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ORIGINAL ARTICLE

Evaluation of Heat Stress Tolerance Indices in Maize Inbred Lines

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Abstract

The heat stress during flowering, pollination and grain filling stages affect the productivity of maize. Twenty maize inbred lines were evaluated in field and plastic houses using alpha lattice design with two replications during spring season of 2016 at National Maize Research Program, Rampur, Chitwan, Nepal. Five stress tolerance indices namely stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), geometric mean productivity (GMP) and mean productivity (MP) were applied to identify superior heat stress tolerant lines. The results showed that STI, SSI, GMP and MP indices were the more accurate criteria for selection of heat tolerant and high yielding lines. The positive and significant correlation of GMP and MP with grain yield under both conditions revealed that these two indices were more applicable and efficient for selection of inbred lines. The biplot analysis identified the groups of tolerant and sensitive inbred lines. The maize inbred lines namely RL-140 and RML-91 found high yielding and low stress susceptibility in both conditions. These results suggest that the inbred lines namely RL-140 and RML-91 should be use a source of heat tolerance for maize breeding program.

Keywords: Biplot, Correlation, Heat stress, Maize and Tolerance index.

Introduction

Maize is a very high yielding potential than any other cereals and thus popularly known as the 'queen of cereals' (Dhaka *et al.,* 2010). Maize is a source of food and income for small farmer in developing countries. Beside direct consumption as food, it is also an important source of industrials raw materials example the manufacture a starch, dextrose, oil sugar, syrup, enzymes, adhesive paper and plastic (REF). Heat stress reduces maize grain yield and any further rise in temperature reduces the pollen viability and silk receptivity and longer anthesis silking interval resulting in reduction up to 70% (Khodarahmpour *et al.,* 2011).

High temperature at time of fertilization reduces pollen viability resulting in poor seed set and reduced grain yield (Rowhani *et al.,* 2011). Grain yield and biomass production was affects by heat stress but mechanism was varying with crop stage. Stress in pre-anthesis stress leading to barrenness in plants, while absorption of fertilized structure and reduced ear growth rate lead to reduction in kernel number and ultimate affect crop yield (Cicchino *et al.,* 2010). Each 1°C increase in temperature above optimum (25°C) result in reduction of 3 to 4% in grain yield (Shaw, 1983). Maize crop cannot be sown earlier than January due to lower temperature which is unfavourable for germination and growth and late sowing is affected by high temperature at reproductive stage.

Therefore, in comparison to agronomic management genetic management of heat stress tolerance genotypes would be low economic input technology that would be readily acceptable to resource poor, heat affected and small land holding farmer (Saxena and Toole, 2002). In the southern part of Nepal, especially in Terai, one of most important heat stresses in the spring maize growing area. Increasing heat tolerance of hybrids is consequently a challenge for maize breeders. For this, it is necessary for promising inbred lines to be tested under both normal and heat stress conditions.

Maize demand will be double in developing world in 2050 and it predicted as crop of greatest production globally and in developing world by 2025 (Rosegrant *et al.,* 2008). As C_4 plant and its physiological efficiency and wide adoption capacity of it can be cultivated over diverse environment and geographic rang than any other cereals. Climate change such as global warming is major challenge on crop production and identify possible ways that would allow yield ceilings to shift by developing improved thermos tolerant cultivars. Therefore, these efforts are particularly important in south Asia, where current production systems are not sustainable and could be adversely impacted by climate change in the near future (Niyogi *et al*., 2010).

Maize stress responses are very complex and interactions between plant structures function and the environment needs to be investigated at various phases of plant development at the organismal, cellular as well as molecular levels (Barnabas *et al*., 2008). Asian heat tolerant accessions were used to produce populations that can be used to develop improved heat tolerance new cultivars (Zaidi and Cairns, 2011). The present study was conducted to examine the accuracy of different stress tolerance indices in identifying maize inbred lines for heat stress tolerance.

Materials and Methods

Experimental Location

This research was carried out at field of National Maize Research Program (NMRP) of Rampur, Chitwan, Nepal from February 24, 2015 to July 2016. The experimental site was located at 27º 40' N latitude, 84 º 19 E and with 228 meter above sea level (MASL).

This site has sub tropical climate. The soil is sandy silt loam, strongly acidic soil (pH 5.0), medium in total nitrogen (0.130%), high in soil available phosphorous (279 kg/ha), high in soil available potassium (215 kg/ha) and high in organic matter content (2.70%) (NMRP, 2011; Gurung et al., 2018).

Plant Materials

The plant materials were derived from National Maize Research Program (NMRP), Rampur, Chitwan, Nepal. The maize inbred lines used in the experiment was listed in Table 1.

Table 1: List of maize inbred lines with their pedigree at NMRP, Rampur, Chitwan, Nepal (2016)

S.N.	Maize Inbred Lines	Pedigree	S.N.	Maize Inbred Lines	Pedigree
1	NML-2	CML-430	11	RL–101	UPAHAR-B-20-2-3-1-1
2	RL–111	UPAHAR-B-31-1-1-1-1	12	RML–91	PUTU-19
3	RML–115	PUTU-17	13	RL–140	POOL-21-12-1-2-2-1-1
4	RML–95	PUTU-17	14	RML–57	CLQG6602
5	RL–105	UPAHAR-B-20-2-4-1-1	15	RML–7	CML-413
6	RML–4	CA00326	16	RML–24	CA00304
7	RML–32	CA00320	17	RML–40	CML-427
8	RML–86	PUTU-20	18	RL–107	UPAHAR-B-20-2-4-3-1
9	RML–17	CML-287	19	RML–20	CA-34503
10	RML–96	AG-27	20	RML–76	CLRCYQ007

Experimental Design and Layout

The experiment was conducted using alpha lattice design with two replications. Each replication had four blocks consisting of five plots each. The maize inbred lines were evaluated in field (normal) and plastic house (for heat stress). The plot was of 3 m long and 0.6 m wide. Each plot contained single row with spacing 60 × 20 cm and consisted 15 hills. The two seeds were sown at beginning, one of whose seedlings were removed at the six leaves stage.

Cultural Practices

The chemical fertilizer applied was at the rate of 120:60:40 kg NPK per hectare. Nitrogen fertilizer were applied at three times; at seed sowing, at six leaves stage and at knee high stage of maize. The irrigation was done at knee high stage, tassel in, stage and milking stage. To created heat stress, half of field was covered with plastic house. The plastic house was built up using 120 gsm plastic. This activity was completed in two weeks just prior to the onset of reproductive period up to the crop harvesting.

Maximum mean temperature was recorded as 46.2°C in April in heat stress condition and 37.23°C for normal condition. Similar temperature was observed in May with maximum mean temperature 43.28°C and normal condition of 34.54°C. Mean temperature was higher (8-9°C) in plastic house at time of flowering, pollination and grain filling periods which responsible for heat stress condition (Figure 1). Partial opening top side of tunnel was done for control relative humidity inside tunnel to avoid any possible disease outbreak.

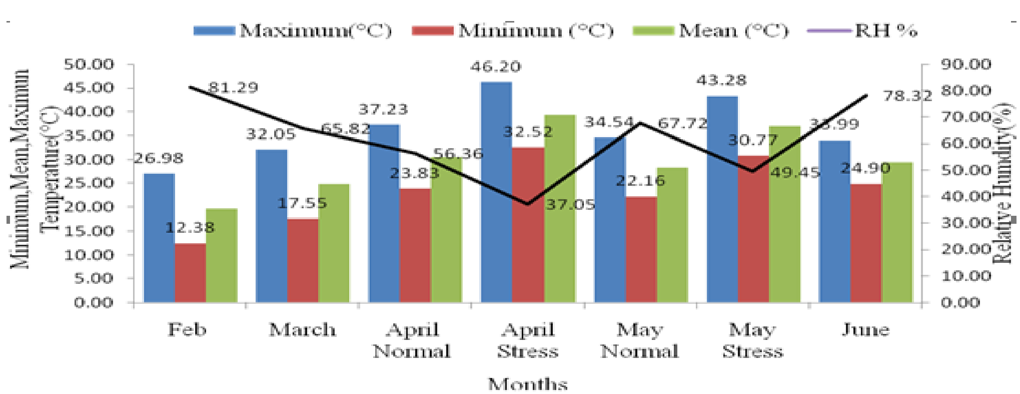

Figure 1: Weather and stress data during the growing period of maize at NMRP, Rampur, Chitwan, Nepal (2016). Please remove the dotted line outside the figure. Also, pt the original picture as this will improve the resolution.

Data Collection and Estimation
Grain yield (kg/ha) at 15% moisture content was calculated using fresh ear weight with the help of the below formula:

$$\text{Grain yield } (\frac{\text{kg}}{\text{ha}}) = \frac{\text{F.W.}(\frac{\text{kg}}{\text{plot}}) \times (100 - \text{HMP}) \times \text{S} \times 10000}{(100 - \text{DMP}) \times \text{NPA}}$$

Where,

F.W. = Fresh weight of ear in kg per plot at harvest
HMP = Grain moisture percentage at harvest
DMP = Desired moisture percentage, i.e. 15%
NPA = Net harvest plot area, m^2
S = Shelling coefficient, i.e. 0.8

This formula was also adopted by Carangal *et al.* (1971), Shrestha *et al.* (2019), Shrestha *et al*. (2018), Gurung *et al.* (2018), Sharma et al. (2019), Sharma *et al.* (2016), Koirala *et al.* (2017), Bartaula *et al.* (2019) and Shrestha *et al.* (2015) to adjust the grain yield (kg ha^{-1}) at 15% moisture content. This adjusted grain yield (kg ha^{-1}) was again converted to grain yield (t ha^{-1}).

The data recorded on grain yield under normal and heat stress were first tabulated in Microsoft Excel (MS-Excel, 2010), then subjected to Restricted Maximum Likelihood (REML) tool in GenStat to obtain ANOVA. Correlation coefficients of different indices were carried out using the formula given by Steel and Torrie (1980) by using SPSS program. The process of estimating the significance of correlation coefficient was also done as proposed by Kothari (2004) and Adhikari *et al.* (2018).

The biplot display was used, which provides a useful tool for data analysis. The collected data were subjected to descriptive statistics and principle component analysis was done using statistical software packages of Minitab ver.17 (Shrestha, 2016). Different indices were carried out using the given formula as given below (Kandel *et al*., 2018):

Stress susceptible index (SSI):
Stress susceptible indices for each genotype were calculated as

$$\text{SSI} = [\frac{1-(\text{YS} \div \text{Yp})}{\text{D}}]$$

Where,
Y_P = Yield of genotype under non-stress condition
Y_S= Yield of Genotype under stress condition
D= Stress intensity = $1-\frac{\text{Meand of XP}}{\text{Means of XS}}$
XP= mean of all genotype yield under normal condition
XS= Means of all genotype yield under stress condition
Genotype was categorized as tolerant and susceptible. Genotype having HIS≤0.50 were highly tolerant, HIS>0.50<1.0 were moderately tolerant and HIS>1.0 were susceptible.

Stress tolerance index (STI):

It was given by Fernandez (1992).

$$STI= \frac{YP \times YS}{(YP)^2}$$

Where,
The symbols have similar meaning as given earlier. High value STI indicates the genotype to be more tolerant to the one with lower value. Selection based on STI resulted in selection of genotype with higher stress tolerance and yield potential.

Tolerance index (TOL):
It was given by Rosielle and Hamblin (1981) as follows

Geometric means productivity (GMP):
It was calculated as given by Fernandez (1992) as follows

$$GMP= \sqrt{YP \times YS}$$

For this genotype with higher value were selected as higher indicate more tolerant genotype

Mean productivity (MP):
It is average of yield under stress and normal environment. It was given by Rosielle and Hamblin (1981). Selection of genotype based on mean productivity was done for those having values which indicate them to be tolerant.

$$MP= \frac{YP+YS}{2}$$

Results

The analysis of variance showed significant difference between inbred lines (Table 2). Among the inbred line RL-140 and RML-91 produced the high grain yield under both stress and normal condition. RML-17 and RML-111 relatively high yield in normal condition, but relatively low yield under stress condition. RL-105 and RML-115 had lower yielded under normal condition and zero yield under stress condition. In contrast, RML-76 and RML-40 had a relatively high yield under heat stress condition but relatively intermediate yield under normal condition. Eight genotypes namely NML-2, RL-105, RL-111, RML-115, RML-24, RML-4, RML-86, RML-95 lines had produces barren cob under stress condition (Table 3).

Table 2: ANOVA of stress tolerance indices and yield in heat stress and normal condition in maize inbred line at NMRPRampur, Chitwan, Nepal (2016)

Source of variance	df	Mean of squares						
		YP	YS	SSI	STI	TOL	GMP	MP
REP.Block	6	28379ns	2462ns	0.00092ns	0.00590ns	24975ns	4467ns	6389ns
Inbred line	19	673050**	169506**	0.167**	0.107**	572894**	519707**	278092**
Error	13	44506	8247	0.005	0.0032	47914	13638	17362

*** *, ns, Significant at 1% levels, respectively, ns= Non-significant. Yp = Yield in non-stress conditions, Ys = Yield in stress conditions, GMP = Geometric Mean Productivity, MP = Mean Productivity, SSI = Stress Susceptibility Index, STI = Stress Tolerance Index, TOL = Tolerance index.*

Table 3: Yield under non-stress, yield under stress, Stress susceptible index, Stress Tolerance index, Tolerance index, Geometric mean productive index and Mean Productive index of maize inbred used at NMRP, Rampur, Chitwan, Nepal (2016)

	Heat indices						
Inbred	**Yp**	**Ys**	**SSI**	**STI**	**TOL**	**GMP**	**MP**
NML 2	1457	0	1.25	0	1457	0	728
RL 101	1469	551.9	0.7713	0.3829	917	898.5	1011
RL 105	366	0	1.25	0	366	0	183
RL 107	1918	264	1.078	0.1373	1654	706.8	1091
RL111	2153	0	1.25	0	2153	0	1077
RL140	2352	702.9	0.876	0.2996	1649	1285.1	1527
RML 115	818	0	1.25	0	818	0	409
RML 17	2201	565.3	0.9269	0.2585	1636	1114.5	1383
RML 20	1213	273.6	0.9587	0.233	939	572.7	743
RML24	1196	0	1.25	0	1196	0	598
RML 32	1130	536.7	0.6474	0.4821	593	777.6	833
RML 4	1287	0	1.25	0	1287	0	644
RML 40	2018	643	0.8488	0.321	1375	1138.3	1331
RML 57	1903	342.6	1.0251	0.1799	1561	807.4	1123
RML 17	582	526.5	0.1189	0.9049	56	553.6	554
RML 76	1689	689.5	0.7376	0.4099	999	1078.7	1189
RML 86	1577	0	1.25	0	1577	0	788
RML 91	2346	716.8	0.8646	0.3083	1629	1294.8	1531
RML 95	1991	0	1.25	0	1991	0	996
RML 96	2090	508	0.9454	0.2437	1581	1029.9	1299
Total	1588	316	0.9899	0.2081	1272	562.9	952

Yp=Yield in non-stress conditions, Ys=Yield in stress conditions, GMP=Geometric Mean Productivity, MP= Mean Productivity, SSI=Stress Susceptibility Index, STI=Stress Tolerance Index, TOL= Tolerance index.

SSI is the ration between the traits at optimum and those at heat stress condition a higher SSI value indicate lowest trait value stressed condition and vice versa. According to Khanna-Chorpa and Vishwanathan (1999), the genotype having SSI value less than 0.5 was highly tolerant, those having values between 0.5 to less than 1 were moderately tolerant and those having values equal or more than 1 were susceptible heat stress.

Based on the STI, lines RML-17 (0.11), RML-32(0.64) and RML-76(0.73) inbred lines showed lowest SSI revealed as highly tolerance to heat stress condition whereas RL-101 (0.7) , RL-140 (0.8) , RML-17 (0.92) , RML-20 (0.95), RML-32 (0.64), RML-40 (0.84), RML-76 (0.40), RML-91 (0.8), RML-96 (0.9) lines having values between 0.5 to less than 1 and revealed as moderately tolerance. Similarly, NML-2, RL-105, RL-111, RML-115, RML-24, RML-4, RML-86, RML-95 all had 1.25, RML-57 (1) and RL-107 (1) those were highly susceptible in nature.

The higher value of stress tolerance index was exhibited by RML-17 (0.90) fallowed by RML-32 (0.48) and RML-76(0.40) whereas zero value of STI was found for NML-2,RL-105,RL-111,RML-115,RML-24,RML-4,RML-86,RML-95.High STI revealed as highest tolerance to heat stress while those with least value are considered to be comparatively susceptible.RML-76 produced the moderately yield in both condition. Based on the TOL index allowed us to select RL-111 (2153) fallowed by RML-95 (1991) and RL-107(1654) inbred as tolerance genotype. RL-111 and RML-95 lines showed zero yield under heat stress. This due to low difference between the two conditions, which decrease the value of the TOL index. Therefore, low TOL does not mean high yielding and genotype yielding should be taken in consideration in addition to this criterion. RML-91(1531) showed highest mean productivity index, followed by RL-140 (1527) were revealed as tolerance (Table 3). Therefore, according to these results selection based on MP will improve mean yield under both condition but does not allow discriminating line having high yield under both stress and line having high yield under normal and lower yield under stress condition. The study of geometric mean productivity showed more comprehensive result. Based on this index, RML-91 (1294.8) followed by RL-140(1285.1) were revealed as tolerance and had high yield under both condition (Table 3).

The result showed that based on MP and GMP inbred line RL-140 and RML-91 were most tolerance while STI and SSI showed RML-17,RML-32 were most tolerance so that need to determine the most desirable stress tolerance criteria, the correlation coefficient between Yp, Ys and quantitative indices of stress tolerance were calculated (Table 4). There were significant correlations between Yp and (MP, TOL, GMP) and between Ys and SSI, STI, GMP and MP and indices GMP and MP consequently appeared as better predictors of Yp and Ys than TOL, SSI and STL. The relationship between both Yp and Ys and MP were consistent with those reported by Fernandez (1992) in Mungbean and Farshadfar and Sutka (2002) in maize. In present study correlation coefficient between SSI and Ys were r= -0.768. The r-value was -0.768 indicate that $(-0.768)^2 \times 100 = 58.98$ % of variation in the mean yield in stress condition is accounted by SSI index. Thus, selection for SSI should give decreased yield under heat stress condition. Likewise, correlation coefficient between STI and Ys r = 0.768 indicate it should give a positive yield response in a hot environment. Therefore, high correlation coefficient between Ys and STI and negative correlation coefficient between Ys and SSI indicated that selection for tolerance based on STI and SSI would be worthwhile only when target environment is heat stressed.

The correlation between GMP and MP yield in stress and normal condition were highly positive response and significant especially under stressed condition. Hence, selection for high GMP and MP should give positive response in both environments. According to the result, the use of STI, GMP and MP indices should help to improve heat tolerance in inbred line. MP that showed high positive correlation with grain yield in both stress and non-stress environment should be more efficient in inbred line selection.

In general, selection of inbred line maize based on GMP might allow us to improve heat tolerance and potential yield under both environments. Based on GMP indices the RL-140, RML-91 appeared as having high yield potential and low stress susceptibility. Based on result of this study inbred RL-140, RML-91 should be use a source of heat tolerance for breeding program.

Table 4: Phenotypic correlation coefficients between maize inbred lines yield in stress and non-stress conditions and heat stress tolerance at NMRP, Rampur, Chitwan, Nepal (2016)

	Yp	**Ys**	**SSI**	**STI**	**TOL**	**GMP**
Ys	0.399^{ns}					
SSI	0.093^{ns}	**-0.768****				
STI	-0.093^{ns}	**0.768****	-1.0**			
TOL	0.867**	-0.111^{ns}	0.519*	-0.519*		
GMP	**0.536***	**0.966****	-0.616*	0.616*	0.056^{ns}	
MP	**0.934****	**0.701****	-0.227^{ns}	0.227^{ns}	**0.631***	**0.794****

** and **, Significant at 5% and 1% levels, respectively, ns: Non-significant. Yp: Yield in non-stress conditions, Ys: Yield in stress conditions, GMP: Geometric Mean Productivity, MP: Mean Productivity, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, TOL: Tolerance Index.*

Table 5: Principal component of first and second tolerance indices and yield in stress and non-stress condition in maize inbred at NMRP, Rampur, Chitwan, Nepal (2016)

Inbred	PC	Eigen Value	Proportion	Cum Proportion	Yp	Ys	SSI	STI	GMP	MP
Line	PC1	3.914	0.559	55.9	-0.26	-0.49	0.38	-0.38	-0.48	-0.39
	PC2	2.807	0.401	96.0	0.5	-0.69	0.36	-0.36	**0.043**	**0.36**

Yp: Yield in non-stress conditions, Ys: Yield in stress conditions, GMP: Geometric Mean Productivity, MP: Mean Productivity, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index.

From Principal component analysis showed that first PC explained 55.9% variation and second PC explained 40.1% (Table 5). Considering the high and negative value of indices of this PCA and high and positive value of indices in this second PC first PC named as low yielding and stress susceptible and second PC as yield potential and heat tolerance.

Thus, selection of genotype having low PC1 and high PC2 were suitable for both stress and normal condition. Thus, inbred RML-91, RL-140, RML-40, RML-17, RML-96, RML-7, RL-107 exhibited low PC1 and high PC2 were superior for both environment conditions. RML-15, RML-115 and RL-105 having high PC1 value and low PC2 value showed poor yield under both environmental conditions. Thus, results obtained from biplot graph confirmed the correlation analysis (Table 4).

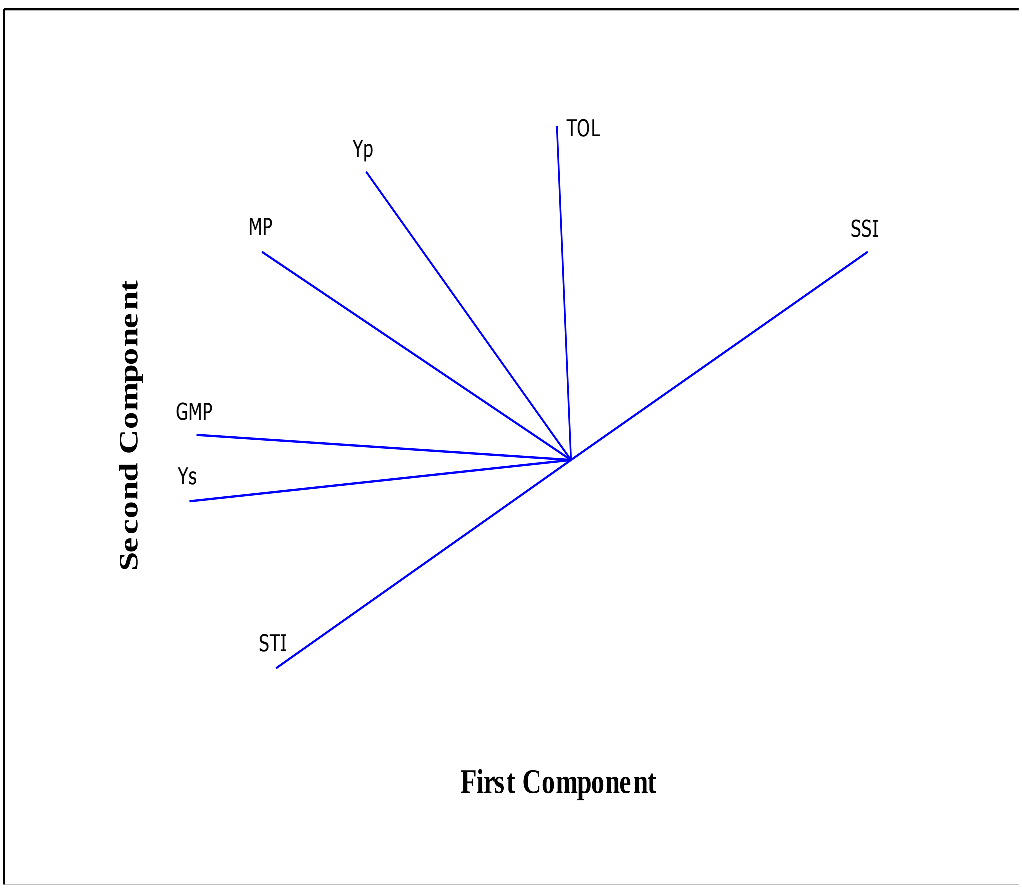

Figure 2: Biplot display of heat tolerance indices

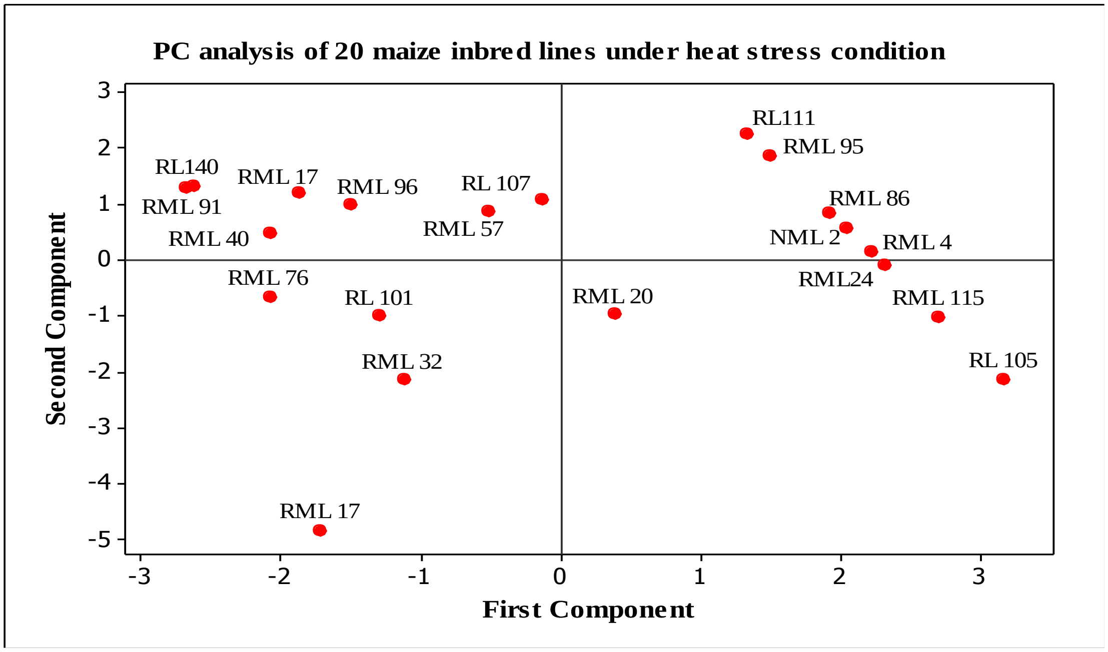

Figure 3: Biplot display of yield in seven heat tolerance based on the first and second main component of maize inbred lines at NMRP, Rampur, Chitwan (2016).

Discussion

The present study showed that GMP and MP were the most accurate criterion in selecting lines with tolerance to heat stress and high yield under both stressed and normal conditions. Inbred lines having high value of GMP and MP were the most tolerant and had high yield under both conditions. Thus, ability of separate heat tolerant from other using GMP index is and susceptible inbred lines. These findings were similar to the results founded by Ahmadzadeh (1997) and Khalili et al. (2004) in maize and Rezaeizad (2007) in sunflower. The similar findings MP will improve mean yield under both conditions were also reported by Moghaddam and Hadizadeh (2002) and Khalili et al. (2004). This due to low difference between the two conditions, which decrease the value of the TOL index. Therefore, low TOL does not mean high yielding and genotype yielding should be taken in consideration in addition to this criterion. These finding were similar to result were reported by Ahmadzadeh (1997) for maize hybrids.

Significant positive correlation between Ys and STI and negative correlation between Ys and SSI. These finding were similar to result to Khaili et al. (2004) in maize and Fernandez (1992). Selection based on a combination of MP and GMP should give high yield under both conditions. This similar result was reported by Ahmadzadeh (1997) in maize and Ghajar Sepanlo et al. (2000) and Sanjari (1998) in wheat. Thus, selection based on a combination of indices may provide a more useful criterion for improving stress tolerance of maize. Ahmadzadeh (1997) in maize, Fernandez (1992) in common bean, Souri et al. (2005) in pea used biplot graph confirmed the correlation analysis for selection heat stress tolerant. Thus, a strong positive association between GMP, MP and STI with Yp and Ys and negative association between SSI and Ys in a biplot display. The results obtained from the biplot graph, confirmed the correlation analysis. Results of this study are in good agreement with Golabadi et al. (2006) in durum for drought tolerance.

Conclusion

The heat stress indices should help to improve heat tolerance in maize inbred lines. The selection based on a combination of indices may provide a more useful criterion for improving heat tolerance and potential yield in different environment.

Based on biplot analysis, lines RML-140 and RML-91 were found high yielding inbred lines with low stress susceptibility in both environments. Therefore RML-140 and RML-91 should be a source of heat tolerance in crosses for hybrid production.

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