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ORIGINAL ARTICLE

Measurement of transpiration restriction under high vapor pressure deficit for sorghum mapping population parents

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Abstract Limiting transpiration rate under high vapor pressure deficit (VPD) and/or progressive soil drying conditions are soil water conservation mechanisms that can play an important drought-adaptive role if water is limiting to support crops at its full potential. In this study, these two important physiological mechanisms were measured on parental pairs of existing Recombinant Inbred Lines (RILs) of sorghum mapping populations; both in experiments run in the glasshouse and growth chambers, and outdoors. In controlled environmental conditions, the RIL1, RIL2, RIL6 and RIL8 showed contrasting transpiration response to increasing VPD. The difference in the soil moisture fractions of transpirable soil water threshold where transpiration initiated a decline were high in RIL1, RIL3 and RIL8 respectively. The exploration of the variation of the evapotranspiration response to VPD was also carried out in a high throughput phenotyping facility in which plants were grown similar to field density conditions. Under high VPD conditions, the RIL parental pairs showed usual transpiration peak during the midday period. At this time period, genotypic differences within parental pairs were observed in RIL1, RIL2, RIL6 and RIL8. The donor parent had lower transpiration than the recurrent parents during the midday/high VPD period. Also, we found variation among parental pairs in leaf area normalized with received radiation and measured plant architecture traits. Across studied genotypes, RIL1, RIL2 and RIL8 showed differences in the plant canopy architecture and the transpiration response to an increasing VPD. Collectively, these results open the opportunity to phenotype the RIL progenies of contrasting parents and genetically map the traits controlling plant water use. In turn, this can act as an important genetic resource for identification and incorporation of terminal drought tolerance components in marker-assisted breeding.

Keywords Transpiration · Canopy · Radiation · Climate change · High throughput phenotyping

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Introduction

Sorghum [*Sorghum bicolor* (*L.*) *Moench*] is one of the important staple food and fodder in the arid and semi-arid regions. Due to its ability to sustain harsh and marginal agro-ecological conditions it has been grown in both possible seasons in semi-arid parts of India: *Kharif* (rainy) and *Rabi* (post-rainy) season. However, despite the high resilience of sorghum to the harsh climate, water stress can dramatically affect grain and stover yield. Water stress at the vegetative and reproductive stage can reduce yield by more than 36% and 55% (Assefa et al. 2010). Post-rainy sorghum yield suffers the harshest environment but is often preferred by the most vulnerable subsistence farming communities in their marginal land in lack of any other alternatives (Witcombe et al. 1998). With about 5 M ha

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cultivated, post-rainy season sorghum is the largest production area of sorghum in India. In such a scenario, it is quite important to focus on yield improvement of these regions by exploiting various physiological and breeding tools.

To screen and breed any drought adapted genotype, identification and understanding key adaptive strategies associated with optimal water use in response to stress is important (Richards and Passioura 1989; Blum 2005; Tuberosa et al. 2007; Vadez et al. 2013, 2014). Unpredictable seasonal variation brings indeed a lot of G × E interactions, and it is quite difficult to identify and quantify adaptive traits that could be used as secondary traits in breeding programs. Two physiological mechanisms involved in water conservation strategies in sorghum have been proved to enhance dryland sorghum yield under terminal stress (Kholová et al. 2014). First of these mechanisms is the expression of a limited transpiration rate under increasing vapour pressure deficit (VPD) conditions and the second is a decrease in transpiration at high soil moisture conditions when subjected to progressive soil drying conditions. These two mechanisms were first studied in a few genotypes of sorghum (Gholipoor et al. 2012; Choudhary et al. 2013), where significant genetic variation was found among the genotypes. This phenomenon was also experimented in a series of crops by several researchers and existence of genetic variation for transpiration rate at different vapor pressure deficit levels (Fletcher et al. 2007; Devi et al. 2010; Kholova et al. 2010; Gilbert et al. 2011; Zaman-Allah et al. 2011). These mechanisms determining the plant water usage at canopy level might be possibly associated with the canopy establishment during the plant growth and development (Kholova et al. 2010 and Vadez et al. 2013).

Various simulation studies (Sinclair et al. 2005, 2010; Kholová et al. 2014) have shown the possibility of an increase in sorghum or soybean crop yield for the semi-arid environment by utilizing the physiological mechanism of a limited transpiration trait. The decrease in transpiration rate would result in conservation of soil moisture for critical post-flowering stages like grain filling and help in terminal drought tolerance (Richards and Passioura 1989; Sinclair et al. 2005). The transpiration response to soil drying has received less attention in sorghum, although genotypic differences have been identified in other crops like pearl millet (Kholova et al. 2010), chickpea (Zaman-Allah et al. 2011), soybean (Vadez and Sinclair, 2001). Overall both these traits are complex and breeding for it would benefit from marker-assisted selection, provided reliable markers can be found for it. Some of the drought tolerance components such as stay green in sorghum, panicle harvest index in pearl millet, drought avoidance root traits in chickpea and transpiration efficiency in groundnut have been confirmed phenotypically and utilized into marker-assisted breeding (Haussmann et al. 2002; Hash et al. 2003; Serraj et al. 2015). This will lead to pyramiding of drought tolerance mechanisms for the development of locally adapted drought-tolerant varieties. However, the first step towards this would be to identify sorghum inbred parents contrasting for these two traits, possibly using highly diverse and representative materials. Therefore, the main objectives of the present study were to evaluate the variation among the sorghum inbred parents of existing mapping populations for possible variation in (a) transpiration response in increasing VPD conditions (b) transpiration response to soil drying conditions. In addition, crop canopy development under natural conditions varying in VPD was also assessed. This work was done in different crop growing season, for which an additional aspect of the research dealt with understanding the relationship between the crop accumulated biomass and intercepted solar radiation.

Materials and methods

Plant genetic material

Fourteen sorghum genotypes were selected for the present study from existing eight Recombinant Inbred Lines (RILs) mapping populations available at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad. The details of the genotypes are furnished in Table 1. The seeds of these genotypes were obtained from Sorghum breeding, ICRISAT.

Plant growth conditions

Five experiments were conducted with the objective to study the plant growth and transpiration response (TR) at ICRISAT, Patancheru (17°30 N; 78°16 E; altitude 549 m), India. For controlled environmental experiments, seeds were sown in 7-inch plastic pots (22 cm diameter × 20 cm height) filled with approx. 5.5–6 kg of Black soil: sand in the ratio of 1:1 with Complete Randomized Design. During soil filling, nitrogen and potassium source such as Diammonium Phosphate (DAP) and Muriate of Potash (MOP) was added at the rate of 0.3 g per kg of soil. In each pot, three hills were raised and finally thinned to a single plant per pot. The temperature and humidity were recorded with the help of data loggers (USB data loggers, Lascar Electronics) at regular intervals throughout the crop growth period.

For exploring the effects of a natural increase in the VPD conditions, the experiments were conducted outdoors in a high throughput phenotyping platform, LeasyScan,

Table 1 Characteristic and pedigree of sorghum lines along with widely adapted post rainy sorghum cultivars

Parental pairs	Genotype	Races	Origin	Pedigree
RIL 1	296B	Kafir-durra	DSR, India	IS3922 × Karad local
	PVK801-P23	–	Parbhani, India	–
RIL 2	BT × 623	Kafir × Zera	USA	BT × 3197 × SC170-6-4
	S35	Caudatum	Ethiopia	–
RIL 3	N13	Durra	Nandyal, India	Nandyal selection
	E36-1		Ethiopia	Landrace
RIL 4	ICSV745	Guinea-Caudatum	ICRISAT, India	Pedigree selection from ICSV197 × A6250
	PB15220-1	–	–	
RIL 5	ICSV745	Guinea-Caudatum	ICRISAT, India	Pedigree selection from ICSV197 × A6250
	PB15881-3	Kafir-Caudatum	ICRISAT, India	(296B × IS18579)-2-3-4-1
RIL 6	SP2417-P3	–	–	
	IS41397-3-P6	Guinea-Caudatum	ICRISAT, India	
RIL 7	ICSB370-2-9	–	ICRISAT, India	
	IS8219-P1	Caudatum	ICRISAT, India	Germplasm
RIL 8	ICSV93046-P1	–	ICRISAT, India	Pedigree selection from ICSV700 × ICSV708
	S35	Caudatum	Ethiopia	–

available at ICRISAT (Vadez et al. 2015). The seeds were sown in plastic trays (60 × 40 × 41.5 cm) filled with approx. 80–85 kg of black soil standing on the high accuracy analytical scales (FieldScales, PSX, Herleen, NL). The soil was fertilized with DAP at the rate of 0.3 g per kg of soil. Six hills were raised at the time of seed sowing and finally thinned to four plants per tray. Four replications per genotype were maintained with an alpha lattice design. Necessary crop protection measures were taken to ensure the uniform crop growth and maintenance.

The response of TR to evaporative increasing VPD

Two experiments were conducted for the study of TR response to increasing VPD during February–March 2016 (Experiment 1) and April–May 2016 (Experiment 2) under controlled environmental conditions. At five to six fully developed leaf stage, uniform potted plants of each genotype were selected for the measurement of TR to increasing VPD, which was tested inside the growth chamber (Conviron, Controlled Environments, Winnipeg, MB, Canada). Before the day of the experiment, the selected plants were watered abundantly and allowed to drain excess water to reach field capacity. Soil evaporation was restricted by covering the soil surface around the stem with a plastic sheet, itself covered with a 2-cm layer of plastic beads. The restriction of the evaporation by this method is described earlier by Kholova et al. (2010). The packed pots were shifted from the glasshouse to the plant growth chamber and let to acclimatize to the growth chamber for 1 day before the experiment. A conviron plant growth chamber was programmed to set increasing VPD conditions in the range of 0.7–3.9 kPa during 1-h step intervals with 15 min transition time. For that, the daytime temperature was increased from 27 °C to 38 °C while the relative humidity was decreased in the range of 40 to 80% respectively. The lower level humidity was retained using a dehumidifier (Daikin, India). The night time temperature was 26 °C and 80% relative humidity level. The light flux 450–500 µ mol m^{-2} s^{-1} was maintained above the canopy level. The TR subjected to increasing VPD was measured as described in the earlier studies in sorghum by Gholipoor et al. (2010), Choudhary et al. (2013) and Shekoofa et al. (2014). During the transition time, the pots are measured by a gravimetric method using 0.01 precision balances (FBK, Kern & Sohn GmbH, Balingen, Germany). The first weight was considered as the field capacity weight, and the pots weights were taken at every 1-h interval for each VPD level to access the transpiration weight. The fresh leaves were separated from the stem part, and total leaf area was measured using a leaf area meter (LI-3100, Li-Cor, Lincoln, Nebraska, USA) to express transpiration rate per unit leaf area.

The response of TR to natural increasing VPD

LeasyScan, a scanner to plant concept continuously captures canopy development traits combined with gravimetric transpiration measurements (Vadez et al. 2015). PlantEye scanner (PlantEye F300, Phenospex, Heerlen, Netherlands) captures 3D point clouds of crop canopy at 2 h intervals, from which 3D leaf area (3D LA cm^2) and other plant

canopy traits are generated. The other plant canopy traits include Plant Height (PH, mm), Projected Leaf Area (PLA), Leaf Area Index (LAI), Digital Biomass (DB), Leaf Angle (LA), Leaf Inclination (LI), Light Penetration Depth (LPD). To serve this purpose genotypes were grown during December–January 2016 (Experiment 4) and April–May 2017 (Experiment 5). The purpose of these two experiments was to study the leaf growth development in response to different VPD conditions. These periods are known for naturally moderate and high VPD environmental conditions. The calculation of leaf growth response to natural VPD was based on the method explained by Alimagham et al. (unpublished). In short, under field conditions, factors such as temperature, radiation and VPD affect leaf area expansion and makes it complex to analyse the sole effect of VPD on leaf area expansion. In this method, thermal time was used to normalise the temperature effect while saturated radiation point was used to normalized radiation effect on leaf area expansion. After normalizing temperature and radiation effect on leaf area expansion, it became possible to assess VPD effect on leaf area expansion. There was no available hourly time step weather data (temperature and radiation) which was needed to normalize the radiation effect on leaf area. In this case, the method explained by Monteith and Unsworth (1990) was used to calculate hourly time step data from daily data. The method had been used by Sinclair et al. (2005). The calendar time was converted into thermal time with a base temperature of 10 °C and optimal temperature of 26–34 °C. The window of 35–173 thermal time for Experiment 4 and 35–273 thermal time for Experiment 5 were taken for normalization.

The evapotranspiration response of the recombinant inbred parental lines was also studied during hot summer April–May 2017 (Experiment 5). The trays were irrigated and maintained under well-watered conditions throughout the experiment. Successive tray weights were used to calculate evapotranspiration. The two consecutive days of measurements were taken for calculation based on the stage of the plant i.e. V6–V7 (Vegetative). The evapotranspiration data were expressed in per unit leaf area using 3D leaf area generated from PlantEye scanner.

The response of TR to progressive soil drying

For the progressive soil drying experiment to measure TR, uniform potted plants were selected. Five biological replicates were assigned for the well-watered (WW) treatment and six biological replicates for the water stress (WS) treatment (Experiment 3). For both the treatments, the plants were maintained under irrigated conditions up to 30 days of crop growing period. Before the initiation of the experiment, the pots were well saturated to soil capacity level and allowed excess water to drip off. Care was taken to restrict the soil water loss evaporation by covering the pots with plastic sheets with itself a layer of plastic beads. On the day of the experiment, the pot weights were taken using 0.01 precision kern balance (FBK, Kern & Sohn GmbH, Balingen, Germany). The initial pot weight was considered as field capacity weight and succeeding days on every morning all the pots were weighted to measure the daily transpiration. The water to be added was calculated based on the day transpiration values of the individual plants. The well-watered treatment plants were maintained at 80% field capacity level and water stressed treatment plants were exposed to gradual water stress imposition. The key aspect of the experiment was to have the soil water content decrease at a similar rate in all pots during the gradual stress imposition process, irrespective of the size of the plants. The daily transpiration was calculated as the difference in weight of the successive days. The transpiration data points were normalized (NTR) as described by Gholipoor et al. (2012) in sorghum crop and Kholova et al. (2010) in pearl millet to reduce the daily environmental variations. The experiment was concluded when the water stress plants transpiration decreased to below 10% of the well-watered plants, i.e. 0.1 NTR value. The total transpirable available soil water in pots was calculated from the difference in initial weight to the last weight (Sinclair and Ludlow 1986). The fraction of transpirable soil water (FTSW) represents the changes in soil moisture during progressive soil drying cycle and is used as the indicator of stress intensity (Ritchie 1981). The FTSW was calculated from the initial pot weight minus last weight divided by total transpirable soil water of the individual pot.

Statistical analysis

Analysis of variance (ANOVA) was carried out with SAS 9.3 (SAS Institute, Inc., Cary, NC, USA) using PROC GLM followed by least significant difference (LSD) test to find the actual differences between the parental pairs. The transpiration rate to increasing vapour pressure deficit and NTR versus FTSW data were subjected to two-segmented linear regression analysis using GraphPad Prism version 6.03 (Graph Pad Software, Inc., San Diego, California, USA) from which the slope, breakpoint (BP) value and R^2 are taken. The value of BP was the breakpoint between the two linear regression equation. The slope of the two linear regressions was statistically compared ($P < 0.05$). If the slope was statistically different, the response was best represented by a nonlinear regression model whereas if the slope was not significantly different; the response was best represented by a simple linear regression model.

Table 2 Results of two segmental linear regression in recombinant inbred sorghum lines for transpiration response to atmospheric increasing vapor pressure deficit conditions

SN	Genotype	Experiment I						Experiment II		
		LA (cm^2)	Slope ± SE	Slope1 ± SE	BP (X_0) ± SE	Slope2 ± SE	R^2	LA (cm^2)	Slope ± SE	R^2
RIL 1	296B	559.69		16.8 ± 1.26	2.7 ± 0.3	7.9 ± 2.0	0.89	410.19[a]	18.4 ± 1.6	0.75
	PVK 801-P23	553.23	13.9 ± 0.9				0.81	674.80[b]	14.3 ± 1.1	0.80
RIL 2	BT × 623	418.84 a	14.2 ± 1.1				0.72	479.82	13.0 ± 1.2	0.73
	S35	591.21 b		18.1 ± 2.3	2.5 ± 0.4	5.6 ± 3.8	0.65	488.41	15.3 ± 1.4	0.74
RIL 3	N13	467.76		17.5 ± 1.4	2.9 ± 0.4	4.0 ± 5.9	0.86	506.99	15.3 ± 1.4	0.71
	E36-1	566.85		17.0 ± 1.5	2.7 ± 0.3	6.4 ± 2.5	0.85	499.64	16.2 ± 1.4	0.75
RIL 4	ICSV745	478.07		16.7 ± 1.3	2.8 ± 0.6	7.0 ± 5.5	0.85	476.06	14.5 ± 1.8	0.60
	PB 15220-1	469.90		19.7 ± 1.9	2.4 ± 0.5	12.0 ± 2.6	0.86	428.97	18.1 ± 1.8	0.69
RIL 5	ICSV745	478.07		16.7 ± 1.3	2.8 ± 0.6	7.0 ± 5.5	0.85	476.06	14.5 ± 1.8	0.60
	PB 15881-3	436.51		20.5 ± 2.3	2.5 ± 0.3	5.9 ± 3.9	0.75	412.16	16.2 ± 2.3	0.53
RIL 6	SP 2417	512.97	18.9 ± 1.1				0.87	439.82	17.8 ± 2.2	0.64
	IS41397-3-P6	498.73		16.9 ± 1.7	3.4 ± 0.2	− 1.7 ± 7.2	0.72	480.84	18.8 ± 3.1	0.45
RIL 7	ICSB 370-2-9	415.98	12.4 ± 1.2				0.59	472.73 a	16.4 ± 1.5	0.73
	IS 8219-P1	364.56	16.0 ± 1.1				0.75	302.71 b	15.8 ± 2.6	0.45
RIL 8	ICSV93046	365.12 a	13.0 ± 0.8				0.83	289.88[a]	21.6 ± 1.9	0.75
	S35	591.21 b		18.1 ± 2.3	2.5 ± 0.4	5.6 ± 3.8	0.65	488.41[b]	15.3 ± 1.4	0.74
	Grand Mean	477.19						454.67		
	F value	2.61						5.62		
	Pr > F	0.0036						< .0001		

[a,b]Differences among the parental pairs ($P < 0.05$)

Results

Genotypic variation in the transpiration response to VPD

The Analysis of variance (ANOVA) test showed that there was a significant difference ($P < 0.0036$ and $P < 0.0001$) in the final leaf area in the range of 365–591 cm^2 and 289–674 cm^2 in experiment 1 and 2 respectively. The population parental pair RIL8 showed variation for the final leaf area in both experiments. The parental pairs such as RIL 2 showed significant variation in the final leaf area in experiment 1 whereas RIL1 and RIL7 showed differences in experiment 2. The daytime maximum temperature and humidity recorded during crop growing period are 27.07 °C and 61.25% (1.39 kPa) in case of exp 1 and 28.74 °C and 73.58% (1.00 kPa) in case of exp 2.

In experiment 1, eight genotypes out of fourteen expressed limited transpiration rates under high VPD conditions with the breakpoint (BP) ranging between 2.4 and 3.4 kPa. Among all the genotypes, IS41397-3-P6 resulted in a negative slope above the BP and recorded the highest BP value at 3.4 kPa. The slope of the segmented linear response above the BP was lower than the slope of the genotypes that did not express a BP. Also, the percentage decrease in transpiration rate above the breakpoint ranged from 22.8 to 60.9%. The genotype PB15220-1 recorded the highest percentage decrease in transpiration.

In this controlled experiment, RIL1, RIL2, RIL6 and RIL8 parental pairs showed the contrast in their response to VPD whereas in another four parental pairs RIL3, RIL4, RIL5 and RIL7 both parents showed a similar transpiration rate response to increasing VPD. In the former RILs, the parents 296B, S35 and IS41397-3-P6 showed a breakpoint in the range of 2.7 kPa, 2.5 kPa and 3.4 kPa. In the latter RILs, RIL3, RIL4 and RIL5 both parents showed an expression of limited transpiration rate whereas in RIL 7 both parents showed a linear increase in transpiration rate with increasing VPD and exhibited no breakpoint. The same set of genotypes was repeated a second time with an identical range of VPD, and all resulted in a linear transpiration response with a slope value of 13.0 to 21.6. The leaf area and transpiration rate of genotypes in response to increasing VPD were illustrated in Table 2.

Table 3 Plant canopy architecture traits measured during moderate VPD crop growing season

SN	Genotype	PH (mm)	LAI	LI	DB (mm^3)	LA (θ)	PLA (cm^2)	LPD (mm)
RIL 1	296B	97.04	0.02[a]	1.39	1,455,673.03	46.08	101.82	74.11
	PVK 801-P23	82.14	0.06[b]	1.37	1,396,718.82	46.87	120.83	64.75
RIL 2	BT × 623	107.25[a]	0.05[a]	1.39	1,189,384.59[a]	46.16	76.21[a]	74.69[a]
	S35	143.38[b]	0.07[b]	1.39	2,765,280.44[b]	46.18	131.01[b]	121.59[b]
RIL 3	N13	102.18	0.04	1.39	1,207,734.64	45.93	83.57	81.04
	E 36-1	96.09	0.04	1.38	2,026,749.35	46.27	150.42	77.31
RIL 4	ICSV745	94.52	0.06	1.39	1,564,422.66	45.85	113.22	66.33
	PB15220-1	97.64	0.06	1.40	1,185,850.20	45.75	84.06	71.50
RIL 5	ICSV745	94.52	0.06[a]	1.39	1,564,422.66	45.85	113.22[a]	66.33
	PB15881-3	86.46	0.04[b]	1.41	904,101.49	45.13	69.88[b]	62.98
RIL 6	SP 2417-P3	72.06[a]	0.04	1.38	958,078.54[a]	46.49	95.88[a]	55.27
	IS41397-3-P6	115.56[b]	0.04	1.38	2,366,766.29[b]	46.54	144.50[b]	81.74
RIL 7	ICSB370-2-9-P2	101.62	0.06	1.38	1,691,617.48	46.63	112.22	73.99
	IS8219-P1	104.72	0.05	1.38	1,547,218.85	46.73	99.86	77.33
RIL 8	ICSV93046-P1	101.57[a]	0.03[a]	1.41	1,565,984.45[a]	45.37	104.09	57.78[a]
	S35	143.38[b]	0.07[b]	1.39	2,765,280.44[b]	46.18	131.01	121.59[b]
	Grand mean	100.25	0.049	1.387	1,536,415	46.15	104.27	75.073
	F value	4.33	3.06	0.39	3.50	0.40	3.38	4.87
	Pr > F	0.0004	0.0053	0.9617	0.0021	0.9571	0.0026	0.0001

PH plant height, *LAI* leaf area index, *LI* leaf inclination, *DB* digital biomass, *LA* leaf angle, *PLA* projected leaf area, *LPD* light penetration depth

[a,b]Differences among the parental pairs ($P < 0.05$)

High throughput phenotyping for canopy conductance traits

Canopy architecture measured by PlantEye scanner

The differences in plant canopy traits studied during moderate and high VPD season are given in Tables 3 and 4. The pictorial representation of the weather data and the leaf area normalised with solar radiation subjected to different VPD crop growing season are given in Fig. 1. Differences between parents in the normalised leaf area was observed for RIL1 and RIL8 in both moderate and high natural VPD season. The RIL2 showed the difference between parental pairs in the high VPD season only (Experiment 2). These RIL parental pairs also showed a difference in the final leaf area in the controlled environmental conditions (Experiment 1 and 2). The RIL1, 2 and 8 which showed the difference in the normalised leaf area also showed a difference in other canopy traits. In RIL2, parents showed the difference in all canopy traits measured during high VPD season. The RIL1 also showed the difference in LAI in both outdoors experiments and RIL8 shown the difference in PH, LAI, DB, PLA and LPD in the high VPD season respectively.

Evapotranspiration dynamics by FieldScales

To study the evapotranspiration pattern, the FieldScales data were taken for two consecutive days with high VPD conditions. The two consecutive days recorded maximum VPD range of 6.88 and 6.51 kPa respectively. Among the studied parental pairs RIL combination, the RIL1, RIL2, RIL6 and RIL8 showed ample variation between parents during midday. Among these RILs, the parent 296B showed the highest increase in rate during midday than any other studied genotypes. In the studied RILs, the Parent A also showed a higher increase in evapotranspiration rate during midnoon than Parent B., i.e. Recurrent parent showed higher evapotranspiration rate under the high VPD conditions than the donor parent. The genotypic variation of RILs are given in Fig. 2. These parental pairs RIL1, RIL2, RI 6 and RIL8 also showed the contrast in the transpiration response subjected to increasing VPD under controlled conditions.

Difference in the transpiration response to soil drying (FTSW threshold)

The FTSW thresholds of the studied RILs population parental pairs are given in Table 5. Across all the genotypes, the estimated FTSW thresholds upon soil drying

Table 4 Plant canopy architecture traits measured during high VPD crop growing season

SN	Genotype	PH (mm)	LAI	LI	DB (mm^3)	LA (θ)	PLA (cm^2)	LPD (mm)
RIL 1	296B	141.45	0.08[a]	1.39	3,219,018.61[a]	45.97	145.78[a]	74.45
	PVK 801-P23	163.39	0.16[b]	1.37	7,001,516.23[b]	46.91	299.01[b]	88.65
RIL 2	BT × 623	175.11[a]	0.10[a]	1.42[a]	4,755,271.93[a]	44.83[a]	172.23[a]	76.67[a]
	S35	204.30[b]	0.16[b]	1.37[b]	8,749,690.09[b]	46.73[b]	298.80[b]	129.23[b]
RIL 3	N13	167.58	0.10[a]	1.39	4,939,932.30	45.89	189.21[a]	89.39
	E 36-1	171.48	0.13[b]	1.38	6,029,123.58	46.41	244.65[b]	106.28
RIL 4	ICSV745	203.91	0.12	1.40	6,689,639.01	45.49	220.17	108.33
	PB15220-1	194.50	0.10	1.43	5,733,412.64	44.36	187.21	122.73
RIL 5	ICSV745	203.91	0.12	1.40	6,689,639.01	45.49	220.17	108.33
	PB15881-3	190.82	0.10	1.41	5,571,346.50	45.17	187.11	102.72
RIL 6	SP 2417-P3	152.71[a]	0.12	1.42	4,909,499.26[a]	44.77[a]	214.36	78.17[a]
	IS41397-3-P6	194.78[b]	0.13	1.38	6,834,908.65[b]	46.60[b]	241.17	108.23[b]
RIL 7	ICSB370-2-9-P2	181.90	0.09	1.42	4,514,845.59	44.87	161.67	88.06
	IS8219-P1	178.97	0.09	1.41	4,792,744.20	45.11	173.31	98.91
RIL 8	ICSV93046-P1	170.33[a]	0.09[a]	1.40	4,539,414.51[a]	45.77	173.79[a]	76.37[a]
	S35	204.30[b]	0.16[b]	1.37	8,749,690.09[b]	46.73	298.80[b]	129.23[b]
	Grand mean	178.40	0.112	1.39	5,603,854	45.65	207.62	96.62
	F value	4.06	6.62	2.32	4.85	2.53	7.58	5.59
	Pr > F	0.0003	< .0001	0.0203	< .0001	0.0118	< .0001	< .0001

PH plant height, *LAI* leaf area index, *LI* leaf inclination, *DB* digital biomass, *LA* leaf angle, *PLA* projected leaf area, *LPD* light penetration depth

[a,b]Differences among the parental pairs ($P < 0.05$)

varied from 0.22 to 0.41, and the correlation coefficients for the FTSW threshold value determination ranged above 0.70. The highest FTSW threshold value was noted by the genotype S35 which is donor parent in the RIL2 and 8 respectively. Among the eight studied RIL parental pairs, RIL1, RIL3 and RIL8 showed the highest difference in FTSW threshold value between parents. In these three pairs, the donor parents PVK801-P23, E36-1 and S35 of RIL showed a higher FTSW threshold than the recurrent parent of RIL. This parental pair which showed the difference in FTSW threshold is shown in Fig. 3.

Discussion

The overall objective of the present work was to study the genetic variation between parents of mapping populations in the expression of a limited transpiration rate under increasing VPD and in the development of the canopy and to examine if measurements for controlled environments and outdoors platform (LeasyScan) would generate consistent results. The underlying hypothesis is that transpiration response to increasing vapour pressure deficit results in increasing transpiration and that a restriction in the transpiration would save water and contribute potentially to drought adaptation. The plant material for this study included eight combinations of existing sorghum RIL mapping parental population pairs. We found a difference in the transpiration response to VPD between two set of controlled environmental experiments. In the first experiment, eight parents expressed a breakpoint in response to increasing VPD and in the repeated experiment none of the genotypes expressed a breakpoint. This difference in results might be due to the difference in environmental conditions inside the glasshouse during the crop growing period. In experiment 1, four contrasting RILs, RIL1, RIL2, RIL6 and RIL8 contrasted in their transpiration response to increasing VPD. The donor parent of RIL2, RIL6 and RIL8 showed VPD sensitive response to atmospheric increasing VPD. By contrast, both the parents in RIL3, RIL4 and RIL5 showed VPD sensitive response to increasing VPD. Similarly, genotypic variation for limited transpiration trait was also reported by Gholipoor et al. (2010), Choudhary et al. (2013), Shekoofa et al. (2014) in sorghum crop. The existence of genotypic variation for limited transpiration response in various other crop species is also well documented by Sinclair et al. (2017).

In controlled environmental conditions, phenotyping for the transpiration response involves gravimetric measurements of water loss in response to variation in VPD. This approach involves single plant measurements grown in the pots. Measurements of water loss in a controlled

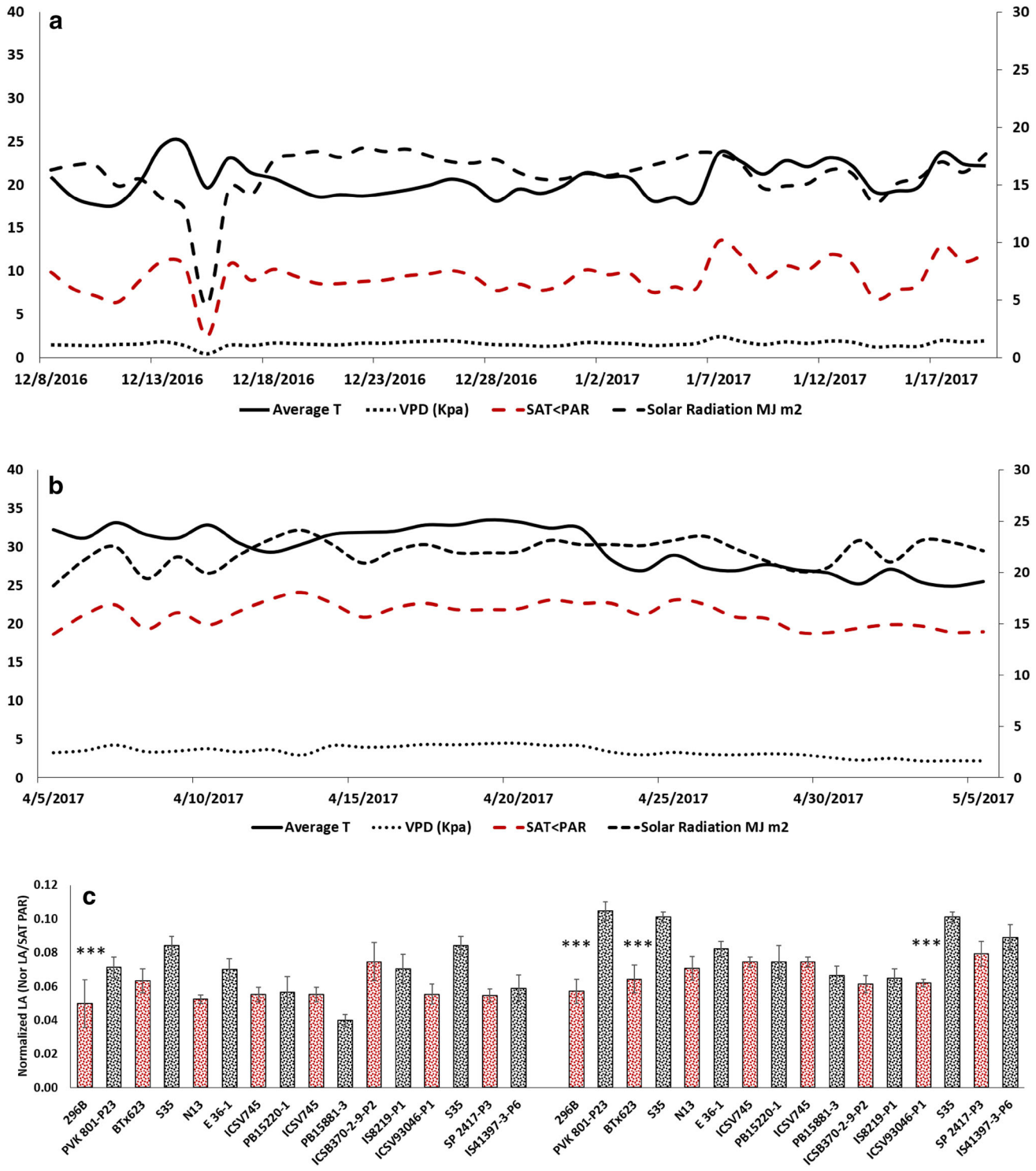

Fig. 1 **a** Weather data for the period Dec–Jan 2016. **b** Weather data for the period April–May 2017; **c** differences in leaf area normalized with received radiation in response to VPD (Red color- Parent A; Black color- Parent B) (colour figure online)

environment needs special care to maintain temperature and humidity inside the conviron plant growth chamber. To overcome this limitation, Vadez et al. (2015) designed high throughput phenotyping platform in which plants are grown in trays similar to field density conditions.

In the LeasyScan experiment, under outdoor high VPD conditions, the outcome of the study showed genotypic differences within parental pairs in the midday evapotranspiration rate of RIL 1, RIL2, RIL6 and RIL8 respectively. Among these, RIL1 showed an ample amount of variation

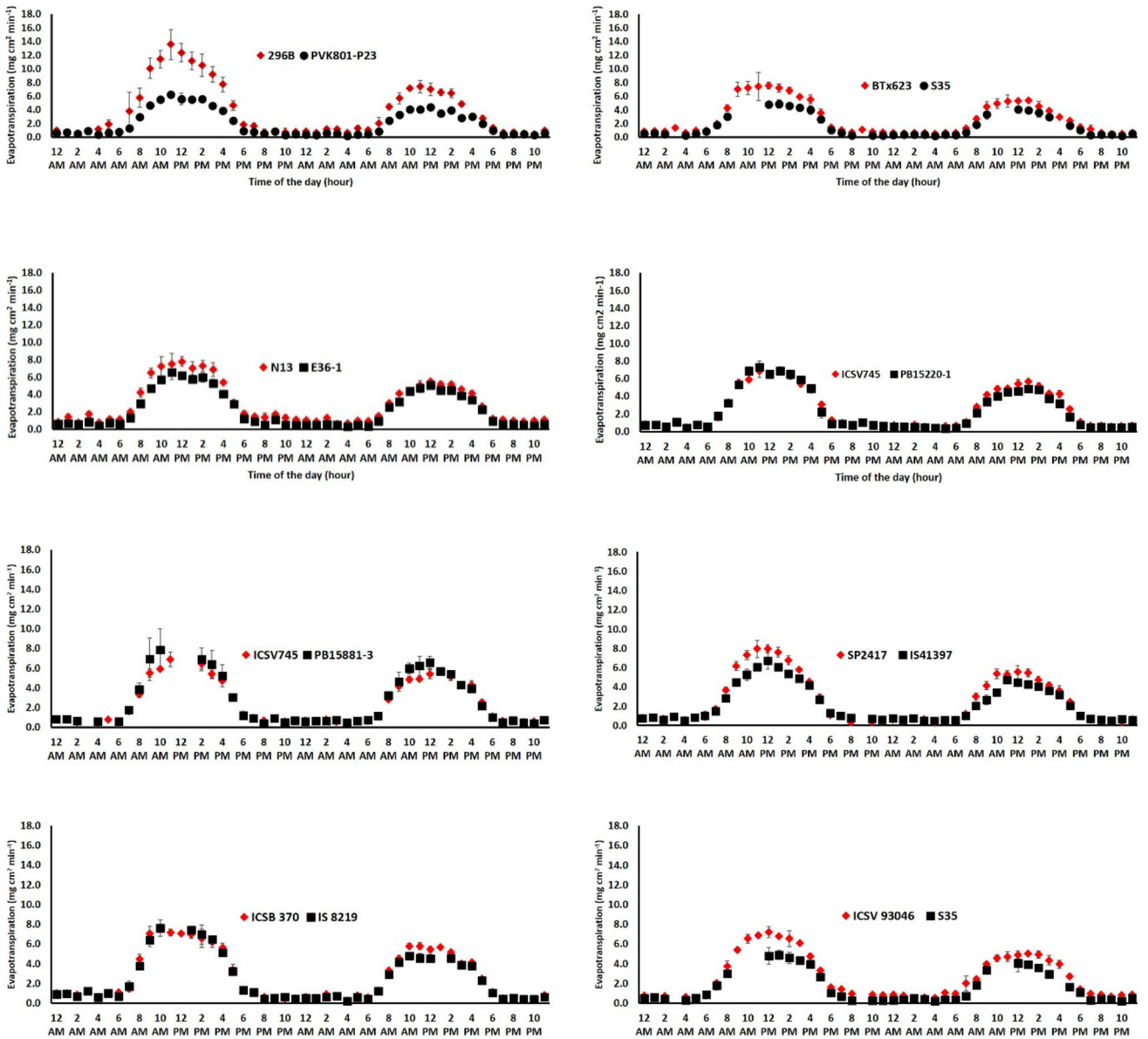

Fig. 2 Midday evapotranspiration rate variation for two consecutive days across RIL 1–8 sorghum mapping population parents (Red color-Parent A; Black color-Parent B) (colour figure online)

among the parents. These four RILs also showed contrasting transpiration rate to increasing VPD in controlled environmental conditions. The donor parent of these RILs, S35 and SP2417 was VPD sensitive, and it shows that donor parent could conserve soil moisture when midday VPD was high. Similar kind of experimental data was also obtained by Vadez et al. (2015) in VPD sensitive and insensitive sorghum, mapping population parents of PRLT and H77 pearl millet and cowpea genotypes; Xu et al. (2015) identified the genotypic variation in transpiration pattern during mid-day in *vigna unguiculate* species. The restriction in transpiration under high VPD allowed by partial stomata closure saves soil moisture at the early vegetative stage, which can increase moisture availability for reproductive stages under the rainfed condition and can enhance yield (Richards and Passioura 1989; Sinclair et al. 2005). The restricted transpiration rate is likely due to the result of low hydraulic conductance located in the leaves of this genotype between the xylem and into the guard cells. Choudhary et al. (2013, 2014) studied the limitation of hydraulic conductance in leaves and roots in sorghum and maize genotypes.

Additionally, other plant canopy traits also explored to study the difference in plant growth development in response to VPD differed within parental pairs. The RIL2 showed differences in all the studied plant canopy traits in

Table 5 Transpiration response of sorghum recombinant inbred lines subjected to progressive soil drying conditions

SN	Genotype	Average transpiration		FTSW BP	TTSW	Confidence interval	R^2
		Well water	Water stress				
RIL 1	296B	100.3 ± 5.49	42.95 ± 0.94	0.25	896.9	0.2196–0.2807	0.95
	PVK 801-P23	103.1 ± 8.60	43.51 ± 0.88	0.40	899.5	0.2790–0.3238	0.96
RIL 2	BT × 623	91.6 ± 9.96	43.50 ± 0.73	0.32	899.7	0.2929–0.3514	0.94
	S35	108.4 ± 10.99	44.28 ± 1.36	0.41	899.1	0.2751–0.3793	0.92
RIL 3	N13	108.9 ± 10.05	43.09 ± 1.34	0.26	910.7	0.2249–0.2951	0.90
	E 36-1	134.8 ± 15.69	44.85 ± 3.81	0.42	818.2	0.2790–0.3410	0.97
RIL 4	ICSV745	87.6 ± 3.98	43.09 ± 0.52	0.30	908.8	0.2715–0.3230	0.94
	PB15220-1	96.4 ± 3.77	43.51 ± 1.31	0.35	896.1	0.2371–0.3201	0.92
RIL 5	ICSV745	87.6 ± 3.98	43.09 ± 0.52	0.30	908.8	0.2715–0.3230	0.94
	PB15881-3	103.9 ± 3.47	42.78 ± 0.73	0.37	893.4	0.2545–0.3199	0.93
RIL 6	SP 2417-P3	105.20 ± 9.81	42.26 ± 0.79	0.25	884.2	0.2135–0.2958	0.93
	IS41397-3-P6	78.10 ± 6.74	40.37 ± 0.74	0.27	844.4	0.2028–0.2935	0.77
RIL 7	ICSB370-2-9-P2	99.2 ± 7.27	43.25 ± 0.45	0.29	893.4	0.2653–0.3046	0.98
	IS8219-P1	85.8 ± 10.24	41.85 ± 1.08	0.22	870.3	0.1684–0.2651	0.58
RIL 8	ICSV93046-P1	87.7 ± 7.28	43.04 ± 0.30	0.24	898.9	0.2135–0.2733	0.94
	S35	108.4 ± 10.99	44.28 ± 1.36	0.41	899.1	0.2751–0.3793	0.92

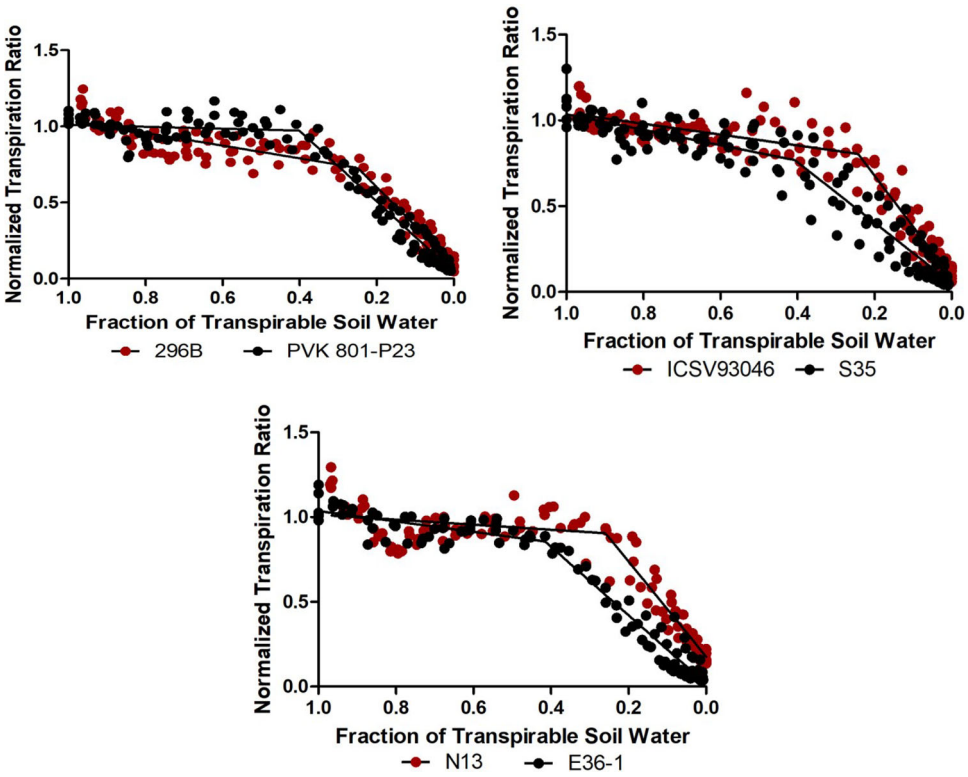

Fig. 3 Normalized transpiration ratio (NTR) as a function of fraction of transpirable soil water (FTSW) for RIL, RIL 3 and RIL 8 sorghum population parents (Red color-Parent A and Black color-Parent B). The solid line in each graph is the regression fit using two segmental linear regression; the intersection indicates the FTSW threshold for decline in transpiration (colour figure online)

response to high VPD season. Under moderate VPD conditions, the RIL2 showed non-significant differences among the parents for the trait leaf angle and leaf inclination angle among the studied traits. This RIL2 also showed a difference in transpiration response of both controlled and outdoor environmental conditions. Kholová et al. (2014) studied that canopy development traits are tightly related with the plant water use in response to VPD.

The transpiration response to soil drying cycle hypothesised that the genotypes which exhibit high FTSW

threshold value saves water early on during the progressive exposure to soil drying and has a conservative behaviour with plant water use. In this study, the FTSW threshold varied in the range from 0.22 to 0.41 and the highest FTSW threshold difference was found in the RIL 1, RIL 3 and RIL 6 respectively. In these RILs, the donor parents PVK801-P23, S35 and E36-1 which exhibited the highest FTSW threshold are likely to conserve water when the soil dries and where the transpiration rate will be restricted. The genotype S35 and E36-1, which expressed breakpoint under high VPD conditions, also expressed high FTSW threshold where transpiration declined upon soil drying. The genotypic variation in response to progressive soil drying condition studies was also demonstrated in various crops by Devi et al. (2010), Kholova et al. (2010) and Gholipoor et al. (2012). Devi et al. (2010) and Gholipoor et al. (2012) also found a clear mean difference in FTSW threshold between lines that either showed a VPD breakpoint or no breakpoint in genotypes of peanut and maize genotypes. That is, they also found that genotypes with higher FTSW thresholds had a breakpoint in their transpiration response to increasing VPD. This could be explained by the fact that the genotypic variation for water conservation properties of early decreases in transpiration response with soil drying and of limitation on transpiration response at high VPD are hypothesized to have a common plant hydraulic conductance mechanism. Therefore, further work would be needed to test the hydraulic characteristics of genotypes that contrast in response to soil drying and increasing VPD.

In summary, RIL 1, RIL 2, RIL 6 and RIL 8 would be suited for the mapping of the transpiration response to increasing VPD and the phenotyping of these populations could be done outdoors under naturally high VPD conditions in the LeasyScan platform. Interestingly, under high VPD conditions the RIL2 showed the difference in all studied plant canopy and transpiration trait among the parental pairs. Also, phenotyping of crop sensitivity measure to progressive soil drying cycle could be advantageous in RIL1, RIL3 and RIL6 populations. These RILs would contribute to water saving mechanisms in the soil when water is limiting factor.

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