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Evaluation of stay green induced maize hybrids for green fodder and grain yields under variable moisture regimes

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Abstract

About 80% kharif area of maize cultivation in India is under rainfed condition where uncertainty of rainfall is a ubiquitous phenomenon which limiting its productivity. Primary and secondary stress responsive traits are mainly on quantitative loci, which make the direct selection of traits difficult. The present experiment aimed to identify the maize hybrids suitable for dual purpose under low moisture stress condition, identification of secondary traits associated with fodder and grain yield and calculation of correlation which could be useful for effective selection for fodder and grain yield. Using 37 maize genotypes, evaluated under 3 moisture regimes for green fodder and grain yield. Association analysis was done among the secondary traits and yield. Among the genotypes significant variability was showed for all characters. The hybrids BAUIM-2 x HKI-1532 and BAUIM-3 x HKI-1532 found suitable for dual purpose exhibited maximum GFY/P under irrigated, rainfed and stress (-50kPa) conditions while, hybrid BAUIM-5 x HKI-1532 found suitable for fodder yield. Hybrids BAUIM-4 x HKI-335 and BQPM-4 x HKI-1532 suitable for stress conditions. The traits stomatal frequency, stay green, tassel blast and green fodder yield per plant showed normal probability distribution; whereas, relative leaf water content, anthesis silk interval, leaf area index, leaf senescence, leaf firing, plant bareness, leaf rolling and grain yield per plant non-normal distribution. Bartlett test for homogeneity of variance was non-significant for LA-3 and LA-cob. The correlation coefficient indicated that, traits GY/P with SG (0.46); TB (-0.39); LR (-0.40); LAI (0.57), BP (-0.57). GFY/P with SG (0.48); TB (-0.39); LR (-0.41), BP (0.48) showed significant association among each other's.

Keywords: Green fodder, Hybrids, Maize, Physiological parameters, Secondary traits, *Zea mays*

Abbreviations: ASI: Anthesis and silk interval; **BP:** Barrenness percentage; **GY/P:** Grain yield per plant; **GFY/P:** Green fodder yield per plant; **IK:** Irrigated; **R:** Rainfed; **S-50:** Managed stress; **LA-3:** Leaf angle of 3rd leaf from top of the plant; **LA-cob:** Leaf angle cob leaf; **LAI:** Leaf area index; **LF:** Leaf firing; **LR:** Leaf rolling; **LS:** Leaf senescence; **RLWC:** Relative leaf water content, **SG:** Stay green; **SL:** Stomata lower surface; **SU:** Stomata upper surface; **TB:** Tassel blast; **E. Index:** Environmental index

Introduction

Maize (*Zea mays* L.) is a major cereal crop worldwide after wheat and rice, serving as staple food for both human consumption, animal forage and feed (Rani *et al*., 2015; Pandit *et al*., 2016; Chaudhary *et al*., 2016). Rain-fed cropping systems provide over 40% of the world's temperate maize production. Uncertainty of rainfall creating low moisture stress is a ubiquitous in random stress cropping systems and often limits maize yields. Low moisture stress causes osmotic stress and inversely influences plant performance. Under comparable water reduction (approximately 40%), maize experienced approximately 39% yield reduction (Daryanto *et al.,* 2016). The quantity of forage and grain yield loss depends on plant growth stage as well as the duration and the severity of the stress. Drought reduces plant growth and reproductive behaviour of plant by influencing the delayed silk extrusion leading to high anthesis silk interval (ASI) (Sah *et al*., 2015); leaf senescence and area, induced barrenness, reductions in kernel number due to poor pollination, early kernel abortion and yield (Edmeades *et al*., 2000; Araus *et al*., 2002; Messmer, 2006). These morphological and physiological parameters ultimately contribute to grain and forage yield.

Hybrids of maize are considered as more stable in performance, high yielding, more uniform in maturity and resistant stresses (Sah *et al*., 2014). For forage and grain yield improvement in maize under soil moisture deficit conditions we need to identify stress tolerant maize genotypes with associated physiological traits/ secondary traits. Phenotyping for critical traits like short ASI, crop growth duration and leaf area, reduced barrenness and epinasty or leaf rolling, stay green etc. are among the obvious secondary traits. Conventional breeding showed that primary and secondary stress-tolerance traits are mainly quantitative loci which make the selection of traits difficult. The higher green forage yielding genotypes along with stay green traits may confer the stress tolerant mechanism due to higher carbohydrates accumulation.

Secondary traits help to overcome the low heritability of yield under low moisture stress condition, which is due to the small genetic variance and the occurrence of poorly understood genotype-by-environment interactions (G x E). To determine best selection method, mean values, components of variance and heritability of the traits is important. Besides that, also is very important to confirm relationship between traits. The information regarding the secondary traits and association behaviour under multiple environmental conditions for all the characters is scanty. Keeping in view this experiment was conducted to identify the hybrid(s) of maize suitable for dual purpose under stress condition and association study among forage yield, grain yield and important secondary traits.

Materials and Methods

Plant materials and experimental designing: Thirty-seven maize genotypes, 24 hybrids, 11 parents and 2 checks *i.e*., Bio-9637 and HQPM-1 were evaluated in sandy loam soil of humid sub-tropical climate at the research farm of the Birsa Agricultural University, Ranchi, India (23.35°N, 85.33°E; 651 m), during July to November, 2013. The hybrids were developed by using drought tolerant parents (HKI-1532, HKI-335 and HKI-488) which transmitted various degree of stay green in hybrids. The experiment was conducted in randomized block design under three sets of environmental conditions. Set-I (IK): irrigated condition under open field (irrigation at -30 kPa soil moisture potential); Set-II (R): random stress condition (exposed to natural rainfall during the cropping period) and Set-III (S-50): managed stress, under rainout shelter (irrigation at -50 kPa soil moisture potential). The stress level was measured with tensiometer installed at root zone depth as described by Bänziger *et al*. (2000).

Observation procedure: Observations were recorded on ten randomly selected plants averaged for GFY/P, GY/ P. Thirteen secondary traits were measured as per the method suggested for RLWC: by Pask *et al*. (2012); ASI and LAI by Amanullah *et al*. (2007); SU; SL; SG: by Jiang *et al*. (2004) with a slight modification on 1 to 10 scale and converted to percentage. A rating of 1 indicated complete or nearly complete leaf death, while rating 10 corresponded to a complete green leaf; LS: scored at one week after 50% male flowering using 1-10 scale (1 = 10% and 10 = 100% dead leaf area) as suggested by Zaidi *et al*. (2008); LF: measured with modification of method suggested by Bänziger *et al*. (2000). The reading was taken on 1 to 9 scales (Kaur *et al*., 2010); LA-3; LA-cob; TB: Tassels dried due to stress in plants. The reading was done with some modification of method suggested by Bänziger *et al*. (2000). 1-9 scale was used i.e., 1 is healthy tassel and 9 is tassel dried completely; LR: measured in 1 to 5 scale; BP: the number of plants with no cob or if cob is present but with no seed set or very few seeds then it were considered as barren plant.

Statistical analysis: Analysis of variance, simple linear correlation was computed for all environments separately for all the 15 traits using SPSS 17.0 software. For testing the equality of variances under different environments Bartlett's and Leven's tests were used. The change in the environmental condition(s) association leads to change in the degree and direction. So for these situations, it is difficult to choose the exact correlation value and direction as well. A single and actual value of 'r' which should be used as selection criteria of significantly associated variables under changing environments were computed by following Gomez and Gomez (1983).

Results and Discussion

Interaction and mean performance: The variance due to G, E and G x E (interaction) were significant for all the traits (Table 1). Mean value of traits showed differential response of genotypes for different traits under different environments. Similar results also reported by fodder traits by Sah *et al*. (2016). It was found that traits *i.e*. RLWC, ASI, LAI, LS, LF, TB, BP, LR and GY/P showed significant test indicating violation from normal distribution (Table 1). Bartlett's and Levenes's test indicated that the traits showing significant test were heterogeneous in the variance. The traits LA-3 and LA-cob showed non-significant variance hence, their variance was equal over all set of experiments. The performance of maize entries under the irrigated condition is higher in comparison to rainfed and stress trial indicating that plant performance decreases if moisture stress increases. Similar findings were also observed by Alfi and Azizi (2015). The RLWC, LAI, SG, GY/P and GFY/P were decreased while increasing the stress, whereas the ASI, LS, LF, TB, LR and BP increased under stress (Table 2-4). The hybrids BAUIM-2 x HKI-1532 and BAUIM-3 x HKI-1532 found suitable for dual purpose exhibited maximum GFY/P under IK, R and S-50 conditions while, hybrid BAUIM-5 x HKI-1532 found suitable for fodder yield. Hybrids BAUIM-4 x HKI-335 and BQPM-4 x HKI-1532 were good for average response under stress conditions.

Association study: The magnitude of correlation of traits will depend on expression genes, which contributes to variation (Chakraborty and Sah, 2012). The positive significant correlation coefficient was observed for LAI with SG and GY/P under the irrigated condition and SU with SL, SG with LR, LAI with GY/P and GFY/P under random stress condition (individual environment correlation was not presented). Similar results have been reported in case of SG and grain yield by Golbashy *et al*. (2010) and Shoa Hoseini *et al*. (2007); LAI and grain yield by Wannows *et al*. (2010). Flowering time is an essential character to determine response of the plants toward the environmental changes. The differences in appearance of male and female flowers (ASI) also indicated the plant performance stability under different environments. These differences were generally negative with grain yield since it reduced the pollen availability during stigma receptivity and seed setting (Khayatnezhad *et al*., 2010). Under the managed stress situation ASI with LR, LF with LR, LR with BP showed positive significant correlation with each other. In all the three environments, the positive association were also observed for LA-3 and LA-cob and GY/P and GFY/P. The traits LAI with TB under random stress condition and the traits SG with LR, ASI with GY/P and GFY/P, which was also reported by Mohan *et al*. (2000). LR with GFY/P was predominant under stress condition showed significant negative association among them. Similarly, GFY/P with BP has negative association in all the three environments. The traits significantly correlated in irrigated condition have shown non-significant correlation under stress condition and *vice versa*. This indicates that the change in the environment is also changing the degree of expressivity of the traits. However, the traits SG with LR, showed both types (direction) *i.e*., positive under random stress situation and negative under managed stress situation, it may be due to differential expression of a gene under different environment or expression of different genes under different environment for single traits. Under such variation of associated traits, the correlation value (r) calculated by using Gomez and Gomez, (1983) may give better indication for selection of traits.

Table 1. ANOVA and normal probability distribution test for GFY/P, GY/P and different morpho-physiological parameters

SV	df	RLWC	ASI	LAI	SU	SL	SG	LS
Env. (E)	2	1558.48**	1812.08**	431007871**	3936.81**	312.80**	6345.51**	158.83**
Genotypes (G)	36	107.33**	17.21**	11022351**	1685.84**	1909.99**	3905.48**	25.77**
E * G	72	110.87**	15.07**	2795364**	734.25**	939.67**	137.02**	18.05**
P. Error	216	1.13	0.36	399811	15.34	11.05	12.48	0.73
Kolmogorov-Smirnov[a]		0.00	0.01	0.17	0.20[p]	0.20[p]	0.20[p]	0.01
Shapiro-Wilk		0.00	0.00	0.01	0.84	0.06	0.27	0.00
Bartlett's		17.453**	35.832**	87.12**	63.966**	17.026**	10.489**	69.103**
Levene's		79.476**	67.344**	43.933**	1.393ns	2.186ns	4.587*	7.107**

SV	LF	LA3	LA-cob	TB	LR	BP	GY/P	GFY/P
Env. (E)	148.35**	195.49**	190.97**	117.23**	0.75**	15231**	95673**	159.26**
Genotypes (G)	2.45**	1250.16**	711.63**	7.01**	11.25**	710.16**	1828.18**	2061.44**
E * G	2.20**	1.30**	2.70**	1.89**	3.73**	363.99**	436.06**	0.49*
P. Error	0.54	1.10	2.10	0.73	0.15	31.17	10.15	
Kolmogorov-Smirnov[a]	0.00	0.20[p]	0.20[p]	0.01	0.01	0.01	0.01	0.20[p]
Shapiro-Wilk	0.00	0.35	0.88	0.00	0.00	0.01	0.01	0.35
Bartlett's	56.641**	0.24ns	0.088ns	-59.041ns	17.36**	19.72**	167.81**	42.67**
Levene's	37.782**	0.214ns	0.137ns	121.869**	32.48**	50.651**	116.634**	24.11**

*$p \leq 0.05$, **$p \leq 0.01$, I-irrigated, R-rainfed, S-stress; [a]. Lilliefors Significance Correction, [p].This is a lower bound of the true significance.

Table 2. Mean performance of selected entries of maize for morpho-physiological traits under irrigated, rainfed and managed stress conditions

Genotypes	RLWC			ASI			LAI			SU			SL		
	IK	R	S-50	IK	R	S-50	IK	R	S-50	IK	R	S-50	IK	R	S-50
BAUIM-2 x HKI-1532	94.34	89.11	77.85	4.50	4.00	4.00	3.76	3.47	0.69	88.33	91.67	90.67	131.00	118.00	117.00
BAUIM-3 x HKI-1532	93.95	89.86	81.89	4.00	4.00	4.00	3.43	2.73	1.20	84.67	115.67	114.67	134.00	153.00	141.67
BAUIM-4 x HKI-335	92.40	91.29	78.66	3.50	3.00	4.00	2.81	3.01	0.78	88.67	82.00	67.33	125.67	124.00	161.67
BQPM-4 x HKI-1532	94.31	87.35	78.47	4.00	4.00	4.00	2.36	2.72	0.98	100.00	94.00	91.33	194.00	124.00	117.00
BAUIM-5 x HKI-1532	93.37	89.33	78.99	4.00	4.00	5.00	2.48	2.96	0.89	67.33	77.33	93.00	138.00	128.00	145.33
Bio-9637-Check-1	94.37	92.85	80.50	4.00	5.50	12.00	2.52	2.38	0.65	97.33	91.33	90.33	119.67	113.67	112.67
HQPM-1-Check-2	94.00	91.50	86.64	4.00	6.50	8.00	1.27	2.03	1.06	101.33	95.33	92.67	141.00	135.00	140.00
Mean Hybrids	93.80	91.61	81.04	4.13	4.17	5.48	2.71	2.61	0.74	97.35	98.46	88.61	141.10	138.61	138.39
E. Index	9.64	7.41	-3.07	-2.23	-2.07	0.52	0.70	0.62	-0.93	2.43	1.83	-8.17	5.51	4.58	3.18
C.D. 5%	1.72	1.33	2.16	1.17	1.09	3.66	0.59	0.55	0.17	4.93	3.62	5.00	4.44	4.78	6.80
C.V.	1.13	0.89	1.64	16.56	14.90	17.97	15.59	15.23	14.87	2.97	2.19	3.36	1.91	2.07	2.97

Table 3. Mean performance of selected entries of maize for morpho-physiological traits under irrigated, rainfed and managed stress conditions

Genotypes\ En.	SG			LS			LF			LA-3			LA-cob		
	IK	R	S-50	IK	R	S-50	IK	R	S-50	IK	R	S-50	IK	R	S-50
BAUIM-2 x HKI-1532	92.50	77.50	75.00	2.00	2.00	3.67	0.33	0.00	2.64	26.47	26.00	24.53	31.92	31.42	29.65
BAUIM-3 x HKI-1532	79.00	60.00	42.50	2.00	2.00	5.00	0.67	0.00	3.95	52.32	51.75	48.83	32.12	31.62	29.83
BAUIM-4 x HKI-335	42.50	45.00	42.50	2.00	2.50	4.67	0.89	0.90	1.23	26.42	25.95	24.49	35.92	35.41	33.42
BQPM-4 x HKI-1532	74.00	57.50	37.50	3.00	3.00	5.00	0.41	1.00	1.16	34.82	34.30	32.36	38.67	38.14	35.98
BAUIM-5 x HKI-1532	37.50	42.00	37.50	2.00	2.00	5.67	1.00	0.42	2.21	47.32	46.76	44.11	35.47	34.96	32.98
Bio-9637-Check-1	21.00	18.00	15.04	2.33	3.00	4.17	0.56	1.25	2.60	42.13	41.60	39.26	27.91	27.43	25.88
HQPM-1-Check-2	26.33	23.67	23.76	2.00	3.00	3.83	1.00	1.25	3.25	39.08	38.56	36.39	46.91	46.34	43.71
Mean Hybrids	46.19	38.09	28.08	2.42	2.96	5.01	0.59	0.80	2.74	39.25	38.72	36.54	36.56	36.04	34.01
E. Index	3.67	-3.33	-11.44	-1.67	-1.12	0.76	-0.87	-0.56	1.49	5.99	5.47	3.47	4.99	4.49	2.51
C.D. 5%	5.70	4.59	6.76	0.80	0.98	1.99	0.75	0.69	0.75	1.90	1.87	1.80	2.68	2.72	2.63
C.V.	9.17	9.04	17.98	19.14	19.10	19.18	17.08	17.01	15.59	3.25	3.25	3.30	4.64	4.77	4.88

Table 4. Mean performance of selected entries of maize for morpho-physiological traits under irrigated, rainfed and managed stress conditions

Genotypes\ En.	TB			LR			BP			GY/P			GFY/P		
	IK	R	S-50	IK	R	S-50	IK	R	S-50	IK	R	S-50	IK	R	S-50
BAUIM-2 x HKI-1532	1.00	1.00	1.00	1.50	1.92	5.00	0.59	1.17	11.07	131.61	98.19	42.44	568.14	493.52	266.27
BAUIM-3 x HKI-1532	1.00	1.33	1.67	0.50	1.54	5.00	0.63	0.00	10.74	122.20	76.69	44.94	408.65	400.64	169.24
BAUIM-4 x HKI-335	1.00	1.67	6.33	0.00	0.77	5.00	1.00	4.50	16.34	103.07	84.83	35.28	265.20	267.09	140.99
BQPM-4 x HKI-1532	1.00	1.00	1.67	1.25	2.12	4.00	0.85	4.92	10.00	89.13	65.50	43.22	315.91	247.47	169.36
BAUIM-5 x HKI-1532	1.00	2.33	5.00	0.50	1.15	5.00	0.00	5.01	17.29	125.20	76.80	34.19	374.58	354.49	181.04
Bio-9637-Check-1	1.00	1.67	4.00	1.06	2.36	8.00	1.15	3.58	16.03	124.60	69.53	34.09	282.57	274.86	76.33
HQPM-1-Check-2	1.00	1.00	3.33	1.20	2.46	8.00	0.33	3.42	13.52	123.54	62.87	40.78	319.01	273.06	126.06
Mean Hybrids	1.00	1.54	2.60	0.95	2.03	5.33	0.79	2.99	14.16	104.33	67.84	37.06	641.53	624.07	216.39
E. Index	-1.85	-1.07	0.19	-2.20	-1.12	2.74	-10.65	-8.01	3.23	38.16	3.99	-20.28	5.47	2.97	-1.81
C.D. 5%	0.12	1.20	2.10	0.97	0.80	1.28	1.36	3.23	5.26	6.31	6.30	1.09	27.64	30.41	21.77
C.V.	4.39	19.10	20.00	19.91	18.60	13.00	19.15	16.21	19.87	4.13	6.49	1.89	13.58	16.39	11.65

Table 5. Correlation coefficient over environments (r_p) using weighted z mean & co-heritability (BS) between 15 traits

Traits	RLWC	ASI	LAI	SU	SL	SG	LS	LF	LA-3	LA-cob	TB	LR	BP	GY/P	GFY/P
RLWC	0.86	0.05	-0.12	0.10	0.05	-0.08	0.12	0.15	0.19	0.09	0.08	0.08	0.04	-0.11	-0.03
ASI	1.01	0.98	-0.12	0.02	-0.01	-0.33	0.10	0.01	-0.21	-0.13	0.13	0.28	0.28	-0.37	-0.31
LAI	0.98	1.00	0.96	-0.25	-0.32	0.57*	0.18	0.13	0.23	-0.05	-0.41	-0.26	-0.37	0.57*	0.45
SU	0.98	1.05	1.05	0.79	0.41	-0.18	-0.02	-0.14	0.02	0.07	0.21	0.02	0.18	-0.25	-0.23
SL	1.06	1.02	0.75	0.89	0.75	-0.19	0.15	-0.08	-0.09	-0.02	0.21	0.05	0.12	-0.32	-0.24
SG	0.95	0.99	0.95	1.74	0.81	0.85	0.05	-0.11	0.08	-0.03	-0.38	-0.37	-0.38	0.46	0.46
LS	0.99	0.99	1.04	0.93	0.93	1.08	0.83	0.03	-0.11	-0.17	-0.05	-0.20	-0.04	-0.01	-0.02
LF	1.02	1.00	1.00	1.00	1.02	1.01	0.97	0.93	0.14	0.04	-0.04	0.32	0.04	-0.05	-0.06
LA-3	0.84	0.96	0.99	1.23	1.05	1.05	0.86	0.69	0.86	0.60*	-0.04	0.03	-0.16	0.33	0.31
LA-cob	0.61	0.98	1.08	0.26	1.12	1.12	0.91	1.05	0.86	0.73	0.06	0.11	-0.05	0.11	0.11
TB	0.97	1.00	1.00	1.09	0.93	0.97	1.00	1.00	1.06	1.10	0.92	0.18	0.37	-0.39	-0.37
LR	0.99	1.00	1.00	1.00	1.08	0.99	1.00	0.99	1.03	1.08	1.00	0.99	0.29	-0.40	-0.40
BP	1.01	0.99	1.01	0.89	0.41	0.98	1.00	0.98	1.01	0.97	0.99	0.99	0.92	-0.57*	-0.60*
GY/P	1.00	1.00	0.99	1.06	0.89	1.00	0.97	1.00	0.97	0.98	1.00	1.00	1.00	0.99	0.91*
GFY/P	0.98	1.00	1.00	0.94	0.94	1.00	0.94	1.00	0.97	0.96	0.99	1.00	0.98	0.99	0.95

Above diagonal: simple correlation coefficient over environment from weighted Z value (Z_w); Diagonal value: heritability (broad sense); Below diagonal: co-heritability (BS) among the 15 traits, (heritability: e™0.6- high heritability, 0.3-0.6 medium heritability and d™0.3- low heritability)

Analysis of weighted Z value and Chi-square (Gomez and Gomez, 1983): According to calculated Z value, none of the traits showed significant value for chi-square test which indicated that the hypothesis of homogeneity cannot be rejected. Therefore, they were further analysed for pooled correlation coefficient over environments (Table 5). The high 'r' value was observed for correlation of LAI with SG (r=0.57) and GY/P (r=0.57); LA-3 with LA cob (r=0.60); BP with GY/P (r=-0.57) and GFY/P (r=-0.62) while low and negative 'r' value was observed for relation of ASI with SG (r=-0.33), GY/P (-0.37) and GFY/P (r=-0.33). The trait LAI with TB (r=-0.41), BP (r=-0.37) and GFY/P(r=0.48); SU with SL (0.41); SG with TB (r = -0.38), LR (r = -0.37), BP (r = -0.37), GY/P (r = 0.46) and GFY/P (r = 0.48); TB with BP (r = 0.37), GY/P (r = -0.39) and GFY/P (r = -0.39); and LR with GY/P (-0.40) and GFY/P (r = -0.41) were found to be associated with each other's. The correlation value (r) may be at least as mentioned above between traits for selecting the correlated traits. Such correlations would be useful for the effective indirect selection of traits under different soil moisture condition.

Conclusion

The pooled correlation method as suggested by Gomez and Gomez (1983) can be used for pooled association study to draw a representative value of 'r'. The association between fodder and grain yield were highly significant. The secondary traits LAI, BP, SG and GFY/p had desirable association with grain yield whereas, LAI, SG and GY/p had desirable association with fodder yield under variable moisture regimes in maize. The expression of the traits was affected by the different level of moisture stress. Under irrigated condition the performance of the lines was higher in comparison to rainfed and stress trial. The RLWC, LAI, SG, GY/P and GFY/P decreased under stress whereas, ASI, LS, LF, TB, LR and BP increased under stress. The hybrids BAUIM-2 x HKI-1532 and BAUIM-3 x HKI-1532 was good for dual purpose because of high grain and fodder yield, however, BAUIM-5 x HKI-1532 can be used especially for fodder production due to high green biomass and BAUIM-4 x HKI-335 and BQPM-4 x HKI-1532 were good for average performance under stress conditions.

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