soil moisture depletion was assayed in the same genotypes. Drought-tolerant genotypes had a lower canopy conductance under fully irrigated conditions at the vegetative stage; this trend was reversed at the early pod filling stage. While two sensitive genotypes had high early growth vigour and leaf development, there was a trend of lower growth in tolerant genotypes under progressive soil drying than in sensitive ones. Tolerant genotypes also exhibited a decline of transpiration in wetter soil compared to sensitive genotypes. Canopy conductance could be proxied by measuring leaf temperature with an infrared camera, although the relationship lost sensitivity at the pod filling stage. This work suggests that some traits contribute to water saving when water does not limit plant growth and development in drought-tolerant chickpea. It is hypothesised that this water would be available for the reproduction and grain filling stages.

Additional keywords: early vigour, infrared thermography, leaf conductance, transpiration, vapour pressure deficit.

Introduction

For crop species like chickpea (*Cicer arietinum* L.) that face terminal stress conditions, water availability during the grain filling period is critical. While deeper rooting can increase water extraction, as it has been hypothesised for almost three decades (Saxena 1984; Johansen *et al.* 1994; Krishnamurthy *et al.* 1998; Kashiwagi *et al.* 2005), water availability during the grain filling period could also be explained by a more conservative use of water earlier during the cropping cycle. Therefore, understanding the regulation of leaf water losses, first when there is no water limitation and secondly when plants are progressively exposed to water deficit, are likely to be equally critical to roots for achieving high chickpea yield under terminal drought.

Recent data indicate that terminal drought-tolerant pearl millet (*Pennisetum glaucum* (L.) R. Br.) genotypes had lower canopy conductance under non-limited water conditions, which would save water in the soil profile and make it available for later stages of development (Kholova *et al.* 2010a). Whether differences in canopy conductance exist under non-limited water conditions in chickpea, and whether such putative differences could relate to the sensitivity of chickpea to terminal drought has not been tested. Limiting transpiration under high evaporative conditions (high vapour pressure deficit (VPD)) when water is not limited in the

soil could also contribute to water conservation under terminal drought. In a simulation analysis, Sinclair *et al.* (2005) demonstrated that the imposition of limited maximum transpiration rates increased sorghum (*Sorghum bicolor* (L.) Moench) yields in 76–90% of seasons in a semi-arid environment. Recent evidence indicates that terminal drought-tolerant pearl millet has transpiration rates restricted at a VPD above 2 kPa (Kholova *et al.* 2010b), and similar findings were reported in peanut (*Arachis hypogaea* L.; Devi *et al.* 2010) soybean (*Glycine max* L.; Fletcher *et al.* 2007) and sorghum (Gholipour *et al.* 2010). This trait has so far not been tested in chickpea.

Limitation of the transpiration rate would restrict the evaporative cooling of leaves and increase leaf temperature, which would be highest when atmospheric VPD is greatest (Isoda and Wang 2002). Therefore, proxying transpiration rates from the canopy temperature of plants has great potential as a tool for improved crop management, provided close relationships between the transpiration rate and leaf temperature are found (Jones 1999; Jones *et al.* 2002; Merlot *et al.* 2002; Leinonen and Jones 2004). Thermal imaging was tested here to assess possible relationships with canopy conductance under well-watered conditions in chickpea.

Under progressive exposure to water deficit, leaf area restriction and the transpiration response to soil drying are also key parameters of plant water use (Sadras and Milroy 1996). Leaf area expansion decreases upon water deficit to balance sink demand and plant assimilatory capacity while conferring, to some extent, a conservative pattern of water use (Alves and Setter 2004). In addition, stomata progressively close upon exposure to water deficit, responding to a reduction of leaf water status, to restrict water loss further. The reduction or stoppage of leaf expansion, the closure of stomata at high soil moisture thresholds or both these responses would slow down soil water depletion, and would be beneficial in the case of long drought spells. Genotypic differences exist in leaf gas exchange response to water stress in several crops such as maize (*Zea mays* L.) (Ray and Sinclair 1997), soybean (Vadez and Sinclair 2001; Hufstetler *et al.* 2007) and peanut (Bhatnagar-Mathur *et al.* 2007), although data in chickpea indicate it does not (Leport *et al.* 1999).

The objective of this work was to assess a set of characteristics related to plant water use in chickpea genotypes that have comparable phenological characteristics and contrasting seed yields under terminal drought stress in the field (Krishnamurthy *et al.* 2010). Specifically, the work aimed at: (i) assessing plant growth response to progressive exposure to water deficit; (ii) assessing canopy conductance and the response of canopy conductance to an increase in VPD; (iii) developing and testing a method to assess canopy conductance from thermal imagery and measure canopy conductance differences in contrasting lines at the vegetative and reproductive stages; (iv) develop a matrix of traits discriminating tolerant from sensitive genotypes.

Materials and methods

Plant material and growth conditions

Eight genotypes of chickpea (*Cicer arietinum* L.) with comparable phenology (92 ± 4 days to maturity) and among the most contrasting for seed yield under terminal drought stress in 3 years of field testing (Table 1) were selected from the International Crops Research Institute for Semiarid Tropics (ICRISAT) mini-core collection (Krishnamurthy *et al.* 2010) for characterisation of traits related to water use. The plants were grown in pots of 20 cm diameter and 18 cm height filled with 4 kg of a Vertisol collected from the ICRISAT farm under glasshouse (day:night temperature: 32°C:25°C; relative humidity: 40–80%) and outdoor conditions (max:min temperature: 32.1–27.7°C:16.8–13.8°C; min:max relative humidity: 29.8–42.8%:87.4–94.3%) at ICRISAT, Patancheru,

India (17°30'N; 78°16'E; altitude 549 m) within a period of 3 months starting from early December 2009. This period is the regular chickpea growing season and the outdoor trial was carried out to assess traits related to plant water use under atmospheric conditions that were close to those in the field. Outdoors, the pots were set on benches with the possibility of protecting the pots from rain. In each environment, 25 pots were prepared for each genotype. Three seeds were sown per pot and 10–15 days after sowing, each pot was thinned to a single plant. Pots were kept well-watered for 6 weeks.

Assessment of leaf transpiration rate under different VPD conditions

A measurement of leaf transpiration rate ($\text{g cm}^{-2} \text{h}^{-1}$) was done at 42 days after sowing (DAS) when the plants were at the late vegetative stage, in outdoor conditions over the course of an entire clear day and under natural changes in atmospheric VPD conditions, by sequentially weighing potted plants at regular time intervals, starting in the morning when the VPD was low and until the afternoon when the VPD decreased following the midday peak. Five plants per genotype, grown in outdoor conditions, were saturated 2 days before starting the experiment and allowed to drain overnight. They were bagged the following day with a plastic bag wrapped around the stem to avoid soil evaporation. Plant transpiration was estimated from the loss in weight of each pot. Pots were weighed with a 0.1 g precision scale PM16 (Mettler-Toledo, Schweiz – GmbH, Germany) every hour from 0715 hours to 1740 hours. To calculate atmospheric VPD, temperature and relative humidity were recorded every 15 min using a temperature and relative humidity recorder (Gemini Tinytag Ultra 2 TGU-4500 Dataloggers (UK) Ltd, Chichester, UK), which was positioned within the crop canopy.

At the end of the day, the plants were transferred to a growth chamber where their transpiration response to increasing VPD was assessed the following day under controlled conditions, using a scale of increasing VPD conditions ranging from 0.45 to 3.4 kPa, with an exposure of 45 min at each VPD. Transpiration of each genotype was estimated from the loss in pot weight after the 45-min exposure to a given VPD. It took ~5 min to weigh the pots and that time was used to increase the VPD to the next level on the scale. Both measurements (outdoors and growth chamber) were made under well-watered conditions in five homogenous plants of each genotype. The radiation to which plants were exposed varied in outdoor conditions, while it was lower but constant in the growth chamber ($\sim 600 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Another measurement of the leaf transpiration rate ($\text{g cm}^{-2} \text{h}^{-1}$) was performed outdoors at 66 DAS when the

Table 1. Variation of phenology (50% flowering and maturity, days) and drought tolerance index across chickpea genotypes, contrasting for terminal drought tolerance

Data are means of 3 years of field experiments (Krishnamurthy *et al.* 2010). The drought tolerance index represented the residual yield variations that were not explained by differences in flowering time and yield potential. T, tolerant; S, sensitive

	Genotypes							
	ICC867 T	ICC3325 T	ICC3776 S	ICC4814 S	ICC7184 S	ICC8058 S	ICC14778 T	ICC14799 T
Average 50% flowering	44.5	48.1	47.1	48.8	51.8	45.9	51.2	48.1
Maturity	87.6	89.9	92.4	92.1	96.3	95.6	92.0	89.9
Drought tolerance index	0.75	0.69	-0.70	-0.54	-0.9	-0.80	0.90	0.60

plants were at the early podding stage. The measurements were made over the course of an entire clear day under the natural changes in atmospheric VPD conditions.

The plants were harvested at the end of the transpiration measurement. Leaf area was determined by detaching each individual leaflet before scanning and analysing with WinRhizo software (WinRhizo, Regent Ltd, Quebec, Canada). Shoot, root and leaf DWs were recorded after placing the samples for 48 h in a 70°C oven. The transpiration rate (g water loss cm⁻² h⁻¹) was computed by dividing the transpiration by the total leaf area.

Canopy temperature

In the transpiration rate measurements at 42 DAS and 66 DAS, canopy temperatures of the genotypes were measured from thermal images obtained with an infrared (IR) FlexCam S (Infrared Solutions, Plymouth, MN, USA) with a sensitivity of 0.09°C and an accuracy of ± 2%. The images were taken outdoors at the highest atmospheric VPD of the day and in the growth chamber at the highest imposed VPD. SmartView 2.1.0.10 software (Fluke Thermography Everett, WA, USA) was used for the analysis of the thermal images and the estimation of canopy temperatures.

Estimation of canopy conductance

The index of canopy conductance (I_g) was used as an indirect estimation of the absolute canopy conductance (Jones 1999, modified). From the canopy temperature, I_g was estimated as:

$$I_g = \frac{T_{\text{dry}} - T_{\text{canopy}}}{T_{\text{canopy}} - T_{\text{wet}}},$$

where T_{wet} is the temperature of a wet surface, T_{dry} is the temperature of a non-transpiring surface and T_{canopy} is the canopy temperature measured with the infrared camera. T_{wet} was measured on green leaves after soaking them with water for 5 min and T_{dry} was the temperature of dry leaves. These temperatures were measured under outdoor conditions after the end of the experiment, using green and dried leaves from extra plants of all genotypes, which were pooled to make the measurements.

Transpiration response to progressive soil water depletion

Two dry-down experiments were initiated in the glasshouse and outdoors at 42 DAS to estimate whether the soil moisture threshold where transpiration declines varied with genotypes. Late in the afternoon of 17 January 2010, all pots were saturated with water and allowed to drain overnight. The following morning, each pot was enclosed in a white plastic bag that was wrapped around the base of the stem, and pots were subsequently weighed. The experimental design was a randomised complete block design with two water treatments (well-watered and water stress) as the main factors and genotypes as the sub-factors with five replications. Each morning, the pots were weighed. Five pots of each genotype were maintained in a well-watered condition by watering the soil daily to return the soil to ~80% of pot capacity. Five pots of each genotype were allowed to dry progressively over approximately a 2-week period. Water was added to the drying pots if needed so that there was only a maximum of 70 g net

loss of water each day. The transpiration values were normalised as described previously (Kholova *et al.* 2010a) to facilitate comparison. In short, a transpiration ratio (TR) was obtained by dividing each individual transpiration value by the mean of the transpiration of the well-watered control, and this was done for each genotype. Then a normalised TR (NTR) was obtained by dividing each TR value by the average of the TR values obtained in the second, third and fourth days of the experiment, before plants were stressed (the first day of transpiration was quite erratic, probably because of recent pot saturation, and was not used). The experiment was terminated for each plant subjected to water deficit when the NTR was less than 0.1. At the end of the experiment, plants were harvested for measurement of the green leaf area and the DWs of shoots, roots and leaves, including the few leaves that were shed in the water stress treatment. After harvest, the fraction of transpirable soil water (FTSW) for each day of the experiment was calculated. The FTSW values represent the portion of the remaining volumetric soil water available for transpiration on each day of the experiment, and were used as the indicator of stress (Ritchie 1981).

FTSW on Day n was calculated as:

$$\text{FTSW} = \frac{\text{Weight}_n - \text{Weight}_{\text{final}}}{\text{Weight}_{\text{initial}} - \text{Weight}_{\text{final}}}.$$

Transpiration efficiency

Transpiration efficiency (TE) was calculated by dividing the increase in biomass during the dry-down experiment by the total water transpired during the same period of time. Plant biomass increase was obtained by subtracting the biomass of plants used for the transpiration rate response to VPD and harvested before the beginning of the dry-down from the biomass of plants at the end of the dry-down experiment. The total transpiration was obtained by adding all daily transpiration values.

Statistical analysis

For plant growth parameters, one-way ANOVA was carried out to test for genotypic differences within each treatment. For the analysis of dry-down data and the calculation of the FTSW threshold analysis, SAS software (SAS Institute, Cary, NC, USA) was used. Values of NTR and FTSW obtained during the dry-down experiments for all plants within each genotype were combined to calculate the FTSW thresholds where NTR initiates its decline, using a plateau regression procedure as described previously (Ray and Sinclair 1998). The plateau regression procedure carried out iterations of the NTR data, starting at FTSW=1 (wet soil) and fitted them to a $y=1$ equation. After the FTSW level where $y=1$ was no longer the best fit for NTR, data were fitted to a linear decline equation. The FTSW threshold (with confidence interval) where NTR begins to decline is then taken as the intersection between the plateau ($y=1$) and linear decline equations. The transpiration response to VPD in the growth chamber was analysed with the split line regression of GENSTAT 9.0 (VSN International Ltd, Hemel Hempstead, UK), which provides a break point value where the slope of the fitted regression changed significantly.

Results

Effect of water stress exposure on growth parameters and transpiration efficiency

Under glasshouse conditions

At 42 DAS (before dry-down), growth parameters varied significantly among the genotypes (Table S1, available as an Accessory Publication to this paper). ICC8058 had the highest shoot biomass followed by ICC4814 and ICC3776. The four drought-tolerant genotypes were among those with the smallest shoot biomass. There was no important variation for root DW, although ICC8058 and ICC3325 tended to show the highest values. Leaf DW was the highest in ICC8058, followed by ICC867, ICC3325 and ICC3776.

In well-watered plants (control) the shoot DW of ICC867, ICC3325 and ICC7184 was lower than that of ICC3776 and ICC8058 (Table S1). For root and leaf biomass, there was no important genotypic variation. Similarly, the total transpiration did not show any important variation, except that ICC8058 had the highest water uptake. Transpiration efficiency was highest in ICC14778 and ICC14799, and lowest in ICC3325 and ICC7184; the remaining genotypes had similar values.

Under water stress, ICC14799, ICC14778 and ICC3325 had lower shoot DW than ICC4814 and ICC8058. For root biomass, there was no important genotypic variation between genotypes, except that ICC3776 and ICC4814 had lower root biomass than the remaining genotypes. The relatively highest leaf growth restriction due to water stress was recorded mostly in ICC14778, ICC14799, ICC4814 and ICC7184 (28–41% of leaf biomass reduction). The total transpiration did not show any

important variation, except for ICC8058, which had a lower water uptake than all other genotypes. There was no significant variation of TE. The leaf expansion rate during the duration of the dry-down varied largely among genotypes (Table S1).

Therefore, in the glasshouse, under well-watered and, to some extent, under water stress conditions, there was a trend of higher shoot biomass in drought-sensitive genotypes than in the tolerant ones.

Under outdoor conditions

At 42 DAS (before dry-down), ICC4814, ICC14778 and ICC14799 had lower shoot, root and leaf DW than all other genotypes, whereas ICC8058 had the highest shoot and root DW, followed by ICC3325, ICC3776 (except for root DW), ICC867 and ICC7184 (Table 2). ICC8058 and ICC867 had the highest leaf DW values, followed by ICC3325.

In control plants, the highest biomass was recorded in ICC7184, followed by ICC8058 and ICC3325. Root and leaf DW did not show any important variation among the genotypes. Transpiration efficiency was the highest in ICC867 and ICC3325, and the lowest in ICC8058 and ICC14778. Although total TR changed little among the drought-tolerant genotypes, ICC867, ICC3325, ICC14778 and ICC14799 tended to have the lowest values. The specific leaf area (SLA) of well-watered plants at the end of the dry-down (56 DAS) tended to be higher in the sensitive than in the tolerant genotypes (Table 2).

Under water stress, all sensitive genotypes except ICC7184 had higher shoot biomass than the tolerant genotypes (Table 2). The lowest root development was recorded in ICC4814, ICC7184

Table 2. Dry mass (g per plant) of shoots, roots and leaves, specific leaf area ($\text{cm}^2 \text{g}^{-1}$), transpiration efficiency (g biomass kg^{-1} water transpired), total water transpired during the dry-down (kg per plant) and leaf expansion rate (cm^2 per day) of chickpea genotypes contrasting for terminal drought tolerance grown outdoors under well-watered (control) and water stress conditions

Values are means of five replicates for each genotype. Genotypes followed by same letter are not significantly different. LER, leaf expansion rate; SDW, shoot dry weight; RDW, root dry weight; LDW, leaf dry weight; SLA, specific leaf area; TE, transpiration efficiency; total TR, total water transpiration; T, tolerant; S, sensitive

	ICC867 T	ICC3325 T	ICC3776 S	ICC4814 S	ICC7184 S	ICC8058 S	ICC14778 T	ICC14799 T
<i>Pre-dry down</i>								
SDW (g)	5.22 ^b	5.29 ^b	5.27 ^b	4.35 ^c	5.21 ^b	6.14 ^a	3.72 ^d	4.26 ^c
RDW (g)	2.23 ^{ab}	2.37 ^a	1.68 ^{cd}	1.18 ^e	2.48 ^a	2.47 ^a	1.30 ^{de}	1.88 ^{bc}
LDW (g)	2.95 ^b	2.69 ^{bc}	2.48 ^{cd}	2.17 ^{ef}	2.44 ^{cde}	3.29 ^a	1.96 ^f	2.36 ^{de}
<i>Control</i>								
SDW (g)	16.34 ^c	17.03 ^b	16.70 ^{bc}	15.93 ^c	20.76 ^a	17.75 ^b	12.15 ^d	15.83 ^c
RDW (g)	6.62 ^{ab}	7.86 ^a	6.12 ^{ab}	5.73 ^b	5.97 ^{ab}	6.73 ^{ab}	7.09 ^{ab}	5.78 ^b
LDW (g)	7.22 ^{bc}	7.50 ^{bc}	7.15 ^c	6.94 ^c	8.11 ^{ab}	8.69 ^a	6.66 ^{bc}	7.69 ^{bc}
SLA	194.97 ^{ab}	177.52 ^b	172.62 ^b	224.53 ^a	212.66 ^{ab}	236.10 ^a	176.55 ^b	190.39 ^{ab}
TE	5.52 ^{ab}	5.69 ^a	4.38 ^c	4.92 ^{bc}	4.93 ^{bc}	4.17 ^d	4.79 ^{bc}	4.86 ^{bc}
Total TR	2.86 ^c	3.09 ^{bc}	3.66 ^{ab}	3.35 ^{abc}	3.88 ^a	3.90 ^a	3.01 ^{bc}	3.16 ^{bc}
<i>Water stress</i>								
SDW (g)	8.71 ^d	10.38 ^c	12.12 ^b	11.68 ^b	10.30 ^c	13.20 ^a	9.67 ^c	8.70 ^d
RDW (g)	2.99 ^{abc}	3.61 ^a	3.58 ^a	2.83 ^c	2.67 ^c	3.50 ^{ab}	2.75 ^c	2.85 ^{bc}
LDW (g)	4.41 ^c	5.36 ^{bc}	5.78 ^b	5.59 ^{bc}	4.92 ^{cde}	6.87 ^a	5.17 ^{bcd}	4.52 ^{de}
TE	1.89 ^c	2.94 ^b	3.86 ^{ab}	4.28 ^a	2.27 ^c	3.38 ^{ab}	3.05 ^b	2.21 ^c
Total TR	2.48	2.26	2.37	2.16	2.34	2.51	2.50	2.35
LER	14.13 ^d	19.29 ^d	40.46 ^b	66.09 ^a	62.60 ^a	66.89 ^a	22.34 ^{cd}	31.57 ^{bc}

ICC14778 and, to some extent, ICC14799, whereas leaf DW was the highest in ICC8058 and ICC3776. The highest TE was recorded in ICC4814, ICC8058 and ICC3776, and the lowest in ICC867, ICC7184 and ICC14779. Total TR did not show any significant variation. In addition, tolerant lines had a lower leaf expansion rate than in the sensitive ones during the course of the dry-down (Table 2).

Overall, in outdoor conditions, growth at 42 DAS, total water used for transpiration under well-watered conditions and growth upon progressive exposure to water deficit were lower in the drought-tolerant genotypes than in the sensitive ones.

Response of leaf gas exchange to progressive exposure to water deficit

In the glasshouse, the transpiration started declining at FTSW values ranging between 0.35 and 0.63 (Table 3; Fig. S1) – a fairly high range of variation. ICC14778 had the lowest threshold among genotypes (0.35) followed by ICC4814, ICC3776 and ICC867, whereas ICC8058 showed the highest FTSW threshold (0.63). However, there was no discrimination for this parameter between the tolerant and susceptible genotypes. Unlike in the glasshouse, a lower range of FTSW threshold values was recorded under outdoor conditions (0.25–0.43) (Table 3; Fig. S1). However, the sensitive genotypes had lower FTSW thresholds (below 0.31) than the tolerant ones, except for ICC7184 and ICC14778. The lowest value was recorded in ICC4814, followed by ICC3776 (0.25 and 0.30 respectively).

Response of leaf transpiration to changing VPD

Under outdoor conditions at the vegetative stage

Calculated atmospheric VPD varied between 0.23 and 5.1 kPa during the course of the day, with the highest recorded at around

Table 3. Statistical analysis of data showing the FTSW threshold where transpiration declines upon exposure to progressive water deficit in chickpea genotypes contrasting for terminal drought tolerance grown under glasshouse and outdoor (ambient climatic conditions at ICRISAT, Patancheru) conditions

T, tolerant; S, sensitive; SE, standard error; CI, confidence interval

Environment	Genotype	FTSW threshold	Approximate SE	95% CI
Glasshouse	ICC867 (T)	0.402	0.0233	0.356–0.447
	ICC3325 (T)	0.558	0.0291	0.500–0.616
	ICC3776 (S)	0.406	0.0281	0.350–0.462
	ICC4814 (S)	0.453	0.0376	0.378–0.528
	ICC7184 (S)	0.498	0.0250	0.449–0.548
	ICC8058 (S)	0.631	0.040	0.511–0.631
	ICC14778 (T)	0.347	0.0312	0.285–0.409
	ICC14799 (T)	0.501	0.0313	0.439–0.564
Outdoors	ICC867 (T)	0.414	0.0313	0.353–0.475
	ICC3325 (T)	0.427	0.0321	0.366–0.489
	ICC3776 (S)	0.304	0.0223	0.260–0.349
	ICC4814 (S)	0.253	0.0199	0.213–0.293
	ICC7184 (S)	0.341	0.0196	0.302–0.380
	ICC8058 (S)	0.299	0.0171	0.265–0.334
	ICC14778 (T)	0.362	0.0219	0.318–0.406
	ICC14799 (T)	0.369	0.0238	0.352–0.417

1530 hours (Fig. 1a). There was genotypic variation in TR across all the VPD conditions. Clearly, there was a tendency to have a higher overall TR in sensitive genotypes (ICC4814, ICC8058 and ICC3776) than in tolerant ones (ICC867, ICC14799 and ICC3325). At a VPD of 3.48 kPa, ICC4814 showed a higher TR (21.6 mg H₂O cm⁻² h⁻¹) than ICC867 and ICC14799 (13.3 and 12 mg H₂O cm⁻² h⁻¹, respectively). Likewise, the TR of ICC8058 was higher than that of ICC14799. The remaining genotypes presented quite similar transpiration rates. At a VPD of around 4 kPa, sensitive ICC4814 and ICC3776 had a higher TR than the tolerant ICC867 and ICC14799, with an increase of 19% and 24% respectively. Additionally, the TR of sensitive ICC4814 was higher than in the tolerant ICC3325. ICC4814 had the highest TR among all genotypes except ICC8058 and ICC14778. The largest variation was recorded at 1310 hours, the VPD being just above 4 kPa. During this period, the TR of ICC4814, ICC8058 and ICC14778 was higher than that of ICC867 and ICC14799. Additionally, ICC4814 transpired more than all the genotypes except ICC8058 and ICC14778. After 1310 hours, the TR of all the genotypes decreased, and ICC867, ICC14799 and, to some extent, ICC3325 had a lower TR than ICC4814. Similar observations were made at 1510 hours.

Under controlled conditions at the vegetative stage

Over the whole range of tested VPD, the transpiration rate was higher in the sensitive genotypes ICC8058 and ICC4814 (above 27 mg H₂O cm⁻² h⁻¹) than in the tolerant ICC867 and ICC14799 (less than 17 mg H₂O cm⁻² h⁻¹; Table 4). With increasing VPD, the transpiration rate of all the genotypes showed an increase that varied with genotypes and the applied VPD. For all genotypes except ICC4814 and ICC8058, there was no break point in the transpiration response to VPD (Fig. 2). Genotypes having no break point had similar slopes of increase of transpiration to VPD, except ICC14778, which had a steeper slope. The slope of the transpiration response to VPD below the break point was steeper in the sensitive genotypes ICC4814 and ICC8058 than in the other genotypes. At the lowest VPD (0.45 kPa), differences in TR among genotypes were small. By contrast, at the highest VPD (3.4 kPa), the range of variation in TR was from 22 mg cm⁻² h⁻¹ for ICC14799 to 37 mg cm⁻² h⁻¹ for ICC4814. In accordance with the outdoor measurements, the tolerant genotypes ICC14799, ICC867 and ICC3325 had lower transpiration rates, on average, across the VPD conditions than the sensitive ICC4814, ICC8058 and, to some extent, ICC3776 (Table 4).

Under outdoor conditions at the early podding stage

The transpiration rate was measured under naturally increasing VPD in outdoor conditions that varied between 0.30 and 5.48 kPa (Fig. 1b). Unlike the experiment done at the vegetative stage, tolerant genotypes tended to have higher TRs than the sensitive ones. Over the whole range of VPD values, sensitive ICC8058, ICC7184 and, to a lesser extent, ICC4814 had the lowest TR. The remaining genotypes exhibited a transpiration rate higher than 18 mg cm⁻² h⁻¹. The largest variation was recorded with the VPD ranging between 3.87 and 5.15 kPa. The TR of the tolerant genotypes was, in fact, fairly similar to the level of the vegetative stage assessment,

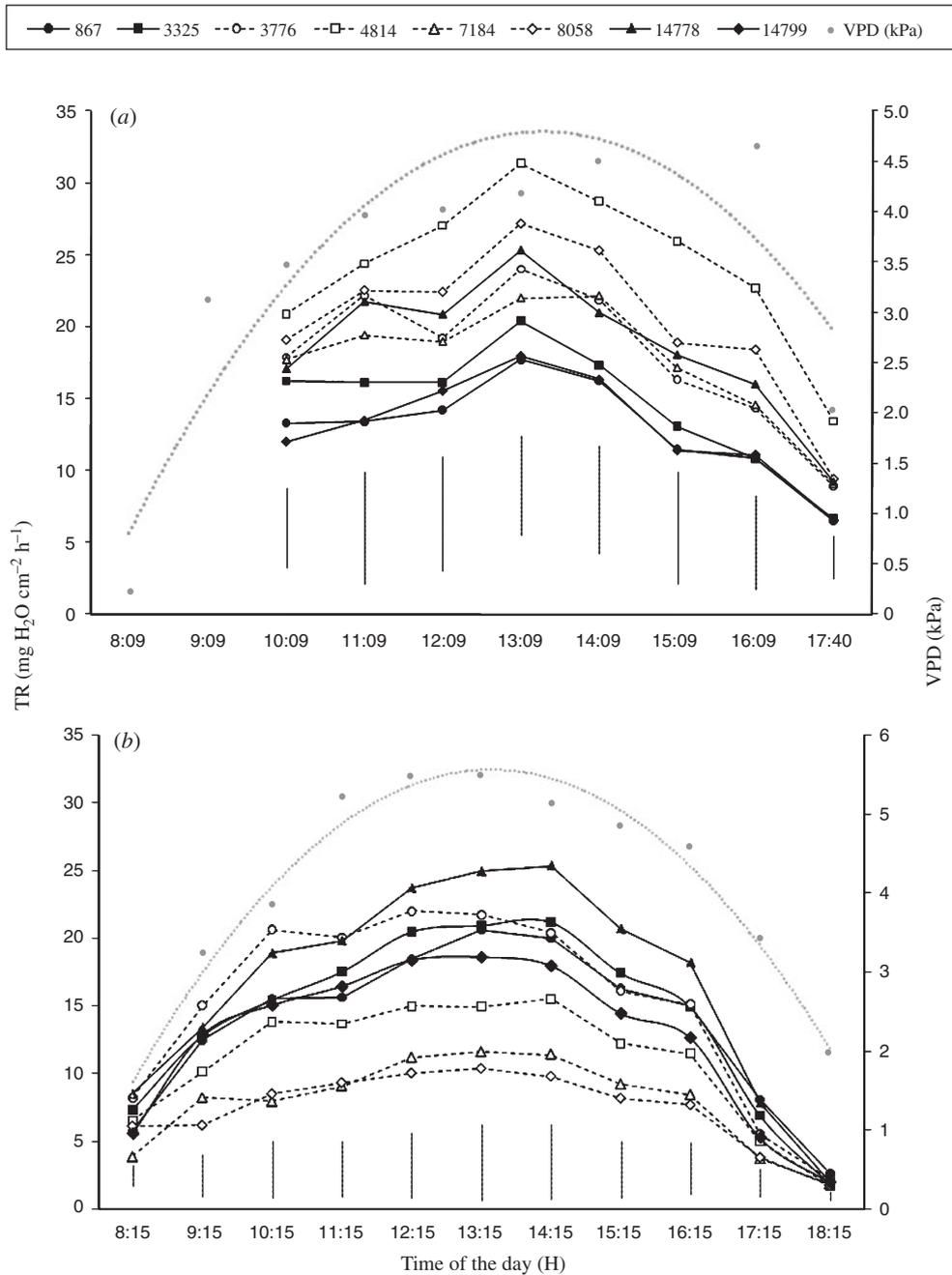


Fig. 1. Transpiration rate (TR; $\text{mg H}_2\text{O cm}^{-2} \text{h}^{-1}$) under well-watered conditions in chickpea genotypes contrasting for terminal drought tolerance (tolerant, black symbols and solid lines; sensitive, open symbols and dashed lines) exposed to the variation of atmospheric vapour pressure deficit (VPD) regimes over an entire day. Plants were grown outdoors and assessed at the (a) vegetative (42 DAS) and (b) early podding stages (66 DAS). The bar at each measurement time indicates the LSD for genotypic means. The dotted line represents the fitting of VPD over the course of the day.

i.e. $\sim 15\text{--}20 \text{ mg cm}^{-2} \text{h}^{-1}$ on average. The TR of sensitive lines decreased from $20\text{--}25 \text{ mg cm}^{-2} \text{h}^{-1}$ to $10\text{--}15 \text{ mg cm}^{-2} \text{h}^{-1}$.

Canopy conductance assessment from thermal imagery

It is difficult to assess the leaf area in crops like chickpea, and a method was developed to assess the canopy conductance

through a comparison of canopy temperature differences between genotypes. The first step was to separate the temperature range of interest (leaves) from the background thermal image (Fig. 3a). This consisted of obtaining a temperature distribution of all areas in the image. It was considered that the temperature distribution across the leaves would follow a normal distribution. A temperature threshold

Table 4. Regression results for the transpiration response of 42-day-old chickpea genotypes, contrasting for terminal drought tolerance to increasing VPD conditions in the growth chamber under well-watered conditions

Genotypes were found to fit either a two-segment linear regression or a linear regression model with no break point. SE, standard error; Slope a, regression at low VPD below break point; Slope b, regression at high VPD above break point; T, tolerant; S, sensitive; TR, transpiration ratio

Genotypes	Mean TR (mg H ₂ O cm ⁻² h ⁻¹)	Breakpoint		Segmented regression				R ²
		Value (kPa)	SE	Slope a		Slope b		
		Value (kPa)	SE	Value (mg H ₂ O cm ⁻² h ⁻¹ kPa ⁻¹)	SE	Value (mg H ₂ O cm ⁻² h ⁻¹ kPa ⁻¹)	SE	
ICC4814 (S)	27.22	2.540	0.411	10.77	1.91	1.61	4.43	0.77
ICC8058 (S)	29.11	2.553	0.250	11.49	2.06	-4.69	4.79	0.81
		Linear regression		Value (mg H ₂ O cm ⁻² h ⁻¹ kPa ⁻¹)		SE		
ICC867 (T)	16.17	No break point	-	4.91	0.42	-	-	0.74
ICC3325 (T)	19.95	No break point	-	5.91	0.61	-	-	0.71
ICC3776 (S)	26.38	No break point	-	5.39	0.65	-	-	0.63
ICC7184 (S)	21.81	No break point	-	5.82	0.48	-	-	0.81
ICC14778 (T)	21.83	No break point	-	7.18	0.62	-	-	0.80
ICC14799 (T)	16.81	No break point	-	4.50	0.49	-	-	0.68

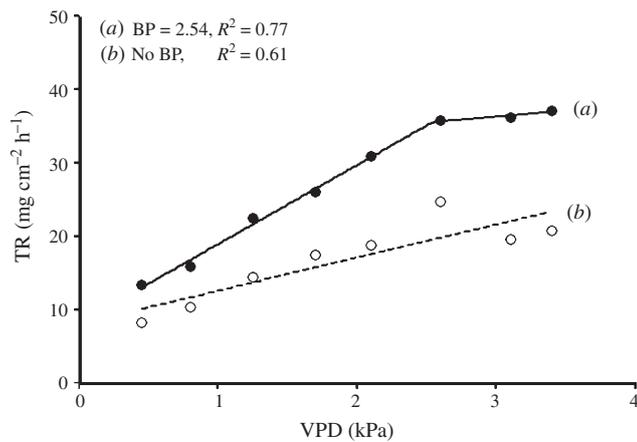


Fig. 2. Transpiration rate (TR; mg H₂O cm⁻² h⁻¹) under well-watered conditions of (a) sensitive ICC4814 and (b) tolerant ICC14799 chickpea genotypes exposed to increasing VPD regimes under controlled conditions in a growth chamber. Plants were assessed at the vegetative stage and each point represents the mean of four replicates.

was then taken past this normal distribution (Fig. 3b), and this colour threshold was used to remove background colours (Fig. 3c). As can be seen in Fig. 3c, the remaining pixels corresponded mostly to the canopy. The repartition of the number of pixels relating to the range of canopy temperatures was then used to compute an average canopy temperature.

Based on the distribution of the thermal image pixels (after removing the background temperature range) with temperature, the average temperature was calculated as follows:

$$T_m = \sum T_i \frac{PX_i}{PX_t}$$

where PX_i is the number of pixels for a given temperature T_i and PX_t is the total number of pixels for all the temperatures in the range covering the whole canopy (after removing the background temperature range).

Sensitivity test

To test the level of precision of the method, the upper limit of the range of temperatures covering the whole canopy was moved by one or two units on the temperature scale, and the estimation of the canopy temperature was recalculated and compared with the first one. This test was performed for five randomly chosen genotypes (Table S2). Increasing the colour threshold by one or two units on the temperature scale increased the temperature by 0.15 to 0.22°C in these genotypes, thus keeping the ranking of genotypes very similar. Therefore, the method was fully valid to compare genotypes for their canopy conductance based on their canopy temperatures.

Estimation of canopy conductance under ambient climatic conditions

Thermal images were used to estimate the canopy temperatures across the genotypes grown in outdoor conditions at 42 DAS and 56 DAS. At 42 DAS, the canopy temperatures ranged between 28.2°C for ICC4814 and 31.0°C for ICC3325 (Fig. 4a). The genotypes ICC8058, ICC14778, ICC7184, ICC3776 and, to a greater extent, ICC4814 were relatively cooler than the remaining genotypes. The canopy temperature differed between ICC4814 and ICC3325 (by 3.2°C), and between ICC4814 and ICC867 or ICC14799 (2.2°C). A quite similar trend was recorded at 56 DAS, with the sensitive genotypes showing lower canopy temperatures overall (data not shown). The canopy temperatures ranged between 27°C and 30.5°C (data not shown).

The index of canopy conductance (I_g) used as an indirect estimation of the absolute canopy conductance ranged between 1.8 in ICC3325 and 3.6 in ICC4814 (Fig. 4b). The tolerant genotypes ICC14799, ICC867 and ICC3325 had a lower I_g (<2.1) than the remaining genotypes (>2.5). Among the tolerant genotypes, only ICC14778 had an I_g in the range of the sensitive genotypes.

Estimation of canopy conductance under controlled conditions

Despite the possible problem of infrared reflection in closed environments, and the risk that the temperature reading from

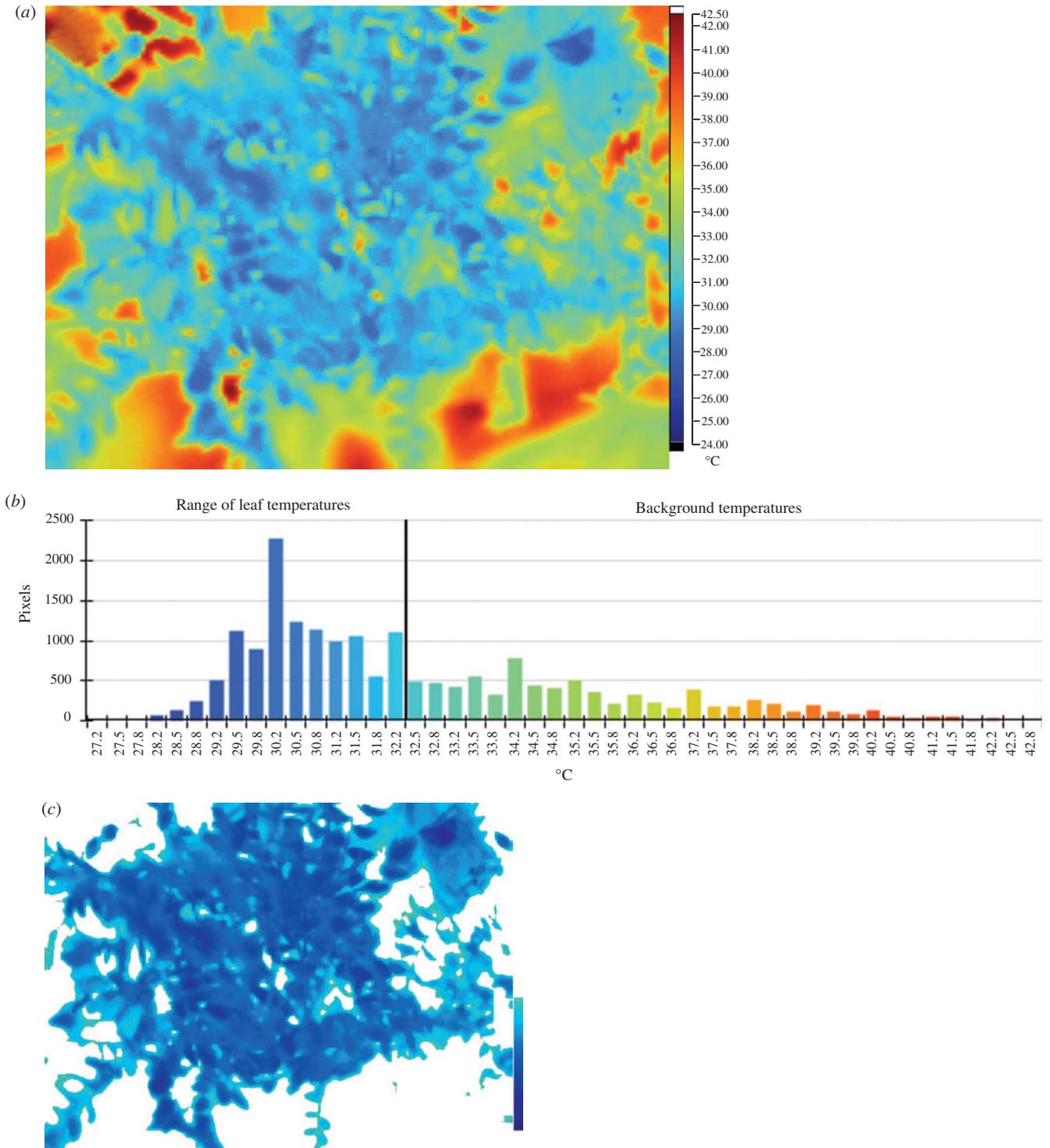


Fig. 3. Thermal image of chickpea canopy (a) before and (c) after removing the background using a colour threshold and (b) pixels distribution for the range of temperatures.

leaves might be ‘contaminated’ by reflection, canopy temperatures were also measured in the growth chambers, using 42-day-old plants. Variation among genotypes was quite different from that recorded under natural conditions, but the

genotypes ICC4814, ICC8058 and, to a lesser extent, ICC3776 and ICC7184 were cooler than the remaining genotypes (data not shown). The difference in canopy temperature ranged between 1.8°C and 5.8°C. The highest I_g values were recorded in ICC4814

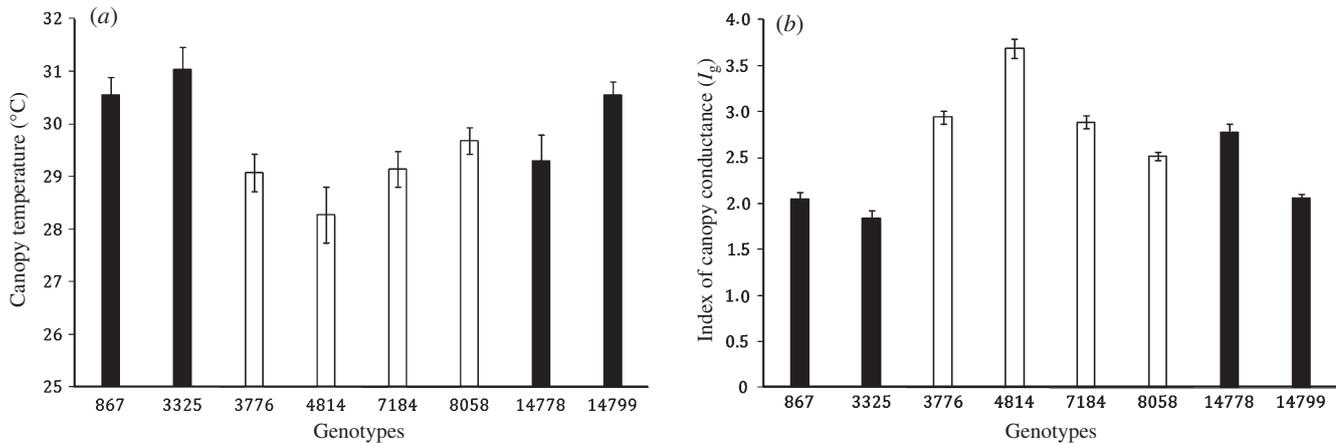


Fig. 4. Variation of (a) canopy temperature (°C) and (b) index of canopy conductance (I_g) in chickpea genotypes contrasting for terminal drought tolerance (tolerant, black bars; sensitive, white bars) grown outdoors under well-watered conditions. Measurements were made at 42 DAS under outdoor conditions at the time of the day that had the highest VPD conditions.

and ICC8058, followed by ICC3776, ICC7184 and ICC14778 (data not shown).

To assess the viability of the thermal approach for deriving genotypic variation in canopy conductance, the transpiration rate was plotted against the canopy temperature (Fig. 5a), and a significant negative correlation was found both at 42 and 56 DAS, although the relationship at 56 DAS was weaker than at 42 DAS ($R^2 = 0.46$) (Fig. 5b).

The transpiration rate also correlated positively with the estimated index of canopy conductance (I_g data not shown at 56 DAS).

Estimation of canopy conductance at later stages of plants grown under well-watered conditions

Measurements were made when the plants were at the early podding stage (66 DAS) (Fig. S2). The canopy temperatures ranged between 29.22°C and 32.06°C. ICC8058 and ICC7184 had relatively hotter canopy temperatures (above 31.5°C) compared with the remaining genotypes, particularly ICC867 and ICC14778, whose canopy temperatures were below 30°C. These results contrast with those obtained at the vegetative stage, where ICC867, ICC14799 and ICC3325 were the genotypes

having hotter canopy temperatures. However, at that stage, there was a much weaker relationship between measured canopy temperature and transpiration rates ($R^2 = 0.21$).

Discussion

Under glasshouse conditions, none of the traits related to plant water use discriminated drought-tolerant from sensitive lines; however, assessment in outdoor conditions revealed several discriminatory traits. Outdoors, tolerant genotypes had a lower canopy conductance under fully irrigated conditions during the vegetative stages than sensitive ones, and this trend was reversed at the early pod filling stage. Upon progressive exposure to water deficit, tolerant genotypes also had a decline of transpiration in wetter soil than sensitive genotypes. While two sensitive genotypes clearly had high early growth vigour and leaf development, all tolerant genotypes (except one) had lower growth under progressive soil drying than sensitive ones. Canopy conductance could be proxied by measuring leaf temperature with an infrared camera, although the relationship lost sensitivity at later stages, especially the early pod filling stage. Genotypes behaved somewhat differently in glasshouse conditions compared to outdoors. While there is no clear

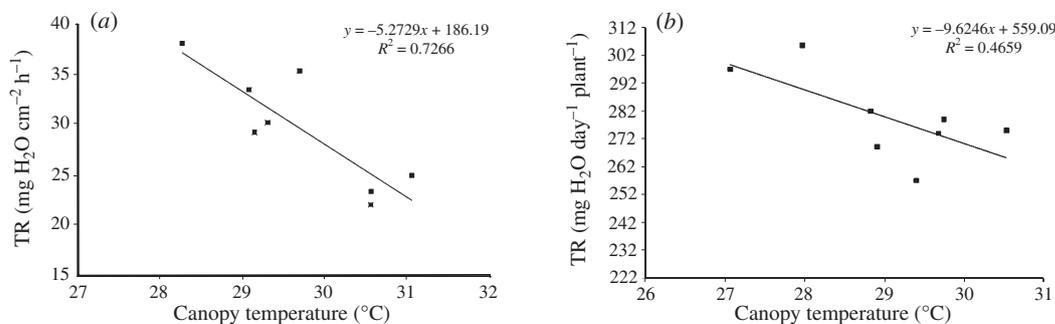


Fig. 5. Relationship between transpiration rate and canopy temperature (°C) in chickpea genotypes contrasting for seed yield and drought tolerance index in the field. Plants were assessed at (a) 42 DAS and (b) 56 DAS under outdoor conditions and at the highest VPD conditions on these two respective days.

explanation for this, light may have limited the growth of chickpea in the glasshouse. Nevertheless, the discrimination of genotypes for traits related to water use occurred under outdoor conditions similar to those in the nearby fields.

Variation in canopy conductance under well-watered conditions

Leaf conductance is one of the factors determining plant water loss and is therefore crucial for crops grown under terminal stress, i.e. with a limited amount of available water. Indeed, the sensitive ICC4814, ICC8058 and ICC3776 genotypes had higher overall transpiration rates than the tolerant ICC867, ICC14799 and ICC3325 at the vegetative stage and under well-watered conditions (Table 4). These data are in agreement with similar findings of lower conductance in terminal drought-tolerant genotypes of pearl millet introgressed with a terminal drought tolerance quantitative trait locus (QTL) (Kholova *et al.* 2010a), and is similar to the low early vigour and high water use efficiency (WUE) during the seedling stage in wheat (*Triticum aestivum* L.) (Condon *et al.* 2004). These lower canopy conductance differences were confirmed in the growth chamber, especially at high VPDs, where ICC14799, ICC867 and ICC3325 had a lower TR than ICC4814, ICC8058 and ICC3776. However, a break point in the transpiration response to high VPD occurred in two sensitive lines (ICC4814 and ICC8058) only, which had also the highest slope of response of transpiration to VPD. These data showed, for the first time, the existence of genotypic differences for the sensitivity of stomata to VPD in chickpea, as previously reported in other crops (Fletcher *et al.* 2007; Devi *et al.* 2010). However, this trait was not found in tolerant lines, as it was in the case of pearl millet (Kholova *et al.* 2010b). This break point could be explained by the high slope of TR response to VPD in the two sensitive lines (ICC4814 and ICC8058). The water savings associated with lower leaf conductance and limited maximum transpiration rates would be especially important in legumes like chickpea where N₂ fixation rates are particularly sensitive to water deficits (Sinclair *et al.* 1987; Guafa *et al.* 1993) and may have a significant impact on the final yield. Another interesting finding regarding the regulation of leaf water loss was that tolerant genotypes exhibited a trend of

higher transpiration rate at the early podding stage (Fig. 1b). This behaviour would result in a differential pattern of water use between tolerant and susceptible genotypes and may, to some extent, explain the difference of sensitivity to terminal stress; plants may leave water available in the soil profile and using it for the reproduction and grain filling stages. In addition, leaf expansion under well-watered conditions was lower in the tolerant genotypes during the dry-down period (Table 2).

Growth under stress conditions

Besides a limitation of transpiration, altered growth upon progressive exposure to water deficit would further limit plant water use. Growth was indeed more limited in the tolerant than in the sensitive genotypes at the end of the dry-down under drought conditions. This might relate to the higher soil moisture thresholds where transpiration declined in tolerant genotypes, and this related well with the lower aboveground biomass under water stress (Table 2). Similar findings have been reported in several crops including millet (Kholova *et al.* 2010a). Soltani *et al.* (2000) previously reported that the FTSW threshold for the decline in leaf transpiration was lower than the threshold for leaf expansion. Since leaf development seemed to discriminate tolerant genotypes from the sensitive ones, more work may be needed to assess whether leaf expansion stops at different moisture thresholds in tolerant and sensitive lines, and understand the extent that this could contribute to terminal stress tolerance in chickpea.

Differences in FTSW thresholds

The FTSW thresholds were lower (below 0.31) in most sensitive genotypes compared with the tolerant ones under outdoor conditions, unlike in the glasshouse (Table 3). These data are consistent with those published in chickpea (Soltani *et al.* 2000). Therefore, transpiration dropped upon progressive soil drying in relatively dryer soil in the sensitive lines than in the tolerant ones. Genotypic differences in the decline in transpiration has also been reported in several crops including soybean (Vadez and Sinclair 2001; Hufstetler *et al.* 2007) and peanut (Bhatnagar-Mathur *et al.* 2007). Our results here were, however, different from those in pearl millet, where tolerant lines had a

Table 5. Summary of the different possible traits that contribute to terminal stress tolerance (rating was made on the basis of outdoor experiment data), and their low vs high ranking among chickpea genotypes, contrasting for terminal drought tolerance

Information is provided only when significant trait differences between tolerant and sensitive entries were found. GC, growth chamber; T, tolerant; S, sensitive; TR, transpiration ratio; DAS, days after sowing

Genotype	Well-watered conditions						Water stress		
	Leaf conductance		Canopy temperature 42 DAS	Shoot DW		Leaf expansion	Total TR 42–56 DAS	FTSW threshold 56 DAS	Shoot DW 56 DAS
	42 DAS outdoor	42 DAS GC		42 DAS	56 DAS				
ICC867 (T)	Low	Low	Hot	–	Low	Low	Low	High	Low
ICC3325 (T)	Low	Low	Hot	–	–	Low	Low	High	Low
ICC3776 (S)	–	High	Cool	–	–	–	High	Low	High
ICC4814 (S)	High	High	Cool	–	Low	High	–	Low	High
ICC7184 (S)	–	–	Cool	–	High	High	High	–	Low
ICC8058 (S)	High	High	Cool	High	High	High	High	Low	High
ICC14778 (T)	–	–	–	Low	Low	Low	Low	High	Low
ICC14799 (T)	Low	Low	Hot	Low	Low	Low	Low	High	Low

decline of transpiration in dryer soils than sensitive lines (Kholova *et al.* 2010a). In any case, under progressive exposure to water deficit, the closure of stomata at high soil moisture in tolerant lines would slow down soil water depletion and it is hypothesised that this would retain water in the soil profile for later stages. Although Soltani *et al.* (2000) have shown with crop simulation modelling in two rain-fed environments that such a trait (a high FTSW threshold for transpiration decline) would contribute to only marginal yield increase under long terminal stress, another study has shown that it would impact yield positively in maize (Sinclair and Muchow 2001). The discrepancy may be explained by the fact that in Mediterranean conditions, chickpea depends on incoming rainfall and may need to maximise water use (Blum 2005). A decline in transpiration at high soil moisture would probably have more importance in environments where the crop depends on stored soil moisture. Here, that trait would add to the lower transpiration rate at the vegetative stage and would collectively contribute to conservative water use. Work is now needed to test the effect of such a trait using crop simulation modelling across representative target locations of chickpea.

Assessing conductance from infrared measurement

Plant temperature is a widely measured variable because it provides insight into plant water status. Although thermal imaging does not measure canopy conductance directly, in any given environment, stomatal variation is the dominant cause of changes in canopy temperature (Jones 2004). Differences in canopy temperature were reported in several crops, including wheat (Zhang 1990, 1997; Zhang and Wang 1999). From our data, differences in canopy temperature among the genotypes were recorded under well-watered conditions and related to leaf conductance with a highly significant correlation between the two parameters at 42 DAS (Fig. 5a). Tolerant genotypes had a warmer canopy under well-watered conditions at the vegetative stage. Furthermore, canopy conductance was found to vary according to the stage of development, and tolerant genotypes had higher canopy conductance at the early pod filling stage than sensitive ones. However, at this stage, the relationship with canopy temperature was not as close (Fig. S2). This could be due to differences in canopy structure, which is another critical determinant of plant canopy temperature and may affect the proportion of sunlight and shaded leaves in relation to the direct solar beam (Jones *et al.* 2009). Thus the monitoring of canopy temperature through thermal imaging would help in understanding the patterns of water uptake and use by the crop, provided this monitoring is carried out when the correspondence to canopy conductance is good, as in the vegetative stage here, under fully irrigated conditions, at the time of the day when the VPD is high. It also opens the prospect of using canopy temperature to select materials under field conditions.

Strategies for drought tolerance

Under terminal drought tolerance, water availability during the grain filling period is crucial because water shortage during flower and pod production has a dramatic negative impact on final seed yield (Leport *et al.* 2006; Fang *et al.* 2010). While preventive

strategies in early phenology can be successful, they also limit the overall crop duration and hence limit light capture and yield. Therefore, water conserving mechanisms during the cropping cycle are needed in medium duration materials like those tested here. A comparison of consistently tolerant and sensitive lines showed that several traits contributed to water savings under a terminal stress: (i) low leaf conductance under non-limited water conditions during the vegetative stage, which could be measured by a warmer canopy; (ii) higher FTSW thresholds for the decline in transpiration to avoid rapid soil water depletion; (iii) a low leaf expansion rate when soil moisture is still non-limited for plant growth and a restriction of plant growth under progressive exposure to stress (Table 5). Not all the tolerant genotypes had each of these traits. It appeared also that the natural conditions outdoors were those allowing the clearest expression of trait differences between contrasting genotypes.

Conclusion

Under terminal stress, sustained water use and transpiration into the reproductive growth stage is crucial for reproductive success (Merah 2001; Kato *et al.* 2008). While a profuse and deep root system has been thought to be the solution to this in many studies over the past three decades or so, our results indicate that the regulation of leaf water loss under both well-watered conditions and progressive soil drying also appear to be important. Generally, tolerant genotypes had lower canopy conductance at the vegetative stage, lower early vigour in two genotypes, more limited early leaf development and a higher soil moisture threshold for a decline in transpiration. These water-saving traits were not all present in a single genotype, suggesting that terminal tolerance breeding of chickpea may imply the pyramiding of several beneficial traits. Transpiration efficiency did not discriminate tolerant from sensitive materials. Although further investigation is needed, these traits could be used as reliable indicators of terminal stress tolerance, therefore offering new opportunities to develop phenotyping platforms that enable rapid screening of genotypes, especially using infrared canopy imaging.

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