Transpiration Efficiency of Amaranth (*Amaranthus sp.)* in Response to Drought Stress

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Abstract

Drought is a major abiotic stress responsible for severe crop losses worldwide. Development of new crop varieties with increased drought tolerance is one way to increase crop productivity. The aim of the present study was to characterize the diversity of nine accessions belonging to Amaranthus *tricolor* and *A. cruentus*, in response to drought stress using a dry-down protocol to characterise the transpiration efficiency (TE). Plants were subjected to either a gradual dry down or well-watered conditions. Results showed that TE was significantly higher ($P<0.01$) in water-deficient (WD) plants compared to water-sufficient (WS) plants, 2.40 g kg^{-1}-7.13 g kg^{-1} and 2.19 g kg^{-1}-4.84 g kg^{-1}, respectively. There was no significant difference in the fraction of transpirable soil water (FTSW) threshold decline between the amaranth genotypes. TE was highly correlated with yield under both WS ($r = 0.89$, $P<0.001$) and WD conditions ($r = 0.662$, $P<0.001$), and negatively correlated with root to shoot ratio under both WS ($r = -0.488$, $P<0.05$) and WD conditions ($r = -0.460$, $P<0.05$). Significant genotypic differences were seen for growth rate and stress susceptibility index (SSI). The result obtained in this investigation underline the need to identify genotypic variation in water use efficiency in amaranth.

Key words

Abiotic stress, amaranth, transpiration efficiency, food security, underutilised crops, water use efficiency.

Abbreviations

FTSW: Fraction of transpirable soil water; NTR: normalized transpiration rate; SSI: Stress susceptibility index; TE: transpiration efficiency; WHC: water holding capacity; WD: water-deficient; WS: water-sufficient.

Introduction

Drought is a serious threat to agriculture, resulting in high annual crop yield losses worldwide. There is a global trend for the increasing frequency and severity of droughts which are expected to become more serious in the next 30-90 years (Dai, 2013). Rain-fed agriculture is particularly vulnerable to unpredictable rainfall patterns and drought, with higher yield losses recorded when compared to irrigated systems (Kurukulasuriya and Ajwad, 2007; Magombeyi and Taigbenu, 2008). Plants have various mechanisms to withstand drought, and their different morphological and physiological strategies for avoiding drought stress are reviewed in Kumar et al. (2012), and Chatterjee and Solankey (2015).

One strategy to mitigate yield losses in a changing climate is diversification of crops away from reliance on staples such as maize (*Zea mays*), wheat (*Triticum spp.*) and rice (*Oryza spp*.), to include species that are better adapted and tolerant to environmental stress, such as minor and underutilized crops (Mayes et al., 2011). Among these crops, amaranth (*Amaranthus spp*.) is increasingly being recognized as having the potential to grow in marginal lands and improve food security due to its adaptability to various environmental conditions and high degree of phenotypic plasticity (Achigan-Dake et al., 2014; Alemayehu et al., 2014). Amaranth is a food crop consumed as both a grain and leafy vegetable in large parts of Africa and Asia (Tucker, 1986; Brenner et al., 2000). It is popular among subsistence farmers because of its fast growth habit and ability to grow in a diverse range of soils and in varied climates

(Palada and Chang, 2003). Compared with staple crops, it has higher nutritive value and better quality of protein (Saunders and Becker, 1983), with a well-balanced amino acid profile (Gamel et al., 2006). Amaranth is distributed globally however most species predominate in the warm temperate and tropical regions (Sauer, 1967). They are C4 plants and are adapted to bright light and warm conditions with a temperature range of 25ºC-30ºC (Schippers, 2000). More than 87 species of amaranth have been described, of which at least 17 edible leaf species and three grain species have been characterized (Mujica and Jacobsen, 2003; Grubben and Denton, 2004). Partly as a consequence of their C4 photosynthesis, amaranth species are able to tolerate relatively high heat and drought, and have been shown to recover well from soil-water deficit by minimizing transpiration through wilting to conserve water (Whitehead and Singh 1992; Myers, 1996; Luoh et al., 2014). Grain amaranth has shown a 40-50% reduced water requirement compared with both wheat and maize (Kauffman and Weber, 1990), whilst vegetable amaranth plants have been grown at 85% field capacity without a significant reduction in yield (Masariramb et al., 2012).

Genetic Phenotypic variation for drought tolerance has been exploited successfully in major crop species such as peanut (Reddy et al., 2001), wheat (Valkoun, 2001), maize (Bänziger et al., 2004) and rice (Zhang et al., 2006) to produce cultivars with improved yield under drought stress. Amaranth shows considerable genetic variability and plasticity for drought tolerance (Slabbert and Van der Hoever, 2007) with a high level of intra species variation compared to staple crops (Erum et al., 2012; Shukla, et al., 2010). The crop displays drought-tolerance mechanisms, such as osmotic adjustment (Liu and Stutzel, 2002a) and high root to shoot ratio (Liu and Stutzel, 2004) which could be exploited given the high level of genetic variability that exists between and within the species in the genus *Amaranthus*. Understanding the genotypic differences in amaranth responses to water deficit is crucial for

developing new water-use efficient cultivars. A deeper understanding of the different mechanisms of drought tolerance is also required.

Transpiration efficiency has been shown to constitute a large source of yield variation in crops subjected to water deficit (Ratnakumar et al., 2009) and has been recognized as a key component of yield variation under drought stress in many crops, including bean (Ehleringer et al., 1991), peanut (*Arachis hypogaea* L.) (Krishnamurthy et al., 2007), grain sorghum (*Sorghum bicolor* (L.) Moench) (Thevar et al., 2010) and banana (*Musa* spp.) (Kissel et al., 2015). Yield and biomass have been shown to be positively correlated with high water-use efficiency (WUE) in wheat (Ehdaie et al., 1991) and breeding for improved WUE has produced improved drought-tolerant genotypes (Condon et al., 2002). Relatively little data is available on the WUE and mechanisms of drought tolerance in amaranth. However, Liu and Stutzel (2004) reported that WUE in vegetable amaranth was unaffected by drought stress.

A detailed investigation into the water relations of vegetable amaranth in response to water deficit is needed to understand mechanisms of drought tolerance in this C4 plant. In this study, we sought to determine if genetic variation for transpiration efficiency under conditions of drought stress existed in nine vegetable amaranth genotypes.

Materials and Methods

Plant Material, Site Description and Growing Conditions

The plant material consisted of nine genotypes; which included three Tanzanian landraces of *Amaranthus cruentus* (B1: Black-seeded amaranth, B2: White-seeded amaranth and B3: Mixed-seeded amaranth). Six genotypes of *Amaranthus tricolor*, of which three were local Malaysian red-leaf vegetable varieties (C1: *Amaranthus* perfect red (var. BBS014), C2: Red amaranth and C3: Red amaranth (var. BBS027)), and three were local Malaysian green-leaf vegetable varieties (D1: Dark green pointed leaf (var. Bamboo Dance 008), D2: Green Special Round Leaf (var. 388) and D3: Green amaranth var. BBS024).

Plants were grown under greenhouse conditions at The University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia (latitude 2.940°N, longitude 101.8740°E) with an average daytime temperature of 36°C and average night-time temperature of 28°C, and average daily relative humidity of 66%. Seeds were sown in 14 × 10 cell trays (54 × 36 cm). Eleven days after emergence, seedlings at the 3rd to 4th leaf stage were transplanted into plastic pots (16 cm × 12.5 cm × 14.5 cm) containing 2 kg of a black peat moss, mix (Holland Brand, Malayisa), with one plant per pot; 5 g fertilizer (N: P: K) per pot was applied once during establishment.

Experimental Design

The experimental treatment was conducted, and completed at the vegetative growth stage. Two treatments were imposed at 15 days after transplantation: drought stress (water-deficient, WD) and well-watered control (water-sufficient, WS). The experimental design was split plot in a randomized complete block design, with one initial set (T0) and two water treatments (water-sufficient, WS and water-deficient, WD) as main plot and nine amaranth genotypes as sub-plot with four replications and three biological repeats. Prior to the onset of drought treatment, plants were irrigated daily to field capacity. On the first day of transpiration-efficiency

assessment, the initial set of plants was destructively harvested to estimate above-ground dry weight; this date was designated as time 0. The remaining plants were watered to maximum soil water-holding capacity (WHC) and allowed to drain freely for 24 hours. WHC was calculated as:

(Weight of saturated-drained soil (24h) - Weight of dry soil)/Weight of dry soil.

After 24 hours, the pots were sealed with a plastic bag to prevent water loss, except by transpiration (Ray and Sinclair, 1998). Pots were then weighed and initial weight was recorded. Subsequently, pots were weighed every 72 hours. After each weighing, water was added back to the WS plants to return them to their maximum WHC. For the WD treatment, no further water was added to induce drought stress. Transpiration efficiency (TE) was calculated for each plant using the following equation:

TE = (Mean shoot biomass at time 0 - Mean shoot biomass at time of harvest) / [(Initial pot weight - Weight of the pot at harvest) + Water added back to the pot].

Soil water status in the individual pots was expressed as fraction of transpirable soil water (FTSW). The daily value of FTSW was estimated as the ratio between the amount of transpirable soil water remaining in the pot and total transpirable soil water. Daily FTSW was calculated based on Ray and Sinclair (1998) as follows:

FTSW = (Daily pot weight - Final pot weight) / (Initial pot weight - Final pot weight)

Two normalizations were carried out to minimize daily variations in transpiration, according to Devi et al. (2009) and the experiment continued until the normalized transpiration rate fell below 0.1.

Chlorophyll analysis

Total leaf chlorophyll content was measured at 2, 8 and 14 days after the imposition of drought treatment (DAT) (starting at 28 days after emergence when the plants were at the vegetative stage) using a portable Minolta Chlorophyll Meter SPAD-502 (Konica Minolta, Langenhagen, Germany). Readings were taken on the 3rd most fully expanded leaflet, avoiding the midrib section. Three readings were taken per leaf and averaged to give a final reading. As reference, chlorophyll content was determined destructively on $2cm^2$ leaf sections according to Bruinsma (1963). The absorbance of extracts was evaluated at 663.6 nm (A663.6) and 646.6 nm (A646.6) with a UV-VIS spectrophotometer (Perkin-Elmer, Lambda 5, Massachusetts, USA) according to the equations given in Porra et al. (1989) as follows:

$$[\text{Chl a}] = 12.25\ E^{663.6} - 2.55\ E^{646.6}$$

$$[\text{Chl b}] = 20.31\ E^{646.6} - 4.91\ E^{663.6}$$

$$[\text{Chl Total}] = 17.76\ E^{646.6} + 7.34\ E^{663.6}$$

A linear function between chlorophyll content and SPAD values was established and used to calculate leaf chlorophyll content. Days to wilting were recorded as days after initiation of drought-stress treatment and wilting was recorded pre-dawn.

Growth analysis and stress susceptibility index

Once the normalized transpiration rate fell below 0.1 plants were destructively harvested and separated into leaves, stem and roots and total leaf area (TLA) was measured using a LI-3100 Area Meter (LICOR, Lincoln, Nebraska, USA). Dry weights were determined

after drying at 80°C in an oven for 72 hours. Specific leaf area (SLA) was then calculated using the following formula:

SLA = Leaf area (cm^2) / Leaf dry weight (g)

Root to shoot ratio (R/S) was calculated as follows:

R/S = Root dry weight (g) / (Leaf + stem dry weight (g))

Yield was calculated as follows:

Yield = Leaf fresh weight (g) + stem fresh weight (g)

A stress susceptibility index (SSI) for yield was determined as the difference between the results obtained under WD and WS conditions. The SSI was calculated according to Fischer and Maurer (1978) using the following equation:

SSI = [1- (Yp / Ys)] / Stress intensity

Stress intensity = 1- (MYs/MYp)

where Yp is the mean value for yield under WS conditions, Ys is the mean value for yield under WD conditions, MYs is the mean yield value for all genotypes under WD conditions, and MYp is the mean yield value of all genotypes under WS conditions.

Data Analysis

The effect of water treatments and genotypes was analysed using Genstat for Windows 16th edition (VSN International 2011). The data were subjected to analysis of variance (ANOVA) and correlation analysis with a split plot design. Mean separation among genotypes was carried out using Tukey's pairwise comparison and significant differences were identified with letters and Least Significant Difference (LSD). The FTSW threshold at which NTR began to decline was calculated using a plateau regression procedure according to the methods of Ray and Sinclair (1998).

Results

Influence of Drought Stress on Growth and Physiology

There were significant differences between the amaranth genotypes for leaf and stem fresh and dry weights under both WS and WD treatments ($P<0.05$). Genotypes B2 and B3 had the highest leaf fresh weight in both WS (25.01, 26.80 g respectively) and WD treatments (3.69, 4.12 g respectively). These two genotypes also recorded the highest percentage loss in fresh weight under WD treatments for all genotypes. In comparison, genotype C3 had the lowest reduction in leaf fresh weight under WD treatment (2.62 g) compared with the WS treatment (8.6 g) (Table 1). There was a significant difference in fresh weight of leaf, stem and root partitioning of individual genotypes in WS and WD treatments. For example, the fresh weight of genotype C3 was primarily partitioned into stem (20.28 g), followed by root (15.22 g) and leaf (8.61 g), under WS treatment, and primarily partitioned into roots (3.30 g), followed by leaf (2.62 g) and stem (2.23 g) under the WD treatment (Table 1).

The root to shoot (R/S) ratio did not change significantly with the WD treatment compared with the WS treatment (P=0.256). Genotypes under the WS treatment did not differ significantly with respect to R/S ratio, whereas there was significant difference recorded among genotypes under the WD treatment (P<0.05), with D2 recording the highest R/S ratio (0.80) and B1 the lowest (0.36) (Table 1).

Total leaf area of WD plants was reduced by two-thirds compared with the WS plants (Table 2) (P<0.001). The highest reduction in TLA was in genotype D2 with an 85% reduction (611.35cm^2 in WS to 76.90cm^2 in the WD treatment), whilst the lowest was genotype C3 with a 58% reduction (403.53cm^2 in WS to 168.43cm^2 in the WD treatment). The reduction in SLA in WD plants was approximately 50% of the SLA of WS plants (P=0.003), with the exception of genotype D3, which was not significantly reduced under the WD treatment relative to the WS treatment (Table 2).

Genotypes did not differ significantly with respect to days to pre-dawn wilting (ranging from 6 to10 days) (Fig. 1). The WD plants started to wilt at 6 DAT when the portion of remaining volumetric soil water available for transpiration dropped to 40% compared with WS plants as shown in FTSW (Fig. 2). The FTSW reached zero transpiration at 14 days after imposition of drought treatment for all genotypes.

Total chlorophyll content did not differ significantly between amaranth genotypes under either WS or WD treatments at 2, 8 and 14 DAT (Table 3). However, the total chlorophyll content was reduced significantly (P<0.001) between 2 DAT and 14 DAT for both treatments. Chlorophyll-a content was higher than chlorophyll-b content in both WS and WD treatments at 2, 8 and 14 DAT. Under severe water deficit conditions (14 DAT), significant genotypic differences existed for chlorophyll-b content, with genotype B3 having the highest (13.85 µgcm^{-2}) and genotype C1 the lowest (4.14 µgcm^{-2}).

The SSI varied significantly among genotypes, with the most drought- tolerant genotype, C3, recording the lowest SSI (0.83) ($P<0.001$), and the most drought susceptible genotype, D2, recording the highest SSI value (1.10) ($P<0.001$) (Fig. 3).

Genotypic Variation in TE in Response to Soil-Water Deficit

The total water transpired was significantly reduced under WD conditions compared with WS conditions in all nine genotypes ($P<0.001$) (Table 4). However, there were no genotypic differences for total water transpired under either treatment. The TE increased significantly for all genotypes in the WD treatment relative to the WS treatment ($P<0.001$) with the exception of D3 genotype where the TE was similar under both water treatments. There were no significant differences among genotypes with respect to final weight of soil water available for transpiration in pots at the end of WD treatment as FTSW reached zero with a range of 0.48-0.53 kg. The relationship between NTR and FTSW for each amaranth genotype is shown in Fig. 4. The genotypes showed the same overall pattern for soil drying and there was no significant difference in the FTSW threshold of the NTR decline (Table 4).

Correlations

Correlation coefficients for all traits measured for WS and WD treatments are shown in Table 5. Under WS treatments, TE was positively correlated with leaf fresh weight ($r=0.801$, $P<0.05$), root dry weight ($r=0.709$, $P<0.001$) and total yield ($r=0.89$, $P<0.001$), and negatively correlated with R/S ($r=-0.488$, $P<0.001$). Under WD treatments, TE was positively correlated with leaf fresh weight ($r=0.536$, $P<0.001$), leaf dry weight ($r=0.841$, $P<0.001$), stem fresh weight ($r=0.549$, $P<0.001$), stem dry weight ($r=0.790$, $P<0.05$) and root dry weight ($r=0.661$,

P<0.001), and negatively correlated with R/S (r=-0.46, P<0.05), SLA (r=-0.668, P<0.001) and days to wilting (r=-0.525, P<0.001).

Discussion

This study was designed to determine the influence of water relations on adaptive strategies to drought in different amaranth genotypes. There is a need to resolve whether the genotypic variation in TE is an inherent consequence of basic physiological changes regardless of soil drying and subsequently identify suitable surrogate traits for TE as a drought-tolerance selection criterion in amaranth species. Liu and Stutzel (2002a) reported that in vegetable amaranth, transpiration during water deficit was regulated through the reduction of leaf expansion and stomatal conductance, and thus prevented leaf dehydration. Leaf area expansion in vegetable amaranths was identified as more sensitive to soil drying when compared with transpiration and stomatal conductance (Liu and Stutzel 2002b).

In this experiment, total water transpired by the plants directly affected the TE value as higher total water transpired reduced the TE. There was a similar pattern of total water transpired in both WS and WD treatments among the nine amaranth genotypes, with WD plants showing a lower value for total water transpired. This was reflected in higher TE values in WD plants compared with WS plants with the exception of genotype D3 which had similar TE under both water treatments. The similar amount of total water transpired among all genotypes under both water treatments suggested that there were other physiological traits that influenced the variation in TE. Sinclair et al. (1984) stated that two critical variables accounted for variation in TE in WS plants, which were a difference in the composition of plant products and / or the CO_2 concentration maintained in the leaves.

The response of transpiration to soil water deficit has previously been described using a linear plateau model (Devi et al., 2009), which identified the critical soil water content at which transpiration rate started to decline. The FTSW represents the portion of remaining volumetric soil water available for transpiration, and at which threshold, the plants' physiological processes start to decline (Liu and Stutzel, 2002a). In the present study, there was a wide range of FTSW threshold values at which the transpiration rate began to decline among the amaranth genotypes indicating genotypic differences in relation to soil drying. The range of FTSW threshold decline in *A. cruentus* in this experiment (0.32-0.38) was very similar to the range (0.22-0.48) reported by Liu and Stutzel (2002b). In contrast, a large difference was found in red *A. tricolor* (0.29-0.56) and green *A. tricolor* (0.41-0.52) in this experiment compared with the range of 0.29-0.44 recorded for *A.tricolor* by Liu and Stutzel (2002b), possibly as a consequence of the different genotypes used in these two studies.

This linear plateau model has also been used as an indicator of stress (Ritchie, 1981). In a study of genotypic responses to transpiration in chickpea, despite no genotypic difference in total water extracted, differences in the pattern of water extraction from the soil profile were observed which consequently affected the pod yield (Ratnakumar et al., 2009). The present study showed that there was a difference in the pattern of water extraction, which influenced the TE value. For example, genotype C2 had a high FTSW threshold (0.56) with restricted transpiration during early soil drying which allowed the plants to conserve more water under water deficit conditions and produce a low TE value (4.01 g kg^{-1}). In comparison, genotype B1 had a low FTSW threshold (0.38) and transpiration continued with further soil drying, producing a high TE value (7.13 g kg^{-1}). Genotype C1 had the lowest FTSW threshold (0.29) among all genotypes, indicating that transpiration declined upon progressive soil drying under relatively drier conditions. However, it is important to note that genotype D3 had high FTSW threshold decline (0.51), but also had similar TE and SLA values for both WS and WD

treatments, and a high R/S ratio under both WS and WD conditions. A possible explanation is the greater root density of D3 compared to the other genotypes, allowing it to sustain high water uptake at low soil-water content. D3 was able to extract higher amounts of water while sustaining an increased transpiration rate at low soil-water content under the WD treatment and resulted in a similar TE value under the WS treatment.

Plants that perform better under water-deficit conditions are likely to have a high TE value and could be associated with a high threshold for decreased NTR (Devi et al., 2009). A higher FTSW threshold could allow the plants to conserve more soil water, better positioning them to endure drought stress (Johnson et al., 2009). In the present study, genotypes with a high FTSW threshold might have had an opportunity to fully utilize the soil-water content and maximize growth before the experiment was terminated. Genotypes such as these are positioned to conserve water during soil drying to the point where transpiration rate is restricted, (Gholipoor and Sinclair, 2012). In this study, it is difficult to conclude whether a high FTSW threshold gave a high TE value, as the value of FTSW did not correlate with the TE values. Hence, there is a need to understand the role of TE as a component of the genotypic differences in the FTSW threshold.

In the present study, a high FTSW threshold was associated with increased drought tolerance, as genotype D3 showed a similar TE value under both WS and WD treatments. Genotype D3 had a high FTSW threshold decline, with a lower TE value for WS and WD plants compared to the other genotypes. This implied that genotype D3 maximized water-use efficiency instead of utilizing the water for maximizing growth. Genotype D3 appeared to have a different mechanism for growth, as soil drying did not significantly alter the TE compared with the WS plants. One explanation could be that lower transpiration under WS conditions led to lower daily transpiration, which would logically drive the transpiration rate of drought

stressed plants upward, and consequently the NTR (Bhatnagar-Mathur et al., 2007; Kholova et al., 2010). Therefore, the maintenance of NTR under drought conditions at similar levels to WS plants results in a lower value for the FTSW threshold at which transpiration begins to decline. Alternatively, this may simply be a consequence of the lower rate of water loss per unit leaf area in the WS plants.

The drought tolerance of the amaranth genotypes was expressed as SSI, (Fisher and Maurer, 1978). The tolerance of a genotype to drought stress is predicted to be higher if the SSI value is low (Zdravkovic et al., 2013). Despite genotype D3 displaying drought tolerance characteristics, it was considered susceptible to drought stress as it had a high SSI value and low yield. In comparison, genotype C3, which also had high FTSW threshold (0.51) and TE value under WD conditions, was considered tolerant to drought, as it had a low value of SSI for yield, which can be explained by the low reduction in TLA. The most susceptible genotype was D2, which had the highest reduction in TLA. Thus, a low or high FTSW threshold may not necessarily produce a desired amount of crop yield. Kholova et al. (2010) reported that two different hybrid lines of pearl millet had low FTSW thresholds. However, one hybrid line also had low yield similar to drought-sensitive lines, compared with high yield achieved by the drought-tolerant lines.

Jomo et al. (2016) reported that the total chlorophyll content of amaranth was significantly reduced in response to soil water deficit with *A. tricolor* recording the lowest reduction in chlorophyll compared to other amaranth species. However, the present study showed no significant difference in total chlorophyll between WS and WD plants after 14 days of drought-stress treatment. Drought stress has been shown to alter the ratio of chlorophyll-a and chlorophyll-b content (Anajum et al., 2011). In the current study, chlorophyll-a content was higher than chlorophyll-b content under both water treatments, which was comparable to the

results of Jomo et al. (2016). Schlemmer et al., 2005, reported no effect of drought stress on chlorophyll content in maize, however in contrast, O'Neil et al. (2006) reported that chlorophyll was the only measurement affected by drought in maize. Therefore, it could be a trend for amaranth species to react differently to water deficit conditions and might be an adaptation strategy of C4 photosynthesis.

Liu and Stutzel (2004) reported a negative correlation between WUE and SLA in amaranth. In the present study, the reduction of SLA in WD plants was similar for all genotypes, except for D3, demonstrating that SLA was not conclusively responsible for the differences in TE among the amaranth genotypes.

Conclusions

The FTSW threshold at which transpiration declined upon progressive soil drying influenced water relations in differing ways for the nine genotypes suggesting different adaptive strategies to drought. It is interesting to note that amaranth species have similar growth performance relative to transpiration efficiency under water-sufficient and water-deficit conditions and high TE may not necessarily the best indicator for drought tolerance selection traits in amaranth genotypes.

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Figure Captions

Fig. 1 Days to pre-dawn wilting (DTW) for nine amaranth genotypes in water-deficient conditions (WD). The error bars indicate ± standard error of mean (SE) with n=6.

Fig. 2 Fraction of transpirable soil water (FTSW) reached zero in water-deficient plants (WD) indicating no soil water was available for transpiration after 14 days imposition of drought stress. The error bars indicate ± standard error of the mean (SE) with n=6.

Fig. 3 Stress susceptibility index (SSI) for yield under drought for nine amaranth genotypes. An SSI>1 above-average susceptibility to drought stress.

Fig. 4 A plateau regression to show the relationship between the normalized transpiration rate (NTR) and the fraction of transpirable soil water (FTSW) of nine amaranth genotypes. The FTSW threshold is indicated by the breakpoint of the plateau where transpiration starts to decline. R^2 indicates the coefficient of determination between NTR and FTSW.

Table 1 Mean of fresh weight (FW) (g) and dry weight (DW) (g) of leaf, root and stem, and root to shoot (R/S) ratio of nine genotypes of amaranth under water-sufficient (WS) and water-deficient (WD) conditions, respectively with ± standard error of means (SE).

Genotype	Leaf FW (g)		Leaf DW (g)		Root FW (g)		Root DW (g)		Stem FW (g)		Stem DW (g)		R/S (g)	
	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD
B1	18.09±1.04ab	3.26±0.27ab	3.87±0.15abc	2.79±0.29a	18.14±2.75a	4.45±1.57a	3.67±0.09ab	2.35±0.27ab	31.15±5.99ab	6.41±0.85a	6.72±1.52a	±3.950.50a	0.37±0.06a	0.36±0.05c
B2	25.10±1.84a	3.69±0.31ab	4.78±0.45ab	2.91±0.22a	19.59±3.91a	4.51±0.35a	4.03±0.57a	2.37±0.29ab	32.79±2.44a	5.36±0.64ab	6.73±0.44a	2.98±0.51ab	0.35±0.03a	0.40±0.04bc
B3	26.80±1.16a	4.12±0.26a	5.47±0.38a	3.35±0.27a	20.31±2.42a	4.00±0.40a	4.20±0.16a	2.86±0.33a	27.71±3.61ab	4.30±0.48abc	6.26±0.19ab	3.00±0.16ab	0.36±0.02a	0.46±0.08bc
C1	19.39±1.76ab	3.06±0.38ab	3.95±0.38abc	2.57±0.33ab	17.93±2.11a	3.56±0.44a	2.86±0.32ab	1.54±0.15b	23.14±1.79ab	3.54±0.49bc	3.19±0.55bc	1.55±0.30bc	0.40±0.01a	0.38±0.03bc
C2	18.24±3.23ab	3.61±0.35ab	3.74±0.70abc	2.52±0.47abc	19.14±4.40a	3.41±1.12a	2.72±0.47ab	1.56±0.43b	18.27±3.61ab	2.69±0.76c	2.35±0.68c	1.33±0.36c	0.49±0.09a	0.39±0.04bc
C3	8.61±2.04b	2.62±0.28ab	1.94±0.18c	2.17±0.32abc	15.22±2.45a	3.30±0.86a	1.96±0.23b	1.63±0.20ab	20.28±2.27ab	2.23±0.32c	2.98±0.40c	1.38±0.17c	0.39±0.03a	0.46±0.03bc
D1	18.43±2.52ab	2.52±0.53ab	3.62±0.45abc	1.79±0.47abc	18.89±1.91a	5.52±0.80a	3.05±0.32ab	2.16±0.26ab	25.31±3.11ab	3.20±0.53bc	4.06±0.46bc	1.93±0.24bc	0.40±0.02a	0.59±0.03abc
D2	17.43±4.72ab	1.43±0.44b	2.76±0.54bc	0.97±0.21c	19.06±3.42a	5.11±1.11a	3.24±0.62ab	2.15±0.08ab	20.36±2.13ab	3.57±0.21bc	2.73±0.29bc	1.72±0.10bc	0.60±0.12a	0.80±0.05a
D3	16.56±4.37ab	2.97±1.25ab	3.17±0.50bc	1.19±0.30bc	18.88±2.79a	3.61±0.56a	3.14±0.58ab	1.42±0.20b	16.76±2.99b	3.63±0.24bc	3.02±0.35c	1.18±0.26c	0.51±0.09a	0.62±0.10ab
SED	2.854		0.556		3.242		0.512		3.349		0.733		0.077	
LSD	5.737		1.116		6.548		1.047		6.723		1.471		0.158	
P	<0.001		0.002		0.002		0.019		<0.001		0.002		0.256	

Table 2 Mean of total leaf area (TLA) (cm^2) and specific leaf area (SLA) (cm^2g^{-1}) and total yield (g) ± standard error of means (SE) of nine amaranth genotypes under water-sufficient (WS) and water-deficient (WD) conditions.

	TLA (cm^2)		SLA (cm^2g^{-1})		Yield (g)	
Genotype	WS	WD	WS	WD	WS	WD
B1	470.84±119.91a	129.87±29.57a	121.31 ± 29.96ał	45.96±8.12a	121.31±29.96a	45.96±8.12a
B2	784.27±54.25a	164.69±12.43a	165.96±10.68a	57.55± 5.79ab	165.96±10.68a	57.55±5.79a
B3	800.66±30.82a	170.47±22.89a	147.48±6.12a	53.40± 10.59abc	147.48±6.12a	53.40±10.59a
C1	739.23±82.72a	210.79±12.75a	186.79±8.16ab	85.77±12.06abc	186.79±8.16a	85.77±12.06a
C2	714.72±115.82a	215.66±21.46a	243.04± 95.63ab	91.51± 11.31abc	243.04±95.63a	91.51±11.31a
C3	403.53±73.82a	168.43±25.46a	202.29± 26.41b	79.91±12.56c	202.29±26.41a	79.91±12.56a
D1	677.12±90.75a	140.71±42.30a	189.94± 20.67ab	97.71±42.79bc	189.94±20.67a	97.71±42.79a
D2	611.35±156.89a	75.90±26.00a	218.84±45.69ab	73.27± 8.29c	218.84±45.69a	73.27±8.29a
D3	540.72±157.66a	158.91±54.38a	168.71±40.62ab	128.55± 25.67abc	168.71±40.62a	128.55±25.67a
SED	112.0		44.94		5.266	
LSD	225.2		90.21		10.573	
P	<0.001		0.003		<0.001	

† TLA is total leaf area and SLA is specific leaf area
‡ SED is standard errors of difference between two means of water treatments
§ LSD is least significant differences of means of water treatments
¶ P is probability (P-value) of the water treatments significantly different at $P<0.05$
# Values identified with same letter are not statistically different among genotype based on Tukey's Pairwise method ($P<0.05$)

Table 3 Days to pre-dawn wilting with ± standard error of means (SE) of nine amaranth genotypes in water-deficient conditions (WD).

Genotype	Days to wilting
	WD
B1	9.00±0.00a
B2	7.00±0.71a
B3	6.00±0.87a
C1	8.00±0.58a
C2	9.00±1.00a
C3	9.00±1.44a
D1	8.00±1.41a
D2	10.00±1.31a
D3	10.00±1.31a
SED	1.517
LSD	3.112
P	0.192

† SED is standard errors of difference between two means of water treatments
‡ LSD is least significant differences of means of water treatments
§ P is probability (P-value) of the water treatments significantly different at P<0.05
¶ Values identified with same letter are not statistically different among genotype based on Tukey's Pairwise method (P<0.05)

Table 4 Mean of total chlorophyll content, chlorophyll-a and chlorophyll-b (µgcm^{-2}) ± standard error of means (SE) of nine genotypes of amaranth under water-sufficient (WS) and water-deficient (WD) conditions at 2 days after treatments (DAT), 8 DAT and 14 DAT

	2 DAT		8 DAT		14 DAT	
			Total chlorophyll content (µgcm^{-2})			
	WS	WD	WS	WD	WS	WD
B1	38.81±2.36a	33.25±2.43a	29.17±1.28a	18.36±2.37a	29.63±1.55ab	17.98±11.02a
B2	37.01±1.95a	42.17±2.19a	31.70±2.59a	23.88±5.25a	20.10±4.36ab	22.48±5.56a
B3	37.67±2.00a	39.67±1.06a	37.51±2.50a	42.47±2.67a	34.86±2.64a	39.50±9.77a
C1	37.55±4.02a	42.28±2.33a	28.21±3.87a	31.52±10.59a	18.27±3.27ab	26.25±12.65a
C2	45.95±1.96a	41.42±1.55a	38.09±3.76a	37.62±5.83a	24.37±2.95ab	43.41±5.99a
C3	41.63±2.87a	38.74±1.86a	34.14±2.18a	34.35±7.12a	24.85±3.18ab	34.73±11.21a
D1	37.26±1.98a	43.58±3.03a	37.74±3.97a	38.42±5.11a	31.12±2.12b	36.89±6.93a
D2	33.14±2.47a	37.04±6.09a	32.86±2.29a	38.63±11.09a	30.63±3.47ab	32.61±17.16a
D3	35.08±5.67a	40.47±3.17a	40.99±7.35a	35.44±11.61a	31.62±3.75ab	36.16±8.45a
SED	4.386		8.420		10.47	
LSD	8.879		16.91		21.21	
P	0.467		0.707		0.636	
			Chlorophyll a (µgcm^{-2})			
B1	23.69±1.37a	20.45±1.42a	18.08±0.74a	11.78±1.38a	18.35±0.90ab	11.57±6.42a
B2	22.65±1.13a	25.65±1.28a	19.55±1.51a	15.00±3.05a	12.80±2.54ab	14.19±3.23a
B3	23.03±1.16a	24.19±0.62a	22.94±1.46a	25.83±1.55a	21.39±1.54a	24.09±5.69a
C1	22.96±2.34a	25.71±1.36a	17.52±2.25a	19.45±6.17a	11.73±1.90ab	16.38±7.36a
C2	27.85±1.14a	25.21±0.90a	23.27±2.19a	23.00±3.39a	15.28±1.72ab	26.37±3.49a
C3	25.33±1.67a	23.65±1.08a	20.98±1.27a	21.10±4.14a	15.57±1.85ab	21.32±6.53a
D1	22.79±1.15a	26.47±1.77a	23.07±2.31a	23.47±2.98a	19.22±1.24b	22.57±4.03a
D2	20.39±1.44a	22.66±3.55a	20.23±1.33a	23.59±6.46a	18.93±2.02ab	20.08±9.99a
D3	21.52±3.30a	24.66±1.84a	24.96±4.28a	21.73±6.76a	19.51±2.18ab	22.15±4.92a
SED	2.554		4.902		6.093	

LSD	5.169		9.847		12.35	
P	0.467		0.707		0.636	
			Chlorophyll b (µgcm^{-2})			
B1	16.15±1.38a	12.90±1.42a	10.52±0.75a	4.19±1.39a	10.79±0.90ab	10.42a
B2	15.10±1.14a	18.12±1.28a	11.99±1.52a	7.42±3.07a	7.16±2.32ab	9.62±1.71a
B3	15.49±1.17a	16.66±0.62a	15.40±1.46a	18.30±1.56a	13.85±1.54a	16.56±5.71a
C1	15.42±2.35a	18.18±1.36a	9.96±2.26a	11.89±6.20a	4.14±1.91b	15.37±5.94a
C2	20.33±1.15a	17.68±0.91a	15.73±2.20a	15.46±3.41a	7.71±1.72ab	18.84±3.50a
C3	17.80±1.68a	16.12±1.09a	13.43±1.27a	13.55±4.16a	7.99±1.86ab	13.77±6.56a
D1	15.25±1.16a	18.94±1.77a	15.53±2.32a	15.93±2.99a	11.66±1.24ab	15.03±4.05a
D2	12.84±1.44a	15.12±3.56a	12.68±1.34a	16.05±6.49a	11.37±2.03ab	29.49±5.31a
D3	13.97±3.31a	17.13±1.85a	17.43±4.30a	19.15±6.54a	11.95±2.19ab	14.60±4.94a
SED	2.565		4.924		6.121	
LSD	5.192		9.891		12.41	
P	0.467		0.707		0.636	

† SED is standard errors of difference between two means of water treatments
‡ LSD is least significant differences of means of water treatments
§ P is probability (P-value) of the water treatments significantly different at P<0.05
¶ Values identified with same letter are not statistically different among genotype based on Tukey's Pairwise method (P<0.05)

Table 5 Mean of total water transpired (kg) and transpiration efficiency (TE) of nine genotypes of amaranth under water-sufficient (WS) and water-deficient (WD) conditions with ± standard error of means (SE), and mean of amount of soil water content in a pot at FTSW=0 of WD plants ± SE . FTSW threshold values for nine amaranth genotypes were calculated using the linear plateau regression model with ± SE and 95% confidence limit of the threshold.

Genotype	Total water transpired (kg)		TE (gk^{-1})		Soil water (kg) when FTSW=0 of WD	FTSW threshold decline of WD	95% CI for FTSW decline of WD
	WS	WD	WS	WD			
B1	2.28±0.05	0.94±0.026a	4.62±0.61ab	7.13±0.69a	0.52±0.02a	0.38±0.04a	0.31-0.51
B2	2.41±0.10a	0.89±0.079a	4.79±0.34a	6.74±0.73a	0.52±0.01a	0.37±0.05a	0.29-0.59
B3	2.42±0.09a	0.92±0.041a	4.84±0.19a	6.91±0.44a	0.49±0.01a	0.32±0.05a	0.24-0.47
C1	2.46±0.12a	1.08±0.055a	2.92±0.40bc	3.78±0.36b	0.48±0.02a	0.29±0.01a	0.25-0.37
C2	2.19±0.08a	0.95±0.031a	2.73±0.47c	4.01±0.77b	0.53±0.02a	0.56a	0.37-0.67
C3	2.25±0.09a	0.98±0.003a	2.19±0.07c	3.60±0.28b	0.46±0.01a	0.51±0.09a	0.29-0.78
D1	2.23±0.05a	0.92±0.048a	3.42±0.33abc	4.09±0.80b	0.49a	0.41±0.04a	0.33-0.52
D2	2.36±0.18a	0.92±0.057a	2.34±0.25c	2.96±0.28b	0.49±0.01a	0.52±0.07a	0.39-0.79
D3	2.43±0.08a	0.97±0.099a	2.41±0.46c	2.40±0.22b	0.51±0.04a	0.51±0.01a	0.06-0.79
SED	0.137		0.667		0.021	0.1067	
LSD	0.274		1.360		0.044	0.2193	
P	<0.001		<0.001		-	-	

† SED is standard errors of difference between two means of water treatments
‡ LSD is least significant differences of means of water treatments
§ P is probability (P-value) of the water treatments significantly different at P<0.05
¶ Values identified with same letter are not statistically different from each other based on Tukey's Pairwise method (P<0.05)

1

2 **Table 6 (a) Correlation coefficients (r) for traits associated with the water-sufficient (WS) treatment for the nine amaranth genotypes.**

Trait	LeafFW (g)	LeafDW (g)	StemFW (g)	StemDW (g)	RootFW (g)	RootDW (g)	R/S	TLA (cm^2)	SLA (cm^2g^{-1})	TLC2 (μgcm^{-2})	TLC8 (μgcm^{-2})	TLC14 (μgcm^{-2})	TWT (kg)	TE (gk^{-1})	Yield (g)
LeafFW	1														
LeafDW	0.88**	1													
StemFW	0.559**	0.533**	1												
StemDW	0.562**	0.571**	0.857**	1											
RootFW	0.435*	0.551*	0.49*	0.482*	1										
RootDW	0.594**	0.642**	0.629**	0.724**	0.651**	1									
R/S	-0.269	-0.325	-0.364	-0.435	0.029	0.105	1								
TLA	0.701**	0.587**	0.368	0.353	0.152	0.376	-0.001	1							
SLA	-0.204	-0.424	-0.407	-0.474	-0.744**	-0.6*	0.106	0.201	1						
TLC2	-0.325	-0.146	-0.195	-0.264	-0.085	-0.199	0.017	-0.305	-0.09	1					
TLC8	-0.11	0.002	-0.157	-0.124	-0.28	-0.116	-0.022	0.188	0.092	0.312	1				
TLC14	0.093	-0.021	0.038	0.099	0.029	0.042	0.232	0.251	-0.074	-0.208	0.087	1			
TWT	0.111	0.247	0.51*	0.368*	0.332	0.374	-0.128	-0.034	-0.435*	-0.023	-0.063	-0.009	1		
TE	0.801*	0.837	0.739	0.867	0.515	0.709**	-0.488**	0.491*	-0.463	-0.223	-0.086	0.021	0.157	1	
Yield	0.874**	0.816**	0.857**	0.788	0.502**	0.687	-0.382*	0.595**	-0.321	-0.278	-0.176	0.026	0.312	0.89**	1

Table 6 (b) Correlation coefficients (r) for traits associated with the water-deficient (WD) treatment for the nine amaranth genotypes.

Trait	LeafFW (g)	LeafDW (g)	StemFW (g)	StemDW (g)	RootFW (g)	RootDW (g)	R/S	TLA (cm^2)	SLA (cm^2g^{-1})	TLC2 (μgcm^{-2})	TLC8 (μgcm^{-2})	TLC14 (μgcm^{-2})	TWT (kg)	TE (gk^{-1})	Yield (g)	DTW
LeafFW	1															
LeafDW	0.678**	1														
StemFW	0.318	0.398	1													
StemDW	0.304	0.484**	0.688**	1												
RootFW	-0.057	0.163	0.383*	0.21	1											
RootDW	0.144	0.389*	0.510**	0.808**	0.320	1										
R/S	-0.572**	-0.649**	-0.126	-0.142	0.138	0.262	1									
TLA	0.577**	0.392*	-0.059	-0.149	-0.089	-0.189	-0.351	1								
SLA	-0.105	-0.546**	-0.461**	-0.655**	-0.236	-0.542**	0.286	0.474**	1							
TLC2	0.049	-0.001	-0.250	-0.160	-0.131	-0.078	-0.007	0.069	0.117	1						
TLC8	-0.094	-0.158	-0.282	-0.287	0.252	-0.274	0.017	-0.009	0.220	-0.014	1					
TLC14	-0.013	-0.089	-0.277	-0.273	0.268	-0.161	0.120	0.126	0.288	0.087	0.668**	1				
TWT	-0.053	0.124	-0.123	0.022	-0.038	-0.024	-0.191	0.198	-0.038	0.170	-0.106	-0.246	1			
TE	0.536**	0.841**	0.549**	0.79**	0.197	0.661**	-0.46**	0.136	-0.668**	-0.072	-0.248	-0.110	-0.091	1		
Yield	0.755**	0.625**	0.815**	0.600*	0.219	0.404*	-0.408*	0.335*	-0.328	-0.145	-0.262	-0.220	-0.080	0.662**	1	
DTW	-0.173	-0.424**	-0.184	-0.413**	-0.138	-0.55**	0.003	-0.238	0.238	-0.115	-0.039	-0.168	-0.042	-0.525**	-0.232	1

Figure 1

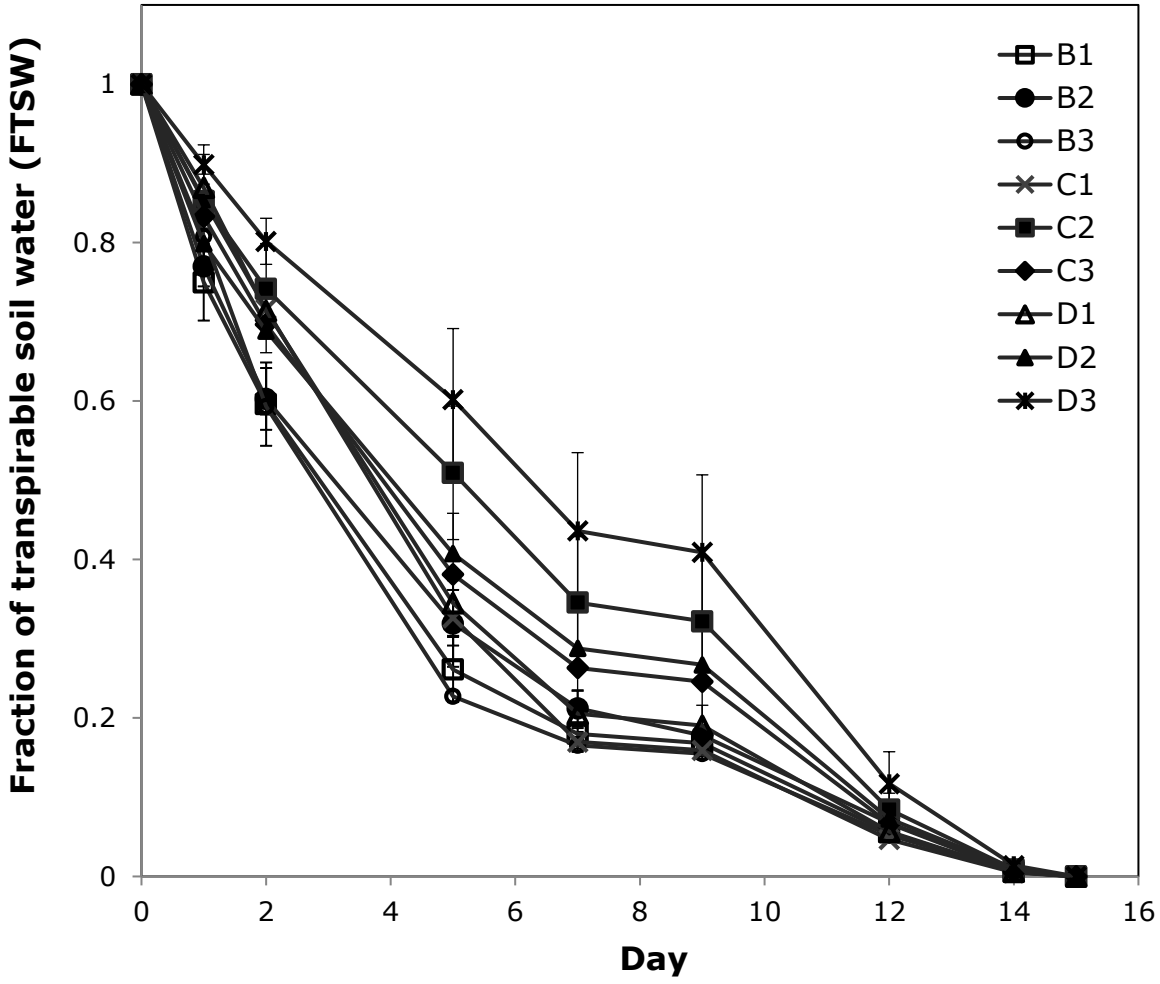
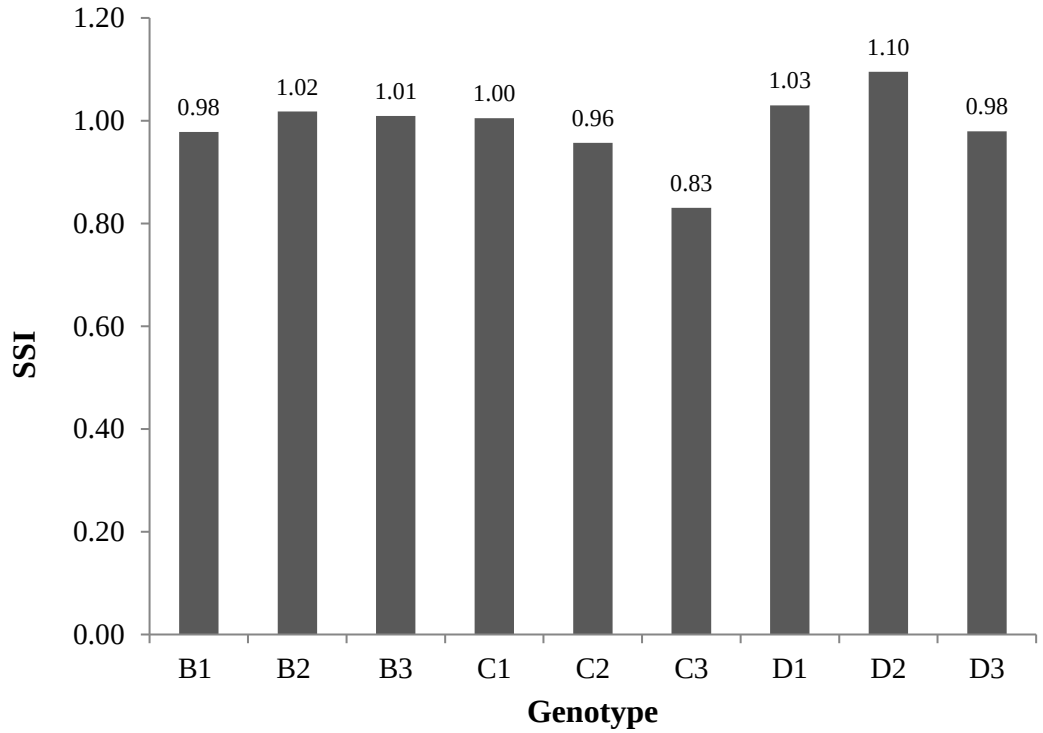

Fraction of transpirable soil water (FTSW)
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
Day
B1
B2
B3
C1
C2
C3
D1
D2
D3

Figure 2


1.20
1.00
0.80
0.60
0.40
0.20
0.00
SSI
0.98
1.02
1.01
1.00
0.96
0.83
1.03
1.10
0.98
B1
B2
B3
C1
C2
C3
D1
D2
D3
Genotype

10 **Figure 3**

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