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Effect of Early Moisture Stress in Recombinant Inbreds and Identification of Moisture Stress Tolerant Groundnut Lines based on Statistical Approaches

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ABSTRACT: Groundnut (*Arachis hypogaea* L.) is an important oil seed crop mainly grown under rainfed situation. Pod yields are generally hampered due to erratic rainfall and frequent drought during the crop growth period under rainfed conditions. Drought during critical crop growth stages is crucial for loss in yield of groundnut varieties. But tolerant genotypes may give better yield due to maintenance of physiological responses that were triggered during drought. Drought tolerance enhancement requires dependable estimate of drought tolerance potentials among the genotypes. In order to appraise drought stress in groundnut, a mapping population of 147 recombinant inbred lines (RILs) derived from the cross NRCG 12568 × NRCG 12326 were evaluated under moisture stress and non-stress field environments. The stress was imposed by withholding irrigation from 30-45 DAS to investigate relationships and repeatability among fifteen drought tolerance indices. Based on correlation and discriminating ability of the indices, four indices namely stress tolerant index (STI), mean productivity (MP), harmonic mean productivity (HMP) and geometric mean productivity (GMP) showed highly significant and positive correlation with pod yield under well-watered and water stress conditions. These indices aid as successful selection criteria in identifying the RILs for their performance under various soil moisture levels. Principal component analysis (PCA) as indicated by PC1 and PC2 components justified 85.60% of variations for moisture stress among RILs under water stress. The PCA were interpreted through biplot analysis and revealed STI, GMP, MP, and HMP are effective for identifying drought tolerant genotypes with high pod yield. Based on rank sum method, involving the four indices distinguished RIL 541, RIL 245, RIL 158 and RIL 175 as the most tolerant RILs for drought. The identified lines shall be evaluated in varied rainfed environments to exploit their stable drought tolerance and yield potential. The validated lines shall be utilized as improved cultivars for rainfed or drought prone environments under changing climate.

Keywords: Moisture stress, Drought tolerant indices, Principal component analysis, Groundnut.

INTRODUCTION

Groundnut, the king of oil seeds is one of the important legume crops cultivated predominantly under rain-fed conditions in the tropical and semi-arid tropical countries including India, where it provides a major source of oil, carbohydrates and proteins (Pasupuleti *et al.*, 2016; Ondulla, 2020). The seed is used mainly for edible oil and contains nearly half of the essential vitamins and one-third of the essential minerals. It is one of the most nourishing foods available in the world (Fonte *et al.,* 2019; Samtiya *et al.,* 2020). Groundnut is cultivated predominantly in the tropics and subtropics, where the availability of water is a major constraint on yield (Viramani and Singh, 1986; Happy *et al.,* 2018). The crop is subjected to water deficit stress at one stage or another leading to drastic reduction in productivity. This necessitates development of cultivars which can withstand water stress and still can be productive. Reduction in peanut yield resulting from drought has been well documented (Nageswara Rao *et al.,* 1989; Carvalho *et al.,* 2017). Drought during the pod and seed forming stages has been shown to reduce pod yield of peanut by 56-85% (Vincent and Pasal, 2016).

Due to lack of rapid, reproducible water stress screening methodologies, combined with ineffectiveness creating defined and repeatable water stress conditions to evaluate huge plant populations efficiently, makes it all the way more difficult to breed for drought tolerance (Ramirez and Kelly, 1998). To decipher the physiological and genetic basis of drought tolerance, quite a few number of vegetative and reproductive growth phase's traits are identified to be used as surrogates to identify drought tolerant genotypes (Foolad, 2005). Hence it is imperative to identify a selection criteria to distinguish desirable water stress resistant genotypes with the less resistant ones. But variation in yield potential could arise from factors related to adaptation rather than to drought tolerance *per se*.

In order to assess the response of genotypes under moisture stress conditions, estimation of drought tolerance indices should be based on the plant performance (in terms of yield, dry matter yield, and/or other quantitative traits) in stress/non-stress environments. Therefore, various researchers have proposed numerous selection indices as surrogates to identify/classify genotypes performing well under limited moisture stress conditions. Often, a comparison of loss of yield under drought-conditions to normal conditions is used as an important measure of drought tolerance to identify drought-tolerant genotypes (Mitra, 2001). These indices are either based on drought tolerance or susceptibility of genotypes (Fernandez, 1992). Yield reduction under drought stress is the most common measure of drought susceptibility of a genotype (Blum, 1988) whilst the values are confounded with differential yield potential of genotypes (Ramirez and Kelly, 1998).

The differences in yield between the stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp is defined as stress tolerance (TOL) by Rosielle and Hamblin (1981). Stress susceptibility index (SSI) for cultivars was proposed by Fischer and Maurer (1978). To identify genotypes producing high yield under both stress and non-stress conditions, STI= stress tolerance index was proposed by Fernandez (1992). Geometric mean (GM), mean productivity (MP) and TOL are the various yield based estimates of drought resistance. Often plant breeders use geometric mean as it helps in assessing relative performance (Ramirez and Kelly, 1998). A selection criteria can be regarded as optimal if it is able to distinguish genotypes that express uniform superiority in both stress and non-stress environments from the ones that are favorable only in one environment. A lower TOL and SSI are favored, as it indicates less sensitivity to stress. When selection is dependent on the above mentioned criterion, it favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. While, selection based on STI and GMP shall result in selecting genotypes with high stress tolerance and yield potential (Fernandez, 1992).

The main task of plant breeder is to understand the association of indices with drought tolerance in cultivars under environmental stress conditions to exploit the genetic variation to improve the stress tolerant cultivars. Several researchers across the crop groups have used these indices to quantify the responses and degree of tolerance of the genotypes to abiotic stresses. To quote a few, Safavi *et al.*, (2015) in sunflower, Uday *et al.*, (2016) in chickpea, and Bennani *et al.*, (2016) and Bennani *et al.* (2017); Zahra Moradi (2015) in bread wheat have used different combinations of the indices to quantify the degree of tolerance of genotypes to abiotic stress. Several drought tolerant indices (DTIs) are suggested to differentiate the degree of drought tolerance between different genotypes. The present study attempted to understand the responsiveness of RILs in water stress at flowering to 50 *per cent* flowering period. Moreover, to assess the selection criteria for identifying drought tolerant RILs using fifteen drought tolerant indices based on pod yield plant per plant under water stress and non-stress field conditions.

MATERIAL AND METHODS

The experiment was conducted at University of Agricultural Sciences, Bangalore, in medium red sandy loamy soil. The material comprised of 147 RILs derived from the cross NRCG 12568 × NRCG 12326 along with two check varieties (GKVK 5 and TMV 2). The experiment was taken up in Augmented design (Federer, 1956) during *summer* 2018, under control and stress condition. Water stress was imposed at 30 days after sowing (DAS) to all the RILs by withholding irrigation in stress plot for fifteen days while the control plot was given regular irrigation once in a week. Harvesting was carried out at about 120 DAS. The maturity of pods was assessed by the *per cent* blackening of the internal inner parenchyma (Miller and Burns, 1971).

Five randomly chosen plants in each RIL were labeled and used for recording pod yield plant^{-1}(g), yield related, physiological and agronomical traits. Fifteen drought tolerant indices, *viz*., tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HAM), stress tolerance index (STI), relative drought index (RDI), abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI), stress non-stress production index (SNPI), yield index (YI), yield stability index (YSI), modified stress tolerance index calculated by pod yield plant^{-1} under normal condition (K_1STI), modified stress tolerance index calculated by pod yield plant^{-1} under stress condition (K_2STI), drought resistance index (DI) and stress susceptibility index (SSI) were calculated based on pod yield plant per plant under stress (Y_S) and control (Y_P) condition to screen for drought tolerance.

Correlation analysis was performed using the software SPSS *ver*. 16. Microsoft Excel along with XLSTAT 2014 was used to compute principal component analysis, rank correlation matrix and biplot analysis as suggested by Iqbal *et al.*, (2014). Based on the value of each DTI of all the groundnut RILs, a rank for each RIL was classified and rank sum (RS) was calculated [RS= Rank mean (Rm) + Standard deviation of rank (SDR) as conducted by Farshadfar *et al.*, (2012)]. The above mentioned procedure was implemented to identify best tolerant genotypes.

RESULTS AND DISCUSSION

According to the existing situation, there is a greater need to develop such crop varieties which shows resistance against the abiotic stresses to ensure food security. Improvement in groundnut for drought tolerance requires reliable assessment of drought tolerance variability among genotypes/RILs. Water stress during crop growth period at certain interval considerably affects pod yield of the RILs. The RILs varied widely for pod yield plant per plant under moisture stress relatively to that under well watered condition (Fig. 1). However, pod yield in water stress differentiated RIL 245 and RIL 439, as they out-performed even after exposing to stress at early stages of the crop growth in comparison with the performance of the same RIL in well watered conditions. Higher magnitude of result was expected for pod yield per plant against RILs under well watered conditions. Few RILs in the population performed better even under stress conditions, while few couldn't cope up with the stress limits. This genic action is majorly due to exposure of the RILs to intermittent moisture stress. However, drought stress largely inhibits the growth and development of crops. To tolerate this condition plant going through osmotic adjustment in which plants reduce the cellular osmotic potential through the formation of different solutes and inorganic ions like K+. All these accumulated solutes help the plant cells to be hydrated under the prolonged deficiency of water and provide some resistance against water stress and helps to maintain the structure of the cell membrane. The relative adaptability of RILs reflects their degree of moisture stress tolerance (Yaman and Prasann, 2021).

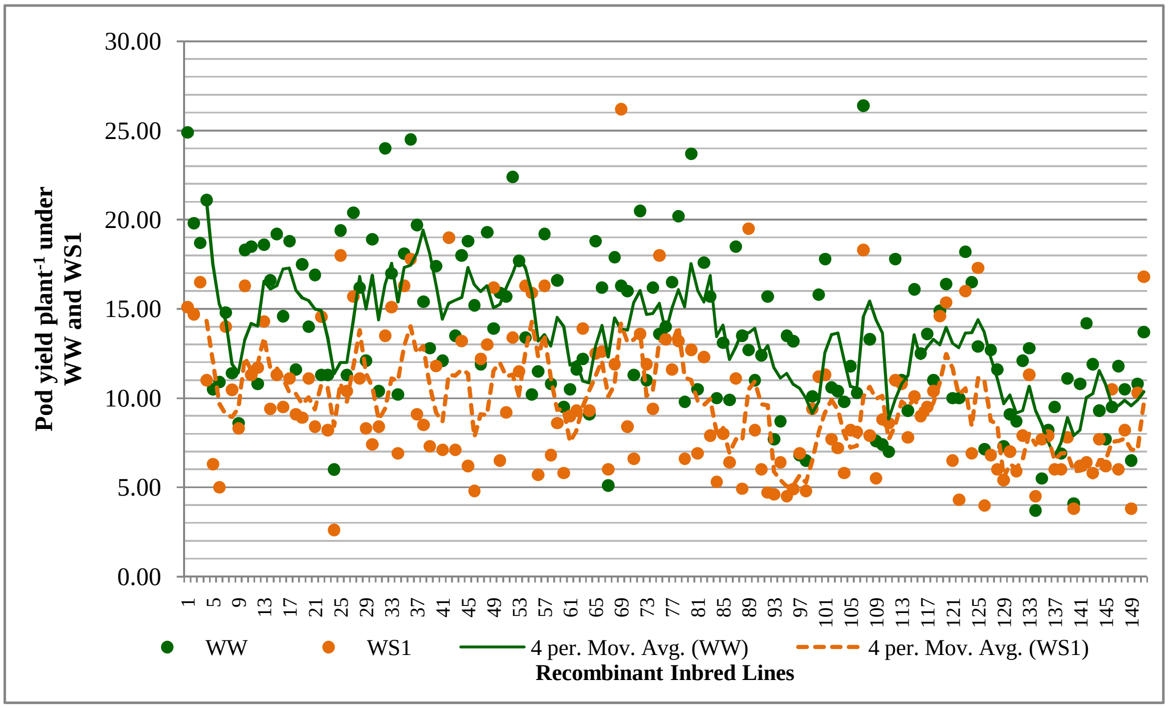


Fig 1. Comparative analysis of the RILs for pod yield plant^{-1} under well watered and water stress condition in groundnut during summer 2018.

Loss of yield is the main concern of plant breeders and they hence emphasize on yield performance under stress conditions. In the absence of special mechanisms to quantify the tolerance, the quantification of drought tolerance should be based on yield under stress and non-stress environments that can lead to selection of high yielding genotypes under stress conditions (Punithavathi *et al.*, 2021). Since, the response of selection under non-stress condition is maximal and heritability of the yield under these conditions is high (Shirinzadeh *et al*., 2010 and Geravandi *et al*., 2011). Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra, 2001). These indices are based on drought resistance or susceptibility of genotypes (Fernandez, 1992).

Good discrimination of genotypes by the DTI's helps in effective selection of most desirable RILs for moisture stress environment. The indices that discriminate the RILs for responses to moisture stress environment assume importance in this context. In the present study, only eight of the 15 indices namely TOL, STI, ATI, SSPI, K_1STI, K_2STI, SNPI and SSI showed better ability to discriminate the RILs for their responses to moisture stress environments than other indices as indicated from high magnitude of estimates in both standardized range (SR) and Phenotypic coefficient of variation (PCV) under water stress (Table 1). It is desirable to preferentially use these indices for screening the genotypes for responses to moisture stress environment in groundnut. Bennani *et al.* (2016 and 2017) have also suggested one of these five indices, *i.e.*, STI for discriminating bread wheat genotypes for their responses to drought stress. However, they used significant mean squares attributable to indices as criteria to identify those with better discriminating ability.

Table 1: Estimates of variability for various drought tolerant indices based on pod yield plant^{-1} under moisture stress environment.

Indices	Pod yield plant^{-1} (g)	
	Standardized Range	PCV (%)
Tolerance index (TOL)	3.81	62.30
Mean productivity (MP)	1.80	32.72
Geometric mean productivity (GMP)	1.88	33.61
Harmonic mean productivity (HMP)	2.07	35.52
Stress tolerance indices (STI)	3.42	70.09
Relative drought index (RDI)	1.75	78.37
Abiotic tolerance index (ATI)	3.03	78.58
Stress susceptibility percentage index (SSPI)	3.81	62.30
Yield index (YI)	2.62	44.90
Yield stability index (YSI)	1.75	78.37
Modified STI (K_1STI)	2.18	63.68
Modified STI (K_2STI)	2.26	70.09
Drought resistant index (DI)	1.78	64.43
Stress non-stress production index (SNPI)	11.61	58.43
Stress susceptibility index (SSI)	3.46	55.02

A. Correlation and Rank sum method

Suitable stress tolerant indices are indicators, which have relatively high significant positive correlation with yield in both non-stress and stressed condition (Kandalkar, 2014 and Subhani *et al.,* 2015). Correlation analysis between pod yield per plant and DTIs can be a good criterion for identifying the best RILs and indices used. Y_S was significantly and positively correlated with MP, GMP, HMP, STI, RDI, YI, YSI, K_2STI and DI and negatively correlated with TOL, ATI, SSPI, SNPI and SSI. Y_P was significant and positively correlated with all drought indices, except RDI, YSI and DI. The drought tolerant indices, MP, GMP, HMP and STI were significantly positively correlated with both Y_P and Y_S (Table 2) indicating that these indices are more effective in identifying high yielding RILs under different water regimes. In bread wheat, GMP, MP, STI, K_1STI, K_2STI, YI, DRI (drought response index), DI, SNPI, RDI and YSI were the most suitable indices for screening drought tolerant genotypes (Farshadfar and Elyasi (2012)). Thi *et al*., (2015) and Abejide *et al.*, (2017) also identified similar findings in tomato and bambara groundnut, respectively, that MP, GMP, HMP and STI are good indicators or parameters to be used for screening and selection of drought tolerant genotypes under well watered and water stress condition. To determine the most desirable drought tolerant RIL, rank mean, SDR and RS for each RIL were calculated based on rank of three DTI's (MP, GMP and STI) which are correlated with pod yield under control and stress condition. The best tolerant genotypes were identified as having low rank mean, SDR and least in RS (Farshadfar *et al.*, 2012). The RILs with low rank values among all DTIs denoted as drought tolerant. In consideration to all indices, RIL 126, RIL 249, RIL 133, RIL 145, RIL 129, RIL 539, RIL 248, RIL 233, RIL 258 and RIL 139 recorded the lower rank mean and standard deviation of ranks under well-watered and water stress condition, Hence rank sum method effectively combines all the three indices into one to select water stress tolerant RILs (Table 3). The same procedures have been used for screening quantitative indicators of drought tolerance in wheat (Mohammadi *et al*., 2012), groundnut (Savita, 2017; Eradasappa, 2018), tomato (Suresh, 2018) and Dolichos (Balaraju, 2018).

Table 2: Correlation coefficients of the RIL population for drought tolerant indices and pod yield plant plant^{-1} under well- water and water stress condition in groundnut.

Variables	TOL	MP	GMP	HMP	STI	RDI	ATI	SSPI	YI	YSI	K_1STI	K_2STI	DI	SNPI	SSI	Y_p	Ys
TOL	1.00	0.10	0.02	-0.05	0.04	-0.78*	0.73*	0.98*	-0.63*	-0.78*	0.68*	-0.60*	-0.72*	0.53*	0.78*	0.71*	-0.63*
MP		1.00	0.97*	0.92*	0.95*	-0.02	-0.05	0.10	0.71*	-0.02	0.72*	0.69*	0.22*	-0.03	0.02	0.77*	0.71*
GMP			1.00	0.99*	0.97*	-0.05	0.01	0.02	0.75*	-0.05	0.61*	0.71*	0.16*	-0.04	0.05	0.70*	0.75*
HMP				1.00	0.95*	-0.07	0.05	-0.05	0.76*	-0.07	0.51*	0.70*	0.12	-0.05	0.07	0.62*	0.76*
STI					1.00	-0.05	0.01	0.04	0.72*	-0.05	0.64*	0.72*	0.16	-0.04	0.05	0.70*	0.72*
RDI						1.00	-0.88*	-0.78*	0.54*	0.96*	-0.36*	0.52*	0.93*	-0.39*	-0.90*	-0.51*	0.54*
ATI							1.00	0.73*	-0.56*	-0.88*	0.34*	-0.62*	-0.96*	0.43*	0.88*	0.43*	-0.56*
SSPI								1.00	-0.63*	-0.78*	0.68*	-0.60*	-0.72*	0.53*	0.78*	0.71*	-0.63*
YI									1.00	0.54*	0.08	0.96*	0.68*	-0.40*	-0.54*	0.10	0.90*
YSI										1.00	-0.36*	0.52*	0.93*	-0.39*	-0.90*	-0.51*	0.54*
K_1STI											1.00	0.09	-0.21*	0.27*	0.36*	0.94*	0.08
K_2STI												1.00	0.69*	-0.43*	-0.52*	0.10	0.96*
DI													1.00	-0.39*	-0.93*	-0.31*	0.68*
SNPI														1.00	0.39	0.31*	-0.40*
SSI															1.00	0.51*	-0.54*
Y_p																1.00	0.10
Y_s																	1.00

*Significance at P=0.05 Y_p- yield under non stress Y_s- Yield under stress

Table 3: Rank sum (RS) method to identify tolerant RILs in groundnut based on hypothetical magnitude of three indices.

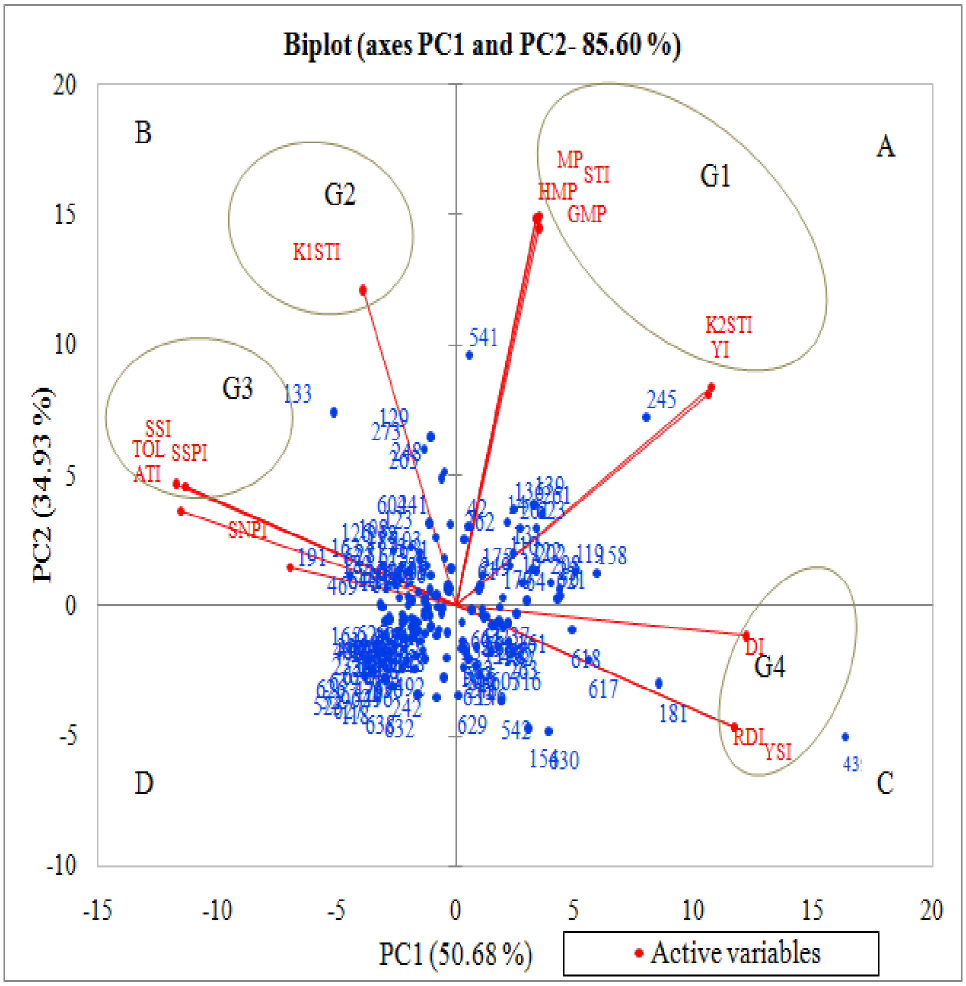
RILs	MP	Rank	GMP	Rank	STI	Rank	Rank Mean (RM)	Standard deviation of ranks (SDR)	Rank sum (RS) (RM +SDR)
126	22.82	1	22.67	1	0.69	1	1.00	0.00	1.00
249	20.16	3	19.90	2	0.53	2	2.33	0.58	2.91
133	20.73	2	19.24	4	0.50	4	3.33	1.15	4.49
145	19.54	5	19.54	3	0.52	3	3.67	1.15	4.82
129	19.72	4	18.72	5	0.47	5	4.67	0.58	5.24
539	17.93	6	17.89	6	0.43	6	6.00	0.00	6.00
248	17.42	9	17.41	7	0.41	7	7.67	1.15	8.82
233	17.32	10	17.31	8	0.41	8	8.67	1.15	9.82
258	17.45	8	16.94	10	0.39	10	9.33	1.15	10.49
139	17.14	11	16.92	11	0.39	11	11.00	0.00	11.00
231	17.10	12	17.08	9	0.39	9	10.00	1.73	11.73
128	16.61	13	16.54	12	0.37	12	12.33	0.58	12.91
529	16.54	14	16.53	13	0.37	13	13.33	0.58	13.91
237	16.49	15	16.29	14	0.36	14	14.33	0.58	14.91
541	17.52	7	16.02	15	0.35	15	12.33	4.62	16.95
631	15.69	18	15.47	16	0.32	16	16.67	1.15	17.82
201	15.89	17	15.38	18	0.32	18	17.67	0.58	18.24
245	15.60	19	15.41	17	0.32	17	17.67	1.15	18.82
108	15.49	20	15.07	19	0.31	19	19.33	0.58	19.91

MP- Mean Productivity GMP- Geometric Mean Productivity STI- Stress Tolerance Index

B. Principal component and biplot analysis

In order to further investigate the inter-relationships and repeatability among drought selection indices, as well as to distinguish tolerant RIL, principal component (PC) analysis was performed and corresponding biplot has been drawn which shows overview of inter-relationship among drought tolerant indices using XLSTAT *ver.* 2017. Graphical representation of the relationships among different indices is displayed in biplot of PC1 and PC2 (Fig. 2). The PC1 and PC2 axes which justify 85.60 *per cent* of total variation, mainly distinguishes the indices in four groups. Of which, group 1 consisted of major indices MP, GMP, HMP, STI, YI and K_2STI are strongly correlated with yield under both conditions indicating that these criteria are suitable for identification of drought tolerant accessions.

Generally, indices belonging to the same group distinguish drought tolerant accessions in similar way. Most reliable indices to identify drought tolerant cultivars is the one which has relatively high correlation with grain yield in both stress and non-stress condition (Farshadfar *et al.,* (2001)). Higher score of PC1 were in accordance with the higher rank of drought tolerance, whereas low score for PC2 showed drought sensitive genotypes. Therefore, first component was named as drought tolerant. The relationship between RILs and tolerance to drought in the form of biplot was used for identification of drought tolerant RILs. The higher scores for PC1 and lower scores for PC2 were in accordance with the higher rank of drought tolerance. RILs were categorized into four groups (A, B, C and D) based on their performance in well-watered and water-stressed environments: Group A - accessions expressing uniform superiority in well-watered and water-stressed conditions, Group B - accessions that perform favourably only in well-watered conditions, Group C - accessions that gives relatively higher yield only in water-stressed condition and Group D - accessions perform poorly in both the conditions. Biplot analysis (Fig. 2) showed that RILs *viz*., RIL 541, RIL 245, RIL 126, RIL 139, RIL 262, RIL 131, RIL 42, RIL 529 and RIL 128 as most drought tolerant (Group A). Zare (2012) used same method for identification of drought tolerant genotypes in barley. Similarly in safflower by Bahrami *et al.* (2014), in Tomato by Thi (2016) and Suresh (2018) and in groundnut by Savita (2017). PCA guide to organize RIL population similar to grouping based on PCA provided on the basis of three-dimensional graph. Since the method allows simultaneous evaluation of the RILs and the interpretation of inter-relationships among the indices, it may be recommended as a method of choice for data analysis in further studies on drought tolerance in groundnut.



The numbers, *viz.*, 133,... indicating code of RILs as shown in Table 3; **G-group of DTIs**: G1, 2, 3 and 4= Group 1, 2, 3 and 4 respectively.

Fig. 2. Biplot analysis based on first (PC1) and second (PC2) principal components.

CONCLUSION

There is a greater need to produce a high amount of food grains to sustain food security to feed the increasing world's population. During the plant growth period, it faces several stresses, which reduces its growth and development. Both biotic stress and abiotic stress have the potential to cause a huge reduction in crop yield and crop quality. A prolonged period without rain leads to water stress. Due to the water deficiency, plant also fails to uptake the nutrients because there is a disturbance in the transpiration process. The groundnut population included in this study differed significantly in terms of pod yield plant per plant determined at the stage of intensive vegetative growth in conditions of optimal and limited irrigation, as well as in terms of the calculated drought tolerance indices. Grouping of RILs in terms of drought tolerance was similar when carried out via PCA which additionally allowed the interpretation of the relationships among the indices. Alas, we identified four drought tolerant indices *viz.*, MP, GMP, HMP and STI positively significant correlated with pod yield planr^{-1}under stress and control condition indicating that these indices were more effective in identifying high yielding accessions under different irrigated conditions. Based on biplot diagram and ranking method calculated from suitable drought indices, RIL 541, RIL 245, RIL 158 and RIL 175 were identified as the most drought tolerant RILs which could be used for developing high yielding hybrids or developing pure line varieties. Further, evaluation of identified promising genotypes in varied rainfed environments is required to exploit the drought potential of these lines for climate smart agriculture. Thus, by developing and utilizing the moisture stress tolerant crop varieties promises their future perspective for maintaining better crop production and stabilizes the income of farmers even under adverse environmental conditions.

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Conflict of interest. The authors state that they have no interest in conflicts.

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