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Analysis of genetic diversity among the maize inbred lines (*Zea mays* L.) under heat stress condition

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ABSTRACT

High temperature adversely affects the plant physiological processes: limits plant growth and reduction in grain yield. Heat stress is often encountered to spring sowing of maize in spring season. Twenty maize inbred lines were studied for days to 50 % anthesis and silking, anthesis–silking interval, leaf firing, tassel blast, SPAD reading and leaf senescence, plant and ear height, leaf area index, ear per plant, cob length and diameter, number of kernel/ear, number of kernel row/ear, number of kernel row, silk receptivity, shelling percentage, thousand kernel weight and grain yield in alpha lattice design at National Maize Research Program at Rampur, Chitwan,Nepal with the objective to identify superior heat stress tolerant lines. Analysis of variance showed significant difference for all the traits. Result of multivariable analysis revealed that twenty inbred lines formed four clusters. The resistance inbred lines and susceptible inbred lines formed different clusters. The members of cluster 4 were found to be tolerant to heat stress due to they had lowest value of tassel blast, leaf firing, and leaf area index with highest value of cob diameter and length, ear per plant, number of kernel row/ear, number of kernel/ear, number of kernel row, shelling percentage, silk receptivity and grain yield whereas as members of cluster 1were found most susceptible due to they had longer anthesis silking interval, with maximum tassel blast and leaf firing along with no grain yield under heat stress condition. From this study inbred lines RL-140, RML-76, RML-91 and RML-40 were found most tolerant to heat stress. These inbred lines belonging to superior cluster could be considered very useful in developing heat tolerant variety and other breeding activities.

Keywords: Maize (*Zea mays L.*), Genetic divergence, Heat stress, Multivariable analysis

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INTRODUCTION

Maize (*Zea Mays* L.) is an important cereals crop belongs to the tribe Maydeae, of the grass family, Poaceae and popularly known as the 'queen of cereals' (Dhaka et al., 2010).It is important cereal crop worldwide after wheat and rice in worldwide and second most staple food crop both in term of area and production after rice in Nepal. It is grown in 8, 91,583 ha producing 2.2 million tons, with an average yield of 2500 kg/ha (MoAD, 2016).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. 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).

Climate change effects 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). Heat and drought stress have emerged as a common problem worldwide which can reduce maize crop productivity (Ali et al., 2015).A record drop in maize production was reported in many maize-growing areas of the world (Van der Velde et al., 2010).It is predicted that maize yield might be reduced up 70 % due to increasing temperatures (Khodarahmpour et al., 2011).

Genetic diversity analysis is imperative in crop improvement and can be studied through morphological, biochemical and molecular markers. Morphological characterization for genetic divergence among genotypes is considered an initial step (Khan et al., 2014).Therefore morphological data has play key role in management of genetic resources. To management of genetic resource study relationships and description and classification of germplasm the morphological characterization is the first step (Smith & Smith, 1989).Cluster analysis is frequently used to classify maize accessions and can be used by breeders and geneticists to identify subsets of accessions which have potential utility for specific breeding or genetic purposes (Rincon et al., 1996).

The main aim of using a cluster technique in plant breeding trials is to group the varieties into several homogeneous groups such that those varieties within a group have a similar response pattern across the locations. Many researchers have used principal component analysis to assess genetic variability among maize genotypes because it retrieves small numbers of components that account for most of the variations in the data (Asare, 2016).The objective of the research was to identify superior heat stress tolerant inbred lines after clustering them based on their response to heat stress condition.

METERIALS AND METHOD

The research was conducted at National Maize Research Program (NMRP) of Rampur, Chitwan Nepal during spring season from February 24, 2015 to July 2016, geographically located at 27º 37' North Latitude and 84 º 29' East longitude at an altitude of 225 meter above sea level. This site contains only sandy loam soil with acidic reaction. This research location is characteristics of subtropical climate. The plant materials were collected from National Maize Research Program

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(NMRP).The list of inbred lines along with pedigree information included in the study is presented in Table 1.

Table 1: Names and pedigree information of maize inbred lines used for heat stress research at NMRP Chitwan (2016).

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

Field experiment was conducted in alpha lattice design. There were two conditions: normal and plastic house (for heat stress), each condition replicated twice. Each replication comprised four blocks consisting of five plots each. Each plot was 3 meter in length 0.6 meter wide. Each plot had one row with spacing 20 cm between rows, inter block gap was 0.5 m was maintained. Each plot contained single row with spacing 60×20 cm and consisted 15 hills, each of two seed were sown, one of whose seedling were removed at the six leaves stage. The dose of chemical fertilizer applied was 120:60:40 kg NPK per hectare. Fertilizer were applied prior to sowing at rate of N 60 kg/ha, P 60 kg/ha and K 40 kg/ha and additional side dressing of 30 N kg/ha were applied at the two times in six leaves stage and knee high stage of maize. The irrigation was done three important stage, knee high stage, tasseling stage and milking stage. To created heat stress condition maize study half of field was controlled heating imposed using two plastic (120gsm) houses were used two week just prior to the onset of reproductive period up to the crop harvesting. Maximum mean temperature 46.2°C in April in heat stress condition whereas as normal condition was 37.23°C and similarly for May month in maximum mean temperature was 43.28°C whereas in normal condition 34.54°C means mean temperature 8-9°C higher in plastic house at time of flowering, pollination and grain filling periods responsible for creates heat stress condition as shown in Fig 1.Partial opening top side of tunnel was done for control relative humidity inside tunnel to avoid any possible disease outbreak.

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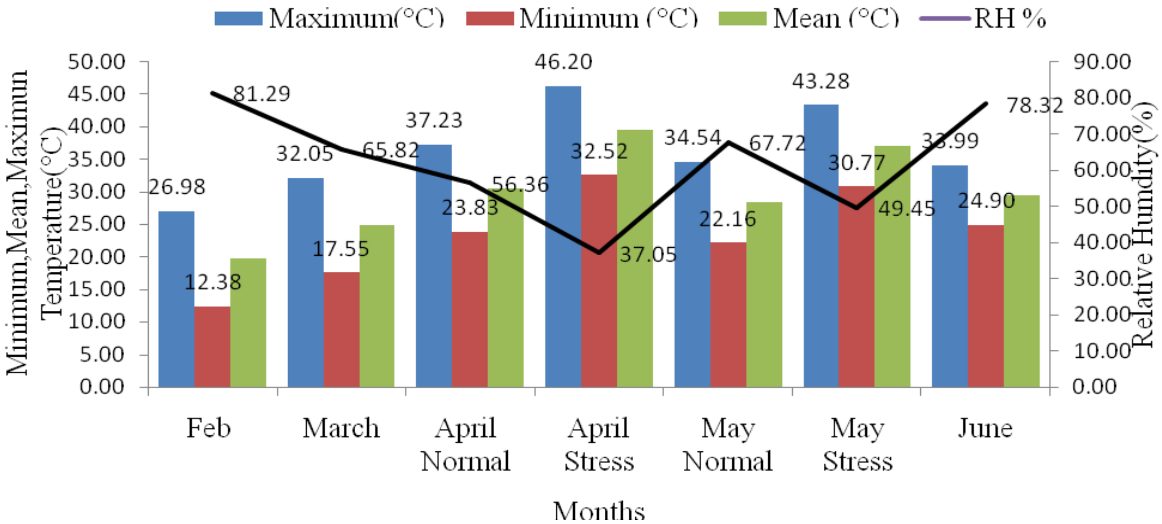

Figure 1: Weather and stress data during the growing period of maize at NMRP, Chitwan (2016).

Data collection

Data on days to 50% anthesis, days to 50% silking and ears per plot, leaf firing, tassel blast, leaf senescence were recorded on plot basis. Whereas, ear height (cm), number of kernels/cob, number of kernel row, number of kernel row/ear, SPAD reading, leaf area index, silk receptivity, thousand kernel weight (g) and shelling per cent were recorded on five selected representative plants. The sample cobs were shelled, cleaned and grain weight and shank weight were recorded to calculate the shelling percent. Thousand kernel weight was measured by counting 1000 grains from the bulk of each plot after shelling and weighed in grams after the moisture was adjusted to 15%. Anthesis-silking interval (ASI) was calculated by subtracting the number of days taken for 50% anthesis from the number of days taken to50% silk emergence. Leaf area index was calculated by total leaf area divided by land area and multiply by correction factor (0.75). Silk receptivity was recorded by total number of fertilized grains per ear divided by number of potential grain per ear. Leaf firing was obtained by the counting the number of plants that showed leaf firing symptoms (younger leaves near tassel burnt or dried) in the total number of plants in a particular plot and was expressed in percentage. Tassel blast was obtained by the counting the number of plants that showed tassel blast symptoms (tassel dried with partial or no pollen shedding) in the total number of plants in particular plot was expressed in percentage. Grain yield was calculated using formula adopted by Carangal et al. (1971) and Shrestha et al. (2015) by adjusting the grain moisture at 15% and converted to the grain yield per hectare.

Statistical Analysis

The data recorded on different parameters from in heat stress field were first tabulated and processing in Microsoft excel (MS- Excel, 2010), then subjected to restricted maximum likelihood (REML) tool in GenStat to obtain ANOVA. These collected data were subjected to cluster analysis (average linkage method) and principal component analysis using statistical software packages of Minitab ver.17.

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RESULTS

The present study genetic diversity for heat stress tolerance was analyzed among 20 maize inbred lines on the basis of 19 agro morphological traits. The result of descriptive analysis (Table1) showed that leaf firing percentage had highest variation (52.3) fallowed by tassel blast percentage (22.93), anthesis silking interval (21.9), SPAD (14.22).Among trait plant physiological maturity showed the lowest variation (1.5). Significant variation among inbred lines differences for grain yield and anthesis silking interval, SPAD reading and leaf senescence, tassel blast and leaf firing percentage, plant and ear height, leaf area index, ear per plant, cob length and diameter, number of kernel/ear, number of kernel row, number kernel/ear, silk receptivity, shelling percentage, thousand kernel weight under heat stress condition. The mean value of observed traits anthesis silking interval, leaf area index, leaf firing, tassel blast, leaf senescence, SPAD, plant height, ear height, plant maturity, cob length, cob diameter, number of kernel row/ear, number kernel/ear, number of kernel row, silk receptivity, shelling percentage, thousand kernel weight and grain yield as presented in Table 1.Inbred lines RML-91 (716.8 kg/ha) fallowed by RL-140 (702.9 kg/ha) and RML-76 (689.5 kg/ha) produces maximum yield whereas as inbred lines NML-2, RL-105, RL-111, RML-115, RML-24, RML-4, RML-86, and RML-95 produces barren cob under heat stress condition.

Table 1: Descriptive statistics of agro morphological traits of 20 maize inbred lines at NMRP, Rampur, Chitwan (2016 spring)

Statistics	Mean	F –test	CV%	LSD	Statistics	Mean	F –test	CV%	LSD
ASI	3.8	*	21.9	1.8	CD	1.677	**	14.66	0.532
LAI	2.64	*	12	0.7	CL	6.89	**	12.97	1.931
LF	18	*	52.3	20.8	NKRE	6.64	**	7.2	1.037
TB	35.6	**	22.93	17.64	NKE	42.1	**	21	19.21
LS	63.8	**	12.4	17.2	NKR	7.82	**	13.52	2.284
SPAD	38.16	*	14.22	11.723	SR%	17.23	**	11.57	4.31
PH	129.9	*	7.91	22.21	SP%	26.79	**	14.7	8.56
EH	66.6	**	8.13	11.7	TKW	164.8	**	6	21.61
PM	106.5	**	1.5	3.432	GY	316	**	8.62	58.89
EPP	1.168	*	15	0.379					

* and **, significant at 5 % and 1 % probability level.

Cluster Analysis

All the inbred lines were clustered using day to 50 % tasseling and silking, anthesis–silking interval (ASI), leaf firing, tassel blast, SPAD reading and leaf senescence, plant and ear height, leaf area index, ear per plant, cob length and diameter, number of kernel/ear, number of kernel row, number kernel/ear, silk receptivity, shelling percentage, thousand kernel weight at heat stress condition as variables. The dendrogram reveled four clusters with minimum of 22.47 % similarity level in UPGMA Clustering. The distance between the clusters centroid was found highest between clusters 1 and 4 and lowest between clusters 2 and 4 is presented in Table 2.The clusters were divided into two groups: group A and Group B. Group A consisted of one cluster named as cluster one whereas group B consisted of three clusters namely Cluster2, Cluster 3 and cluster 4. Cluster 1 consisted of 8 lines named as NML-2, RL-105, RML-24, RL-111, RML-4,

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RML-86, RML-95 and RML-115 which represent 40% of total lines. Inbred lines grouped in this cluster had longer anthesis silking interval, with maximum tassel blast and leaf firing along with zero value for grain yield including cob length, cob diameter and length, number of kernel row/ear, number of kernel/ear, number of kernel row, shelling percentage, silk receptivity and thousand kernel weight. The lines of this cluster are most susceptible to heat stress. Cluster 2 consisted of 5 lines named as RL-101, RML-17, RML-32, RML-96 and RML-7, 25% of total lines was characterized with had highest leaf senescence fallowed by thousand kernel weight and lowest for cob diameter and length, ear per plant, number of kernel row/ear, number of kernel/ear and number of kernel row. The inbred lines categorized into cluster 3 were RL-107, RML-20 &RML-57, 15 % of total lines had shorter plant and ear height, late physiological maturity and highest for ear per plant. Cluster 4 consisted of 4 lines named as RL-140, RML-76, RML-91 and RML-40, 20 % of total lines were characterized by lowest value of tassel blast, leaf firing, leaf area index with highest value of cob diameter and length, ear per plant, number of kernel row/ear, number of kernel/ear, number of kernel row, shelling percentage, silk receptivity and grain yield in heat stress condition. Since this cluster of lines had superior trait value for heat stressed condition, these lines may be of interest to researchers.

Table 2: Distance among the different cluster centroid of maize under heat stress at NMRP, Rampur,Chitwan (2016).

	Cluster1	Cluster2	Cluster3	Cluster4
Cluster 1	0	614.977	398.599	750.397
Cluster 2		0	247.245	158.825
Cluster 3			0	399.583
Cluster4				0

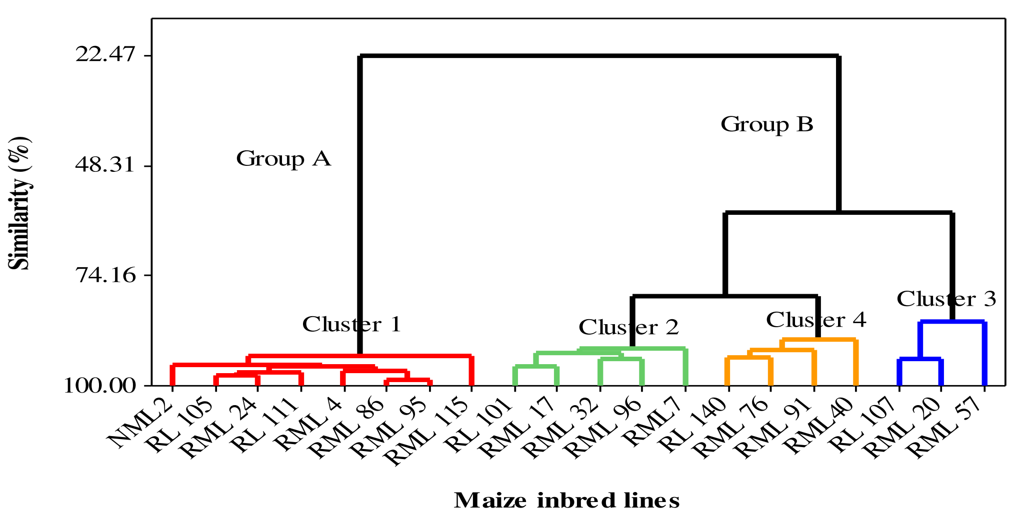

Figure2: Cluster analysis of 20 maize inbred lines evaluated for agro-morphological traits under heat stress at NMRP, Rampur, Chitwan (2016).

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Table 3: Mean of Clustering of 20 Maize inbred lines under heat stress condition at NMRP, Rampur, Chitwan (2016).

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Centroid
No. of inbred lines	8	5	3	4	20
Anthesis silking interval	5.06	2.8	2.66	3.37	3.8
Leaf area index	2.83	2.55	2.53	2.47	2.64
SPAD Chlorophyll	33.209	41.31	42.93	40.56	38.16
Leaf firing	26.35	14.88	11.6	5.75	17.13
Tassel blast	47.51	28.64	27.33	26.825	35.63
Plant height	131.25	130.78	125.133	129.45	129.85
Ear height	71.06	65.74	59.833	63.8	66.59
Physiological maturity	107.563	105	104.16	107.75	106.47
Leaf sensense	58.75	72	68.33	60	63.75
Ear per plant	1.18	1.066	1.323	1.143	1.16
Cob diameter	0	2.69	2.77	2.93	1.677
Cob length	0	11.46	8.43	11.64	6.37
Number of kernel row per ear	0	11.04	10.76	11.35	6.64
Number of kernel row	0	12.26	13.26	13.825	7.82
Number of kernel per ear	0	56.46	49.73	102.37	42
Silk receptivity	0	27.1	21.76	35.95	17.23
Shelling percentage	0	51.17	25.44	50.89	26.78
Thousand kernel weight (g)	0	285.32	260.467	271.825	164.76
Grain yield (kg ha^{-1})	0	537.68	293.4	688.05	316.04

Principal Component Analysis

The PCA showed close resemblance with clustering and partitioned the total variance into 4 PCs having eigen value >1 explanining 85.9 % of total vaiation with eigen value between 10.935 to 1.293, among 20 lines of maize under heat stress,However, the remaning componnent contributed only 14.4 % towards total diversity for this set of maize lines.The first three pricipal component (PC1) which explained 79.2 % was associated mainly by anthesis silking interval (0.257),tassel blast(0.229), leaf firing (0.216) and leaf area index (0.120) with negative loading with grain yield, cob diameter (-0.295),cob length (-0.275),number of kernel/ear (-0.275),number of kernel/row (-0.297),number of kernel/ear (-0.298),shelling percentage(-0.276),silk receptivity (-0.295) and thousand kernel weight(-0.297) due to some heat susceptible lines in this cluster. Second principal (PC2) was responsible for about 13.4 % was mainly related to positively for leaf firing (0.239), physiological maturity (0.258). SPAD(0.145) and anthesis silking interval (0.106)with negatively for leaf area index (-0.273),tassel blast (-0.176),ear height(-0.538),ear per plant(-0.258),leaf senescence(-0.149) and grain yield(-0.008).PC3 contributed 8.3%with major positive contributor are leaf senescence (0.585) fallowed by leaf firing (0.218) whereas negatively associated with anthesis-silking interval (-0.104) leaf area index(-0.283),plant height(-0.147),ear height(-0.186),physiological maturity(-0.565) and grain yield(-0.135).PC4 accounted 6.8 % of the total variation was mainly negative association with, leaf area index (-0.489),leaf firing (-0.024),tassel blast (-0.139), leaf senescence(-0.397),SPAD(-0.201),shelling percentage(-0.058) and thousand kernel weight(-0.009) and positive association with anthesis-silking interval (0.154), grain yield (0.024), ear per plant (0.708),silk receptivity (0.030), plant height (0.708),

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number of kernel row/ear (0.026), number of kernel/ear (0.087) and number of kernel/row (0.087).Thus positive relation with grain yield, anthesis silking interval, number of kernel row/ear, number of kernel/ear and number of kernel row, etc. and negative association with tassel blast, leaf firing, leaf senescence, ear height, thousand kernel weight, shelling percentage lead to this principal component had variability and selection within this is importance for heat stress condition as shown in Table 3.The present research revealed that these genotype formed in cluster four in heat stress condition were found most tolerant to heat stress. The finding PCA supported the result obtained by cluster analysis and PCA score plot was shown in figure 3.

Table 3: The first four principal components of traits used for cluster analysis and PCA and the eigen analysis of the correlation matrix at NMRP, Rampur,Chitwan (2016).

	Principal components			
Variable	PC1	PC2	PC3	PC4
Eigenvalue	10.935	2.545	1.569	1.293
Proportion	0.578	0.133	0.083	0.068
Cumulative	0.578	0.71	0.792	0.860
Anthesis silking interval	0.257	0.106	-0.104	0.154
Leaf area index	0.120	-0.273	-0.283	-0.489
SPAD Chlorophyll	-0.200	0.145	-0.216	-0.201
Leaf firing	0.216	0.239	0.218	-0.024
Tassel blast	0.229	-0.176	0.074	-0.139
Plant height	0.03	-0.591	-0.147	0.039
Ear height	0.097	-0.538	-0.186	-0.035
Physiological maturity	0.103	0.258	-0.565	-0.009
Leaf senescence	-0.086	-0.149	0.585	-0.397
Ear per plant	0.025	-0.265	0.141	0.708
Cob diameter	-0.295	-0.031	0.046	-0.023
Cob length	-0.275	-0.032	0.001	-0.011
Number of kernel row per ear	-0.297	-0.039	0.019	0.026
Number of kernel row	-0.298	-0.003	-0.000	0.048
Number of kernel per ear	-0.275	-0.052	-0.192	0.087
Silk receptivity	-0.295	-0.02	-0.093	0.030
Shelling percentage	-0.281	-0.016	-0.008	-0.058
Thousand kernel weight	-0.297	-0.015	0.070	-0.009
Grain yield (kg/ha)	-0.286	-0.008	-0.135	0.022

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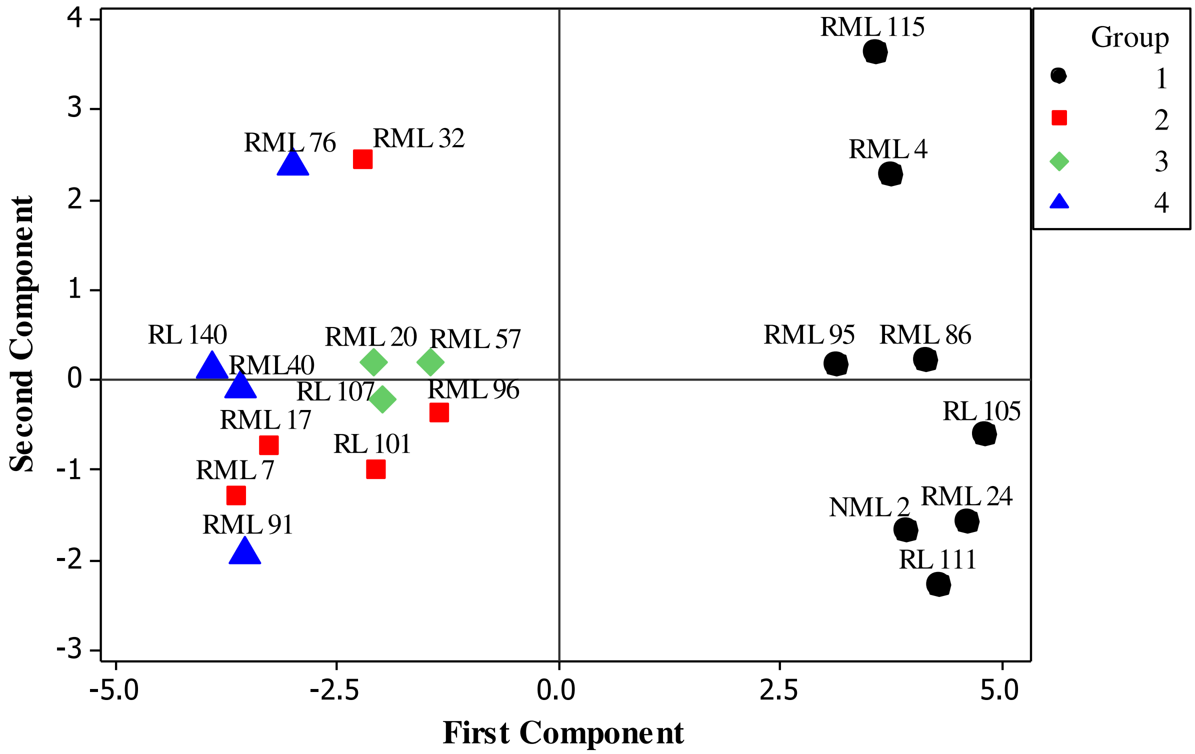

Fig 3: The score plot of first two components of maize inbred lines under heat stress at NMRP Rampur, Chitwan (2016).

DISCUSSION

In present study there is sustainable genetic diversity in grain yield and heat tolerance trait. Khodarahmpour et al. (2011) reported similar finding reduced grain yield up to 70% under heat stress condition in maize inbred lines. Grain yield reduction was associated with poor pollen viability under heat stress condition reported by Rowhani et al. (2011).Pre-anthesis stress in plants absorption of fertilized structure and reduced ear growth rate lead to reduction in kernel number leading to barrenness and ultimate affect crop yield reported by Cicchino et al., (2010b). There were four considerable genetic divergence groups due to genetic factor also subjected to environmental factor. Shrestha (2016) also reported that significant amount of genetic diversity for in maize inbred line for their different morphological traits. Krupakar et al. (2009) also have assessed the range of variability of 16 genotypes for 14 traits in maize. The presence of divergent cluster indicated their superior trait value for heat stressed condition; these inbred lines may be of interest to researcher for abiotic stress breeding. Souza and Sorrels (1991) also reported estimation of genetic diversity and relationships among germplasm accessions facilitates the selection of parents with diverse genetic background which is very essential for breeding program. Thus similar finding about presence of genetic diversity in maize yield and its component were also reported by Singh and Chaudharai (2003).In present study cluster 4 were most heat stress tolerance this finding similar by Ali et al. (2008) who reported that cluster analysis can be useful for finding high yielding genotypes. In this study of principal component use to reduces of original variables into four principal component and information about each variable which support cluster analysis result. These findings were similar to the results founded by Syafii et al. (2015) and Kamara et al. (2003).In present finding multivariable analysis help to selection of inbred lines for heat stress tolerance breeding: similar to the results reported by Akte ret al., (2009).

CONCLUSION

The genetic diversity was observed in inbred lines differences for grain yield and anthesis silking interval, SPAD reading and leaf senescence, tassel blast and leaf firing percentage, plant and ear height, leaf area index, ear per plant, cob length and diameter, number kernel ear^{-1}, number of kernel rowear^{-1}, number kernel row, silk receptivity, shelling percentage, thousand kernel weight under heat stress condition. UPGMA revealed that inbred lines formed four distinct clusters. The resistant lines and susceptible lines formed different clusters. The member of cluster 4 was found to be tolerance to heat stress where as members of cluster 1 were found most susceptible to heat stress. From this study inbred lines RL-140, RML-76, RML-91 and RML-40 were found most tolerant to heat stress as shown by lower reduction in grain yield and heat tolerance trait tassel blast, leaf firing and shorter anthesis siliking interval. The PCA showed close resemblance with clustering. The presence of high level of diversity among the inbred line for heat stress tolerance grouped into divergent cluster indicated their superior trait value for heat stressed condition; these inbred lines may be of interest to researcher for abiotic stress breeding.

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AUTHOR CONTRIBUTIONS

S.K.G., B. R.O. and J. S. designed the experiment in research field research. M. K. performed experiments, analyzed data and wrote manuscript. S. K. G. and J. S. gave final approval of the manuscript version to be published.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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