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Regression of Grain Yield of Maize Inbred Lines and Their Diallel Crosses on Elevated Levels of Soil-Nitrogen

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RS and MMA managed the analyses of the study. Author THK performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Maize grain yield response to elevated levels of soil nitrogen is dependent upon genotype of the cultivar. Thus the optimum rate of N-fertilizer differs from maize genotype to another according to its nitrogen use efficiency (NUE). The main objective of this study was to determine the optimum N-rate for each studied inbred and hybrid that maximize grain yield. Six inbred lines of maize differing in their productivity under low-N were crossed in a diallel fashion to produce 15 F1´s. Parents and F1´s were evaluated in two seasons (2012 and 2013) using a split-plot design in randomized complete blocks arrangement with 3 replications. Main plots were allotted to four N-rates, i.e. 0, 80, 160 and 240 kg N/fed for N1, N2, N3 and N4, respectively. The sub-plots were assigned for the genotypes. Reducing N-level from 204 to 160, 80 and 0 kg N/feddan (fed) [one fed = 4200 m2] caused an increase in days to silking (DTS), anthesis silking interval (ASI), barren stalks (BS), economic NUEe and biological NUEb NUE and a decrease in the remaining studied traits including grain yield and its component. Maximum increase and decrease in traits occurred at N1 level (0 kg N/fed). The inbred lines L17, L18 and L53 proved to be tolerant (T), while L29, L54 and L55 inbred lines were sensitive (S) to N stress. The most tolerant crosses to low-N stress and the most responsive crosses to elevated levels of nitrogen were identified. Only two crosses (L18 × L53 and L18 × L55) showed high tolerance to low-N stress and responsiveness to high-N expressed in grain

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yield per plant (GYPP) and per fed (GYPF). The T×T group of crosses exhibited better performance in most studied traits than T×S and S×S groups of crosses under the low and high N levels. The three inbred lines L53, L18 and L17 and the three groups of crosses [inefficient responsive (IR), efficient non-responsive (ENR) and inefficient non-responsive (INR)] showed a quadratic response to the elevated levels of nitrogen with an optimum N level of 180 kg N/fed, while the three inbred lines L54, L29 and L55 and the efficient responsive (ER) group of crosses (L18 × L53 and L18 × L55) showed near linear response to elevated N levels.

Keywords: Zea mays; Low-N; tolerance; responsive; NUE; prolificacy; ASI.

1. INTRODUCTION

Maize is an important cereal crop that is grown as food for human and feed for livestock. Its grain constitutes about 9.7396 % grain protein, 4.85% grain oil, 9.4392% grain crude fibre, 71.966% grain starch, 11.77% embryo while fodder contains 22.988% acid detergent fibre, 51.696% neutral detergent fibre, 28.797% fodder cellulose, 40.178% fodder dry matter, 26.845% fodder crude fibre, 10.353% fodder crude protein and 9.095% fodder moisture [1-2]. Nitrogen is the most important nutritive element for the production of maize. One of the reasons responsible for low productivity of maize is using lower rates of nitrogen fertilizer than that recommended by the Ministry of Agriculture. Most Egyptian farmers use low-N fertilizer rates because of high price ratio between fertilizer and grain. Limited availability of N fertilizers and low purchasing power of farmers continued to be an important yield limiting factor in farmer's field. In that context, Gerner and Harris [3] revealed that price ratios between fertilizer and grain are high where fertilizer is not subsidized, and the supply of fertilizer often limits its use. Hybrid maize breeding programs in Egypt and allover the world concentrated their activity in the last decades on developing high- yielding hybrids under high soil-N conditions, *i.e.*, hybrids of high N-responsiveness. Current breeding programs should pay attention to develop hybrid corn of high tolerance to the low soil nitrogen conditions, prevailing in the lands of poor farmers who cannot afford to spend money for purchasing the recommended amount of nitrogen fertilizer, in addition to, its high-N responsiveness if grown under high-N conditions.

Breeding for tolerance to low-N is a difficult task because the genetic mechanisms that control the expression of such tolerance in crop plants is poorly understood and because of the polygenic nature of such a complicated character [4-5]. Such tolerance to low-N necessitates that plant breeder should improve the nitrogen use efficiency (NUE) under low-N. Significant and consistent differences have been reported in the accumulation and distribution of N to various plant parts among maize lines [5-7]. Laffitte and Edmeades [8] found that the low N tolerant cultivars are superior in the utilization of available N, either due to enhanced uptake capacity or because of more efficient use of absorbed N in grain production. Wiesler et al. [9] reported that high N efficiency was achieved by a combination of high N uptake and high N utilization in maize.

Modern hybrids have shown tendencies to withstand higher levels of stress (*i.e.*, low N), which allow them to better sustain suitable photosynthetic rates, appropriate assimilate supplies, and maintain plant growth rates attributable to enhanced nitrogen use efficiency [10-11]. Along with the prevailing belief that high yields require more N, the idea that different hybrids respond differently to low-N should be considered. Moreover, different hybrids may behave differently in their tolerance to low-N stress [12]. Therefore, the objectives of present study were: (i) To identify tolerant maize hybrids to low-N and of high responsiveness to the elevated levels of N rates, (ii) To study the effects of reducing N level on traits of inbred lines and hybrids under investigation and (iii) To determine the optimum N application for maximizing the grain yield for each of studied genotypes.

2. MATERIALS AND METHODS

This study was carried out during the growing seasons of 2011, 2012 and 2013 at the Agricultural Experiment and Research Station of the Faculty of Agriculture, Cairo University, Giza, Egypt (30° 02' N latitude and 31° 13' E longitude with an altitude of 22.50 meters above sea level). Six maize inbred lines in the 6th selfed generation (Table 1), showing clear differences in performance and general combining ability for grain yield/feddan (fed) under low-N were chosen as parents of diallel crosses. During 2011 crop growing season, all possible diallel crosses (except reciprocals) were made among the six parents, and seeds of the 15 direct F_1 crosses were obtained. Two field evaluation experiments were carried out in 2012 and 2013 years. Each experiment included 15 F_1 crosses, their 6 parents and 5 check cultivars, *viz.,* SC 10 (white grains), SC 128 (white grains) and SC 173 (yellow grains) obtained from the Agricultural Research Center (ARC) and SC 2055 (yellow grains) and SC 2066 (yellow grains) obtained from Hi-Tech Company-Egypt.

Table 1. Designation, origin, and most important traits of 6 inbred lines (L) used for making diallel crosses of this study

Entry designation	Origin	Institution (country)	Productivity under low-N
L17-Y	SC 30N11	Pion. Int. Co.	High
L18-Y	SC 30N11	Pion. Int. Co.	High
L53-W	SC 30K8	Pion. Int. Co.	High
L29-Y	Pop 59	ARC-Thailand	Low
L54-W	SC 30K8	Pion. Int. Co.	Low
L55-W	SC 30K8	Pion. Int. Co.	Low

ARC = Agricultural Research Center, Pion. Int. Co. = Pioneer International Company in Egypt, SC = Single cross, W = White grains and Y = Yellow grains

Evaluation in each season was carried out under four nitrogen levels, *viz.*, N1, N2, N3 and N4 (0, 80, 160 and 240 kg N/fed, respectively). Nitrogen fertilizer was added in two equal doses in the form of Urea before 1st and 2nd irrigations. The preceding crop was the Egyptian clover (*Trifolium alexandrium* L.). Available soil nitrogen in 30 cm depth was analyzed immediately prior to sowing. The available nitrogen to each plant (including soil and added N) was calculated for each nitrogen levels and found to be 3.0, 6.3, 9.6 and 12.9 g N/plant during 2012 season and 2.8, 6.1, 9.4 and 12.7 g N/plant during 2013 season, with an average across the two seasons of 2.9, 6.2, 9.5 and 12.8 g N/plant for the four nitrogen levels (N1, N2, N3 and N4), respectively. A split-plot design in randomized complete blocks (RCB) arrangement with three replications was used. The main plots were devoted to nitrogen levels (N1, N2, N3 and N4). The sub-plots were assigned to 26 maize genotypes (6 inbred parents, 15 F_1 hybrids and 5 checks). Each experimental plot consisted of one ridge of 4 m long and 0.7 m width. Before the 1st irrigation, hills were thinned to one plant/hill. The soil of the experimental site was clayey loam. All other agricultural practices were followed according to the recommendations of ARC, Egypt. Data were collected for days to 50% silking (DTS), plant height (PH), ear position (EP), anthesis-silking interval (ASI), percent of barren stalks (BS), chlorophyll concentration index (CCI) measured by Chlorophyll Concentration Meter, Model CCM 200 as the ratio of transmission at 931 nm to 653 nm through the leaf of top-most ear (http://www.apogeeinstruments.co.uk/apogee-instruments-chlorophyll-content-meter-technical-information/). At harvest, ears per plant (EPP), kernels per plant (KPP), 100-kernel weight (100-KW), grain yield per plant (GYPP), grain yield per feddan (GYPF), total above ground dry matter plant^{-1} (TDM), harvest index (HI), economic nitrogen use efficiency (NUE_e) calculated as follows: $NUE_e = GDM/N_s$, where GDM = grain dry matter and N_s = available soil-N/plant and biological nitrogen use efficiency (NUE_b) as follows: $NUE_b = TDM/N_s$ were measured. The NUE_e and NUE_b were calculated according to Moll et al. [13].

Biometrical Analysis

Combined analysis of variance of the split-plot design across the two years was performed as the homogeneity of variance test was non-significant. LSD values were calculated to test the significance of differences between means according to Snedecor and Cochran [14]. Grouping of genotypes based on tolerance and responsiveness was performed according to Sattelmacher et al. [15] and Worku et al*.,* [16]. For each genotype or group of genotypes, regression function was performed for nitrogen level effects by Microsoft Office Excel 2010 computer software. Rank correlation coefficients were calculated using SPSS 17 computer software between pairs of four nitrogen levels. The significance of the rank correlation coefficient was tested according to Steel et al*.,* [17].

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance was carried out for the studied 26 genotypes (G) of maize (6 inbred lines +15 F_1's + 5 check commercial single-cross hybrids) under the four nitrogen (N) levels (Table 2). Mean squares due to years were significant (P ≤ 0.01) for all studied traits, except for anthesis-silking interval (ASI), plant height (PH), ears/plant (EPP) and 100-kernel weight (100-KW), indicating significant effect of climatic conditions on most studied traits. Mean squares due to N levels and genotypes were significant (P ≤ 0.01) for all studied characters, indicating that each of the two main factors in this study, *i.e.,* N level or genotype has an obvious effect on all studied traits. Mean squares due to the 1st order interaction, *i.e.,* N×Y, G×Y and G×N were significant (P ≤ 0.01) for all studied traits, except for days to silking (DTS) and chlorophyll concentration index (CCI) for N×Y, DTS, ASI, BS, EPP, kernels/plant (KPP), harvest index (HI) and economic nitrogen use efficiency (NUE_e) for G×Y. Mean squares due to the 2nd order interaction G×N×Y were insignificant for all studied traits, except for PH, ear position (EP), BS and grain yield/plant (GYPP), which were significant, indicating that the rank of maize genotypes differ from nitrogen level to another and from one year to another and the possibility of selection for improved performance under a specific soil nitrogen [8,18-23].

3.2 Effects of low-N

The effect of the four levels of nitrogen on the studied traits is presented in Table 3. The highest GYPP was obtained from the N4 which is logic, since available nitrogen for each plant was at maximum (12.8 g N/plant) across seasons and therefore we considered this N level as the best one for GYPP and the percent change, in different studied traits was calculated in relevance to this N level, either in case of increases or decreases.

Table 2. Analysis of variance of split plot design for studied 26 maize genotypes under four levels of nitrogen (N) combined across two years

SOV	df	Mean squares				
		DTS	**ASI**	**PH**	**EP**	**BS**
Years (Y)	1	**	ns	ns	**	**
Nitrogen levels (N)	3	**	**	**	**	**
N×Y	3	ns	**	**	**	**
Error	12	1.69	0.01	308.6	41.4	0.04
Genotypes (G)	25	**	**	**	**	**
G×Y	25	ns	ns	**	**	ns
G×N	75	**	**	**	**	**
G×N×Y	75	ns	ns	*	**	**
Error	400	1.26	0.0006	54.1	23.5	0.002
		CCI	**EPP**	**KPP**	**100-KW**	**GYPP**
Years (Y)	1	**	ns	**	ns	**
Nitrogen levels (N)	3	**	**	**	**	**
N×Y	3	ns	ns	**	*	**
Error	12	53.1	0.02	31899.8	10.6	873.4
Genotypes (G)	25	**	**	**	**	**
G×Y	25	**	ns	ns	**	**
G×N	75	**	**	**	**	**
G×N×Y	75	ns	ns	ns	ns	*
Error	400	6.5	0.007	3228.5	1.5	27.1
		GYPF	**TDM**	**HI**	**NUE_e**	**NUE_b**
Years (Y)	1	**	**	**	**	**
Nitrogen levels (N)	3	**	**	**	**	**
N×Y	3	*	**	**	**	**
Error	12	26.8	725.4	24.1	14.9	12.5
Genotypes (G)	25	**	**	**	**	**
G×Y	25	**	**	ns	ns	**
G×N	75	**	**	**	**	**
G×N×Y	75	ns	ns	ns	ns	ns
Error	400	0.8	23.3	1.4	1.3	1.7

** and ** indicate significance at 0.05 and 0.01 probability levels, respectively, ns = non-significance*

Table 3. Means of studied traits under N1, N2, N3 and N4 (0, 80, 160 and 240 kg N/fed, respectively) and relative change (%) compared to the N4 combined across two seasons

Parameters	N1	N2	N3	N4	N1	N2	N3	N4
	Days to 50% silking (DTS) day				**Anthesis-silking interval (ASI) day**			
Parents	70.7	71.5	72.9	73.8	5.1	2.6	2.3	2.3
Change %	-4.2	-3.0	-1.1	-	125.9	16.0	1.2	-
Crosses	67.6	69.3	68.8	71.8	4.1	1.7	1.4	1.6
Change %	-5.8	-3.5	-4.2	-	162.4	5.7	-12.1	-
LSD 0.05	N = 0.20, G = 0.42				N = 0.01, G = 0.01			
	Plant height (PH) cm				**Ear position (EP) %**			
Parents	177.6	204.0	200.8	195.4	42.0	46.3	49.6	43.6
Change %	-9.1	4.4	2.8	-	-3.6	6.1	13.7	-
Crosses	200.9	233.8	226.6	219.9	43.0	50.0	49.8	48.7
Change %	-8.7	6.3	3.0	-	-11.7	2.6	2.3	-
LSD 0.05	N = 2.65, G = 2.77				N = 0.51, G = 0.99			
	Barren stalks (BS) %				**Chlorophyll concentration index (CCI) %**			
Parents	30.4	13.4	5.8	4.3	28.9	45.0	52.0	56.4
Change %	607.0	212.0	35.0	-	-48.8	-20.3	-7.9	-
Crosses	16.5	0.4	0.0	0.1	33.7	57.9	62.9	64.6
Change %	19249.0	420.0	-100.0	-	-47.8	-10.4	-2.5	-
LSD 0.05	N = 0.03, G = 0.02				N = 0.39, G = 1.28			
	Number of ears per plant (EPP)				**Number of kernels per plant (KPP)**			
Parents	0.9	0.9	1.2	1.2	326.3	505.0	859.9	924.1
Change %	-27.2	-21.4	3.7	-	-64.7	-45.4	-6.9	-
Crosses	1.0	1.1	1.3	1.4	370.7	620.8	908.2	1103.3
Change %	-29.1	-17.5	-5.1	-	-66.4	-43.7	-17.7	-
LSD 0.05	N = 0.02, G = 0.03				N = 26.97, G = 21.43			
	100-kernel weight (100-KW) g				**Grain yield per plant (GYPP) g**			
Parents	27.1	32.0	36.1	40.2	87.8	111.0	124.3	163.8
Change %	-32.6	-20.3	-10.2	-	-46.4	-32.2	-24.1	-
Crosses	27.8	32.9	36.9	39.4	119.5	150.1	175.4	224.5
Change %	-29.3	-16.5	-6.3	-	-46.8	-33.2	-21.9	-
LSD 0.05	N = 0.49, G = 0.46				N = 4.46, G = 1.96			
	Grain yield per feddan (GYPF) ard/fed				**Total above ground dry matter per plant (TDM) g**			
Parents	12.0	20.4	22.6	21.7	211.3	255.9	277.5	322.3
Change %	-44.5	-5.9	4.5	-	-34.4	-20.6	-13.9	-
Crosses	16.2	29.7	34.6	30.0	256.6	307.7	338.3	391.9
Change %	-45.9	-1.0	15.5	-	-34.5	-21.5	-13.7	-
LSD 0.05	N = 0.78, G = 0.34				N = 4.07, G = 1.82			
	Harvest index (HI) %				**Economic nitrogen use efficiency (NUE_e) g/g**			
Parents	35.0	36.1	37.4	42.5	20.8	14.7	10.1	8.9
Change %	-17.6	-15.1	-12.0	-	134.1	65.4	13.9	-
Crosses	39.0	40.9	43.4	48.0	28.2	19.9	14.3	12.2
Change %	-18.7	-14.9	-9.7	-	131.9	63.1	17.2	-
LSD 0.05	N = 0.74, G = 0.44				N = 0.58, G = 0.44			
	Biological nitrogen use efficiency (NUE_b) g/g							
Parents	59.2	40.1	26.7	20.7				
Change %	186.2	93.8	29.2	-				
Crosses	71.9	48.2	32.6	25.2				
Change %	185.6	91.6	29.5	-				
LSD 0.05	N = 0.45, G = 0.50							

N = nitrogen, G = genotype and Change = 100(RE - E1)/E1, RE = Respective environment*

Mean grain yield/plant (GYPP) was significantly decreased due to N1, N2 and N3 by 24.1, 32.2 and 46.4% for inbred lines and 21.9, 33.2 and 46.8% for crosses, respectively. Effects of N1, N2 and N3 levels on the mean performance of GYPP were approximately similar to those effects on grain yield/fed.

It can be observed that the rigidity of the N stress on GYPP was at maximum (46.4 and 46.8% reduction for inbred lines and hybrids, respectively) under the N1 level (0 kg N/fed). Consistent to these results, reduction in grain yield due to N stress was reported by several investigators [6,20,24]. On the contrary, GYPF of both inbred lines and hybrids under the N3 showed a tendency of increase over that under N4. Reductions in grain yield resulted from N stress in both inbred lines and hybrids were associated with reductions in all yield components (EPP, KPP, 100-KW), harvest index, TDM, CCI, PH and DTS. Maximum reductions were exhibited by kernels/plant (64.7 and 66.4%) and CCI (48.8 and 47.8%) for inbred lines and hybrids, respectively under N1 due to severe stress. On the other hand, the low-N stress (0 kg N/fed) caused increases in BS, ASI, NUE_e and NUE_b. Maximum increases appeared by BS followed by NUE_b trait (Table 3). Increases in NUE_e and NUE_b are favorable, while those in BS and ASI are unfavorable. It is believed that under lower-N conditions than optimum, maize plants are forced to improve their NUE ability as means of coping with N-stress conditions, though this increase differs from one genotype to another. In this context, Pandey et al. [25] and Al-Naggar et al. [26], reported also that NUE increased as soil-N decreased. Moreover, elongation of anthesis-silking interval in this study due to N-stress was in full agreement with Monneveux et al. [27] and Al-Naggar et al. [26].

Rank correlation coefficients estimated for pairs of the studied (four) N environments for GYPF and GYPP are presented in Tables 4 and 5, respectively. In general, the magnitude and number of significant correlation coefficients for GYPF and GYPP were much higher in inbred lines than those in hybrids, indicating that the interaction of inbred lines with different environments (four N levels) was much less than that of F_1 crosses. The crosses have therefore higher ability to exhibit the differences between environments than the inbred lines, since heterozygotes are more responsive to improved environments than homozygotes, expressed in grain yield per feddan or per plant. This conclusion was previously confirmed by Rodrigues et al. [28] and Monneveux et al. [27].

Table 4. Rank correlation coefficient between pairs of four N levels for GYPF of parental inbred lines (above diagonal) and F_1 crosses (below diagonal) across two seasons

Environment	N1	N2	N3	N4
N1		0.09	0.03	-0.14
N2	-0.26		0.71*	0.77*
N3	-0.28	0.36		0.94**
E4	-0.01	0.27	0.39	

** and ** significant at 0.05 and 0.01 probability levels, respectively*

Table 5. Rank correlation coefficient between pairs of four N levels for GYPP of parental inbred lines (above diagonal) and F_1 crosses (below diagonal) across two seasons

Environment	N1	N2	N3	N4
N1		0.03	-0.14	0.03
N2	-0.19		0.94**	1.00**
N3	-0.37	0.36		0.94**
N4	-0.15	0.27	0.39	

** and ** significant at 0.05 and 0.01 probability levels, respectively*

In both inbred lines and hybrids, the level N1 (no N application) showed no correlation with any other environment for both GYPF and GYPP. The level N2 was correlated with N4 (the richest environment in N) for GYPF (0.77**) and GYPP (1.00**) and with N3 (high-N) for GYPF (071*) and GYPP (0.94**) for inbred lines. In the inbred lines the level N3 was significantly correlated with the N4 for GYPF and GYPP (0.94**). Maximum number of significant correlation coefficients was shown for GYPF and GYPP by N4 and N2 with two other N levels for inbred lines and hybrids (Table 4 and 5).

3.3 Genotype × nitrogen interaction

Means of each inbred line, cross and check for GYPP and GYPF under different nitrogen levels (0, 80, 160 and 240 kg N/fed) across seasons are presented in Table (6). The highest mean grain yield per plant and per feddan was recorded for the inbred lines L53 followed by L18 and L17 under all N-levels, while the lowest ones were exhibited by L55, L29 and L54, indicating that the first three inbred lines are tolerant (efficient) to low-N and responsive to higher N-levels, while the other three inbred lines could be considered sensitive (inefficient) and of low responsiveness. Results in Table (6) indicate the existence of cross × nitrogen interaction in most studied F_1 crosses for GYPP and GYPF. The rank of crosses for GYPP and GYPF under N1 was markedly changed under N2, N3 and N4 conditions. The most tolerant crosses to low-N for both GYPP and GYPF were L18 × L53, L53 × L29, L17 × L53, L29 × L54 and L18 × L29 in a descending order, while the most responsive crosses to elevated levels of nitrogen were L17 × L54, L29 × L55, L53 × L54, L17 × L18 and L53 × L55. Only two crosses (L18 × L53 and L18 × L55) showed high tolerance to low-N and responsiveness to high-N expressed in GYPP and GYPF. It was also noted that L18 is common to both crosses that were tolerant and responsive. The cross L18 × L53 was significantly superior in GYPF over the best check under N1 (SC 2066) by 6.53%. Superiority in GYPF was shown by L17 × L54, L29 × L55, L17 × L18 over the best check under N2 (SC 2066) by (15.82, 5.38 and 3.80%, respectively) and L17 × L54, L29 × L55 and L53 × L54 by 16.89, 5.63 and 1.9%, respectively over the best check under N3 (SC 2066) and L17 × L54, L29 × L55, L17 × L18 and L53 × L54 by 21.31, 8.23, 8.0 and 7.51%, respectively over the best check under N4 (SC 10).

Table 6. Means of grain yield per plant (GYPP) and grain yield per feddan (GYPF) of inbred lines, crosses and check cultivars under N1, N2, N3 and N4 (0, 80, 160 and 240 kg N/fed, respectively) combined across two seasons

Genotypes	GYPP				GYPF			
	N1	N2	N3	N4	N1	N2	N3	N4
	Inbred lines							
L17	64.5	138.5	145.0	154.5	11.4	22.5	27.1	29.1
L18	68.7	125.9	137.2	158.6	12.1	22.8	26.0	30.4
L53	76.1	148.6	149.7	164.5	13.5	24.4	28.4	31.2
L29	60.1	84.6	89.6	103.6	10.1	12.6	12.8	15.0
L54	67.5	88.5	94.1	116.8	11.3	13.8	14.1	17.3
L55	61.9	80.1	86.8	98.5	10.2	12.6	13.2	14.5
	Crosses							
L17XL18	96.1	178.1	194.2	224.7	16.5	32.8	37.2	44.6
L17XL53	103.3	132.0	142.8	162.8	18.5	25.4	26.9	30.8
L17XL29	92.2	127.6	136.4	147.9	15.9	23.2	25.7	28.0
L17XL54	91.1	202.1	229.2	260.1	16.1	36.6	43.6	50.1
L17XL55	85.0	127.5	138.3	147.5	15.2	22.9	25.8	27.7
L18XL53	116.2	128.1	141.4	196.0	21.2	28.2	26.3	37.0
L18XL29	101.3	132.0	139.6	160.0	18.2	25.0	26.3	30.6
L18XL54	98.9	117.4	142.1	156.3	17.6	24.4	26.7	28.9
L18XL55	101.0	144.3	153.1	201.5	18.2	28.7	29.2	38.6
L53XL29	105.1	152.4	160.4	170.0	18.8	27.2	30.4	32.4
L53XL54	62.7	191.9	199.9	226.7	10.3	30.9	38.0	44.4
L53XL55	70.5	173.2	184.4	208.2	12.4	29.7	35.4	41.3
L29XL54	102.4	123.5	130.1	143.1	18.4	23.2	24.3	27.0
L29XL55	90.9	196.3	209.3	234.5	15.9	33.3	39.4	44.7
L54XL55	95.0	124.5	124.3	150.5	17.0	23.0	23.4	28.5
	Checks							
SC 128	91.6	152.0	153.3	194.5	16.1	27.5	29.4	37.0
SC 173	92.4	156.5	164.5	204.3	16.3	28.8	31.2	38.9
SC 10	105.4	166.3	173.5	218.2	18.6	31.0	33.0	41.3
SC 2055	92.0	160.8	160.1	187.9	16.5	27.6	30.5	35.9
SC 2066	108.5	187.8	201.8	202.3	19.9	31.6	37.3	37.6
LSD 0.05	G = 1.96, N = 3.40, G×N = 4.46				G = 0.34, N = 0.60, G×N = 0.78			

G = Genotypes, N = Nitrogen

3.4 Superiority of Tolerant (T) Over Sensitive (S) Genotypes

The higher absolute GYPF and lower ratio of GYPF under low-N to yield under high-N were considered as an index of tolerance to low-N stress. Based on this index, the low-N tolerant (T) inbred lines were L17, L18 and L53, while the low-N sensitive (S) inbred lines were L29, L54 and L55. The F_1 crosses L18 × L53, L18 × L55 and L18 × L29 were, therefore, considered low-N tolerant and L53 × L54, L17 × L29 and L17 × L18 were considered sensitive crosses to low-N. Data averaged for each of the two groups (T and S) for inbred lines and hybrids differing in tolerance to low-N indicate that GYPF of low-N tolerant (T) was greater than that of the sensitive (S) inbred lines and crosses by 17.1 and 36.3%, respectively under N1 (3.57 g N/plant no N addition) conditions (Table 7).

Superiority of low-N tolerant (T) over sensitive (S) inbred lines in GYPF under low-N was associated with superiority in most studied traits, namely GYPP (10.5%), EPP (14.3%), KPP (39.9%), 100-KW (9.0%), HI (2.7%), NUE_e (10.0%), NUE_b (6.7%), BS (-11.2%), PH (-9.3%) and ASI (-5.8%). Superiority of T over S crosses in GYPF under low-N was due to their superiority in GYPP (28.3%), EPP (23.4%), KPP (8.0%), 100-KW (14.1%), HI (11.6%), NUE_e (32.1%), NUE_b (19.3%), BS (-62.8%) and ASI (-21.7%). The superiority of T over S under low-N for crosses was greater than that for inbred lines. This might be attributed to the high nitrogen use efficiency traits of the hybrids due to heterosis as compared to their inbred parents. These results are in agreement with those reported by Kling et al. [29] and Gama et al. [30]. CIMMYT breeders found that maize grain yield under low-N was closely related to some secondary traits such as improved N-uptake, high plant nitrate content, large leaf area, high specific leaf-N content, more ears per plant, short ASI and late leaf senescence [31-36].These results are in consistency with those reported by Al-Naggar et al. [26]. Reduction in barren stalks and shortening in ASI of tolerant as compared to sensitive inbred lines and hybrids in the present study are desirable and may be considered as important contributors to low-N tolerance [26,33].

3.5 Differential Response of T×T, T×S and S×S Crosses

Mean performance of traits were averaged across three groups of F_1 crosses, *i.e.,* T×T, T×S and S×S groups based on grain yield per feddan of their parental lines under stress and non-stress conditions, *i.e.,* parental tolerance to stress (low-N) and presented in Table 8. Number of crosses was 3, 9 and 3 for the T×T, T×S and S×S groups, respectively. In general, T×T crosses had (higher) favorable values for grain yield and its attributes and lower (favorable) values for ASI and BS than S×S and T×S crosses under low-N. Low-N T×T crosses were generally superior in most studied characters over other groups of crosses; Where T×S crosses were the most inferior (Table 8) under low-N stress conditions.

Table 7. Superiority (%) in some selected characters of the most three tolerant (T) over the most three sensitive (S) inbred lines and crosses to low-N under low-N (0 kg N/fed) combined across two seasons

Trait	Inbred lines			Crosses		
	T	S	% Superiority	T	S	% Superiority
GYPF (ard)♀	12.3	10.5	**17.1**	19.4	14.2	**36.3**
GYPP (g)	69.8	63.2	**10.5**	107.4	83.7	**28.3**
EPP	0.80	0.70	**14.3**	0.95	0.77	**23.4**
KPP	287.9	205.8	**39.9**	299.8	277.7	**8.0**
100-KW (g)	25.8	23.7	**9.0**	29.7	26.0	**14.1**
TDM (g)	187.2	174.3	**7.4**	242.5	207.0	**17.1**
HI (%)	30.9	30.1	**2.7**	37.1	33.2	**11.6**
NUE_e (g/g)	23.0	20.9	**10.0**	36.4	27.6	**32.1**
NUE_b (g/g)	75.5	70.7	**6.7**	99.2	83.1	**19.3**
BS (%)	35.8	40.3	**-11.2**	10.8	28.9	**-62.8**
PH (cm)	173.2	190.9	**-9.3**	206.9	202.7	**2.1**
ASI (day)	6.6	7.1	**-5.8**	4.4	5.6	**-21.7**

% Superiority = 100 × [(T – S)/S] and ♀ one ard = 140 kg of grains

Table 8. Trait differences averaged across 2012 and 2013 seasons for T×T, T×S and S×S groups of F_1 crosses for low-N tolerance under four nitrogen levels

Trait	T × T				T × S				S × S			
	N1	N2	N3	N4	N1	N2	N3	N4	N1	N2	N3	N4
DTS (days)	65.9	66.5	67.2	66.4	66.7	69.1	70.0	70.5	69.0	69.4	69.4	69.9
ASI (days)	4.6	2.3	1.1	1.3	5.3	2.8	1.6	1.5	5.6	2.9	1.6	1.5
PH (cm)	207.1	231.3	237.3	249.4	207.2	225.3	240.5	228.2	213.5	228.7	238.4	234.2
BS (%)	12.3	4.2	0.2	0.0	21.3	7.2	0.4	0.0	19.8	7.0	1.0	0.2
EPP	0.9	1.1	1.2	1.2	0.9	1.1	1.1	1.3	0.8	1.0	1.1	1.2
KPP	342.9	672.0	714.5	958.5	272.4	603.6	620.1	918.2	287.4	600.1	636.1	876.9
100-KW (g)	29.1	33.4	33.7	37.5	26.6	31.7	32.3	36.3	27.6	32.6	33.1	37.0
GYPP (g)	105.2	153.0	159.4	194.5	89.7	147.0	164.8	186.5	96.1	142.2	154.5	176.0
GYPF (ard)♀	18.7	28.8	30.2	37.5	15.9	27.6	31.2	35.8	17.1	26.5	29.0	33.4
TDM (g)	238.8	306.8	322.5	359.0	214.5	294.5	320.7	348.4	224.2	287.1	303.1	334.0
HI (%)	36.8	41.1	41.2	45.3	34.7	40.7	42.8	44.5	35.6	40.5	42.1	43.7
NUE_e (g/g)	35.4	23.6	20.2	15.2	30.1	21.9	20.9	14.6	32.0	21.7	19.4	13.7
NUE_b (g/g)	97.0	60.0	49.2	33.7	87.1	56.2	48.9	32.7	90.7	56.0	46.0	31.3

T = Tolerant, S = Sensitive, N1 = (0 kg N/fed), N2 = (80 kg N/fed), N3 = (160 kg N/fed), N4 = (240 kg N/fed) and ♀ one ard = 140 kg of grains

This indicates that the tolerant cross to low-N should include two tolerant parents and assure that low-N tolerance trait is quantitative in nature, so the tolerant cross accumulates additive genes of low-N tolerance from both parents. Superiority of low-N T×T crosses over S×S and T×S crosses was more pronounced under high-N conditions, indicating that these T×T crosses are tolerant to low-N and responsive to high-N conditions.

Grain yield per feddan of low-N T×T (18.7 ard) was greater than that of S×S (17.1 ard) and T×S (15.9 ard) by 9.36 and 11.32%, respectively. Superiority of low-N T×T over S×S and T×S crosses in GYPF under low-N conditions was due to their superiority in GYPP by 9.1 and 15.5 g, KPP by 55.5 and 70.5, 100-KW by 6.1 and 2.5 g, TDM by 14.6 and 24.3 g, HI by 1.2 and 2.1%, NUE_e by 3.4 and 5.3 g/g and NUE_b by 3.3 and 9.9 g/g, respectively (Table 8). Moreover, the low-N T×T crosses were earlier in DTS by 3.1 and 0.8 day, of shorter ASI by 1.0 and 0.8 day and lesser BS by 7.5 and 9% than S×S and T×S crosses, respectively under low-N conditions.

3.6 Grouping Hybrids Based on Tolerance and Responsiveness

Mean grain yield per plant or per feddan and NUE_e across years of studied crosses under low-N was plotted against same trait of the same genotypes under high-N (Figs. 1 and 2) where numbers from 1 to 15 refer to F_1 hybrids names 1 = L17×L18, 2 = L17×L53, 3 = L17×L29, 4 = L17×L54, 5 = L17×L55, 6 = L18×L53, 7 = L18×L29, 8 = L18×L54, 9 = L18×L55, 10 = L53×L29, 11 = L53×L54, 12 = L53×L55, 13 = L29×L54, 14 = L29×L55 and 15 = L54×L55, which made it possible to distinguish between efficient and inefficient genotypes on the basis of above-average and below-average grain yield under low-N and responsive and non-responsive genotypes on the basis of above-average and below-average grain yield under high-N [14,16,31]. According to tolerance to low-N and responsiveness to high-N, studied crosses were classified into four groups, *i.e.,* N efficient and responsive, N efficient and non-responsive, N non-efficient and responsive and N non-efficient and non-responsive based on NUE_e and GYPF traits.

The F_1 crosses No. 1 (L17 × L18), No. 6 (L18 × L53) and No. 9 (L18 × L55) had the highest NUE_e under high-N and low-N, *i.e.*; they could be considered as the most N efficient and the most N responsive genotypes in this study (Fig. 1). On the contrary, the F_1 crosses No. 5 (L17 × L55) and No. 3 (L17 × L29) had the lowest NUE_e under both high-N and low-N and therefore could be considered inefficient and non-responsive (Fig. 1). Classification of the studied crosses into the previous-mentioned groups based on grain yield/fed (Fig. 2) was similar to that based on NUE_e (Fig.1), except the cross No. 1 (L17 × L18) which was shifted to the inefficient but responsive group. Based on both NUE_e and GYPF traits, the crosses No. 10, 2, 7, 13, 8 and 15 were classified as N-efficient but non-responsive genotypes, while the crosses No. 4, 14, 12 and 11 were classified as responsive to high-N, but N inefficient genotypes (Figs. 1 and 2).

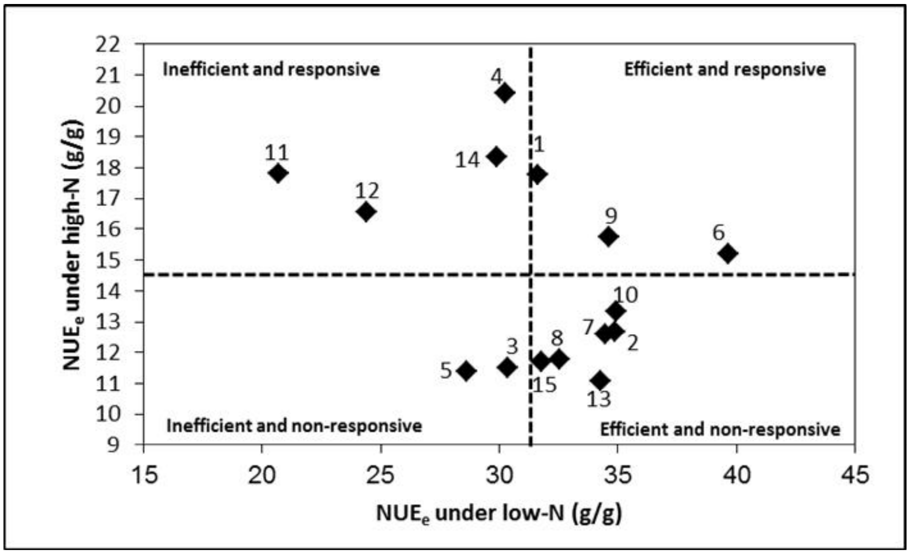

Fig. 1. Relationships between economic nitrogen use efficiency (NUE_e) of 15 F_1 maize hybrids under high- and low-N combined across two seasons. Broken lines represent mean of (NUE_e) (numbers from 1 to 15 refer to F_1 hybrids names)

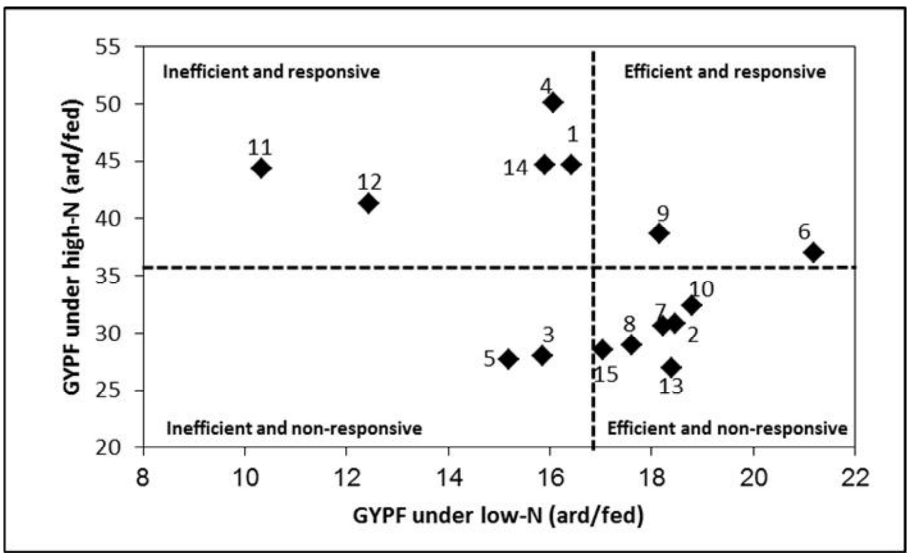

Fig. 2. Relationships between grain yields per feddan (GYPF) of 15 F_1 maize hybrids under high- and low-N combined across two seasons. Broken lines represent mean of GYPF (numbers from 1 to 15 refer to F_1 hybrids names)

3.7 Identifying Optimum Appropriate N Application

Data were reanalyzed to evaluate GYPF responses of inbred lines and hybrids across varying levels of stress *via* regression technique. For each genotype or group of genotypes, quadratic regression function was performed for N rate. The regression functions were used to distinguish which treatments provide optimum value for each genotype (or group of genotypes). The relationship between nitrogen levels and grain yield/fed of the inbred lines across two years is illustrated in Fig. 3. It is obvious that the quadratic response of the three inbred lines L53, L18 and L17 to the increase of N level was much higher than that of the remaining three inbred lines L54, L29 and L55, indicating that the first three inbred lines are more responsive to elevated N levels than the latter inbred lines. The quadratic regression function is clearly expressed for the first three lines (responsive inbred lines to N) compared with that of the latter inbred lines, which showed a weak nearly linear regression (Fig. 3).

The optimum N level for the three responsive inbred lines is about 180 kg N/fed. The latter three inbred lines showed very small linear increase in GYPF by increasing N level up to 240 kg N/fed, but such increase in GYPF is not favorable, because of non-profitability from the economic point of view and the resulting pollution in the environment due to the excess of nitrogen. Fig. 4 shows the relationship between nitrogen levels and GYPF of the four categories of F_1 crosses, previously grouped in Fig. 2, *i.e.*, inefficient and responsive (IR), efficient and responsive (ER), efficient and non-responsive (ENR) and inefficient and non-responsive (INR) across two years. The IR, ENR and INR groups of F_1 crosses showed a quadratic (curvilinear) response to N level, with an optimum N of about 180 kg N/fed. While, the ER group of crosses (L18 × L53 and L18 × L55) showed near linear response to elevated levels of N. The IR group of crosses showed the highest quadratic response to N level, which is logic since these hybrids (L17 × L18, L17 × L54, L53 × L54, L53 × L55 and L29 × L55) are the most responsive ones in this experiment to elevated levels of nitrogen. On the contrary, the ENR and INR groups of crosses showed the lowest quadratic response to N. In this context, the corn grain yield typically exhibits a quadratic response to low-N with a near-linear increase across a range of N-levels, a gradually decreasing rate of yield increase relative to N-levels decrease and finally a yield plateau at some relatively low-N level [37-39].

Most recently Clark [11] mentioned that there was little yield response to N rates above 90 kg N/ha at the low and high densities, as there was a curvilinear increase until yield plateau at the low density (8.1 Mg/ha at 133 kg N/ha) and the high density (5.9 Mg/ha at 102 kg N/ha). He added that response to N was greatest at the middle density (83,980 plants/ ha), as there was a quadratic response with maximum yield at 188 kg N/ha (8.7 Mg/ha). He found that across the low-stress environments, the lowest density (44,460 plants/ha) responded little to N rates above 90 kg N/ha, while there was greater response to N rates at the middle density (13.5 Mg/ha at 162 kg N/ha) and the high density (13.4 Mg/ha at 174 kg N/ha).

He concluded that no support was found for the idea that increasing corn yield requires increases in both plant density and N rate above rates typically used. A recent Indiana study [36] showed that under large ranges of plant density (54,000-104,000 plants/ha) and N rate (0-330 kg N/ha), higher densities required more N. This seems logic, given the prevailing belief that high yields require more plants,

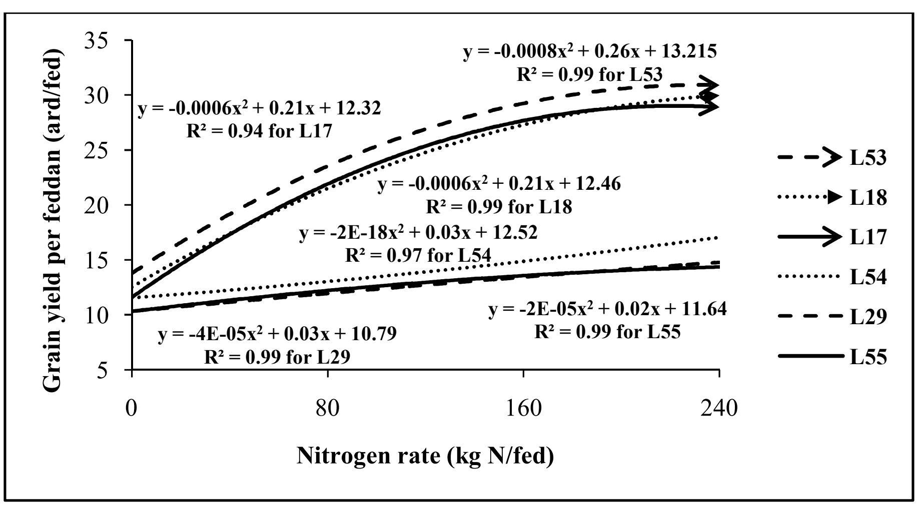

Fig. 3. Relationship between GYPF of inbred lines and nitrogen levels across two seasons

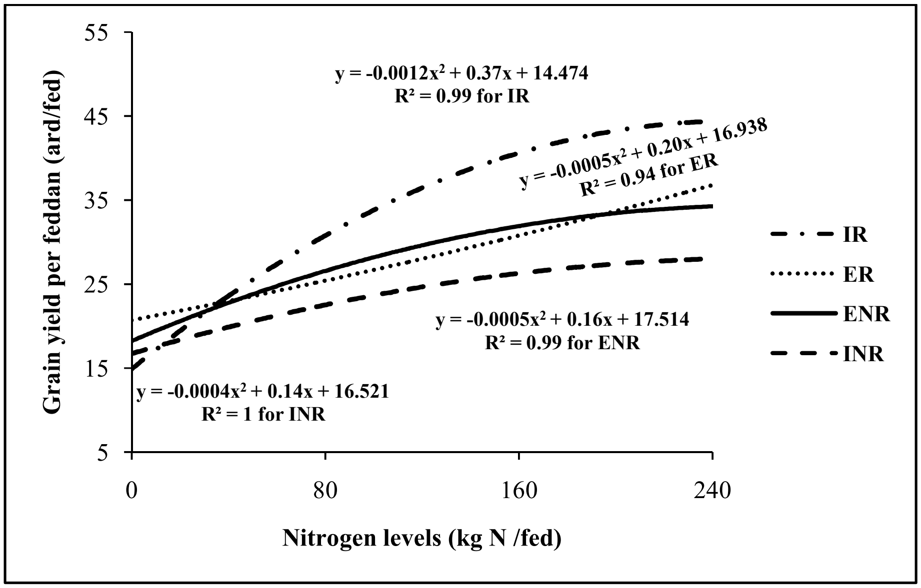

Fig. 4. Relationship between GYPF of four groups of F_1 crosses, namely, five inefficient and responsive (IR), two efficient and responsive (ER), six efficient and non-responsive (ENR) and two inefficient and non-responsive (INR) crosses and nitrogen levels across two seasons

and that more plants require more N. Their and our results advance our understanding of N rate-plant density interaction within contrasting environmental conditions, but understanding the complexities of hybrid interactions with N rate and plant density will require additional work.

4. CONCLUSION

1. The present investigation showed that some crosses were superior over commercial crosses under the four studied N levels, i.e., the cross L18 × L53 was significantly superior in GYPF over the best check under N1 (SC 2066) by 6.53%, the crosses L17 × L54, L29 × L55 and L17 × L18 over the best check SC (2066) under N2 by (15.82, 5.38 and 3.80%, respectively), the crosses L17 × L54, L29 × L55 and L53 × L54 (16.89, 5.63 and 1.9%, respectively) over the best check (SC 2066) under N3 and L17 × L54, L29 × L55, L17 × L18 and L53 × L54 by 21.31, 8.23, 8.0 and 7.51%, respectively over the best check (SC 10) under N4, thus, using these crosses in breeding program will increase the benefit to our society from the reduced of N application and additional cost of farmers.
2. Tolerant genotypes to low-N are characterized by more grain yield/plant, more ears/plant, high harvest index, high total dry matter, short anthesis-silking interval, less barren stalks, less plant height and lower ear position than sensitive genotypes. Identification of low-N stress tolerance-traits would help to plan indirect selection and marker assisted selection for yield under stress.
3. The three inbred lines L53, L18 and L17 and the three groups of crosses (IR, ENR and INR) showed a quadratic response to the elevated levels of nitrogen with an optimum N level of 180 kg N/fed, while the three inbred lines L54, L29 and L55 and the ER group of crosses (L18 × L53) and (L18 × L55) showed near linear response to elevated N levels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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