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Research Article

A comparative analysis of transpiration response to atmospheric increasing vapor pressure deficit conditions in cereal crops

G. Karthika^{1,2}, Sunita Choudhary², Meenakshi ganesan¹, Jana Kholova² and Vincent Vadez^{3,2*}.

¹ Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India.

² International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India.

³ Institut de Recherche pour le Développement (IRD), Université de Montpellier, UMR DIADE, 911 Avenue Agropolis, BP 64501, 34394, Montpellier Cedex 5, France.

*E-Mail: vincent.vadez@ird.fr; v.vadez@cgiar.org

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Abstract

Plant adaptation to drought depends on both inherited and adaptive characteristic of water conservative traits. Expression of limited transpiration rate (TR) under high vapor pressure deficit (VPD) conditions could be one of the potential sources of soil water conservation for drought tolerance. Large genetic variation for limited TR has been identified in the comparison of three major C₄ cereals viz., maize, pearl millet and sorghum under elevating VPD. The total amount of water transpired under elevating VPD by these three cereals not found dependent on leaf area, in fact, it was reflected more by the variation in transpiration rate. Pearl millet showed better adaptation of limitation of TR than maize and sorghum under high VPD regimes.

Keywords

Cereals, transpiration, vapor pressure deficit, breakpoint and variability.

Introduction

Maize, Pearl millet and Sorghum are important cereal crops for food and feed in arid and semi-arid tropics. The C₄ crops are evolved to cope-up with high temperature, low CO₂ and high irradiance environment. However, when it comes to drought (atmospheric and soil), they do suffer equally as the C₃ cereals. Water stress limits the crop yield and plays a significant role in the potential yield gap. Drought tolerance is a complex trait and regulated by many component traits (Monteith, 1995; Vadez *et al.*, 2014). Identification of component traits to drought-tolerance and utilisation into crop breeding program is essential for sustainable agriculture. One such trait identified in recent years is the transpiration response to increasing atmospheric evaporative demand (also called vapor pressure deficit), VPD is the difference between the vapor pressure inside the leaf to saturated air pressure of the atmosphere which drives the transpirational pull. Plant transpiration increases with increasing VPD (Sinclair and Bennett, 1998) but genetic variation has been reported in many crops in the transpiration response to high VPD conditions. Some genotypes indeed restrict their transpiration under high VPD, by partial stomata closure, and then limit their maximum transpiration rate. This trait contributes to soil water conservation, water is conserved in early crop stages and effectively used for later critical stages (Richards and Passioura,

1989; Sinclair *et al.*, 2005). Kholova *et al.* (2010) identified genetic variation for limited transpiration rate in pearl millet which is linked to the water use at the vegetative stage. Gholipour *et al.* (2013) evaluated thirty-five single cross maize hybrids for limited transpiration rate in response to increasing high VPD and reported VPD threshold for limiting TR range of 1.7 to 2.5 kPa. Gholipour *et al.* (2010) and Choudhary *et al.* (2013) identified VPD sensitive and insensitive lines by screening twenty-six sorghum genotypes, and the expression of limited transpiration trait ranged from VPD threshold of 1.6 to 2.7 kPa in sensitive lines. Shekoofa *et al.* (2014) compared the expression of limited transpiration trait in a controlled test environment to the field conditions. This study showed a similar trend of expression and possibility to compare the studied lines under a range of conditions. In particular, it looked at the trade-off between the maximum transpiration trait and the photosynthesis-driven plant growth, i.e. trade-offs between carbon dioxide entry in the plant and water losses at the stomata level. Several attempts have also been made to address these trade-offs between water conservation and biomass accumulation so that utilisation from the soil profile get maximized and no water remains available in the soil profile once the crop has matured. Sinclair *et al.* (2005) conducted a simulation study for the limited transpiration traits

in sorghum and reported 9-13% yield benefits under arid regions with a very minimum penalty under well-watered conditions.

Similarly, Kholova *et al.* (2014) simulated the same traits for post rainy sorghum cultivars reported for grain yield and fodder. The model showed the close relationship between the crop yield and amount of water available at post flowering crop growth stage subjected to limited water conditions. The primary objective of the present study is to compare the genetic variation in three major crops of semi-arid tropics for transpiration response to elevating VPD conditions.

Material and Methods

Two experiments (Exp I and Exp II) were conducted at Controlled Environment Research Facility (CERF), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (17°30 N; 78°16 E; altitude 549m) during March to April and July to August 2015. A total of sixty-six genotypes were selected which comprised of 28 inbred lines of tropical and temperate maize, 19 lines reference collection of tropical sorghum and 19 lines of tropical pearl millet contrast for transpiration efficiency (**Table 1**). Seeds were sown in the 7 "plastic pots filled with approx. 5-6 kg of vertisol:sand in the ratio of 2:1 with Complete Randomized Design (CRD). Before sowing, the soil was well fertilized with Di-Ammonium Phosphate (DAP) and Muriate of Potash (MOP) in the concentration of 0.3g per kg of soil. Three hills were raised at the time of sowing and finally thinned to two plants per pot in Exp I and single plant per pot in case of Exp II. The plants were maintained under well-watered conditions throughout the experiment.

At V5-V7 (vegetative stage of 5-7 fully developed leaves) plant stage, the uniform pots were selected, saturated with water and allowed to drain overnight. Soil evaporation was restricted by covering the soil surface around the stem with a plastic sheet covered with a 2-cm layer of plastic beads. The plants were grown in a glasshouse and shifted to plant growth chamber (Conviron, Controlled Environments, Winnipeg, MB, Canada) for one day. After acclimation, the transpiration response to increasing VPD was assessed in the range of 0.7 kPa to 4 kPa (Exp I) and 0.6 kPa to 3.6 kPa (Exp II) for one-hour intervals (15min transition time). The lower level humidity was maintained with the help of dehumidifier (Daikin, India). The light flux density ranged from 450-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy level in a growth chamber. The protocol for transpiration response to increasing VPD was followed same as described in

the earlier studies by Kholova *et al.* (2010); Gholipour *et al.* (2010); Choudhary *et al.* (2013) and Shekoofa *et al.* (2014) in cereal crops. The transpiration response was measured by a gravimetric method using 0.01 precision balances (FBK, Kern & Sohn GmbH, Balingen, Germany) to five plants per genotype. The first weight was considered as field capacity weight, and the pots were weighted at every one-hour interval for each VPD level to access the transpiration response. The fresh leaves were separated from the plant part at the end of the experiment, and total leaf area was measured using a leaf area meter (LI-3100, Li-Cor, Lincoln, Nebraska, USA).

The gravimetric transpiration measurement of plants was expressed in Transpiration Rate (TR) as transpiration (mg) per unit leaf area (m^2) and per unit of time (s). The VPD was calculated as per equation (given by Monteith and Unsworth, 1990).

$$\text{VPD} = \frac{100 - RH}{SVP} \times SVP$$

Analysis of variance (ANOVA) was done using SAS 9.3 PROC GLM (SAS Institute, Inc., Cary, NC, USA) followed by least significant test (LSD) test to find the significant difference among and between the crop species. The transpiration response to increasing VPD was categorized into three VPD levels as low VPD (<1.5 kPa), medium VPD (1.5 kPa to 2.5 kPa) and high VPD (>2.5 kPa) to find the difference at each level. The transpiration rate to increasing vapor pressure deficit was subjected to two-segmented linear regression equation using GraphPad Prism version 6.03 (Graph Pad Software, Inc., San Diego, CA). The value of (Breakpoint) BP was the breakpoint between the two linear regression equations. The slope of the two linear regressions was statistically compared ($P < 0.05$). If the two slopes are statistically different, the response is best represented by a nonlinear regression model. If the two slopes are not significantly different the response is best represented by a simple linear regression model.

Genetic components like genotypic coefficient of variance (GCV), the phenotypic coefficient of variance (PCV), heritability (h^2 Broad sense) and genetic advance (GA % mean) were estimated as suggested by Johnson *et al.* (1955), Burton, (1952) and Lush (1940). The coefficient of variation (CV %) was categorized into low (0-10), moderate (11-20) and high (>20) as suggested by Sivasubramanian and Madhava Menon, (1973). The heritability % were categorized into low (0-30), medium (30-60) and high (more than 60) as suggested by Robinson *et al.* (1949). The genetic

advance as percent of mean was categorised into low (<10), medium (10-20) and high (more than 20) as suggested by Johnson *et al.* (1955).

Results and Discussion

Analysis of variability revealed significant ($P<0.05$) differences among all the lines for leaf area (LA, cm^2) under investigation in both the set of experiments. In this study, the genetic variability parameters like PCV and GCV were calculated and showed high values for LA, T and TR respectively. In the case of the maize crop, the high heritability and high genetic advance per cent mean were observed for LA, T and TR. Moderate to high heritability and high genetic advance were recorded for LA, T and TR in case of pearl millet and sorghum respectively. The analysis of variance and estimated variability parameters are given in **Table 2 and 3**. The transpiration (T, g) and transpiration rate (TR, $\text{mg m}^2 \text{sec}^{-1}$) showed large variation in the studied genetic material. The variability in canopy development, transpiration and transpiration rate were also discussed. With regard to canopy development, at the first set of experiment (Exp I; March to April), the mean leaf area was recorded highest in pearl millet ($1425\pm 485\text{cm}^2$) followed by sorghum ($1139\pm 437\text{cm}^2$) and maize ($788\pm 316\text{cm}^2$). The LSD test showed a significant difference among three crops ($P<0.05$). Similarly, second set of experiment (Exp II; August-September) showed highest mean leaf area in pearl millet ($547\pm 146\text{cm}^2$) followed by sorghum ($476\pm 87\text{cm}^2$) and maize ($417\pm 128\text{cm}^2$). The leaf area was not statistically different between sorghum and maize in the second set of experiment.

The plant water loss (transpiration) of maize in the course of the day was comparatively less than pearl millet and sorghum under a range of low, medium and high VPD levels in the both sets of experiments. The water loss in maize plant ranged between 9.8 and 13.7 g/day (Low to High VPD) in Exp I, whereas it ranged between 12.4 and 17.6 g/day (Low to High VPD) in the case of Exp II. The water loss in both pearl millet and sorghum plants were comparatively similar in both the experiments and statistically non-significant to each other.

The measurement of transpiration rate in plants was expressed as per unit leaf area. In both the experiments (Exp I and II), the maize TR was higher than sorghum TR under both low and high VPD conditions and was significantly different among the crops ($P<0.005$) used in the study. When the plants exposed to increasing VPD conditions, the maize and sorghum TR were similar, and they were non-statistically different to each other in both the experiments.

Plant adaptation to water deficit depends on both the inherent and adaptive characteristics that condition water uptake. Among these characteristics, the canopy development, the amount and rate of transpiration (water loss per unit of leaf area), and the sensitivity of plants to evaporative demands are important factors that influence plant fitness in a particular environment. Pearl millet has rapid development of canopy size in early vegetative stage compare to sorghum and maize. The differences in canopy size ideally should reflect the differences in total water use. Pearl millet has higher transpiration water loss due to large canopy size; interestingly sorghum has lost a similar amount of water although the leaf area was significantly lower than pearl millet which indicates that the higher canopy conductance in sorghum. Maize seems to be lower in canopy size as well as the transpiration. Several genetic factors can contribute to these interspecies differences in leaf area, including the rate of leaf appearance, or simply the size of individual leaves appearing at different stages. However, environmental interaction effect is also known to play an important role in leaf area development through a combination of hydraulic and metabolic controls which are specific to species (Pantin *et al.*, 2011, 2012; Kudoyarova *et al.*, 2011). In all cases, it is an important to harness the genetic determinants of leaf area development (both the inherent characteristics and the genetic responses to environmental conditions) which subsequently drives the transpiration up to certain extent and understand these species adaptations to specific conditions (Van Oosterom *et al.*, 2001). Van Oosterom *et al.* (2011) reported higher leaf appearance rate in sorghum hybrids which showed reduced tillering, that led to both a reduced leaf area around anthesis and increased yield under water stress. Limiting the size of the transpiring leaves is one way to control plant water losses, but a smaller canopy would also restrict light capture capacity and limit yield under certain conditions (Sinclair and Muchow 2001). Therefore limitation in conductance of the canopy could be another point to compare these three crops for adaptation. Pearl millet showed more limited TR under high VPD and appeared to conserve soil moisture better than sorghum and maize under a high evaporative environment at early vegetative stages.

In both the experiments, a maximum VPD greater than 3kPa was achieved. In the Exp I, the temperature and humidity ranged from 27°C to 38°C and 80% to 40%, respectively whereas in the Exp II, temperature and humidity ranged from 31°C to 34°C and 85% to 30%, respectively. For acclimation, the day and night regime temperature and relative humidity maintained in Exp I and II

were 27°C/65% and 28°C/80% respectively. Due to the difference in temperature and humidity between experiments, the data were analysed individually, and results are discussed as experimental wise under each crop category.

Maize

In Experiment I, among 28 inbred lines of maize, nine lines expressed a limited TR and other 19 lines showed a linear increase in TR under elevating VPD. The VPD threshold at which those nine lines showed a limitation in TR was observed in the range of 1.93 (MBS847 and LP1233) to 2.95 kPa (VL 109150). The same set of inbred lines in Exp II, eight among 28 lines showed BP in range of 2.26 (VL 108320) to 3.10 (EP1) kPa. The lines EA1197, PH207, VL 109150 and VL 12153 showed BP in both the experiments.

Sorghum

Large genotypic variation for limited TR trait under increasing VPD conditions in sorghum lines was noticed. In Exp I, eight lines exhibited a limited TR with VPD threshold range of 2.10 (IS 25910 and IS 8348) to 2.73 (IS 14276) kPa, and six genotypes exhibited VPD threshold in the similar range of 2.22 (IS 3147 and IS 27791) to 2.88 (IS 8348) kPa in Exp II. Among nineteen sorghum lines, three lines (IS 27791, IS 3147 and IS 8348) exhibited a limited TR in both the experiments. The slope above the breakpoint ranged from 2.63 mg m² sec⁻¹ kPa⁻¹ for IS 8348 and 3.19 mg m² sec⁻¹ kPa⁻¹ for IS 3147 in the first experiment. The line IS 8348 had a negative slope in case of the second experiment.

Pearl Millet

Pearl millet lines, tested in two sets of experiment also showed a good range of variation for limited TR traits. Among nineteen lines, twelve lines expressed limited TR at high VPD where VPD threshold ranged from 1.83 to 2.72 kPa and recorded by IP 13520 and IP 7953 respectively. In Exp II only two lines IP 4542 and IP 6179 expressed limitation TR above 2.42 kPa, remaining seventeen lines showed a linear increase in TR with increasing VPD. The lines IP 4542 and IP 6179 were consistent in performance in both the experiments.

Genotypic variation for the sensitivity of transpiration to VPD also found in cereals crop like Pearl millet, *Pennisetum glaucum* (Kholova *et al.*, 2010); Maize, *Zea mays* L. (Yang *et al.*, 2012) and Sorghum, *Sorghum bicolor* (Choudhary *et al.*, 2013). The hypothesis states that 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 and Sinclair *et al.*, 2005). In this study, the restriction of the transpiration rate in crop plants for little or no increase in TR showed sizeable genetic variation. The variation in VPD breakpoint does not differ widely among these three crops. Four lines of maize (EA1197, PH207, VL 109150 and VL 12153), three lines of sorghum (IS 27791, IS 3147 and IS 8348) and two lines of pearl millet (IP 4542 and IP 6179) expressed sensitivity to elevating VPD by limiting TR consistently in both the sets of experiments. These lines may have the ability to conserve more soil water under high atmospheric VPD conditions compared to others. Water stress tolerance results from a complex combination of traits that influence supply and demand for water (Passioura 2012). The ability of a genotype to adapt to a particular water availability level eventually determines the level of tolerance of that genotype. Therefore, lines having a limited TR could further be evaluated for a given environment and selection could be based on the range of breakpoint exhibited. For this study, the heritability and genetic advance were also measured and were high, showing the potential of this trait to be used as an efficient secondary trait in breeding programs for the limited water environment.

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Table 1. Genotype used in this study

S. No.	Genotype	Crop	Origin	S. No.	Genotype	Crop	Origin
1	IP 6179	Millet	Cameroon	34	IS 14556	Sorghum	Cameroon
2	IP 13520	Millet	India	35	IS 15428	Sorghum	Cameroon
3	IP 20349	Millet	Yemen	36	IS 3583	Sorghum	CIRAD, France
4	IP 3110	Millet	India	37	IS 10978	Sorghum	Germany
5	IP 14311	Millet	Cameroon	38	IS 3147	Sorghum	CIRAD, France
6	IP 7953	Millet	India	39	SCMALAWI	Maize	Subtropical
7	IP 15857	Millet	Tanzania	40	KY21	Maize	Dent
8	IP 8647	Millet	Sudan	41	LP1233	Maize	South-American Flint
9	IP 6125	Millet	Cameroon	42	CML245	Maize	Tropical highlands
10	IP 6891	Millet	Kenya	43	MO17	Maize	Dent
11	IP 9651	Millet	Nigeria	44	FV2	Maize	Flint
12	IP 3471	Millet	India	45	PH207	Maize	Dent
13	IP 9391	Millet	Ghana	46	W64A	Maize	Dent
14	IP 13363	Millet	Tanzania	47	ZN6	Maize	Subtropical
15	IP 12395	Millet	South Africa	48	B73	Maize	Dent
16	IP 9351	Millet	Ghana	49	EA1197	Maize	Flint
17	IP 4542	Millet	India	50	W117U	Maize	Dent
18	IP 4979	Millet	Nigeria	51	FV76	Maize	Flint
19	IP 18389	Millet	Namibia	52	MBS847	Maize	Dent
20	IS 393 (411) 659	Sorghum	USA	53	EP1	Maize	Flint
21	IS 8347	Sorghum	USA	54	FC16	Maize	Flint
22	IS 20743	Sorghum	Pakistan	55	CH10	Maize	Flint
23	IS 25910	Sorghum	Cameroon	56	FV252	Maize	Dent
24	SSM 275	Sorghum	USA	57	VL 1018466	Maize	-
25	IS 20763	Sorghum	Pakistan	58	VL 1054	Maize	-
26	IS 30619	Sorghum	South Africa	59	VL 058725	Maize	-
27	IS 14276	Sorghum	Algeria	60	VL 1018550	Maize	-
28	IS 27791	Sorghum	Cameroon	61	VL 1018553	Maize	-
29	IS 29472	Sorghum	South Africa	62	VL 511305	Maize	-
30	IS 31693	Sorghum	Sudan	63	VL 1022	Maize	-
31	IS 16044	Sorghum	Lesotho	64	VL 109150	Maize	-
32	IS 16173	Sorghum	Mali	65	VL 12153	Maize	-
33	IS 8348	Sorghum	Ethiopia	66	VL 1018113	Maize	-



Table 2. One-way ANOVA for leaf area and transpiration traits in studied genotypes.

Exp	Source of variation	Transpiration (g)				Transpiration Rate (mg m ² sec ⁻¹)				
		LA	Low VPD	Medium VPD	High VPD	Total T	Low VPD	Medium VPD	High VPD	Average TR
Experiment I	Crop MS	11937949.0	314.8	831.6	1045.9	43012.8	4972.6	5023.0	5940.3	5318.8
	Error MS	169464.0	9.9	17.8	22.1	933.2	392.1	607.3	501.8	458.9
	variance	70.5***	31.9***	46.7***	47.4***	46.1***	56.2***	8.3***	11.8***	11.6***
	CV%	38.5	27.7	27.7	28.4	26.8	56.2	53.2	44.8	49.2
	SED	56.8	0.4	0.6	0.6	4.2	2.7	3.4	3.1	3.0
	LSD (5% level)	111.7	0.9	1.1	1.3	8.3	5.4	6.7	6.1	5.8
Experiment II	Maize	788.0 a	9.8 a	12.7 a	13.7 a	95.8 a	40.8 b	51.9 b	55.6 b	49.1 b
	Sorghum	1139.7 b	12.7 b	16.9 b	18.7 b	128.0 b	34.4 a	45.4 ab	50.2 b	43.1 b
	P Millet	1425.3 c	12.4 b	17.5 b	18.8 b	128.7 b	27.6 a	38.7 a	41.2 a	35.5 a
	Crop MS	467552.0	138.3	469.2	685.4	392.5	3799.4	6941.6	8518.0	6095.7
	Error MS	558822.0	30.7	55.2	63.8	47.1	652.0	910.7	1180.0	849.1
	variance	8.4***	4.5**	8.5***	10.7***	8.3***	5.8**	7.6***	7.2***	7.2***
	CV%	50.4	41.4	40.9	40.1	40.0	42.6	37.3	38.7	38.0
SED	32.6	0.8	1.0	1.1	0.9	3.5	4.2	4.7	4.0	
LSD (5% level)	64.1	1.5	2.0	2.2	1.9	6.9	8.2	9.3	7.9	
Experiment II	Maize	417.2 a	12.4 a	16.3 a	17.6 a	15.4 a	62.2 b	81.8 b	89.0 ab	77.7 b
	Sorghum	475.6 ab	14.5 b	19.8 b	22.1 b	18.8 b	52.3 a	71.5 a	78.8 a	67.5 a
	P Millet	546.6 b	13.8 ab	19.5 b	21.4 b	18.3 b	63.9 b	88.7 b	98.0 b	83.6 b

Low VPD - <1.5 kPa; Medium VPD – 1.5-2.5 kPa; High VPD - >2.5 kPa.

Significance level – 5%



Table 3. Estimates of the phenotypic coefficient of variation (PCV%), the genotypic coefficient of variation (GCV%) and heritability (h^2) for studied physiological traits.

	Traits	Maize					Pearl millet					Sorghum					
		Mean	GCV	PCV	h^2	GA % mean	Mean	GCV	PCV	h^2	GA % mean	Mean	GCV	PCV	h^2	GA % mean	
Experiment I	T (g)	LA	788.02	0.58	0.68	0.73	101.18	1425.25	0.40	0.52	0.61	65.25	1139.73	0.34	0.52	0.44	46.65
		Low VPD	9.79	0.35	0.46	0.59	56.18	12.39	0.23	0.33	0.51	34.34	12.74	0.33	0.42	0.62	54.04
		Medium VPD	12.68	0.40	0.49	0.68	68.47	17.48	0.18	0.31	0.33	21.51	16.88	0.33	0.41	0.64	53.88
		High VPD	13.68	0.38	0.49	0.61	61.24	18.84	0.23	0.36	0.43	31.31	18.69	0.27	0.36	0.56	42.31
		Total T	95.76	0.37	0.47	0.64	61.24	128.65	0.20	0.32	0.41	26.89	128.03	0.29	0.37	0.61	47.13
	TR	Low VPD	40.75	0.67	0.90	0.55	103.12	27.62	0.51	0.70	0.54	77.84	34.42	0.34	0.53	0.43	46.38
		Medium VPD	51.90	0.64	0.86	0.55	97.87	38.72	0.47	0.67	0.50	69.43	45.38	0.31	0.48	0.40	40.36
		High VPD	55.58	0.54	0.71	0.59	85.54	41.18	0.45	0.63	0.50	65.12	50.24	0.30	0.46	0.41	39.35
		Average TR	49.10	0.60	0.79	0.57	93.62	35.48	0.47	0.65	0.51	68.86	43.09	0.30	0.47	0.41	39.34
Experiment II	T (g)	LA	417.21	0.65	0.78	0.69	111.29	475.64	0.63	0.87	0.53	94.68	546.61	0.31	0.50	0.38	39.18
		Low VPD	12.38	0.58	0.70	0.69	99.25	13.83	0.45	0.62	0.54	68.38	14.50	0.32	0.48	0.46	45.03
		Medium VPD	16.28	0.57	0.68	0.69	97.14	19.51	0.48	0.64	0.58	75.90	19.83	0.27	0.44	0.37	34.22
		High VPD	17.64	0.53	0.66	0.64	87.02	21.41	0.43	0.58	0.54	64.74	22.05	0.28	0.46	0.37	34.90
		Total T	15.44	0.55	0.67	0.68	93.24	18.25	0.45	0.60	0.56	68.88	18.80	0.28	0.45	0.39	36.14
	TR	Low VPD	62.21	0.57	0.69	0.68	96.81	63.96	0.50	0.67	0.56	77.85	52.25	0.38	0.47	0.64	62.18
		Medium VPD	81.81	0.54	0.64	0.69	92.20	88.71	0.46	0.58	0.65	77.19	71.49	0.30	0.41	0.54	45.07
		High VPD	89.00	0.61	0.72	0.71	105.93	98.00	0.48	0.58	0.69	82.03	78.81	0.25	0.37	0.47	35.56
		Average TR	77.67	0.57	0.68	0.70	98.03	83.56	0.47	0.58	0.65	77.88	67.52	0.30	0.40	0.55	45.19

GCV - Genotypic coefficient of variation; PCV - Phenotypic coefficient of variation; h^2 -heritability; GA % mean -Genetic advance % mean
Significance level – 5%