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Identification of Maize Genotypes for Moisture Stress Tolerance

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Abstract—*A total of 100 maize genotypes along with three inbreds as check from different sources (63 from CIMMYT, Hyderabad- India, 37 from IIMR, New Delhi and 3 Checks from BAU, Ranchi) were grown in Augmented design under two environments viz., normal field and moisture stress condition (rainout shelter) for screening the best performing lines based on physiological traits, yield parameters and stress indices. Observations were recorded for 10 morphological characters including per day productivity and 11 stress indices were estimated following standard formulae. Pearson's correlation coefficients were calculated between all the stress indices and adjusted grain yield in stress and non-stress conditions by substituting corresponding variance and covariance in the formulae given by [19].All the genotypes were ranked for morphological traits and stress indices for their good performance to poor performance. Averages were taken from both types of ranking sets and Spearman's rank correlation was estimated for the significance of the agreement between the ranks. Physiological traits (leaf senescence, leaf rolling and stay green characters) were measured in ordinal scale end arc-sine transformation was done. Perusal of ranking based on physiological traits suggested that the leaf senescencing property of most of the test genotype were almost similar. The best yielders in stress condition were found to bear differential extent of leaf rolling, but a closer extent for stay greenness. These physiological parameters could only be the stress coping and survival mechanisms adopted by plants. Based on mean ranks and standard deviation of rank, eight genotypes were identified as drought tolerant.*

1. INTRODUCTION

Maize (*Zea mays* L., 2n = 2x = 20, with ZZ genome) belongs to tribe maydeae and the grass family, graminae. It is believed that the center of origin is North America. It is monoecious plant with protandrous nature and under natural conditions it is cross pollinated as about 95% of the pistillate flowers on a cob receive pollen from nearby other plants and about 5% of the kernels are as a result of self-pollination. Maize plant produces single leaf at each node borne on the principal stalk in two opposite ranks (distichous). Because it has the C4 photosynthetic pathway, it is more efficient than C3 pathway under high temperature and dryland conditions. Maize grain contains about 10% protein, 4% oil, 70% carbohydrate, 2.3% crude fiber, 10.4% albuminoides and 1.4% ash and sufficient quantities of carotenoids and other vitamins.

Maize otherwise known as corn is one of the most important food crops world-wide. Besides large number of commercial products it is used for diversified purposes like human food (25%), poultry feed (49%), animal feed (12%), industrial (starch) product (12%), beverages and seed (1% each). Among cereal crops, maize has the highest average yield per ha and remains third after wheat and rice in total area and production in the world [10]. Maize grows in most parts of the world over a wide range of environmental conditions, with altitudinal ranges of 0 to 3000 meters above sea level (masl) [9]. Maize has become the dominant food and main source of dietary energy and protein for particularly to rural and underprivileged people of developing countries like India.

According to one of the global food supply-demand model, the demand of maize will increase from 526 mt to 784 mt from 1993 to 2020, particularly in developing countries [34]. However, being an efficient moisture user, it requires 500-800 mm of water during life cycle of 80-110 days [8]. Furthermore, under water scarce conditions the growth and yield of maize decrease due to reduction in photosynthetic capacity [7, 28]. These characteristics of maize make it an excellent model plant to examine the physiological basis of water stress tolerance and to identify some key traits in improving drought tolerance [7, 35].

In countries like India, the major maize growing season is *Kharif*, which accounts for about 85% of the total area in the country. Maize crop grown during the *Kharif* season usually faces uneven erratic distribution of rain and in *Rabi* unavailability of water at proper stage of crop growth. Both the season faces a common problem of water stress. The same situation is found in states like Jharkhand. At the genetic level, moisture stress has been considered to be quantitative traits which influence on maximal plant yield and productivity [20]. Changes in water balance and soil available water are crucial to crop yields by directly affecting plant physiological processes and responses [22, 25].

Breeding for moisture stress tolerance used physiological and morphological traits rather than grain yield alone [2]. It remains difficult to identify traits that effectively mark moisture stress tolerant genotypes. Therefore, understanding physiological response of plants under stress condition may result to improvement and production of drought tolerant varieties of crops [23]. Recent studies have given more attention to physiological characters that indicate water status of plant such as leaf relative water content to assess the response of plants to water stress [42, 18], stomatal characteristics [43] and flowering traits, such as synchronization of the male and female flowering (anthesis-silking interval) under drought conditions. Also, better stay green is frequently emphasized as a key element of tolerance to moisture stress [37, 4]. However, it requires the identification of key traits and their incorporation into high-yielding varieties [7, 35].

Researchers also demonstrated that stress indices calculated from the yield performance at different soil moisture condition were also an important parameter for the selection of stress tolerant genotypes.

Keeping these issues and present day trend of corn improvement schemes in view, the present investigation was conducted to identify the drought tolerant maize genotypes.

2. MATERIALS AND METHODS

The experimental materials consisted of 100 test genotypes along with 3 inbreds as control treatments

Field evaluation

All the test genotypes along with the controls were raised in two different moisture level condition, one as normal field condition and the other as stress condition of tensiometer reading of 50 Kpa after the knee height stage of crop in rainout shelter in an augmented design during the *Kharif* 2014. All the control treatments were replicated for 6 times in 6 blocks. The experimental material was sown in rows with plant to plant distance of 20 cm and row to distance of 60 cm in both the field conditions. Recommended package of practices were followed for fertilizer and crop protection measures with irrigation supply as per the experimental and/or crop need. Different plant characters observed during the trial and estimated stress indices are given below with their descriptions:

Table 1: Morpho - physiological characters observed in drought screening experiment

S.N.	Abb.	Characters	Units	Stage of observation
1	Days to anthesis	**DA**	days	The time of anthesis is when 50% of plants have anthers visible in the middle third of the main branch
2	Days to silking	**DS**	days	Number of days from planting when 50% of the plants in a plot have extruded silks
3	Anthesis-silk interval	**ASI**	days	Derived from anthesis date and silking date as follows ASI=DS-DA
4	Days to 75% dry husk	**75% DDH**	days	Recorded as days from sowing to 75% dry husk of upper ear of >50% of plants in the row
5	Kernel yield per plot	**KY/Plot**	g	Recorded for each plot for each entry
6	Leaf rolling	**LR**	scoring	The leaf rolling recorded for stress environment only as per method suggested by [45] with little modification using 1-10 scale in spite of 1-5 scale previously used, and scores were converted to percentage.
7	Stay green	**SG**	%	Measured as independent visual estimation of the retention of the green-area for leaves at 75% dry husk on a 0 to 10 scale. A rating of 0 indicated complete or nearly complete leaf death, while rating 10 corresponded to a complete green leaf. And scores were converted to percentage as in [39].
8	Leaf death rate (leaf senescence)	**LS**	score	Leaf senescence was scored at one week after 50% tasseling using 1-10 scale (1 = 10% and 10 = 100% dead leaf area), [45].
9	100 Kernel weight	**100Kwt.**	g	Recorded for 100 kernels at 15% moisture.
10	Per day grain yield {per day productivity}	**Pd/GY**	g	Calculated as; Kernel yield per plant (g) / Days between silking to maturity

Table 2: Stress indices estimated for drought screening

1	Modified stress tolerance index	**KiSTI**	KiSTI, K1=Yp2/Ȳ p2 K2=Ys2/Ȳ s2 used by [11].
2	Yield index	**YI**	YI=(Ys)/(Ȳ s) used by [15] and [5].
3	Stress susceptibility index	**SSI**	SSI = ((1-(Ys/Yp))/(1-(Ȳ s/Ȳ p)) used by [14].
4	Relative drought index	**RDI**	RDI =(Ys/Yp)/ (Ȳ s/Ȳ p) [13].
5	Stress tolerance index	**STI**	STI =(Ys ×Yp)/(Ȳ p2) used by [12].
6	Geometric mean productivity	**GMP**	GMP =√Ys×Yp used by [12].
7	Tolerance index	**TOL**	TOL = TOL=Ys-Yp used by [38].
8	Mean productivity	**MP**	MP =(Ys+Yp)/2 used by [21].
9	Drought resistance index	**DRI**	DI=(Ys×(Ys/Yp))/Ȳ [2].
10	Yield stability index	**YSI**	YSI=Ys/Yp used by [5].
11	Stress susceptibility percentage	**SSPI**	SSPI=(Yp-Ys/2(Ȳ p))×100 used by [31].
In the above formulae, Ys= yield under stress for each genotype, Yp= yield under non stress for each genotype, ȳs= yield means in stress condition, ȳp = yield means in non-stress conditions for all genotypes.			

Statistical analysis

The genotypes were first analyzed through augmented block design for the comparative study of among the test genotypes and between the test genotypes versus control entries for the screening of lines in different level of soil moisture. Correlation analysis was performed between yield indices and grain yield in two environments.

Analysis of variance (ANOVA) for augmented block design

Analysis of variance was performed using online software from IASRI, New Delhi’s website to detect if significant differences exist among the control genotypes, between test genotypes and test-versus-control genotypes for the yield in both environments. The repeated controls were used to estimate the error mean square and the block effect. The block effect was estimated from the repeated control means and then removed from the means of the test varieties. This reduces error and increases precision somewhat for estimation of adjusted means.

Block adjustment was computed as;

$$a_i = \bar{X}_i - \bar{\bar{X}}$$

Observation adjustment for test entries was computed as;

$$\hat{Y}_{ij} = Y_{ij} - a_i$$

Where, $\mathbf{a_i}$: block adjustment; $\bar{X}_i$: mean of replicated treatments in block ‘i’; $\bar{\bar{X}}$: overall mean of the replicated treatments; $\hat{Y}_{ij}$: adjusted observed value; Y_{ij} : observed value.

Two different ANOVA analyses for estimation of adjusted means of treatments and block effect were performed as:

Table 3: ANOVA for treatment adjusted

Source of variation	**df**	**SS**	**MSS**	**f-value**	**f-prob.**
Block (unadjusted)	**b-1**	**SSB**			
Treatments (adjusted)	**t-1**	**SST**	**M_SST=SST/(t-1)**	**M_SST/M_SSE**	
Error	**(b-1)(c-1)**	**SSE**	**M_SSE=SSE/(b-1)(c-1)**		
Total	**n-1**				

Where, b: no. of blocks; t: no. of genotypes (controls + tests) per block; c: control treatments; n: total no. of plots in all blocks.

Table 4: ANOVA for block adjusted

Source of variation	**df**	**SS**	**MSS**	**f-value**	**f-prob.**
Block (adjusted)	**b-1**	**SSB**	**M_SSB=SSB/(t-1)**	**M_SSB/M_SSE**	
Treatments (unadjusted)	**t-1**	**SST**			

The contrast analyses among controls, among tests and tests-versus-control were done as per the following ANOVA:

Table 5: ANOVA for contrast analysis

Source of variation	df	SS	MSS	f-value	f-prob.
Among controls	**c-1**	**ASSC**	**S_1= ASSC/(c-1)**	**S_1/ M_SSE**	
Among tests	**v-1**	**ASST**	**S_2= ASST/(v-1)**	**S_2/ M_SSE**	
Test versus control	**1**	**SSTC**	**S_3**	**S_3/ M_SSE**	

Different test treatments and control treatments were compared for their significance using respective critical difference values (CD).

Standard errors

Difference between two control varieties; $S_c = \sqrt{2MSE/r}$

Difference between adjusted means of two selections in the same block;

$$S_d = \sqrt{2MSE}$$

Difference between adjusted means of two selections in different blocks;

$$Sv = \sqrt{2(c+1)MSE)/c}$$

Difference between adjusted selection and control mean; $S_{vc} = \sqrt{((r+1)(c+1)MSE)/rc}$

C.D (at 5%) = S.Ed* t (5%) at error df.

Correlation analysis

Pearson's correlation coefficients were calculated between all the stress indices and adjusted grain yield in stress and non-stress conditions by substituting corresponding variance and covariance in the formulae given by [19].

3. RANKING OF GENOTYPES

All the test genotypes/entries were ranked for adjusted mean values of morpho-physiological traits and estimated stress indices for their good performance to poor performance, viz. 1 for best performer and towards 100 for poorer performance. Average ranks were assigned to those entries having same level of observations. Averages of ranks were taken from morphological yield traits (viz. KY/Plot, 100 Kwt. and Pd/GY) from both the environments and stress indices, and Spearman's rank correlation was estimated for the significance of the agreement between the ranks.

$$\text{Rank Correlation coefficient } (r_s) = \frac{SS(X) + SS(Y) - \sum d^2}{2*(\sqrt{SS(X)*SS(Y)})}$$

Where, $SS(X): \left\{\frac{n^3 - n}{12}\right\} - \left\{\sum \frac{t^3 - t}{12}\right\}$;

$SS(Y): \left\{\frac{n^3 - n}{12}\right\} - \left\{\sum \frac{t^3 - t}{12}\right\}$

't' is the no. of ties in ranks in 'X' variable

't' is the no. of ties in ranks in 'Y' variable
'n' is the no. of paired observations.

Such calculated 'r_s' value was tested for its significance using following method for large sample:

$$F(r_s) = \frac{1}{2} \ln \left\{ (1+r_s)/(1-r_s) \right\}$$

$$Z = \sqrt{(n-3)/1.06} * F(r_s)$$

The calculated Z value was then compared with the tabulated Z-score at 5% level of significance.

4. RESULTS AND DISCUSSION

Analysis of variance (ANOVA) and mean performance

The observations taken on seven morpho-physiological traits in non-stress and ten in stress environment were subjected to ANOVA; the mean sum of square (MSS) for each trait in both environment revealed that the trait days to anthesis (DA) was significant among the test entries in non-stress condition, whereas, they were non-significant for stress environment. Contrary to this test entries were significant for anthesis silk interval (ASI) in stress environment but not in non- stress environment The test entries were found insignificant for their performance for the traits; days to silking (DS) and 75% dry husk (DDH) in both the crop environment. Significant differences were observed among the test entries for the rest of all traits, viz. kernel yield per plot (KY/Plot), 100 kernels weight (100 KWt.) and per day grain yield (GY/day) in both the environments. The entries were also found significantly differing for the physiological traits viz. leaf senescence (LS), leaf rolling (LR) and stay green (SG) which were observed in stress environment only (table - 6 and table - 7)

It indicated that genetic variation existed among test genotypes and there lies scope in identification of drought tolerant lines. These findings of significant differences for the yield in both the conditions are in consistence with those reported by [33] in QPM maize and [1] in the evaluation of wheat genotypes.

Table 6: ANOVA (MSS) for all 7 traits in non-stress environment

Trait	Source of Variation	df	DA	DS	ASI	DDH	KY/Plot	100Kwt.	GY/day
Treatment Adjusted	Block	5							
	Treatments	102	22.43**	29.22 ns	6.72 ns	76.42 ns	26222.15**	5.86*	1.09**
	Error	10	6.32	66.53	51.05	48.22	582.13	1.75	0.11
Block Adjusted	Block	5	7.42 ns	55.57 ns	63.26 ns	99.92 ns	192.6 ns	1.09 ns	0.25 ns
	Treatments	102							
Contrast Analysis	Among-Controls	2	247.72**	200.67 ns	40.05 ns	36.22 ns	13701.9**	1.89 ns	1.06**
	Among-Tests	99	17.88*	25.86 ns	6.12 ns	72.05 ns	25781.14**	5.91**	1.09**
	Test-vs-Control	1	22.92 ns	18.33 ns	0.26 ns	626.43**	95691.27**	8.41*	0.45*

Table 7: ANOVA (MSS) for all 10 traits in stress environment

Trait	Source of Variation	df	DA	DS	ASI	DDH	KY/Plot	100Kwt.	GY/day	LS	LR	SG
Treatment Adjusted	Block	5										
	Treatments	102	24.4 ns	38.2 ns	7.54*	27.37 ns	21985.2**	9.57*	0.59**	31.71**	134.73*	307.42*
	Error	10	33.08	38.7	2.99	21.59	1282.6	3.5	0.12	0.0001	50.71	105.99
Block Adjusted	Block	5	19.65 ns	7.2 ns	8.19 ns	51.12 ns	345.06 ns	1.99 ns	0.10 ns	ns	128.22 ns	98.14 ns
	Treatments	102										
Contrast Analysis	Among-Controls	2	64.89 ns	199.5*	38.39**	38.39 ns	2893.52 ns	20.14*	0.01 ns	ns	30.89 ns	1062.19**

	Among-Tests	99	23.72 ns	35.32 ns	6.94 ns	26.85 ns	21929.72**	9.35*	0.60**	0.00001**	136.34*	294*
	Test-vs-Control	1	9.77 ns	0.35 ns	6.4 ns	51.3 ns	65862.65**	10.34*	0.92*	78.33**	205.66 ns	131.83 ns

The reduction in performance of genotypes for KY/plot, 100 KWT and GY/day traits was due to unavailability of optimum moisture during flowering [16], cob development and grain filling [6] when evapo-transpiration request exceeds water supply from the soil [26]. The reduction in yield may also be due to reduction in seed size [34] and number of kernels per ear [3]. The reduced weight of grain may be due to shriveled grain *i.e*., plant unable to maintain an optimal water status under stress condition [44].

Correlation analysis

The estimate indicators of drought tolerance indicated that the identification of drought tolerant lines based on a single criterion may be contradictory. To determine the most desirable drought tolerant criteria, the correlation coefficients between Yp, Ys, and other quantitative indices of drought tolerance were calculated. A suitable index must have a significant correlation with grain yield in both the conditions [29], because it will be able to separate and identify genotypes with high grain yield in both conditions. The significant and positive correlation of yield in both conditions with KiSTI (K1STI & K2STI), YI, STI, GMP, MP and DI indicated that these indices were more effective in identifying drought tolerant genotypes. The observed relations were consistent with those reported [32] in landrace wheat and [19] in durum wheat. However, significant and negative correlation recorded between SSI and yield in stress condition was in accordance with the result of [32]. [41] and [24] also have suggested MP, GMP and STI as suitable indicators for drought screening. The significantly negative correlation for stress susceptibility index (SSI) under drought conditions shows that plant environment has a decisive factor in yield; confirmed by [30].

Performance ranking

The genotypes were identified as superior rankers based on their average rankings, estimated from individual rankings, with consideration of low standard deviation**.**

Yield related traits in both field conditions were first ranked individually and averages were taken foreach test entries. The ranks assigned to each were found to be almost similar for their performance**.** Such similarity in ranking indicated the stability of genotypes for their yield potential, although their performances in stress condition were lower than normal condition. The average ranks assigned to genotypes based on stress indices were also in accordance to the ranking based on yield related traits, which was confirmed by the significant rank correlation coefficient estimated from average ranks of above mentioned criteria.The results for this method can be supported by the results obtained by [32] and [24]. Perusal of ranking based on physiological traits suggested that the leaf senescencing property of most of the test genotypes were almost similar. The best yielders in stress condition were found to bear differential extent of leaf rolling, but a closer extent for stay greenness. These physiological parameters could only be the stress coping and survival mechanisms adopted by plants. [3] have indicated that the leaf rolling could poorly explain the drought tolerance. Associations between foliar stay-green and yield are often weak [3] and reasons for this must be sought in the nitrogen balance of the crop at that growth stage. However, plants that stay green retains green leaves for a longer period of time and produce grain normally [40], for sorghum; [27].

Based on mean ranks and standard deviation of rank, the test genotypes- 95, 62, 39, 30, 18, 89, 20 and 103 could be identified as the drought tolerant (**table-8).**

Table 8: Best drought tolerant genotypes selected

Best 10 from yield performance ranking			**Best 10 from average ranking**	**Best 10 from stress indices average ranking**	**Common in both systems of average ranking (Final selection)**	**Group belonging of identified drought tolerant genotypes for physiological traits (values in parentheses denote the group)**		
Non-stress environment	**Stress environment**	**Common in both condition**				**Leaf senescence**	**Leaf rolling**	**Stay green**
95, 57, 89, 18, 62, 39, 20, 30, 8, 66.	95, 18, 62, 20, 39, 89, 8, 30, 103, 66.	95,18, 62,39, 20, 89, 30, 8, 66.	62, 39, 30, 95, 18, 89, 50, 20, 45, 103, 66.	95, 89, 62, 18, 39, 20, 30, 66, 8, 103, 90, 13.	95, 62, 39, 30, 18, 89, 20, 103, 66.	95, 62, 39, 30, 18, 89, 20, 103, 66- (1).	95, 89- (11), 62 (18), 39 (4), 30, 20- (10), 18, 66 (12), 103 (5).	95,62- (11), 39 (13), 30 (20),18(18,20(16),03, 89-(19),66 (10)

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