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AMMI and GGE biplot analysis to evaluate the phenotypic stability of recombinant inbred lines (RILs) of peanut under mid-season water stress conditions

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Abstract

In a two-year experiment 186 recombinant inbred lines (RILs) derived from TAG-24 and TMV-2NLM cross were grown under water stress (WS) and without water stress (WWS) to identify stable high yielding genotypes and genotypes with high WUE using SLA, SCMR or WSD under drought. Genotypes PBS-40107, PBS-40110, PBS-40114, PBS-40117, PBS-40152, PBS-40159 and PBS-40521 had high yield under both WS and WWS conditions. Mean values for SCMR and WSD increased under WS whereas SLA, HI, PY and KY increased under WWS condition. Genotypes PBS-40616 and PBS-40110 were identified as stable high yielders by AMMI, GGE and simultaneous selection index (SSI) and could be cultivated under drought stress and alternatively could be used in breeding programs.

Key words: Peanut, RILs, water stress, AMMI, GGE biplot

Introduction

Peanut (*Arachis hypogaea* L.) an important legume crop of arid and semi-arid regions of the world is mainly grown under rainfed conditions where yields are commonly affected by intermittent periods of water stress (WS). In India, it is cultivated in about 4.87 m ha area with production and productivity of 7.22 m t and 1543 kg ha^{-1}, respectively during 2014-15 to 2016-17 (Anonymous 2017). Yield losses due to water stress can vary with cultivars, crop growth stage, intensity and duration while the drought stress at pod setting stage cause up to 88% yield loss (Varasoot et al. 2003), whereas at pre-flowering stage increased the yield by 13-19% (Nageswara et al. 1985).

Though sufficient variability exists for drought resistance in peanut (Songsri et al. 2009), but breeding efforts are lagging behind due to significant GE interactions. Rapid progress in drought resistance breeding has been achieved using harvest-index (HI), water-use-efficiency (WUE), specific-leaf-area (SLA) water-saturation-deficit (WSD) and SPAD-chlorophyll-meter-reading (SCMR). The SLA and SCMR have been found to be highly correlated with WUE and have been used as surrogate traits for WUE (Lal et al. 2006). Presence of considerable genotype and GE interactions complicate the selection process and warrant the use of multi-environment trials (METs) to evaluate the relative performance of genotypes over the environments. Stability analysis like most popular Additive Main Effects and Multiplicative Interaction (AMMI) and GGE biplot analysis is commonly used to analyse MET data.

The GGE biplot emphasizes that G and GE are the two sources of variation and must be considered simultaneously to identify test environments (Yan and Tinker 2006). GGE biplot graphically displays GE interaction in two-way table and is very effective tool for mega environment analysis, genotype evaluation and environmental evaluation (Yan et al. 2001). AMMI analysis computes additive main effects of genotype and environment by ANOVA and multiplicative interaction effect of GEI by PCA (Kuchanur et al. 2015; Kannababu et al. 2017). Both GGE and AMMI models don't quantify the stability measure and to overcome this lacuna several stability measures have been proposed such as Modified AMMI stability Index, (MASI, Ajay et al. 2018a). Moreover, stability per se

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alone is not a desirable selection criterion because most stable genotypes would not necessarily give the best yield performance, hence, simultaneous consideration of grain yield and stability in single non-parametric index is essential (Farshadfar et al. 2011). Therefore, simultaneous selection index (SSI) for yield and stability is computed adding the ranks of mean yield and stability index (Rao and Prabhakaran 2005; Farshadfar et al. 2011). Therefore, present investigation was aimed at identifying stable high yielding genotypes in a set of 186 peanut RILs under water stress conditions.

Materials and methods

Field screening was conducted at ICAR-Directorate of Groundnut Research, Junagadh, India during 2010 and 2012 summer seasons (February to May), in a medium black calcareous (17% $CaCO_3$) clayey, VerticUstochrept soil. A total of 188 genotypes consisting of 186 RILs and their parents (TAG-24 and TMV-2NLM) were evaluated using split-plot design under treatments involving water stress treatment (WS) imposed during 40-75 days after sowing by with-holding irrigation and the other without water stress (WWS). All management practices recommended for the region were followed. TAG-24 is a semi-dwarf commercial Spanish cultivar having low SLA (Basu and Nautiyal 2004), high yield and very high harvest index (Patil et al. 1995). TMV-2NLM is a narrow leaf mutant of TMV-2 with medium SLA and low HI (Nigam et al. 2001). Status of soil moisture and temperature in WS and WWS plots were recorded during the pod formation stage of the crop.

Specific leaf area (SLA, $cm^2\ g^{-1}$) and SPAD chlorophyll meter reading (SCMR) were recorded at 55th day after sowing (DAS). Second fully expanded leaf from the apex (from 10 randomly selected plants of each genotype in each replication) was used to record SCMR in the morning (08:00 h-09:30 h) with the help of a Minolta SPAD chlorophyll meter (Minolta Corp., Ramsey, N.J., USA). The same leaf samples were used to record the leaf area with a LI-3100 Area Meter (LI-COR Inc., Lincoln, N.E., USA). These leaves were then oven dried at 60 °C for 48 h. Weight of dried leaves was recorded to compute SLA as:

SLA = leaf area (cm2)/leaf dry weight (g)

Three leaflets each from the 3rd to 5th (top to bottom) leaves were collected from 10 different plants and used immediately for determination of water saturation deficit (WSD). WSD was determined on 74th DAS, just one day before relieving the stressed plants of water stress, whereas the WWS plots were irrigated on 73rd DAS. Immediately after plucking, fresh weight of the leaflets was recorded (FW), then leaflets were immersed in water for six hours at ambient temperature. Weight of these saturated leaflets was recorded (SW). These leaflets were, then, dried at 70°C for two days to get dry weight (DW). WSD was calculated using following formula (Vitamvas et al. 2015):

$$WSD = ((SW-FW)/(SW-DW)) * 100$$

Crop was harvested at maturity and data on, PY (pod yield kg ha^{-1}) and HI (Harvest Index) were calculated.

Statistical analysis

The analysis of variance (ANOVA) on the data was conducted using DSAASTAT (Onofri 2007). Stability analysis involving AMMI and GGE biplot were performed in R (R core team 2015) using package 'agricolae' (de Mendiburu 2017) and 'GGEbiplotGUI' (Bernal and Villardon 2016), respectively. Modified AMMI stability Index (MASI) as described by Ajay et. al. (2018a) was calculated as follows,

$$MASI = \sqrt{\sum_{n=1}^{N'} PC_n^2 \times \theta_n^2}$$

Where, PCn are the scores of nth IPC; and èn is the percentage sum of squares explained by the nth IPC effect. Simultaneous selection index for yield and stability (SSI), as described by Farshadfar et al. (2011) was calculated using following formula,

SSI = Rank of MASI + Rank of PY of genotype

MASI and SSI were computed using package 'ammistability' (Ajay et al. 2018b) available in R.

The yield reduction (YR%) for a genotype was calculated using equation of Steynberg et al. (1989) YR% = ((Yp-Ys))D (Yp*100), Where, Yp and Ys are pod yields under WWS and WS plots

Results and discussion

As expected, moisture content in WS and WWS plots were similar at both the 0-5 cm and 5-10 cm soil depths before imposition of water stress. After stress was imposed, moisture content was more in deeper layer of soil (5-10 cm) averaging 17.68% and 17.76% compared to upper layer (0-5 cm) with average of 12.18% and 13.87% in stressed plots, whereas there were no such differences in irrigated plots. In 2012 stressed plots were more stressed of moisture compared to 2010 season. The soil temperature increased as the day advanced from 8 AM to 4 PM irrespective of the irrigation treatments in both the years. The upper layer of soil was slightly warmer compared to lower layer. Similarly, the temperatures recoded in WS plots were higher compared to those of WWS plots (Fig. 1). As the crop age increased, there was decrease in the soil moisture content irrespective of the irrigation treatments and soil depths which was attributed to enhanced evapo-transpirational losses of water due to increased air-temperature and crop-canopy.

ANOVA revealed significant genotypic (G) differences for all traits (except HI) indicating presence of sufficient variability among RILs and are a source of genetic diversity for breeding purpose (Table 1 and 2). The genotype×environment interaction (GEI) was significant for most of the traits except WSD indicating that genotypic performance varies between WS and WWS treatments. This is in agreement with the earlier findings who have reported significant variability for G and GEI (Lal et al. 2009). Mean and range for different phenotypic traits under WS and WWS treatments are presented in Table 2. Pod yield (PY) ranged from 607 to 4355 kg/ha and 389 to 3045 kg/ha under WS in 2010 and 2012, respectively whereas, under WWS it increased to 1226 to 4750 kg/ha and 945 to 5229 kg/ha in 2010 and 2012, respectively. Genotypes PBS-40107, PBS-40110, PBS-40114, PBS-40117, PBS-40127, PBS-40152, PBS-40159 and PBS-40521 had high yield under both WWS and WS conditions and it supports the findings of Foulkes et al. (2007) that genotypes performing well under optimum conditions

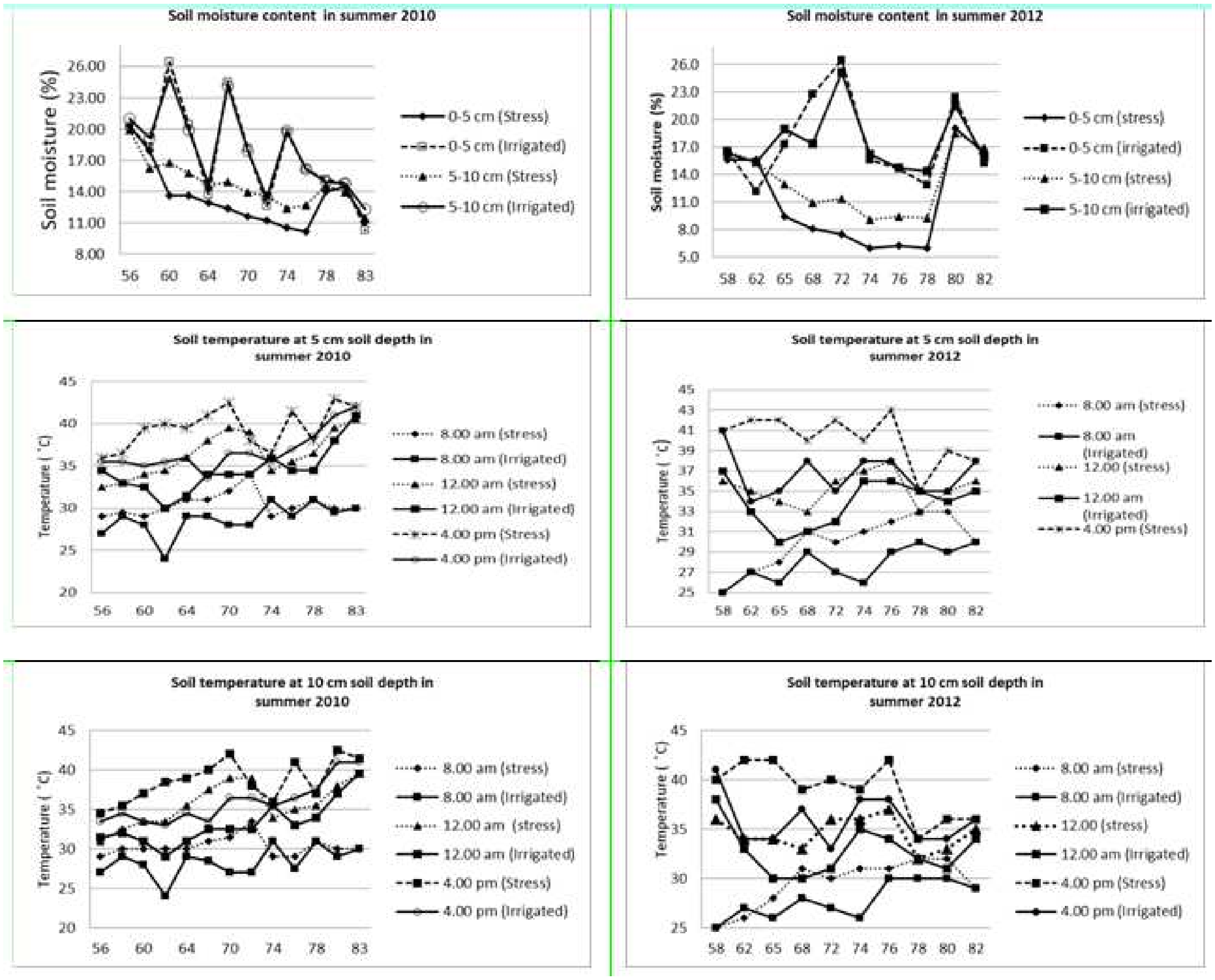

Fig. 1. Soil moisture content and temperatures (8.00 am, 12.00 noon and 4.00 pm) recorded at 0 to 5 cm and 5 to 10 cm soil depths

Table 1. Analysis of variance for yield related traits under drought and optimum conditions

Source of variation	Df	SCMR	SLA	WSD	HI	PY
Environment (E)	3	1904.0	38065.4	3161.5	1551.4	232326249.7
Rep	4	18.0	1025.8	149.6	54.1	2318185.9
Genotype (G)	187	31.1**	673.1**	22.5**	59.1	1946929.6**
G x E	561	7.2**	259.0**	13.3	51.8*	365374.2**
Residual	748	5.5	130.2	12.5	43.7	177144.9
Total	1503	13.1	323.9	20.7	51.7	936664.5
LSD		2.3	11.2	3.46	6.48	413.13

SCMR = SPDA chlorophyll meter reading; SLA = Specific leaf area; WSD = Water saturation deficit; HI = Harvest Index and PY = Pod yield

Table 2. Mean and range for different phenotypic traits of peanut RILs under water stress (WS) and without water stress conditions (WWS) over two years

Trait	Year	Mean		Range		Avgerage reduction (%)
		WS	WWS	WS	WWS	
SCMR	2010	42.59±0.18	39.16±0.22	35.8–50.7	29.7–47.3	-7.28
	2012	39.22±0.15	37.10±0.19	34.2–46.7	31.2–45.2	
SLA	2010	156.3±0.91	172.3±1.24	127.8–197.6	111.4–213.7	6.12
	2012	149.4±0.68	153.4±1.02	122.7–179.5	118.2–207.1	
WSD	2010	19.01±0.24	13.1±0.15	7.7–26.7	7.4–17.3	-25.68
	2012	19.4±0.23	17.5±0.18	12.4–28.3	8.8–22.9	
HI (%)	2010	36.8±0.41	39.3±0.40	20.9–48.1	20.5–47.9	6.55
	2012	34.4±0.34	36.8±0.35	21.0-43.6	18.9–45.0	
PY (kg/ha)	2010	3034.3±51.32	3646.3±46.24	606.8–4355.0	1225.8–4750.0	21.92
	2012	1789.7±30.69	2532.3±48.78	388.5–3045.4	945.2–5228.6	

retain high yield under stress. The HI increased under WS by 6.5% and it ranged from 20.9 to 48.1% and 21.0 to 43.6% in 2010 and 2012, respectively under WWS, whereas under WS it ranged from 20.5 to 47.9% and 18.9 to 45.0% in 2010 and 2012, respectively.

SCMR increased by 7% under WS conditions and it ranged from 29.7 to 47.3 and 31.2 to 45.2 in 2010 and 2012, respectively under WWS whereas under WS range increased to 35.8 to 50.7 and 34.2 to 46.7, respectively in 2010 and 2012. SLA reduced by 6.1% under WS condition and ranged from 127.8 to 197.6 and 122.7 to 179.5 in 2010 and 2012, respectively under WS whereas, under WWS it changed to 111.4 to 213.7 and 118.2 to 207.1 in 2010 and 2012, respectively. WSD increased by 25.7% under drought stress condition and ranged from 7.7 to 26.7 and 12.4 to 28.3 in 2010 and 2012 respectively under WS whereas it ranged from 7.4 to 17.3 and 8.8 to 22.9 in 2010 and 2012, respectively under WWS. Mean values of SCMR and WSD, increased under WS, whereas SLA, HI and PY reduced which has been attributed to reduced availability of soil moisture (Lal et al. 2009). In the present study, GGE analysis was considered satisfactory as the first two principal components of the GGE explained 84% variation. Mean performance and stability of 186 RILs as analysed by GGE biplot is depicted in Fig. 2. Accordingly, RIL 27 (PBS-40127) was high yielder (4044 kg/ha) followed by RIL 7 (PBS-40107) with 3840 Kg/ha but their yields are variable over the environments whereas RIL 190 (TMV-2NLM) and RIL 67 (PBS-40507) were poor yielders. Genotypes PBS-40110 and PBS-40114 had comparatively high yield and were stable across environments.

The results of AMMI ANOVA for PY indicated contribution of G, E and GEI to the tune of 53.1%,

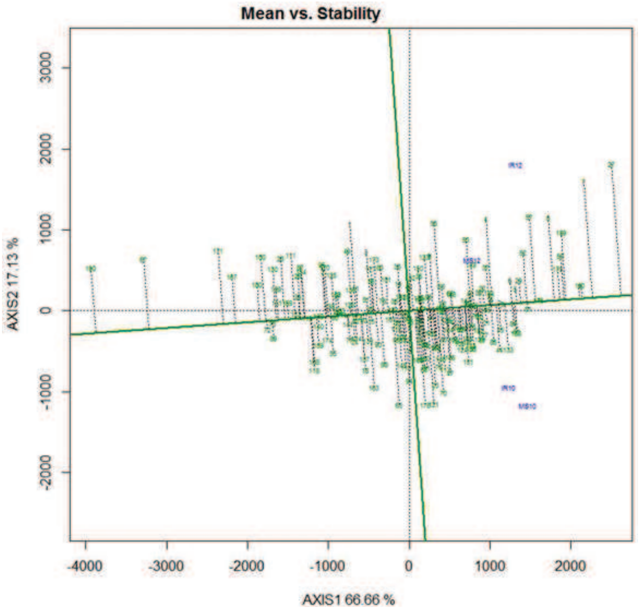

Fig. 2. Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling peanut genotypes evaluated for PY under WS and WWS conditions during 2010 and 2012. IR10 = WWS in 2010; IR12 = WWS in 2012; MS10 = WS in 2010; MS12 = WS in 2012

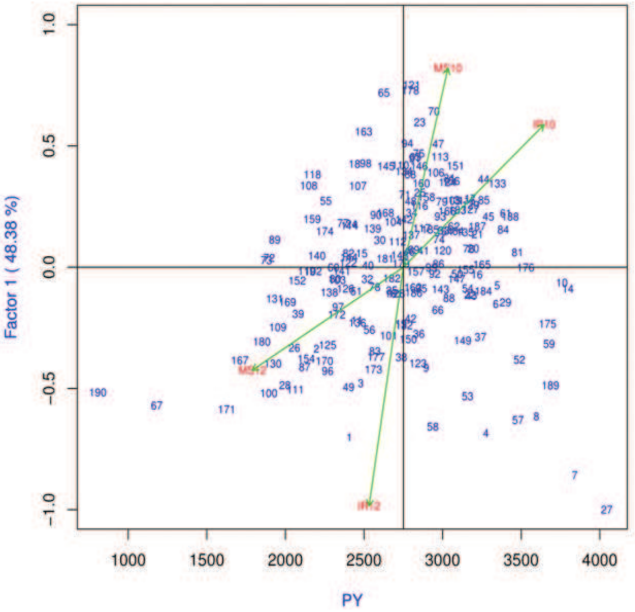

Fig. 3. AMMI biplot showing distribution of genotypes and environment for IPCA1 and PY. IR10 = WWS in 2010; IR12 = WWS in 2012; MS10 = WS in 2010; MS12 = WS in 2012

Table 3. AMMI analysis of variance for pod yield under mid season stress and optimum conditions

Source	Df	SS	MS	F value	% SS
Environment (E)	3	697154253	232384751**	3635.7	53.1
Genotype (G)	187	363856833	1945758**	30.4	27.7
G×E interaction	561	204938849	365310**	5.7	15.6
IPCA1	189	99221706.91	524983**	8.2	48.4
IPCA2	187	67245850.07	359604**	5.6	32.8
IPCA3	185	38471291.84	207953**	3.3	18.8
Residuals	752	48066204.40	63918		3.7

27.7% and 15.6%, respectively. AMMI identified three significant IPCA1 to IPCA3 which explained 48.4, 32.8 and 18.8% of GE sum of squares, respectively (Table 3). AMMI biplot with PY on X-axis and IPCA1 scores on Y-axis is presented in Fig. 3. Genotypes located farthest on the X-axis are high yielders whereas on the Y-axis genotypes whose IPCA1 scores are closer to zero are more stable. The results identified genotypes such as RILs 176 (PBS-40616), 165 (PBS-40605) 155 (PBS-40595), 10 (PBS-40110), 14 (PBS-40114), as stable and RILs 121 (PBS-40561), 178 (PBS-40618), 7 (PBS-40107), and others as unstable. MASI and SSI discriminated RILs 176 (PBS-40616), 10 (PBS-40110), 16 (PBS-40116) and 20 (PBS-40120) as stable high yielders whereas RILs 67 (PBS-40507), 171 (PBS-40611) and 190 (TMV2-NLM) as unstable low yielders (Table 4). Environment and GEI significantly influenced most of the traits under WS. AMMI and GGE-biplot analysis, identified PBS-40116, PBS-40120 and PBS-40616 as stable high yielders under water deficit conditions. Genotypes PBS-40105, PBS-40121 and PBS-40524 were stable high yielders with low WSD. In addition, genotypes with low SLA and high SCMR (PBS-40622 and PBS-40130); and low WSD (PBS-40551, PBS-40550 and PBS-40545) were also identified which could serve as donors for future breeding programs.

Table 4. Modified AMMI stability index (MASI), rank of MASI (rMASI), mean pod yield (PY), rank of PY (rY) and simultaneous selection index (SSI) of stable high yielders and unstable low yielders of peanut RILs

RIL	Genotype	MASI	rMASI	PY	rY	SSI
Stable high yielders						
176	PBS-40616	0.12	1	3528.2	9	10
16	PBS-40116	0.31	2	3218.2	29	31
20	PBS-40120	0.72	8	3195.5	30	38
10	PBS-40110	1.71	39	3759.1	4	43
21	PBS-40121	1.43	24	3222.7	27	51
147	PBS-40587	0.76	10	3089.0	46	56
5	PBS-40105	1.75	42	3347.1	18	60
135	PBS-40575	1.40	22	3143.8	41	63
22	PBS-40122	1.53	29	3173.3	35	64
84	PBS-40524	1.83	49	3386.0	16	65
Unstable low yielders						
49	PBS-40149	4.93	166	2402.3	145	311
3	PBS-40103	5.92	178	2479.3	135	313
1	PBS-40101	6.51	182	2406.9	143	325
28	PBS-40128	4.54	159	1997.5	176	335
130	PBS-40570	4.37	155	1920.4	180	335
111	PBS-40551	5.06	168	2063.3	173	341
100	PBS-40540	4.84	165	1893.4	182	347
190	TMV-2NLM	4.79	164	806.4	188	352
67	PBS-40507	5.60	173	1181.9	187	360
171	PBS-40611	5.61	174	1626.7	186	360

Authors' contribution

Conceptualization of research (CL); Designing of the experiments (CL); Contribution of experimental materials (CL); Execution of field/lab experiments and data collection (BMC, HKG); Analysis of data and interpretation (BCA); Preparation of manuscript (BCA, CL).

Declaration

The authors declare no conflict of interest.

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