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MULTIVARIATE ANALYSIS FOR YIELD AND ITS COMPONENT TRAITS IN MAIZE (*ZEA MAYS* L.) UNDER HIGH AND LOW N LEVELS

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ABSTRACT

A study was carried out using 256 full sib progenies of a large random mating population of maize composite Mahidhawal under two environments of fertility *viz.*, (i) high N (E_1) and (ii) low N (E_2) to identify efficient full sibs under variable N levels. The highest per cent increase in mean values under high N was observed for grain yield plant^{-1} (9.21%) followed by ASI (6.51%) and stover yield plant^{-1} (6.22%). This suggested high N level had more effect on expression of these traits. Principal component analysis on 11 traits, seven principal components had eigen values more than one and accounted for 75.32% of the total variation. Nitrogen efficiency index with N responsiveness at high N showed that 70 and 112 full sibs respectively, were the best full sibs for N-deficient soils of this region. Presence of significant and positive phenotypic correlation was observed for grain yield plant^{-1} with ear length, ear girth, 100-grain weight, number of cobs plant^{-1}, stover yield plant^{-1} and harvest index under both N levels except kernel row ear^{-1} under low N level. The magnitude of phenotypic correlation of grain yield with other traits was numerically higher at low N level than high N level.

INTRODUCTION

Maize (*Zea mays* L.) is the fourth most important cereal crop in India next to rice and wheat. Maize is used like a human food, livestock feed, for producing alcohol and non-alcohol drinks, built material, like a fuel and like medical and ornamental plant (Bekric and Radosavljevic, 2008). Worldwide, nitrogen together with phosphorus is one of the macronutrients that are most limiting to maize grain yield (D'Andrea *et al.,* 2006). Nitrogen (N) fertilization remains an important agronomic practice for maize production to obtain high yield under low nitrogen (LN) conditions or when converting N fertilizer efficiently into yield under high nitrogen (HN) conditions (Sattelmacher *et al.,* 1994). Nitrogen is an important element to maize production as it promotes vegetative growth, maximizes both kernel initiation and kernel set, it is also key in filling the kernel sink (Below, 1997). Santos *et al.* (1997) observed yield losses of 65.8% when an open pollinated variety that was developed under soils of high fertility was grown under low N conditions. Lafitte *et al.* (1995) suggested that a progress for low N environments may achieved by selecting N efficient genotypes. Increased varietal tolerance to low soil N stress offers an effective partial solution to enhance maize production and food security among the resource poor small-scale farmers. Under this strategy, plants are able to tolerate deficiency of N by partitioning more N and carbohydrates to the ear. An appropriate breeding strategy can be used to develop genotypes that tolerate the stress and produce high grain yield under both low soil N and optimal conditions (Miti *et al.,* 2010). Since, the grain yield in maize is quantitative in nature and polygenically controlled, effective yield improvement and simultaneous improvement in yield components are imperative (Bello and Olaoye, 2009). Selection on the basis of grain yield character alone is usually not very effective and efficient. However, selection based on its component characters could be more efficient and reliable (Muhammad *et al.,* 2003). To develop an appropriate breeding strategy in selecting genotypes that tolerate low N conditions, an appropriate knowledge of interrelationships between grain yield and its component traits can significantly improve the efficiency of selection in breeding programme. The correlations between traits is also great importance for success in selections to be conducted in breeding programs, and it is the most widely used one among numerous methods that can be used (Yagdi and Sozen, 2009). Multivariate methods have three main purposes: summarizing information, eliminating "noise" from the data sets and revealing the structure of the data sets (Crossa *et al.*, 1990; Gauch, 1992). Multivariate methods can also be used for determining grain yield stability and identifying genotypic groups possessing desirable traits (Lin *et al.*, 1986). Cluster analysis can identify differences among genotypes for the breeder via classification of genotypes (Sabaghnia *et al.*, 2012). Keeping above in the view, the present investigation was undertaken to study association between yield and yield attributing characters in a large random mating heterozygous maize population of Mahidhawal under two N levels, and other basic characteristics using principal component analysis and cluster analysis under different N levels to classify and identify diverse genotypes with best agronomic characters for nitrogen use efficiency.

MATERIALS AND METHODS

A total 256 full sib families (64 half sib families) developed as per North Carolina Design-I in heterozygous, large random mating Mahidhawal population of maize at the Research Farm of the Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur. To develop full sib and half sib families, each 64 randomly chosen male plants were crossed with five randomly chosen female plants. After harvest, out of the five female plants, four successfully pollinated female plants that had sufficient seed for field evaluation were retained to constitute a male group (a group of four families involving the same male parent). Sixty four such male groups (half sib families) or a total of 256 full sib families were obtained in Mahidhawal population. The 256 full sib families were evaluated in two different environments created by two nitrogen (N) levels *viz.*, (i) High N, 120: 60:00 NPK kg per hectare (E_1 or HN) and (ii) Low N, 60: 60: 00 NPK kg per hectare (E_2 or LN) in incomplete block design with two replication. All the standard crop cultivation practices (such as applica-tion of pesticides and irrigation) were followed for raised the healthy crop. Each family/progeny were grown in a plot of 3 meters length with crop geometry of 60 x 20 cm. The observation were recorded on five randomly selected competitive plants for ear length (cm), ear girth (cm), kernel rows ear^{-1}, 100-grain weight (g), number of cobs plant^{-1}, grain yield plant^{-1} (g), stover yield plant^{-1} (g) and harvest index (%) except for day to 50% tasseling, day to 50% silking, anthesis silking interval (ASI), which were recorded on plot basis. Phenotypic correlation coefficients (r_p) were computed among phenotypic traits using full sib's family means under both N levels (E_1 and E_2 environments) according to Miller *et al*. (1958).

To better understand the relationships, similarities and dissimilarities among the yield and its component traits, Nitrogen efficiency index of maize genotypes was determined as per Pan *et al*. (2008) for phosphorus efficiency index (PEI). Principal component analysis (PCA), based on the rank correlation matrix was calculated by PAST software (Hammer *et al.,* 2009). The principal components whose eigenvalues were more than one were retained and involved in the calculation of *F* value for all 256 full sibs. The relative weight of each principal component was weighed by the corresponding contribution rate accounting for variations of all traits. Consequently, *F* values of different genotypes were calculated according to the retained principal components and their relative weight, namely

$$F = \sum_{i=1}^{256} PCi \times RWi.$$

That value is used for classification of maize full sibs was determined by the Ward's minimum variance cluster method and maize accessions were divided into 3 categories according to the N efficiency index (Fig. 1) and four categories according to N efficiency index in combination with standardized grain yield at high N (Fig. 2).

RESULTS AND DISCUSSION

The absorption of nitrogen by plants plays an important role in their growth. Therefore, nitrogen fertilization has been a powerful tool for increasing the yield of cultivated plants, such as cereals (Gallais and Hirel, 2004). Though, there is the great demand to decrease of N supply in maize production because of the increasing cost of nitrogen fertilizer as well as the environmental pollution. Thus, breeding hybrids with high nitrogen use efficiency is the most economical and effective approach to meet this purpose (Below and Uribelarrea, 2006). To develop efficient hybrid under different N environments, there is need to understand N responsive of full sibs and their relationship under variable N levels. A comparison of full sibs

Table 1: Means ± the standard deviation and responses of yield and other traits under high N (E_1) and low N (E_2) in Mahidhawal population of maize

Character	E_1	E_2	LN/HNratio	Per cent increase
Days to 50% tasseling	51.23 ±1.48	50.75 ± 1.74	0.99	0.94
Days to 50% silking	53.66 ±1.56	53.03 ± 1.82	0.99	1.18
Anthesis silking interval	2.43 ±0.43	2.28 ± 0.39	0.94	6.51
Ear length (cm)	15.29 ±1.14	14.86 ± 1.13	0.97	2.92
Ear girth (cm)	12.06 ±0.49	11.77 ± 0.46	0.98	2.42
Kernel rows ear^{-1}	12.75 ±0.75	12.34 ±0.77	0.97	3.34
100-grain weight (g)	25.28 ±2.39	24.48 ± 2.61	0.97	3.26
Number of cobs plant^{-1}	1.05 ±0.11	1.04 ± 0.08	0.99	0.96
Grain yield plant^{-1} (g)	92.43 ±15.09	84.64 ± 13.03	0.92	9.21
Stover yield plant^{-1} (g)	113.39 ±15.41	106.74 ± 13.77	0.94	6.22
Harvest index (%)	44.75 ±2.12	44.09 ± 1.65	0.99	1.49

Table 2: Principal component analysis of yield and other traits over environment in Mahidhawal population of maize

PC	Eigen value	Variance (%)	Cumulative variance percentage
1	5.35	24.35	24.35
2	3.21	14.59	38.94
3	2.22	10.09	49.03
4	1.71	7.80	56.84
5	1.48	6.73	63.57
6	1.35	6.16	69.74
7	1.22	5.58	75.32

at high and low N supply revealed that days to 50% silking was delayed (1.18%) more than days to 50% tasseling (0.94%) at high N supply (Table 1). This leads to an average prolongation of anthesis silking interval at high N environment by 6.5%. This indicates that days to 50% silking was more affected by high N supply than the days to 50% tasseling. It may be because of that in the early stage the high N is diverted in production of more crop biomass resulting delayed female flowering. But opposite trend was reported by Presterl *et al.* (2002) that silking date was delayed more at reduced N supply compared to days to anthesis. Among the yield component traits, kernel row ear^{-1} (3.34%) and 100-grain weight (3.26%) were the most affected under low N level. The reduction in grain weight may be due to poor seed development after fertilization. This could be the result of limitation in the source of photosynthetic products to seed development, which also reduces grain yield (8% with LN/HN ratio 0.92) much more than stover yield reduction (6% with LN/HN ratio 0.94). Similar magnitude of reduction in grain yield under low N was also reported by Bertin and Gallais (2000); Gallais and Hirel (2004); Li *et al.* (2011). Mean performance under both the environments indicated that high N level environment was more favourable for expression of most of the quantitative traits. Maximum percent increase in mean values under high N was observed in grain yield plant^{-1} (9.21%) followed by ASI (6.51%) and stover yield plant^{-1} (6.22%). Whereas, minimum increase in per cent in mean values was observed for harvest index (1.49%) (Table 1). Since, nitrogen and phosphorus are major essential nutrients for plants affecting directly to crop growth. Hence, low levels of N and P have many other nutrient deficiencies that results decreases in the crop growth rate and ultimately affects biomass and seed yield. The increase in the mean values of grain yield and stover yield could be due to harvest index (HI) may increase when N availability is increased or improved under limiting conditions (Ciampitti and Vyn, 2011), due to the curvilinear relationship between plant biomass and HI (Echarte and Andrade, 2003). It is therefore expectable that HI increases as N supply increases, up to a threshold where a maximum HI is reached.

Principal component analysis (PCA) has been widely used in plant sciences for the reduction of variables and grouping of genotypes. Kamara *et al.* (2003) used PCA to identify traits of maize (*Zea mays* L.) that accounted for most of the variance in the data. Presence of the genetic variability for the characters and identification of superior genotypes are the basic prerequisites for any successful breeding program. In the present study, nitrogen efficiency was assessed using principal component analysis of 11 parameters of the 256 full sibs. The 11 parameters at high N along with 11 indexes at low N relative to high N differed significantly among full sibs. According to principal component analysis on 11 traits, seven principal components (PC) had eigen values more than one and accounted for 75.32% of the total variance in the data (Table 2). The relative weight of each principal component was weighted by the corresponding contribution rate accounting for variation of all the traits.

The N efficiency of maize full sibs was determined by cluster analysis according to the composite parameters (*F* values) of each full sib. As the result, all full sibs were classified into three clusters: cluster 1 (*F* value >0.46) as N efficient full sibs, cluster II (-0.30 < *F* value < 0.4) as medium N efficient full sibs and cluster III (-0.34 < *F* value) as N inefficient full sibs (Fig. 1). On the basis of the above classification, the first cluster consisted

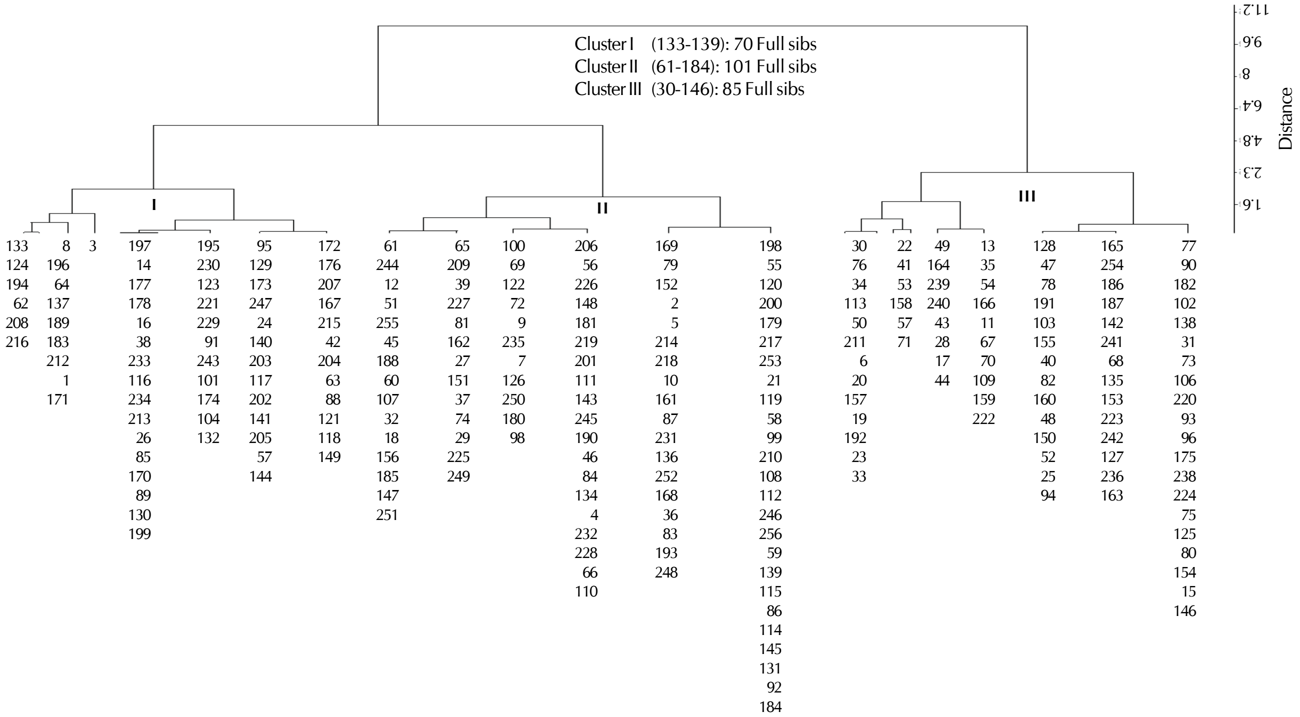

Figure 1: Clustering of 256 full sib's families of maize for N efficiency by Ward cluster method according to the *F* values of each full sib where *F* values are the composite parameters for assessing N efficiency obtained from principal component analysis. Cluster I-III represents high N efficiency, moderate N efficiency and low N efficiency, respectively

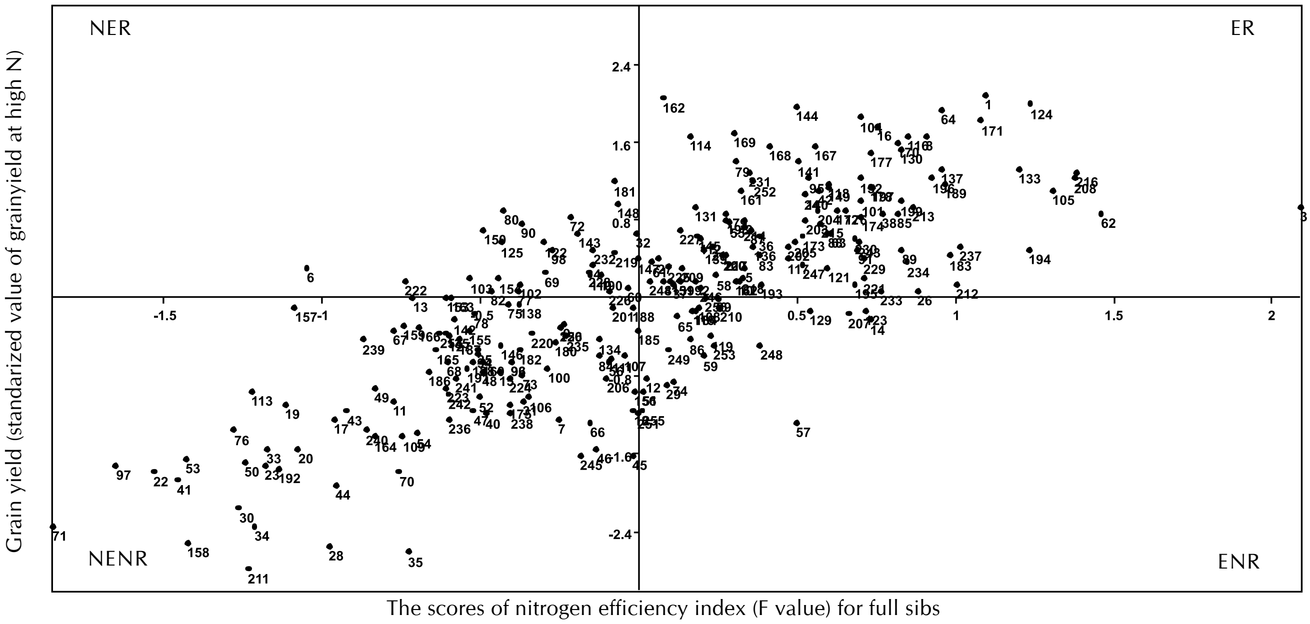

Figure 2: Plotting of 256 full sibs families of maize according to N efficiency index and standardized values of grain yield per plant under high N. N efficiency index obtained from principal component analysis. Standardized values of grain yield plant^{-1} are calculated as the following function: Xs=(X-X)/SD. Categories represented by efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR)

of 70 full sibs were distinguished by a high relative performance in yield and its component traits compared to the low N environment. The second cluster was consisted of 101 moderately N efficient full sibs and the third cluster consisted of 85 N inefficient full sibs. This study revealed that 70 full sibs had the lowest growth and yield reduction and highest level of N efficiency (*F* value >0.46) under low N level and 101 full sibs had the medium growth and yield reduction and high level of N efficiency (-0.30 < *F* value < 0.4) under low N level, while 85 full sibs had the low growth and yield reduction and lowest N efficiency (-0.34 < *F* value) under low N level. This indicated considerable genotypic variability was found in response to N efficiency in these maize full sibs. The most N efficient, 70 full sibs (cluster I) were identified with responsive to increased availability of N in the soil. Hence, the potential of these full sibs could be exploited through isolating inbred line to develop superior hybrids for low and high input farming systems.

The 256 full sibs in both N level environments were further classified into four group according to their N efficiency (*F* values) and yield potential (standardized values of grain yield plant^{-1} at high N) as per Bayuelo-Jimenez *et al.* (2011). The categories were (i) efficient and responsive (ER); (ii) non-efficient and responsive (NER); (iii) non-efficient and non-responsive (NENR); and (iv) efficient and non-responsive (ENR) (Fig. 2). This study indicated that highest number of full sibs (112) were efficient and responsive followed by non-efficient and non-responsive (92), non-efficient and responsive (31) and efficient and non-responsive (21). When the combination of nitrogen efficiency index (NEI) with N responsiveness at high N was considered, 70 full sibs (Fig.1) and 112 full sibs (Fig. 2) were the best full sibs for N-deficient soils of this region. These full sibs were grouped as high N efficient and most N responsive to increased N availability. It was indicated that the N efficient full sibs generally had considerably high yield potential at high N, whereas the N inefficient full sibs did not always had low yield potential at high N level. This study helps in identifying full sibs that was not only efficient and responsive under low N conditions (ER) but also remains high yield potential with applied N fertilizers (ENR). Thus, these N efficient full sibs could be suitable for high and low input farming systems would reduce cost of cultivation in the form of nitrogenous fertilizers.

Phenotypic correlation coefficients (r_p) calculated among the examined character in Mahidhawal population of maize full sibs were presented in Table 3. The sign of positive significant correlation among traits was all most consistent between two N levels. The estimates were either positive under both N levels, or negative, though there were some cases where the correlations were significant under one level but not in the other level. These were most predominant for grain yield plant^{-1} with kernel row ear^{-1} (0.16**) and stover yield plant^{-1} with number of cobs plant^{-1} (0.13*) under low N environment (E_2). However, harvest index was significantly correlated with number of cobs plant^{-1} (0.13*) under high N environment (E_1). This indicated that yield and its component traits significantly affected the magnitude and sign of correlation under both the environments. Hence, sufficient genotypic variability observed for these full sibs for their performance under low and high N level conditions. Sofi and Rather (2007) also observed strong correlation between grain yield and kernel row number.

The correlation coefficients of yield and its component traits revealed the presence of significant and positive phenotypic correlation of grain yield plant^{-1} with ear length (0.59**), ear girth (042**), 100-grain weight (0.42**), number of cobs plant^{-1} (0.13*), stover yield plant^{-1} (0.89**) and harvest index

Table 3: Phenotypic correlation coefficients (r_p) among various traits under high N (E_1, below diagonal) and low N (E_2, above diagonal) environments, and r_p between E_1 and E_2 (diagonal, underlined with bold face) environments for various characters in Mahidhawal population of maize

	DFT	DFS	ASI	EL	EG	KRE	HGW	NCP	GYP	SYP	HI	NEI
DFT	0.685**	0.976**	0.083	-0.081	-0.076	-0.155*	-0.091	0.023	-0.144*	-0.070	-0.209**	0.0009
DFS	0.962**	0.639**	0.297**	-0.089	-0.105	-0.168**	-0.114	0.008	-0.161*	-0.098	-0.195**	-0.016
ASI	0.053	0.323**	0.254**	-0.052	-0.147*	-0.090	-0.122	-0.062	-0.108	-0.140*	0.024	-0.079
EL	-0.093	-0.116	-0.102	0.640**	0.222**	-0.003	0.303**	0.125*	0.494**	0.470**	0.221**	0.560**
EG	-0.012	-0.029	-0.066	0.240**	0.580**	0.364**	0.345**	0.080	0.498**	0.516**	0.165**	0.560**
KRE	-0.005	-0.013	-0.028	-0.030	0.257**	0.687**	-0.140*	0.021	0.169**	0.104	0.195**	0.244**
HGW	-0.129*	-0.129*	-0.025	0.311**	0.303**	-0.205**	0.725**	0.058	0.419**	0.383**	0.222**	0.367**
NCP	0.119	0.110	-0.011	0.123*	0.097	0.042	-0.034	0.681**	0.125*	0.138*	0.013	0.376**
GYP	-0.186**	-0.186**	-0.034	0.591**	0.420**	0.019	0.424**	0.139*	0.560**	0.910**	0.563**	0.826**
SYP	-0.083	-0.106	-0.101	0.555**	0.379**	-0.070	0.404**	0.089	0.898**	0.610**	0.175**	0.744**
HI	-0.257**	-0.214**	0.108	0.268**	0.202**	0.143*	0.174**	0.139*	0.550**	0.147*	0.383**	0.483**
NEI	-0.029	-0.031	-0.010	0.610**	0.476**	0.229**	0.424**	0.366**	0.759**	0.657**	0.452**	-

*significant at $P < 0.05$ level; **significant at $P < 0.01$ level, DFT, days to 50% tasseling; DFS, days to 50% silking; ASI, anthesis silking interval; EL, ear length; EG, ear girth; KRE, kernel rows ear^{-1}; HGW, 100-grain weight; NCP, number of cobs plant^{-1}; GYP, grain yield plant^{-1}; SYP, stover yield plant^{-1}; HI, harvest index; NEI, Nitrogen efficiency index, gained from principal component analysis

(0.55**) under high N level (E_1). But grain yield plant^{-1} and harvest index was significant and negatively correlated with days to 50% tasseling (-0.18**,-0.25**) and days to 50% silking (-0.18**, 0.21**) respectively, under high N level (Table 3). The results are in accordance with Hafny and Aly (2008) observed that at high N level, grain yield of inbred lines negatively correlated with days to 50% tasseling and days to 50% silking and positive significant with harvest index. Bocanski *et al.* (2009) had also reported strong correlations between grain yield and 100-kernel weight.

On the other hand presence of positive significant phenotypic correlation (r_p) of grain yield plant^{-1} with ear length (0.49**), ear girth (0.49**), kernel row ear^{-1} (0.16**), 100-grain weight (0.41**), number of cobs plant^{-1} (0.12*), stover yield plant^{-1} (0.91**), and harvest index (0.56**) under low N level (E_2). But similarly as under high N environment, grain yield plant^{-1} and harvest index was also significant and negatively correlated with days to 50% tasseling (-0.14*, -0.16*) and days to 50% silking (-0.20**, 0.19**) respectively under high N (Table 3). Negative phenotypic correlations between grain yield and male and female flowering dates were also observed by Betran *et al.* (2003). Furthermore, in majority of the cases the magnitude of phenotypic correlation (r_p) of grain yield with other traits was numerically larger at low N level than high N level. This indicated that low N supply significantly affects phenotypic relationship of yield with its attributing traits. It might be due to efficient utilization of available nutrient and other resources in the soil. These results are in accordance with Presterl *et al.* (2002), Zaidi *et al.* (2003), Li *et al.*, (2011) reported that in majority of the cases the magnitude of r_p was numerically larger at LN level than HN level. The r_p between perfor-mances under high N and low N levels were highly signifi-cant ($P < 0.01$) for all the traits. It was highest for 100-grain weight (0.72**), was followed by days to 50% tasseling (0.68**), kernels row per ear (0.68**) and number of cobs plant^{-1} (0.68**) and the lowest for ASI (0.25**) followed by harvest index (0.38**). This indicated moderate to high relationship between both the environments. Similar results have also been reported by Li *et al.*, (2011).

The results of correlation between nitrogen efficiency index (NEI) and other traits indicated that N efficiency was significant positively correlated with ear length (0.61**), ear girth (0.47**), kernel row ear^{-1} (0.22**), 100-grain weight (0.42**), number of cobs plant^{-1} (0.36**), grain yield plant^{-1} (0.75**), stover yield plant^{-1} (0.65**), and harvest index (0.45**) under high N level (E_1). On the other hand, N efficiency index was was significant and positively correlated with ear length (0.56**), ear girth (0.56**), kernel row ear^{-1} (0.24**), 100-grain weight (0.36**), number of cobs plant^{-1} (0.37**), grain yield plant^{-1} (0.82**), stover yield plant^{-1} (0.74**) and harvest index (0.48**) under low N level (E_2). This also indicated that majority of the cases the magnitude of phenotypic correlation (r_p) of NEI with yield and its component traits was numerically larger at low N level (E_2) than at high N level (E_1). This may be because of high mobility of N and photosynthetic assimilates to kernel and stover resulting the magnitude of correlation was larger under low N level. Coque and Gallais (2007) also reported that more N was remobilized under low-N level.

This study conclude that 256 full sibs progenies at high N level seems to fully express their genetic potential for yield and other traits in this population. Maximum per cent increase in mean values under high N was observed in grain yield plant^{-1} followed by ASI and stover yield plant^{-1}. The magnitude of phenotypic correlation of grain yield and nitrogen efficiency index with other traits was numerically higher at low N level than high N level. This indicated that under low N level, studied traits had high association than under high N level. Based on multivariate analysis, available maize full sibs differ considerably in efficiency and responsiveness to N fertilizer. The population Mahidhawal had enough variability for the nitrogen efficiency and responsiveness to N, which can be mobilized in specific hybrid combinations to develop superior hybrids for high yield under N deficient soils and applied N fertilizers in maize.

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