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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 is often exposed to terminal drought, and deep and profuse rooting has been proposed as the main breeding target to improve the terminal drought tolerance. This work was carried out to test whether plant water use at vegetative stage and under non water limiting conditions could relate to the degree of sensitivity of chickpea to terminal drought. Transpiration response to a range of vapor pressure deficits under controlled and outdoors conditions was measured together with canopy conductance (Gs) using gravimetric measurements and thermal imagery in 8 chickpea genotypes with comparable phenology and contrasting seed yield under terminal drought in the field. Additionally, response of plant growth and transpiration to progressive soil moisture depletion was assayed in the same genotypes. Tolerant genotypes had overall a lower canopy conductance under fully irrigated conditions at vegetative stage, and this trend was reversed at early pod filling stage. While two sensitive entries had clearly high early growth vigor and leaf development, there was a trend of lower growth in tolerant genotypes under progressive soil drying than sensitive ones. Tolerant genotypes also exhibited a decline of transpiration in wetter soil than sensitive genotypes. Canopy conductance could be proxied by measurement of leaf temperature with an infrared camera, although the relationship lost sensitivity at pod filling stage. This work strongly suggests that there are traits that contribute to water saving when water is still not limiting plant growth and development in drought tolerant chickpea. It is hypothesized that this water would be available and then critical for the reproduction / grain filling stages.

*Keywords***:** Transpiration, leaf conductance, infrared thermography, vapor pressure deficit, early vigor, terminal drought.

Introduction

For crop species facing terminal stress conditions like chickpea, water availability during the grain filling period is critical. While deeper rooting can increase water extraction, as it has been hypothesized for almost three decades (Saxena *et al.* 1984; Johanssen *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 second 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 genotypes had lower canopy conductance under non-limiting 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-limiting 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 VPD) when water is not limiting in the soil could also contribute to water conservation under terminal drought. In a simulation analysis Sinclair *et al.* (2005) indeed demonstrated that the imposition of limited maximum transpiration rates increased sorghum 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 VPD above 2 kPa (Kholova *et al.* 2010b), and similar findings were reported in peanut (Devi *et al.* 2010) soybean (Fletcher *et al.* 2007) and sorghum (Gholipoor *et al.* 2010). This trait has so far not been tested in chickpea.

Limitation of 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 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; Merlot *et al.* 2002; Jones *et al.* 2002, Leinonen and Jones, 2004). Thermal imaging was then tested here to assess possible relationships with canopy conductance under well-watered conditions in chickpea.

Under progressive exposure to water deficit, the transpiration response to soil drying and the leaf area restriction are also key parameters of plant water use (Sadras and Milroy, 1996). Leaf area expansion decreases upon imposition of 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 further restrict water loss. Both the reduction/stoppage of leaf expansion and/or the closure of stomata at high soil moisture thresholds 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 (Ray and Sinclair, 1997), soybean (Vadez and Sinclair, 2001; Hufstetler *et al.* 2007), and groundnut (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 having 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 increase in VPD; (iii) developing and testing a method to assess canopy conductance from thermal imagery and measure Gs differences in contrasting lines at 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 with comparable phenology (92 ± 4 days to maturity) and among the most contrasting for seed yield under terminal drought stress in three years of field testing (Table 1) were selected from the ICRISAT mini-core collection (Krishnamurthy *et al.* 2010) for characterization 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: 32/25 ºC and relative humidity: 40-80%) and outdoors

conditions (max / min temperature: 32.1-27.7 / 16.8-13.8ºC and the 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. This period is the regular chickpea growing season and the outdoors trial was carried out to assess traits related to plant water use under atmospheric conditions that are close to those in the field. Outdoors, the pots were set on benches and with the possibility to protect the pots from rains. In each environment, twenty five pots were prepared for each genotype. Three seeds were sown per pot and 10 to 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 the leaf transpiration rate (g cm^{-2} h^{-1}) was done at 42 DAS when the plants were at late vegetative stage, in outdoors conditions over the course on 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 is low and until the afternoon when the VPD decreases following the midday peak. Five plants per genotypes, grown in outdoors conditions, were saturated two 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 losses in weight of each pot. Pots were weighed with a 1 g precision scale every hour from 07.15 am until 17.40 pm. 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 Datalogger), which was positioned within the crop canopy.

At the end of the day, the plants were transferred to a growth chamber where, the following day, their transpiration response to increasing VPD was assessed under controlled conditions, using a ladder 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 about 5 min to weigh the pots and that time was used to increase the VPD to the next level on the ladder. 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 outdoors conditions, while it was lower but constant in the growth chamber (circa 600 µmol m^{-2} s^{-1}).

Another measurement of the leaf transpiration rate (g cm^{-2} h^{-1}) was performed outdoors at 66 DAS when the plants were at early podding stage. The measurements were made over the course on 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 analyzing with WinRhizo software (WinRhizo, Regent Ltd, Canada). Shoot, root and leaf dry weights were recorded after placing the samples for 48 h in a 70ºC 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 IR FlexCam S (Infrared solutions, USA) with a sensitivity of 0.09ºC and 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. The software SmartView 2.1.0.10 (Fluke Thermography) was used for the analysis of the thermal images and the estimation of canopy temperatures.

Estimation of canopy conductance

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

$$Ig = \frac{(Tdry - Tleaf)}{(Tleaf - Twet)}$$

Where *T*wet is the temperature of a wet surface, *T*dry is the temperature of a non-transpiring surface, and *Tleaf* is the leaf canopy temperature measured with the IR camera. *T*wet was measured on green leaves after soaking them with water 5 min and *T*dry is the temperature of dry leaves. These temperatures were measured under outdoors 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 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 randomized complete block design with two water treatments (WW and WS) as main factors and genotypes as 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 about 80% of the pot capacity. Five pots of each genotype were allowed to dry progressively over approximately a 2-weeks 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 normalized 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 normalized TR (NTR) consisted in 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 is usually quite erratic likely 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 dry weights of shoot, root and leaves, including the few leaves that 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 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 each day n was calculated as:

$$FTSW = \frac{Pot\ Weight\ day\ n - Final\ Pot\ Weight}{Initial\ Pot\ weight - Final\ Pot\ weight}$$

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 prior to the beginning of the drydown (pre-dry down harvest) 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 treatment. For the analysis of dry-down data and the calculation of the FTSW threshold analysis, SAS (SAS Institute, Inc., 1988, Cary, NC, USA) was used. Values of normalized TR and FTSW obtained during the drydown experiments for all plants within 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 carries out iterations of the NTR data, starting at FTSW = 1 (wet soil) and fits them to a $y = 1$ equation. From the FTSW level onwards where $y = 1$ is no longer the best fit for NTR, data are 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 analyzed with the split line regression of Genstat (9.0), which provides a breakpoint value where the slope of the fitted regression significantly changes.

Results

Effect of water stress exposure on growth parameters and transpiration efficiency

Under glasshouse conditions - At 42 DAS (Pre-dry down), growth parameters varied significantly among the genotypes (Supplementary Table 1). ICC 8058 had the highest shoot biomass followed by ICC4814 and ICC3776. The four tolerant genotypes were among those with the smallest shoot biomass. There was no important variation for root dry weight, although ICC8058 and ICC3325 tended to show the highest values. Leaf dry weight was the highest in ICC8058, followed by ICC867, ICC3325, and ICC3776.

In well watered plants (Control) the shoot dry weight of ICC867, ICC3325, and ICC7184 was lower than that of ICC3776 and ICC8058 (Supplementary Table 1). For root and leaf biomass, there was no important genotypic variation. Similarly, the total transpiration did not show important variation, except that ICC8058 had the highest water uptake. Transpiration efficiency was highest in ICC14778, ICC14799 and lowest in ICC3325 and ICC7184, the remaining genotypes had similar values.

Under water stress, ICC14799, ICC14778 and ICC3325 had lower shoot dry weight 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 with ICC14778, ICC14799, ICC4814 and ICC7184 (28-41% of leaf biomass reduction). The total transpiration did not show important variation, except with ICC8058 that had a lower water uptake than all genotypes. There was no significant variation of TE. The leaf expansion rate during the duration of the dry down varied largely among genotypes (Supplementary Table 1).

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

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

In control plants, the highest biomass was recorded in ICC7184 followed by ICC8058 and ICC3325. Root and leaf dry weight did not show 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 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 tolerant genotype (Table 2). The lowest root development was recorded in ICC4814, ICC7184, ICC14778 and to some extent ICC14799, while leaf dry weight was the highest in ICC8058 and ICC3776. The highest TE was recorded in ICC4814, ICC8058 and ICC3776 and 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).

So overall, in outdoors conditions, growth at 42 DAS, total water used for transpiration under well-watered conditions, and growth upon progressive exposure to water deficit, was lower in the tolerant than in the sensitive genotypes.

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; Supplementary Fig. 1), therefore a fairly high range of variation. ICC14778 had the lowest threshold among genotypes except ICC4814, ICC3776 and ICC867, whereas ICC8058 showed the highest FTSW threshold (0.63). Yet, there was no discrimination for this parameter between the tolerant and susceptible genotypes. Unlike the glasshouse, a lower range

of FTSW threshold values was recorded under outdoors conditions (0.25-0.43) (Table 3; Supplementary Fig. 1). However, the sensitive genotypes had lower FTSW thresholds (below 0.31) than the tolerant one, except for ICC7184 and ICC14778. The lowest value was recorded with ICC4814 followed by ICC3776. (0.25 and 0.30 respectively).

Response of leaf transpiration to changing VPD

Under outdoors conditions at 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 3.30 pm (Fig. 1A). Across all the VPD conditions there was genotypic variation in TR. Clearly, there was a tendency to have a higher overall TR in sensitive (ICC4814, ICC8058, ICC3776) than in tolerant germplasm (ICC867, ICC14799, ICC3325). At a VPD of 3.48 kPa ICC 4814 showed a higher TR (21.6 mg $H_20.cm^{-2}.h^{-1}$) than ICC 867 and ICC 14799 (13.3 and 12 mg $H_20.cm^{-2}.h^{-1}$, respectively). Likewise, TR of ICC 8058 was higher than ICC 14799. The remaining genotypes presented quite similar transpiration rate. At VPD around 4 kPa, sensitive ICC 4814 and ICC 3776 had higher TR than tolerant ICC 867 and ICC 14799, with an increase of 19 and 24% respectively. Additionally, TR of sensitive ICC 4814 was higher than in tolerant ICC 3325. ICC 4814 had the highest TR among genotypes except ICC 8058 and ICC 14778. The largest variation was recorded at 1.10 pm, VPD being just above 4 kPa. There, TR of ICC 4814, ICC 8058 and ICC 14778 was higher than in ICC 867 and ICC 14799. Additionaly, ICC 4814 transpired more than all the genotypes except ICC 8058 and ICC 14778. After 1.10 pm, the TR of all the genotypes decreased and ICC 867, ICC 14799 and to some extent ICC 3325 had the lowest TR as compared to ICC 4814. Similar observation were made observation at 3:10 pm.

Under controlled conditions at vegetative stage - Over the whole range of tested VPD, transpiration rate was higher in sensitive genotypes ICC 8058 and ICC 4814 (above 27 mg H_2O m^{-2} h^{-1}) than in tolerant ICC 867 and ICC 14799 (less than 17 mg H_2O m^{-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 breakpoint in the transpiration response to VPD (Fig. 2). Genotypes having no break point had

similar slope of increase of transpiration to VPD except a higher slope in ICC14778. The slope of the transpiration response to VPD below the breakpoint was higher in the sensitive genotypes ICC4814 and ICC8058 than in the other genotypes. At the lowest VPD (0.45 kPa), differences of TR among genotypes were small. By contrast, at the highest VPD (3.4 kPa) the range of variation in the TR was 22 $mg.cm^{-2}.h^{-1}$ for ICC 14799 to 37 $mg.cm^{-2}.h^{-1}$ for ICC 4814. In accordance with the outdoors measurement, tolerant genotypes ICC 14799, ICC 867 and ICC 3325 had lower transpiration rate, on average across the VPD conditions, than sensitive ICC 4814, ICC 8058 and to some extent than ICC 3776 (Table 4).

Under outdoors conditions at early podding stage - The transpiration rate was again measured under naturally increasing VPD in outdoors conditions that varied between 0.30 kPa and 5.48kPa (Fig. 1B). Contrary to the experiment done at vegetative stage, tolerant genotypes tended to have higher TR than the sensitive ones. Over the whole range of VPD, sensitive ICC 8058, ICC 7184 and to a lesser extent ICC 4814 had the lowest TR. The remaining genotypes exhibited a transpiration rate higher than 18 $mg.cm^{-1}.h^{-1}$. 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, i.e. about 15-20 $mg.cm^{-1}.h^{-1}$on average. The TR of sensitive lines decreased from 20-25 $mg.cm^{-1}h^{-1}$ to 10-15 $mg.cm^{-1}h^{-1}$.

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 in 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 was then taken past this normal distribution (Fig 3B), and this color threshold was used to remove background colors (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 temperatures, the average temperature was calculated as follows:

$$\mathrm{T}m = \sum Ti \frac{PXi}{PXt}$$

Where PXi is the number of pixels for a given temperature Ti and PXt 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 canopy temperature was recalculated and compared to the first one. This test was performed for five randomly chosen genotypes (Supplementary Table 2). Increasing the color threshold by one of two units on the temperature scale increased the temperature from 0.15 to 0.22 ºC in the genotypes, therefore 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 outdoors conditions at 42 DAS and 56 DAS. At 42 DAS the canopy temperatures ranged between 28.2 and 31.0ºC for ICC 4814 and ICC 3325 (Fig. 4A). The genotypes ICC 8058, ICC 14778, ICC 7184, ICC 3776 and to a higher extent ICC 4814 were relatively cooler than the remaining genotypes. The canopy temperature differed between ICC 4814 and ICC 3325 (by 3.2 ºC), and between ICC 4814 and ICC 867 or ICC 14799 (2.2 ºC). A quite similar trend was

recorded at 56 DAS with the susceptible genotypes showing overall lower canopy temperatures (data not shown). The canopy temperatures ranged between 27 and 30.5ºC (data not shown).

The index of canopy conductance (Ig), used as an indirect estimation of the absolute canopy conductance, ranged between 1.8 with ICC 3325 and 3.6 with ICC 4814 (Fig. 4B). The tolerant genotypes ICC 14799, ICC 867 and ICC 3325 had a lower Ig (< 2.1) than the remaining genotypes (> 2.5). Among the tolerant genotypes, only ICC14778 had an Ig in the range of those in the sensitive genotypes.

Estimation of canopy conductance under controlled conditions of a growth chamber

Despite possible problem of IR reflection in closed environments, and the risk that temperature reading from leaves might be "contaminated" by reflection, canopy temperatures was also measured in the growth chambers, using 42 days old plants used above. Variation among genotypes was quite different from the one recorded under natural conditions, but the genotypes ICC 4814, ICC 8058 and to a lesser extent ICC 3776 and ICC 7184 were cooler than the remaining genotypes (data not shown). The difference in canopy temperature ranged between 1.8 and 5.8 ºC. The highest index of canopy conductance were recorded in ICC 4814 and ICC 8058 followed by ICC 3776, ICC 7184 and ICC 14778 (data not shown).

To assess the viability of the thermal approach for derivation of 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 (Ig data not shown at 56 DAS).

Estimation of canopy conductance at late stage of plants grown under well watered conditions

The measurements were made when the plants were at early podding stage (66 DAS) (Supplementary Fig. 2). The canopy temperatures ranged between 29.22 and 32.06 ºC. ICC8058

and ICC7184 had relatively hotter canopy (above 31.5 ºC) as compared to the remaining genotypes, particularly ICC867 and ICC14778 whose canopy temperature was below 30 ºC. This result contrast with the one obtained at the vegetative stage where the two later genotypes were among those having hotter canopy. However, at that stage there was a much weaker relationship between measured canopy temperature and the transpiration rates ($R^2 = 0.21$).

Discussion

While under glasshouse conditions none of the traits related to plant water use discriminated tolerant from sensitive lines, the assessment in outdoors conditions revealed several discrimination traits. There, tolerant genotypes had a lower canopy conductance under fully irrigated conditions at vegetative stages than sensitive ones, and this trend was reversed at 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 entries had clearly high early growth vigor and leaf development, all tolerant genotypes except one had a lower growth under progressive drying than sensitive one. Canopy conductance could be proxied by measurement of 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 the glasshouse conditions compare to outdoors. While there is no clear 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 natural 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 losses and is therefore crucial for crops grown under terminal stress, hence with a limited amount of available water. Indeed sensitive ICC4814, ICC8058, and ICC3776 had higher overall transpiration rate than tolerant ICC867, ICC14799, and ICC3325 at vegetative stage and under WW conditions (Table 5). These data are in agreement with similar finding of lower conductance in terminal drought tolerant

genotypes of pearl millet introgressed with a terminal drought tolerance QTL (Kholova *et al.* 2010a), or to the low early vigor and high WUE during the seedling stage in wheat (Condon et al., 2004). These lower canopy conductance differences were confirmed in the growth chamber, especially at high VPD where ICC 14799, ICC 867 and ICC 3325 had lower TR than ICC4814, ICC8058, and ICC3776. However, a breakpoint in the transpiration response to high VPD occurred in two sensitive lines 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, 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 breakpoint could be explained by the high slope of Tr response to VPD in these two sensitive genotypes. The water savings associated with lower leaf conductance and limiting maximum transpiration rates would be especially important in legumes like chickpea where N_2 fixation rates are particularly sensitive to water deficits (Guafa *et al.* 1993, Sinclair *et al.* 1987) 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 early podding stage (Fig. 1B). This behavior 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, by leaving water available in the soil profile and using it for the reproduction and grain filling. In addition, the leaf expansion under well-watered conditions was lower in the tolerant genotypes during the dry-down duration (Table 2).

Growth under stress conditions

Beside 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 drydown 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 WS (Table 2). Similar finding has 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 would be needed to assess whether leaf expansion stops at different moisture thresholds in tolerant and sensitive lines, and understand the extent 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 to the tolerant ones under outdoors conditions, unlike 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 groundnut (Bhatnagar-Mathur *et al.* 2007). Our results here were, however, different from those in pearl millet where tolerant lines had a 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 hypothesized that this would retain water in the soil profile for later stages. Although Soltani *et al*. (2000) have shown with crop simulation modeling in two rainfed environments that such trait (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 chickpea in Mediterranean conditions depends on in-coming rainfall and may need to maximize water use (Blum, 2005). A decline in transpiration at high soil moisture would likely have more importance in environments where crop depends on stored soil moisture. Here, that trait would also add to the lower transpiration rate at vegetative stage and would collectively contribute to a conservative water use. Work is now needed to test the effect of such a trait using crop simulation modeling across representative target locations of chickpea.

Assessing conductance from IR measurement

Plant temperature is a widely measured variable because it provides insight into plant water status. Although thermal imaging does not directly measure canopy conductance, 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 at 42 DAS (Fig. 5A). Tolerant genotypes had a warmer canopy under well watered conditions at vegetative stages. Besides, the canopy conductance was found to vary according to the stage of development, and tolerant genotypes had higher canopy conductance at early pod filling stage than sensitive ones. However, at this stage, the relationship with canopy temperature was not as close (Supplementary Fig. 2). This could be due to differences in canopy structure, which is another critical determinant of plant canopy temperature and may affect the proportion of sunlit and shaded leaves in relation to the solar direct beam (Jones *et al*. 2009). Thus the monitoring of canopy temperature through thermal imaging would help in understanding the patterns of water uptake / use by the crop, provided they are used when the correspondence to canopy conductance is good, i.e. like at vegetative stage here, under fully irrigated conditions, at the time of the day when the VPD is high. It also opens prospect of using it 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 dramatic negative impact on final seed yield (Leport *et al*. 2006; Fang *et al.* 2010). While escape strategies through early phenology have been successful, they also limit the overall crop duration and hence limit light capture and yield. So, water conserving mechanisms during the cropping cycle are needed in medium duration materials like those tested here. 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-limiting water conditions of the vegetative stage, which could be measured by a warmer canopy, (ii) a 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

limiting for plant growth and a restriction of plant growth under progressive exposure to stress (Table 5). All tolerant genotypes did not have 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, with many studies in the past three decades or so, our results indicates that the regulation of leaf water losses under both well-watered conditions and progressive drying appear to be also important. Generally, tolerant genotypes had lower canopy conductance at vegetative stage, lower early vigor in two of them, more limited early leaf development, higher soil moisture threshold for a decline of transpiration. These water saving traits were several and 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 screenings of genotypes, especially using infrared canopy imaging.

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Table 1. Variation of phenology (50% flowering and maturity, days) and drought tolerance index (DTI) across the studied chickpea genotypes, contrasting for terminal drought tolerance (tolerant, T; sensitive, S). Data are means of three years field experiments (Krishnamurthy et al., 2010). The DTI represented the residual yield variations that were not explained by differences in flowering time and yield potential (details in above mentioned reference).

	Genotypes							
	867 (T)	3325 (T)	3776 (S)	4814 (S)	7184 (S)	8058 (S)	14778 (T)	14799 (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
DTI	0.75	0.69	-0.70	-0.54	-0.9	-0.80	0.90	0.60

Table 2. Dry mass (g.plant^{-1}) of shoots, roots and leaves (SDW, RDW, LDW), specific leaf area (SLA, cm^2.g^{-1}), transpiration efficiency (TE, g biomass kg^{-1} water transpired), total water transpired during the dry down (Total TR, kg plant^{-1}) and leaf expansion rate (cm^2 day^{-1}) of chickpea genotypes contrasting for terminal drought tolerance (tolerant, T; sensitive, S), 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.

		867 (T)	3325 (T)	3776 (S)	4814 (S)	7184 (S)	8058 (S)	14778 (T)	14799 (T)
Pre-dry down	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}
Control	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}
Water stress	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^{e}	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}

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 (tolerant, T; sensitive, S), grown under glasshouse and outdoors (ambiant climatic conditions at ICRISAT-Patancheru) conditions.

Environment	Genotype	FTSW threshold	Approximate SE	95% CI
Glasshouse	867 (T)	0.402	0.0233	0.356- 0.447
	3325 (T)	0.558	0.0291	0.500 - 0.616
	3776 (S)	0.406	0.0281	0.350 - 0.462
	4814 (S)	0.453	0.0376	0.378 - 0.528
	7184 (S)	0.498	0.0250	0.449 - 0.548
	8058 (S)	0.631	0.040	0.511 - 0.631
	14778 (T)	0.347	0.0312	0.285 - 0.409
	14799 (T)	0.501	0.0313	0.439 - 0.564
Outdoors	867 (T)	0.414	0.0313	0.353- 0.475
	3325 (T)	0.427	0.0321	0.366 - 0.489
	3776 (S)	0.304	0.0223	0.260 - 0.349
	4814 (S)	0.253	0.0199	0.213 - 0.293
	7184 (S)	0.341	0.0196	0.302- 0.380
	8058 (S)	0.299	0.0171	0.265 - 0.334
	14778 (T)	0.362	0.0219	0.318- 0.406
	14799 (T)	0.369	0.0238	0.352 - 0.417

Table 4. Regression results for the transpiration response of 42-days-old chickpea genotypes, contrasting for terminal drought tolerance (tolerant, T; sensitive, S), to increasing 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 models with no breakpoint.

Genotypes	Mean TR	Segmented regression						
	(mg H_2O m^{-2} h^{-1})	Breakpoint		Slope a		Slope b		r^2
		**Value*	*SE*	***Value*	*SE*	***Value*	*SE*	
4814 (S)	27.22	2.540	0.411	10.77	1.91	1.61	4.43	0.77
8058 (S)	29.11	2.553	0.250	11.49	2.06	- 4.69	4.79	0.81
		Linear regression		***Value*	*SE*			
867 (T)	16.17	*no Breakpoint*		4.91	0.42			0.74
3325 (T)	19.95	*no Breakpoint*		5.91	0.61			0.71
3776 (S)	26.38	*no Breakpoint*		5.39	0.65			0.63
7184 (S)	21.81	*no Breakpoint*		5.82	0.48			0.81
14778 (T)	21.83	*no Breakpoint*		7.18	0.62			0.80
14799 (T)	16.81	*no Breakpoint*		4.50	0.49			0.68

(*) kPa, (**) mg H_2O m^{-2} h^{-1} kPa^{-1}; (SE) standard error.

(Slope a) regression at low VPD below breakpoint; (Slope b) of regression at high VPD above breakpoint.

Table 5. Summary of the different possible traits that contribute to terminal stress tolerance (rating was made on the basis of outdoors experiments data), and their Low/High ranking among chickpea genotypes, contrasting for terminal drought tolerance (tolerant, T; sensitive, S). Only information is provided when significant trait differences between tolerant / sensitive entries were found (different tables and figures).

	Well-watered conditions							Water stress	
	Leaf conductance		Canopy Temperature	Shoot DW		Leaf expansion	Total Tr	FTSW Threshold	Shoot DW
	42 DAS Outdoor	42 DAS GC*	42 DAS	42 DAS	56 DAS		42-56 DAS	56 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

*GC: growth chamber

Supplementary Table 1. Dry mass (g.plant^{-1}) of shoots, roots and leaves (SDW, RDW, LDW), specific leaf area (SLA, cm^2.g^{-1}), transpiration efficiency (TE, g biomass kg^{-1} water transpired), and total water transpired during the dry down (Total TR, kg plant^{-1}) and leaf expansion rate (LER, cm^2 day^{-1}) of chickpea genotypes contrasting for terminal drought tolerance (tolerant, T; sensitive, S), grown in glasshouse 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

		867 (T)	3325 (T)	3776 (S)	4814 (S)	7184 (S)	8058 (S)	14778 (T)	14799 (T)
Pre-dry down	SDW (g)	4.73^{c}	4.80^{c}	4.89^{bc}	5.33^{ab}	4.98^{bc}	5.57^{a}	3.12^{d}	3.87^{d}
	RDW (g)	1.28^{ab}	1.52^{a}	0.78^{c}	1.05^{bc}	1.47^{ab}	1.54^{a}	1.31^{ab}	1.40^{ab}
	LDW (g)	2.80^{ab}	2.73^{ab}	2.54ab	2.02c	2.09c	3.11a	1.89c	2.14c
Control	SDW (g)	9.43^{b}	9.16^{b}	11.41^{a}	11.37^{ab}	9.36^{b}	11.85^{a}	10.32^{ab}	10.77^{ab}
	RDW (g)	2.44^{ab}	1.93^{b}	2.16^{ab}	2.80^{ab}	1.97^{b}	2.97^{ab}	3.03^{a}	2.77^{ab}
	LDW (g)	5.79^{ab}	4.97^{b}	5.43^{ab}	5.91^{ab}	4.64^{b}	5.95^{ab}	5.73^{ab}	6.57^{a}
	SLA	328.00	366.00	266.76	278.70	349.60	394.32	281.77	373.69
	TE	1.30^{bc}	0.88^{c}	2.20^{b}	2.14^{b}	1.04^{c}	1.49^{bc}	3.47^{a}	2.43^{a}
	Total TR	2.26^{bc}	2.40^{bc}	2.32^{bc}	2.61^{b}	2.39^{bc}	3.12^{a}	2.03^{c}	2.47^{bc}
Water stress	SDW (g)	8.52^{bc}	8.02^{c}	8.12^{c}	9.18^{ab}	8.68^{bc}	9.66^{a}	6.94^{d}	6.25^{d}
	RDW (g)	2.24^{ab}	2.11^{abc}	1.61^{c}	1.89^{bc}	2.47^{a}	2.24^{ab}	2.24^{ab}	2.40^{ab}
	LDW (g)	4.72^{ab}	4.03^{bc}	3.86^{c}	4.26^{bc}	3.93^{c}	5.15^{a}	3.60^{c}	3.88^{c}
	TE	1.23	0.90	1.20	1.66	1.79	1.55	1.68	1.36
	Total TR	1.64^{a}	1.40^{abc}	1.37^{abc}	1.55^{abc}	1.27^{bc}	1.21^{c}	1.57^{ab}	1.35^{abc}
	LER	29.24^{d}	52.78^{bc}	44.77^{cd}	35.66^{cd}	49.88^{cd}	69.69^{ab}	44.39^{cd}	87.58^{a}

Supplementary Table 2. Sensitivity test for the estimation of canopy temperature by moving the threshold of temperature where pixels are considered to be part of the plant

Genotype	Estimated canopy temperature		
	Initial	Limit +1	Limit +2
867	35.81	35.99	36.11
3776	34.40	34.55	34.62
8058	32.74	32.82	32.89
4814	30.92	31.05	31.11
3325	35.82	35.93	36.02

Figures captions

Fig. 1. Transpiration rate (TR, $mgH_2O.cm^{-2}\ h^{-1}$) 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 VPD regimes over an entire day. Plants were grown outdoors and assessed at (A) vegetative (42 DAS) and (B) early podding stages (66 DAS). The bar at each measurement time indicates the LSD for genotypic means. (open symbols and dashed lines: sensitive; closed symbols and plain lines: tolerant). The doted line represents the fitting of vapor pressure deficit (VPD) over the course of the day.

Fig. 2. Transpiration rate (TR, $mgH_2O.cm^{-2}\ h^{-1}$) under well-watered conditions of sensitive (a) 4814 and tolerant (b) 14799 chickpea genotypes exposed to increasing VPD regimes under controlled conditions of a growth chamber. Plants were assessed at the vegetative stage and each point represents the mean of 4 replicates.

Fig. 3. Thermal image of chickpea canopy (A) before and (C) after removing the background using color threshold and (B) pixels distribution for the range of temperatures.

Fig. 4.: Variation of (A) canopy temperature (ºC) and (B) index of canopy conductance (Ig) in chickpea genotypes contrasting for terminal drought tolerance (tolerant, black bars; sensitive, white bars) grown outdoors under well watered conditions and the measurements were made at 42 DAS under outdoors conditions, at the time of the day having the highest VPD conditions.

Fig. 5. Relationship between transpiration rate and canopy temperature (ºC) in chickpea genotypes contrasting for seed yield and DTI in the field. Plants were assessed at (A) 42 DAS and (B) 56 DAS under outdoors conditions and at the highest VPD conditions on these two respective dates.

Supplementary Fig. 1. Relationship between the normalized transpiration ratio (NTR) and the fraction of transpirable soil water (FTSW) of in two chickpea genotypes contrasting for terminal drought tolerance (tolerant, black symbols and solid lines; sensitive, open symbols and dashed lines) grown (A) under glasshouse and (B) outdoors conditions.

Supplementary Fig. 2. Variation of (A) canopy temperature (ºC) and (B) Relationship between transpiration rate and canopy temperature in chickpea genotypes contrasting for terminal drought tolerance (tolerant, black bars; sensitive, white bars) grown under well watered conditions. The measurements were made when the plants were at early podding stage (66 DAS).

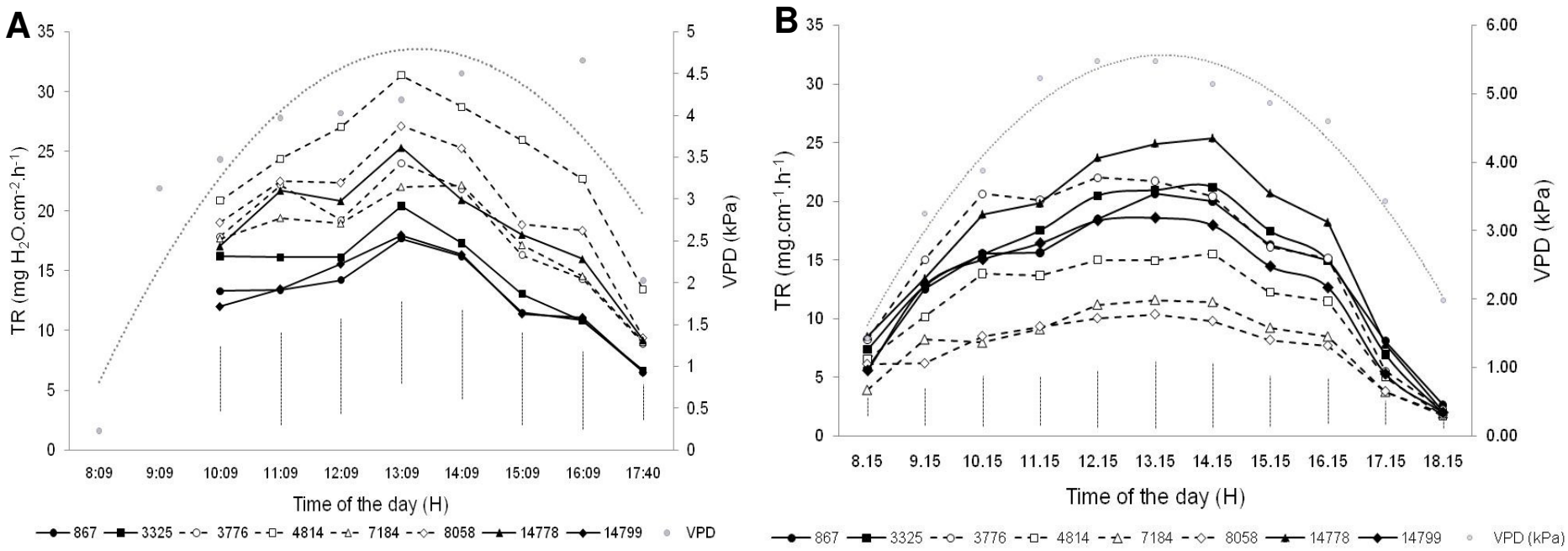
A
TR (mg $H_2O.cm^{-2}.h^{-1}$)
VPD (kPa)
Time of the day (H)
8:09
9:09
10:09
11:09
12:09
13:09
14:09
15:09
16:09
17:40
867
3325
3776
4814
7184
8058
14778
14799
VPD
B
TR (mg.$cm^{-1}.h^{-1}$)
VPD (kPa)
Time of the day (H)
8.15
9.15
10.15
11.15
12.15
13.15
14.15
15.15
16.15
17.15
18.15
867
3325
3776
4814
7184
8058
14778
14799
VPD (kPa)

Figure 1

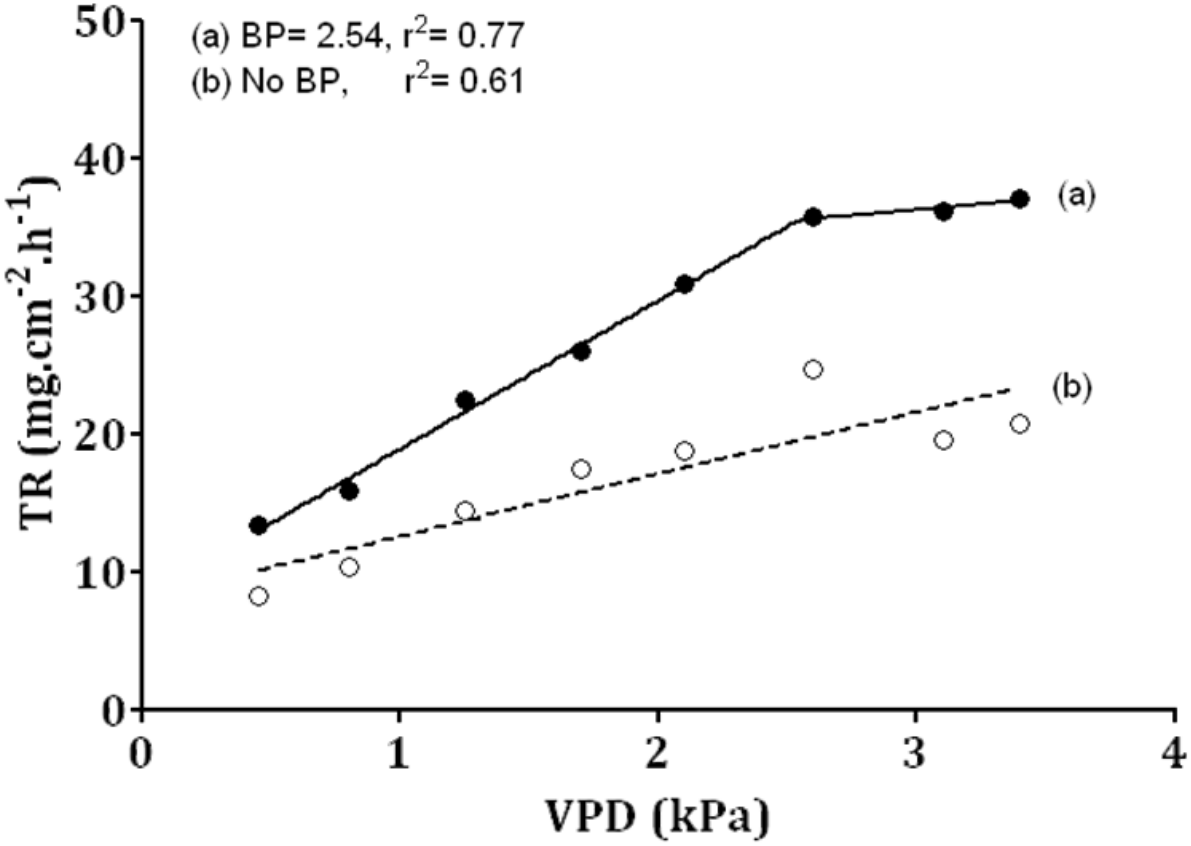
(a) BP= 2.54, r2= 0.77
(b) No BP, r2= 0.61
TR (mg.cm-2.h-1)
VPD (kPa)
(a)
(b)
0
10
20
30
40
50
0
1
2
3
4

Figure 2

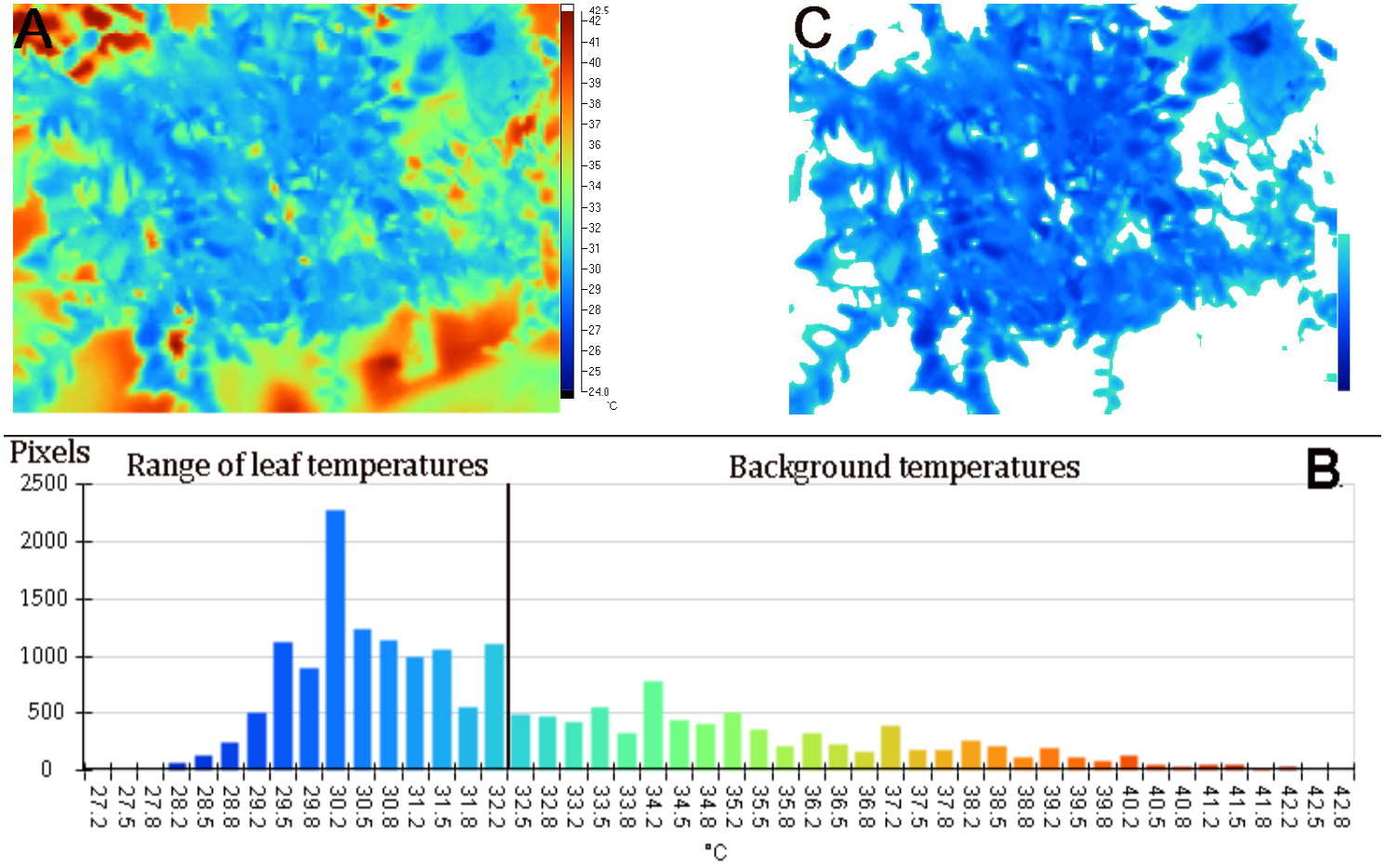
A
42.5
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24.0
°C
C
Pixels
Range of leaf temperatures
Background temperatures
B
2500
2000
1500
1000
500
0
27.2
27.5
27.8
28.2
28.5
28.8
29.2
29.5
29.8
30.2
30.5
30.8
31.2
31.5
31.8
32.2
32.5
32.8
33.2
33.5
33.8
34.2
34.5
34.8
35.2
35.5
35.8
36.2
36.5
36.8
37.2
37.5
37.8
38.2
38.5
38.8
39.2
39.5
39.8
40.2
40.5
40.8
41.2
41.5
41.8
42.2
42.5
42.8
°C

Figure 3

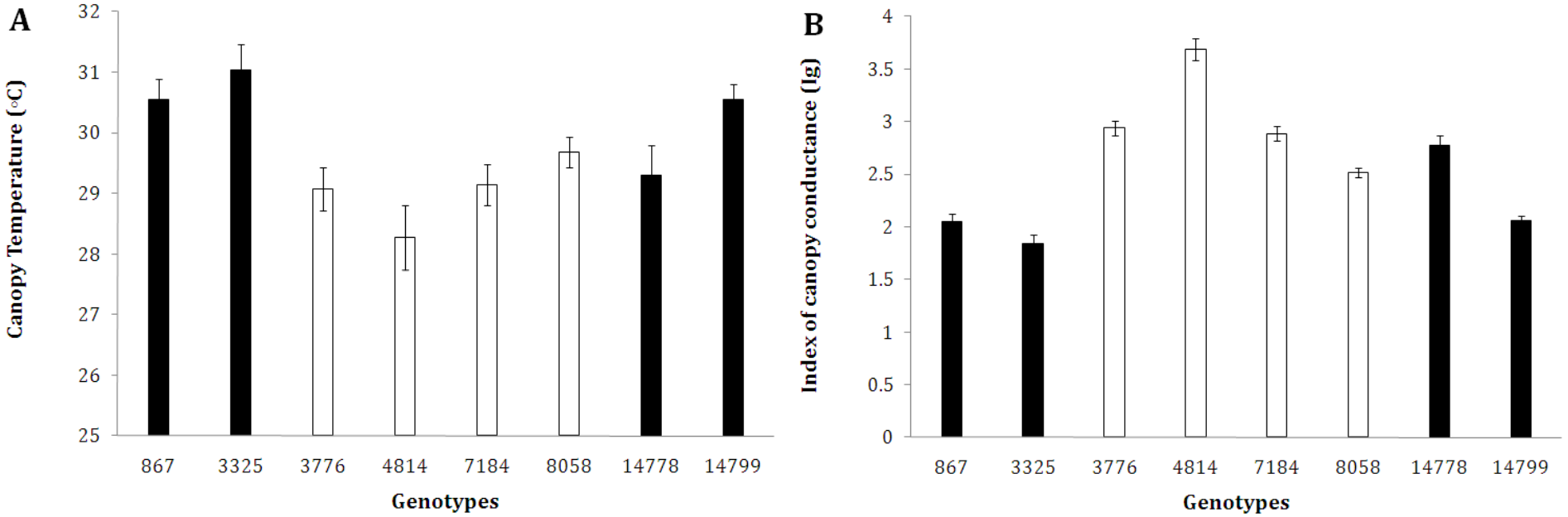
A
Canopy Temperature (°C)
32
31
30
29
28
27
26
25
867
3325
3776
4814
7184
8058
14778
14799
Genotypes
B
Index of canopy conductance (Ig)
4
3.5
3
2.5
2
1.5
1
0.5
0
867
3325
3776
4814
7184
8058
14778
14799
Genotypes

Figure 4

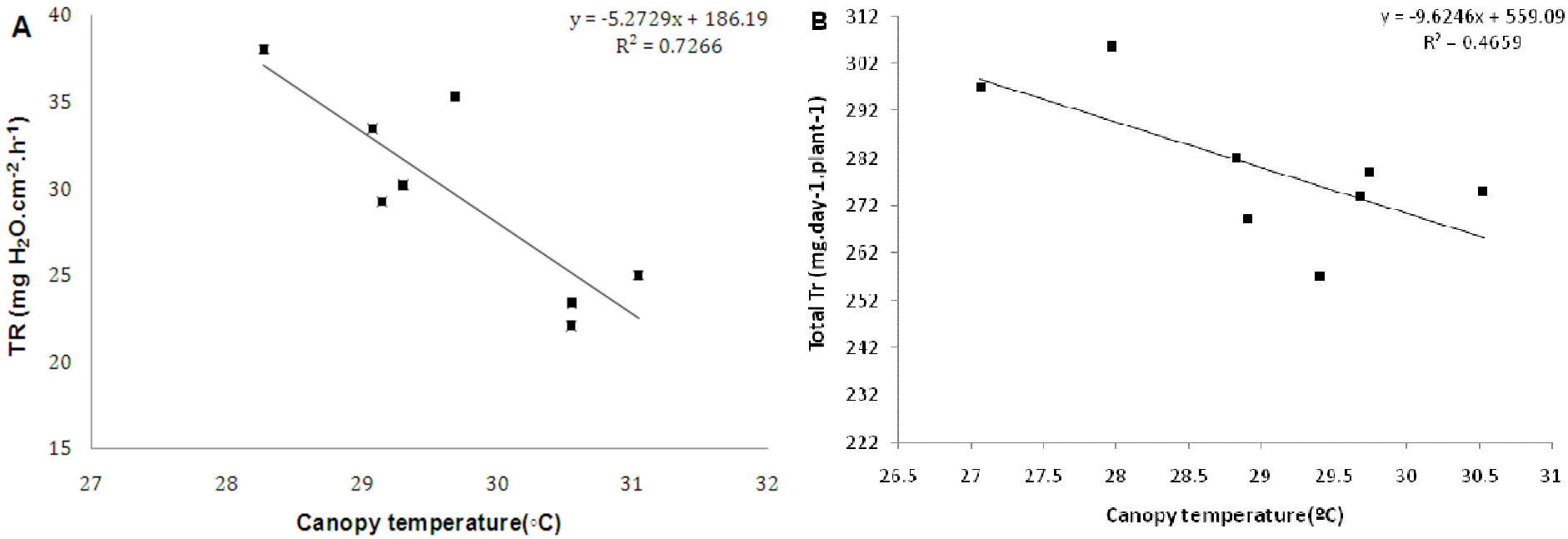
A
y = -5.2729x + 186.19
R² = 0.7266
TR (mg H₂O.cm⁻².h⁻¹)
40
35
30
25
20
15
27
28
29
30
31
32
Canopy temperature(◦C)
B
y = -9.6246x + 559.09
R² = 0.4659
Total Tr (mg.day-1.plant-1)
312
302
292
282
272
262
252
242
232
222
26.5
27
27.5
28
28.5
29
29.5
30
30.5
31
Canopy temperature(ºC)

Fig. 5