

# Genotypes Contrasting for Terminal Drought Tolerance also Contrast for the Developmental Pattern of Water Use in Varying Environmental Conditions

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## ABSTRACT

Current scientific efforts have sharpened the discussion about various water use strategies and their importance for crop drought tolerance. It was previously found that tolerant pearl millet (*Pennisetum glaucum* (L.) R. Br.) genotypes had lower vegetative stage transpiration rate ( $Tr$ ,  $g\ cm^{-2}\ d^{-1}$ ) in well-watered conditions, which would confer a yield advantage under terminal drought. A set of experiments in glasshouse conditions (VPD between 3.6-0.4kPa) and growth chambers set at vapor pressure deficit (VPD) of 1.13 and 2.55kPa were developed to test how leaf area development affects the overall water use under well-watered conditions under high and low VPD and how it relates to transpiration rate ( $Tr$ ) and transpiration efficiency (TE). Tolerant genotype (PRLT 2/89-33) exhibited lower  $Tr$  compared to sensitive (H77/833-2) in all growing conditions, however lower  $Tr$  was linked to higher TE only in VPD 1.13kPa. Tolerant genotype (PRLT 2/89-33) produced higher LA than sensitive H77/833-2 in VPD 1.13kPa and in glasshouse conditions. Also, leaf appearance was delayed in the tolerant genotype, but its leaves grew longer and more rapidly in VPD 1.13kPa and in glasshouse conditions. In VPD 2.55kPa, the tolerant genotype grew shorter leaves and utilized less water compared to H77/833-2 from around 14 DAS (when leaf No.6 was expanding) and this pattern of water use was similar in glasshouse conditions. Under lower VPD there was no difference in the amount of water utilized between both genotypes during observed developmental stages (up to 21 DAS, when leaf No. 9 just emerged). The pattern of water use of H77/833-2 was quite similar in different VPD conditions. We conclude that leaf area development, in relation to water use efficiency and leaf conductance, leads to a pattern of water use for genotypes that is highly environment-specific. These differences, measured under well-watered conditions, could have important consequences on water available for grain filling under terminal water stress. Water saving mechanisms need to be seen from two angles: a) increased water productivity when development takes place at low VPD, and b) lower water use linked to lesser leaf area development but no water productivity advantage when plant development takes place under high VPD.

## INTRODUCTION

Efforts to identify the different component of the adaptation of crops to water limitation have often used the simple concept where  $\text{Yield} = T \times \text{TE} \times \text{HI}$  (T- amount of water transpired, TE – transpiration efficiency, HI – harvest index). According to this formula, yield basically depends on: (i) the amount of water that the plant can make available for transpiration (T), (ii) efficiency of transpired water in terms of carbon fixation through photosynthesis, and (iii) the conversion of biomass into grain (HI). However, this formula overlooks possible interactions effects between the terms of the equation. In particular, it overlooks the fact that there may be stages where water utilization (T) might be critical for some other component of the equation (e.g. HI). Therefore, it appears clearer that, at least for certain crops and conditions, the timing of water utilization throughout plants development might be a component of drought adaptation even more important to consider than the components of the Passioura equation (Sinclair, Hammer *et al.* 2005, Blum 2009, Kholova, Hash *et al.* 2010a, b). For instance, pearl millet genotypes tolerant to terminal stress were able to restrict transpiration rate (TR) before stress conditions occurred and, in so doing, could save water in the soil profile that would be available for grain filling (Kholova, Hash *et al.* 2010a, b).

In these previous studies, the focus was put on understanding the control of leaf water losses under well-watered conditions from the angle of the regulation of stomata opening. Tolerant genotypes were also shown to restrict TR to greater extend when VPD crossed 2kPa. In addition to stomatal conductance aspect, control of leaf water loss depends on the extent of leaf area development. Recent information in maize (Reymond, Muller *et al.* 2003) shows that leaf area development is influenced by the vapor pressure deficit (VPD) and that there was a genotypic variation in this VPD leaf development interaction. As such, genotypes having leaf area development sensitive to VPD would restrict their water use. However, part of that effect could be counterbalanced by the fact that transpiration efficiency is lower at high VPD, TE being in an inverse relationship to VPD (Bierhuizen and Slatyer 1965, Tanner and Sinclair 2003). So, looking at how plant leaf area development and transpiration efficiency combine to determine plant water use and how this varies under low and high VPD conditions is an important complement to previous work on stomatal conductance aspects (Kholova, Hash *et al.* 2010a, b).

Therefore, the aim of this study was to compare the leaf area development characteristics of genotypes known to contrast in the TR restriction in VPD regimes above 2kPa, to assess this putative variation under different VPD regimes, to test transpiration efficiency differences, and assess how these presumed differences could result in different plant water use.

## MATERIALS AND METHODS

### *Plant Material*

Two pearl millet (*Pennisetum glaucum* (L.) R. Br.) genotypes contrasting in tolerance under drought stress (PRLT 2/89-33 (tolerant) and H77/833-2 (sensitive)) were selected for the study based on our previous experiments (Serraj, Hash *et al.* 2005; Yadav, Yash *et al.* 2004), where tolerance/sensitivity was assessed on test-cross hybrids of these inbred parental lines, developed by crossing the inbred parental lines to the most common male sterile line tester 843A (Stegmeier, Andrews *et al.* 1998). Tolerance of these hybrids was based on yield under terminal drought stress in several years of field trials, and on the panicle harvest index (PNHI), a surrogate for the success of spikelet fertility and the degree of grain filling (Bidinger, Mahalkshmi *et al.* 1987). Tolerant genotype PRLT 2/89-33 derives from the ICRISAT Bold Seeded Early Composite, which is an elite breeding population based on Iniadi landrace germplasm from West Africa. PRLT 2/89-33 was shown to tightly restrict water loss especially in VPD higher than 2kPa which was presumed to be a part of its water conservation mechanism and may suggest certain advantages under terminal drought conditions. Sensitive genotype H77/833-2 has a North Indian origin and is the heat resistant parental genotype of many commercially used hybrids of this area. H77/833-2 was shown to use the maximum available soil water at any point of its growth and development and lacked any tight control of transpiration as in the case of the tolerant genotype.

### *Plant Growth Conditions*

Plants were grown individually in 6" and 10" diameter pots for growth chamber/glasshouse experiments in a mixture of Alfisol, sand and manure (5:2:1) and kept well-watered during all developmental stages. Smaller pots were used for early growth stage sequential harvests (see below).

For examination of plant growth and development in optimal conditions (Exp. 1), plants were grown in glasshouse conditions where VPD fluctuated between 3.6/0.4kPa during day/night up to 38 days after sowing (DAS; approximately 14 days after flag leaf appeared; during early grain filling stage). For each genotype, there were 25 replicated pots divided in 5 separate sets (5 replications for each genotype randomized in each set) which were harvested sequentially every week starting at 17 DAS.

For investigation of VPD effect on plant growth and development (Exp. 2), plants were germinated in glasshouse and 5 DAS (in 3 leaf stage) pots were transferred into growth chambers (GCh) under different VPD conditions and same light intensity ( $800 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) during the 12.5 h day cycle. Five replicated pots of each genotype were randomized in a GCh set to low day VPD (1.13 kPa) with combination of 28°C and 70 % RH during day (22°C and 70 %

RH were set for night cycle), whereas five replicated pots of each genotype were randomized in another GCh set to high VPD (2.55kPa) with 30°C and 40 % RH during day (25°C and 70 % RH during night cycle).

### ***Analysis of Growth and Development***

i. Transpiration was monitored daily from the beginning of the experiment under well-watered conditions. Plants were watered to excess and left to drain overnight on the first day of experiments. Next morning, pots were bagged with a plastic bag tightly around the plants stem (Exp. 1) or a thick layer of plastic beads was applied to the soil surface (Exp. 2) to avoid soil evaporation. Therefore any water losses would be mostly due to plant transpiration. Pots were weighed and this first value was considered as “saturated weight” i.e. 100% soil capacity. Pots were then weighted every morning and re-watered up to 80% of saturated weight. Transpiration was monitored throughout the experiment. In experiment 1, the transpiration rate (TR;  $\text{g cm}^{-2} \text{d}^{-1}$ ) was assessed with the set of plants that was harvested weekly and calculated as the average of three days transpiration before plants were harvested divided by the leaf area (LA) at harvest. A similar procedure was used to assess TR in Exp 2.

ii. Transpiration efficiency (TE) was assessed each week in Exp. 1, using the sequential harvests (at 17, 24, 31, 38 DAS) to monitor the biomass increases and the daily transpiration to compute weekly water use. TE was assessed over the entire growth period in Exp 2. For TE estimation in Exp 2, the biomass at the beginning of transpiration monitoring was estimated from an extra plant grown in each pot, which was thinned and its dry weight determined at 5 DAS. In Exp. 2 plants were harvested only at 21 DAS.

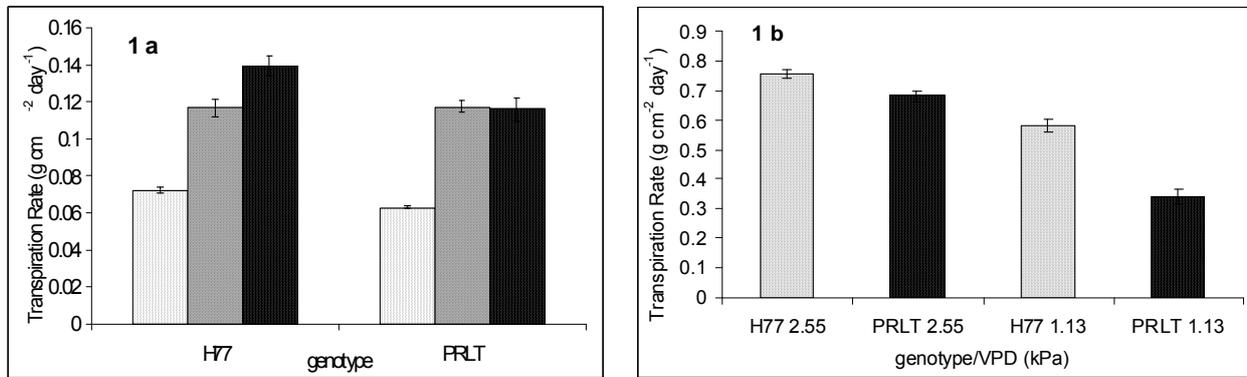
iii. During both experiments, increase in length of all leaves (Exp. 1) and 5-7<sup>th</sup> leaf (Exp. 2) was measured every morning (in Exp. 1) and every morning and evening (in Exp. 2) with a ruler and at the time of harvest, total plant leaf area (LA) was measured with LA meter (model LI-3100 Licor, Lincoln, NE). For expressing leaf growth, time scale in degree days ( $^{\circ}\text{day}$ ) was used (according to Singh, Yoshi *et al.* 1998), with a temperature range 10-45°C.

iv. Amount of water utilized in particular time periods (daily in Exp. 1 and every few days in Exp. 2) was monitored and the slopes of increase in transpiration were expressed in Exp. 1.

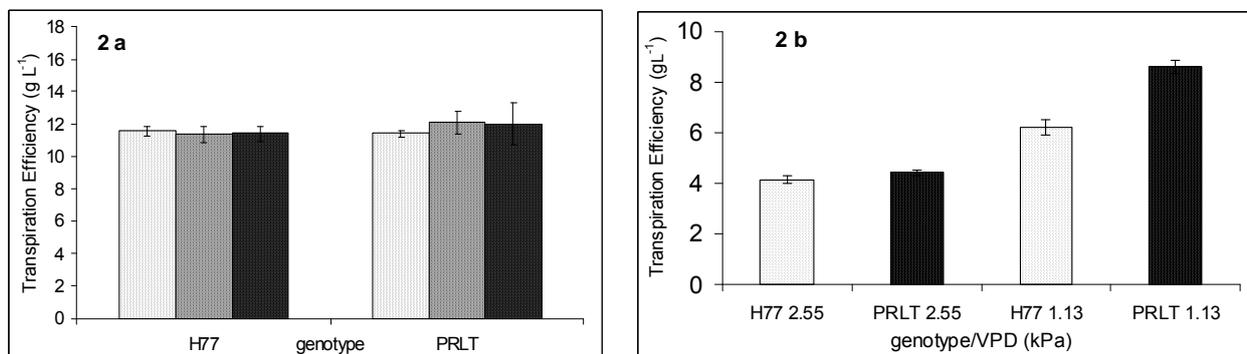
v. Results were statistically analyzed with the program package CoStat version 6.204 (CoHort Software, Monterey, CA, USA). ANOVA analysis and Tukey-Kramer test was performed at the  $p=0,05$  level of significance.

## RESULTS

TR was almost always significantly lower in tolerant genotype (PRLT 2/89-33) compared with the sensitive one, both in Exp 1 and 2 across all environmental conditions (Fig. 1a, b). It is in good agreement with our previous results (Kholova, Hash *et al.* 2010 a, b). Interestingly in Exp. 2 the TR difference between tolerant and sensitive genotype was far smaller in the high VPD regime (2.55kPa) than in the low VPD regime (1.13kPa; Fig. 1b). Also, differences in TR between contrasting genotypes were not reflected in differences in TE in glasshouse conditions (Exp. 1; Fig. 2a) and in high VPD regime (2.55 kPa; Exp 2; Fig. 2 b), confirming similar results in previous studies (Kholova, Hash *et al.* 2010b). By contrast, TE of tolerant genotype (PRLT 2/89-33) was significantly higher in low VPD regime (1.13 kPa) compared to sensitive one (H77/833-2) in Exp. 2 (Fig. 2b). TR of PRLT 2/89-33 was also much lower than in H77/833-2 in those low VPD conditions.

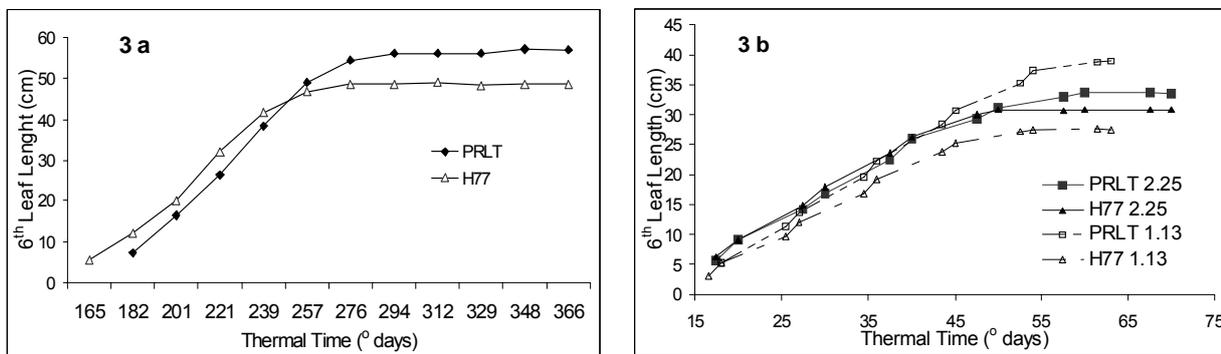


**Figure 1a , b** (a) Transpiration rate of terminal drought tolerant (PRLT 2/89-33 referred as PRLT) and sensitive (H77/833-2 referred as H77) genotypes grown in glasshouse conditions in three different developmental stages (24, 31, 38DAS/ first, second, third column) and (b) in growth chamber conditions with day cycle VPD 2.55 and VPD 1.13 at 21 DAS. With each average  $\pm$  SE for n=5 is shown.



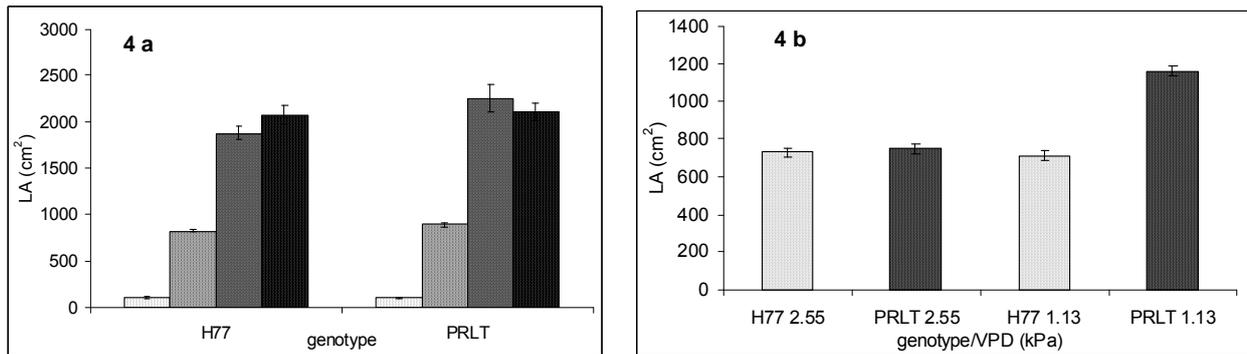
**Figure 2a, b** (a) Transpiration efficiency of terminal drought tolerant (PRLT 2/89-33 referred as PRLT) and sensitive (H77/833-2 referred as H77) genotypes grown in glasshouse conditions between three different developmental stages (17-24, 24-31, 31-38DAS/first, second, third column) and (b) in growth chamber conditions with day cycle VPD 2.55 and VPD 1.13 at 21 DAS. With each average  $\pm$  SE for n=5 is shown.

This TE variation might be related to the leaf appearance and development pattern as shown in Exp. 2. There we found that leaves of tolerant genotype (PRLT 2/89-33) emerged approx. 5°days later compared to sensitive H77/833-2. However, leaves of tolerant genotype (PRLT 2/89-33) grew longer and expanded for longer time period (for approx. 7°days more) compared to sensitive genotype (H77/833-2) under low VPD (1.13 kPa; Fig. 3 b). Contrarily, in high VPD regime (2.55 kPa) the leaves expanded at similar speed for comparable time period in both investigated genotypes (Exp. 2, Fig. 3 b). As such, the leaf length of PRLT 2/89-33 was reduced under high VPD whereas that of H77/833-2 was not and was even slightly increased. Leaves of tolerant genotype PRLT 2/89-33 in Exp. 1 emerged with around 10°days delay (similarly as in low VPD conditions in Exp. 2), but the differences in leaf elongation rate between genotypes were not large (similarly as in high VPD conditions in Exp. 2; Fig. 3a).



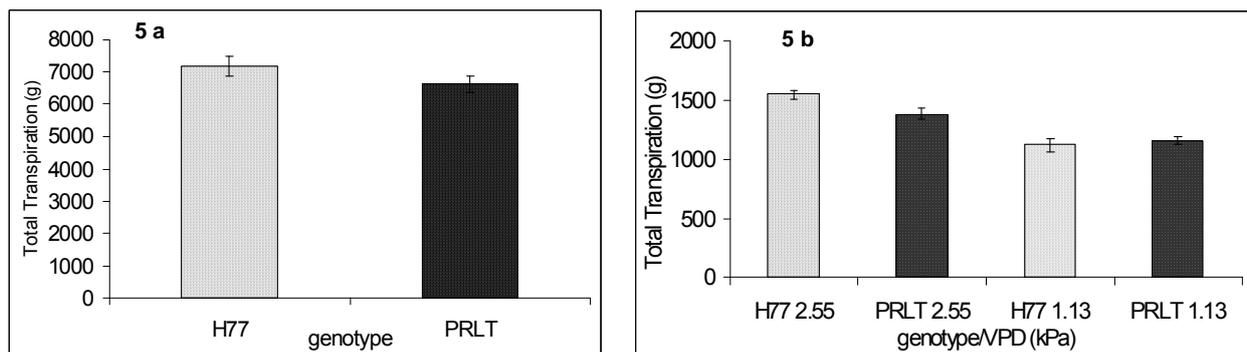
**Figure 3a, b** (a) Average length of 6<sup>th</sup> leaf of terminal drought tolerant (PRLT 2/89-33 referred as PRLT) and sensitive (H77/833-2 referred as H77) genotype grown in glasshouse conditions and (b) in growth chamber conditions with day cycle VPD 2.55 (full line) and VPD 1.13 (dotted line).

Leaf growth pattern related well to the total LA as shown on Fig. 4 a, b. There were constant differences between genotypes in total LA measured at most of the sequential harvests but these differences diminished with plant age in Exp 1.; i.e. PRLT 2/89-33 (tolerant) attained significantly higher LA at most of the points of harvest compared to H77/833-2 (sensitive). However, total LA in Exp. 2 differed significantly between tolerant/sensitive genotypes only in low VPD (1.13 kPa) regime and the differences were not statistically distinguishable in high VPD (2.55 kPa) regime (Fig. 4 b).



**Figure 4a, b** (a) Total leaf area of terminal drought tolerant (PRLT 2/89-33 referred as PRLT) and sensitive (H77/833-2 referred as H77) genotypes in glasshouse conditions during four different developmental stages (17 (pre-harvested plants) 24, 31, 38DAS/first, second, third, fourth column) and (b) in growth chamber conditions with day cycle VPD 2.55 and VPD 1.13 at 21 DAS. With each average  $\pm$  SE for  $n=5$  is shown.

Finally, the total quantity of water used during plant growth and development was similar in tolerant/sensitive genotypes grown in the glasshouse or in growth chamber conditions at high VPD regime (Exp. 2; Fig 5a, b). However, there were genotypic differences in the dynamics in utilization of this available water conditioned by environment, which reflected the leaf area development pattern. In glasshouse environment (Exp. 1) and high VPD regime (Exp. 2), PRLT 2/89-33 (tolerant) utilized more water until around 14/18 DAS in GCh/GH on average compared to H77/833-2 (sensitive; data not shown). However, from this time onwards PRLT 2/89-33 used less water compared to H77/833-2. Contrarily, in low VPD regime in Exp. 2, tolerant genotype (PRLT 2/89-33) utilized similar amount of water during its growth up to 21 DAS (Exp. 2).



**Figure 5a, b** (a) Total amount of water utilized during the plants development by terminal drought tolerant (PRLT 2/89-33 referred as PRLT) and sensitive (H77/833-2 referred as H77) genotypes in glasshouse conditions up to 38 DAS and (b) in growth chamber conditions with day cycle VPD 2.55 and VPD 1.13 up to 21DAS. With each average  $\pm$  SE for  $n=5$  is shown.

## DISCUSSION

Here we confirmed observations of our previous studies (Kholova, Hash *et al.* 2010a, b) that TR of terminal drought tolerant genotype (PRLT 2/89-33) was invariably lower compared to its sensitive counterpart (H77/833-2) across VPD conditions tested. However, the genotypic difference in TR appeared to be smaller under high VPD of the growth environment. This is different from the previous studies where TR differences were higher under high VPD in PRLT 2/89-33 than in H77/833-2. This may be explained by the fact that plants were grown under high VPD conditions. By contrast, plants in the previous study were grown under low VPD and then were transferred to a growth chamber to test the response to increasing VPD. Differences in root hydraulics were hypothesized to be responsible for the differences in the TR response to VPD (Kholova, Hash *et al.* 2010b). It was also found that the differences in TR were not reflected in differences in TE (biomass production per amount of water transpired) in environments where VPD was above 2kPa which was also the case for a substantial part of the day in the glasshouse environment (Kholova, Hash *et al.* 2010b). However, a lower TR in the low VPD environment related to higher TE for tolerant PRLT 2/89-33. This difference may be explained by the higher leaf growth of PRLT 2/89-33 and the similar water use. By contrast, in high VPD environment the leaf expansion rate was comparably decreased in PRLT 2/89-33 while it was unchanged in H77/833-2, leading to less water use in PRLT 2/89-33, despite the smaller differences in TR under high VPD.

## CONCLUSION

The overall aim of this study was to show the importance of environmental conditions for plant development and its further consequences in terms of water use. We conclude that the pattern of water use depends both on the control of stomata opening and on the leaf area development pattern. Both these traits are highly environment-specific and result in differences in the overall plant water use before stress occurs, with direct consequences on plant adaptation to terminal drought stress. Therefore, previously proposed water saving mechanisms need to be seen both from the angle of an increased water productivity when plant development takes place at low VPD, and from a lower water use when development takes place under high VPD, which relates to a restricted leaf area development.

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