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Morpho-physiological and biochemical characterization of maize genotypes under nitrogen stress conditions

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Abstract

Forty maize inbred lines were assessed at seedling, vegetative and flowering plant growth stages for nitrogen use efficiency under nitrogen sufficient and deficient soil conditions. Significant variations were observed in the morpho-physiological and agronomical traits. Inbred lines were classified into two major clusters based on the response of morphological and physiological traits to nitrogen limiting conditions. Cluster 1(C1) I included DMI 4, DMI 5, DMI 22, DMI 27 and DMI 56 inbred lines-derived from the drought or thermal tolerant parents HKI335 and LM17 with less reduction in growth-related parameters as compared to cluster CII inbred lines, which were derived from sensitive genotypes MGUD22 and HKI1015wg8. The enzymatic activities of nitrate reductase (NR) and glutamine synthetase (GS) were observed to be more critical for screening in the early growth stage. The identified inbred lines have the potential for developing maize hybrids with improved nitrogen use efficiency.

Key words: Maize, inbred line, SPAD, Nitrogen Harvest Index, anthesis-silking-interval

Introduction

Maize (*Zea mays* L.) is one of the most important staple food in many countries of the world and the third most important crop in India after rice and wheat. Maize contributes ~45% to global grain production. India is the seventh-largest producer and contributes about 2.6 % of the global maize production (FAO 2018). In India, it constitutes ~10% of the total volume of food grains produced (DAC & FW 2020). Maize is not only used for human food and animal feed but also utilized as a bio-energy crop and in the synthesis of biochemical compounds (fibre, plastic, adhesive, etc.). The C_4 photosynthesis in maize allows very efficient conversion of CO_2 into carbohydrates and finally green biomass and yield, especially under conditions of optimum nitrogen (N) supply (Ghannoum et al. 2010). Nitrogen is an essential nutrient required for the growth and development of plants. It is an essential building block of numerous biological compounds including amino acids, proteins, nucleic acids, chlorophyll and some plant hormones (Kraiser et al. 2009). The genotypic variation exists in all the maize genotypes for nitrogen use efficiency (NUE). The genotypes with contrasting morpho-physiological traits respond to N availability differently (Hirel et al. 2007). Low N availability in most of the agricultural lands of India is an important yield-limiting factor (Bänziger et al. 2000). Further, the cultivation of high-yielding maize hybrids is always associated with the application of large quantities of N fertilizers. The NUE for cereals including maize has been estimated to be less than 50%, consequently, more than half of the applied N is lost from the soil, which severely pollutes the environment in different ways (Raun and Johnson 1999; Sutton et al. 2011). For example, fertilizer-driven nitrous oxide emissions into the atmosphere contribute to the depletion of the ozone layer (Cameron et al. 2013; Fowleret al. 2013). Thus the development of maize hybrids having high NUE is crucial not only to reduce the cost of cultivation but also to reduce the environmental footprint. High NUE hybrids can be developed if we have high nitrogen use efficient inbred lines for hybrid development. In this endeavor, a set of forty inbred lines was evaluated for NUE under N

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sufficient and limiting conditions. Observations were recorded on important morpho-physiological and biochemical parameters. The objectives of the study were to assess the variation in response to N availability, and to identify high-yielding and N efficient maize inbred lines for breeding programs.

Materials and methods

Plant materials and experimental design

A set of forty inbred lines of maize (Table 1) were received from the ICAR-Indian Institute of Maize Research, Ludhiana, Punjab. These inbred lines represent diverse germplasm developed from the crosses of genotypes with varied characteristics. The experiment was conducted under nitrogen sufficient and limiting conditions during *Kharif* 2016, 2017 and 2018 at Research Farm of ICAR-Indian Agricultural Research Institute, New Delhi. The residual soil nitrogen content of the research farm for three years was 62.23% in 2016, 42.19% in 2017 and 38.13% in 2018. The maize inbred lines were allowed to grow under N

Table 1. A list of maize inbred lines studied. (DT and DS represent drought tolerance and drought sensitive, respectively)

S. No	Inbred Line	Source Pedigree	Characteristics of source parent
1.	DMI 2	DTPY C9 F73-2-1 (F) ×HKI1532(M)	DTPY C9 F73-2-1 (F)= DT , HKI1532(M)=DS
2	DMI 4	HKI 1532(F) ×LM13(M)	HKI1532(F)=DS, LM13=Moderately tolerant
3	DMI 5	HKI 1532(F) ×LM17(M)	LM17=DT, HKI1532(F)=DS
4	DMI 8	DTPYC9F46-3-6(F) ×HKI1577(M)	DTPYC9F46-3-6(F)=DT
5	DMI 9	HKI1532(F) ×DTPYC9F46-3-6(M)	DTPYC9F46-3-6(M)= Moderately sensitive
6	DMI 10	CM 140 (J617-61)	CM 140= DT
7	DMI 13	LM 17(F) × HKI 1015wg8 (M)	LM17=DT, HKI 1015wg8=DS
8	DMI 14	CA14514 (F) × HKI 1015wg8 (M)	HKI 1015wg8=DS
9	DMI 22	HKI 335	HKI 335=DT
10	DMI 23	CM 139 (Tarun x Makki Safed1)-Y63	CM 139 = DS
11	DMI 25	BJIM 08-27	Intermediate response to drought stress
12	DMI 26	BJIM 10-36	Intermediate response to drought stress
13	DMI 27	BJIM 10-1	Intermediate response to drought stress
14	DMI 35	MGUD 22	Drought sensitive
15	DMI 44	HM-4	Intermediate response to drought stress
16	DMI 45	Vivek QPM-9	Drought tolerance
17	DMI 46	Bio-9681	Intermediate response to drought stress
18	DMI 47	JH3459	Intermediate response to drought stress
19	DMI 51	HTRIL-063(LM17 ×HKI 1015wg8)	LM17=DT, HKI 1015wg8=DS
20	DMI 55	HTRIL-027(LM17 ×HKI 1015wg8	LM17=DT, HKI 1015wg8=DS
21	DMI 56	HTRIL-093(LM17 ×HKI 1015wg8)	LM17=DT, HKI 1015wg8=DS
22	DMI 60	DTRIL-256 (HKI 335 x MGUD22)	HKI 335=DT,MGUD22=DS
23	DMI 61	DTRIL-123 (HKI 335 ×MGUD22)	HKI 335=DT,MGUD22=DS
24	DMI 62	DTRIL-159 (HKI 335 ×MGUD22)	HKI 335=DT,MGUD22=DS
25	DMI 63	DTRIL-120 (HKI 335 ×MGUD22)	HKI 335=DT,MGUD22=DS
26	DMI 64	DTRIL-156 (HKI 335 ×MGUD22)	HKI 335=DT,MGUD22=DS
27	DMI 66	DTRIL-150 (HKI 335 ×MGUD22)	HKI 335=DT,MGUD22=DS
28	DMI 71	DML 1029	Intermediate response to drought stress
29	DMI 74	DML 1104	Intermediate response to drought stress
30	DMI 75	DML 1112	Intermediate response to drought stress
31	DMI 76	DML 1117	Intermediate response to drought stress
32	DMI 77	DML 1126	Intermediate response to drought stress
33	DMI 81	DML 1230	Intermediate response to drought stress
34	DMI 83	DML 1276	Intermediate response to drought stress
35	DMI 90	DML 1429	Intermediate response to drought stress
36	DMI 95	DML 1610	Intermediate response to drought stress
37	DMI 96	DML 1620	Intermediate response to drought stress
38	DMI 97	DML 1687	Intermediate response to drought stress
39	DMI 98	DML 1722	Intermediate response to drought stress
40	DMI 102	DML 1648-1	Intermediate response to drought stress

sufficient (residual N+180 Kg/ha N added by chemical fertilizer) and N limiting (residual N only) conditions (Table 1). The experiments were conducted in a randomized block design (RBD) with three replications. Each experimental plot was comprised of 2 rows of 5m length with inter and intra row spacing of 60 and 20 centimeters, respectively. The crop was raised following the recommended cultural practices.

Evaluation of maize inbred lines

The soil textures were sandy and loamy types at experimental field of IARI, New Delhi. Organic carbon per cent, pH and electrical conductivity were 4.9 g/kg, 7.9 and 0.35 decisiemens per meter (ds/m) at 0-15 cm soil depth, respectively. Several morphological and physiological parameters were recorded at various plant growth stages, *viz*., seedling, vegetative and flowering. The plant height was measured at each phenological stage using one meter long wooden scale from the base of the main stem to the base of the last unfolded leaf at each stage and expressed in centimeters. Stem girth was measured using vernier calipers at 10 cm above the ground level. The numbers of leaves were counted at each stage. For leaf area, fully opened top-most leaf was used to measure leaf length and width and multiplied by k-shape factor with the value of 0.75 for maize (Montgomery 1911). Leaf color chart (LCC) and SPAD value was measured on the top most fully expanded leaf by leaf color chart and chlorophyll meter (SPAD-502, MINOLTA), respectively. The chlorophyll content was estimated by the Dimethyl sulphoxide (DMSO) method (Hiscox and Israelstam 1979). Freshly removed leaf was finely chopped and a 50 mg portion was dipped in a test tube containing DMSO. Total soluble protein content (mg g^{-1} FW) was measured on 300 mg sample of leaf (Bradford 1976). Fresh leaf samples were used for nitrate reductase (μ moles NO_3^- red h^{-1} g^{-1} FW) and glutamine synthetase (μ moles of glutamine formed min^{-1} g^{-1} FW) enzyme estimation by Nicholas and Nason (1957) and Lea et al. (1990), respectively. Nitrogen content in leaf, stem and root was estimated by using the Micro-kjeldahl method (AOAC 1970). The nitrogen harvest index (NHI) displays the extent of nitrogen translocation from vegetative to reproductive structures and calculated using the Good et al. (2004) method. Anthesis-silking interval was calculated as the number of days between anthesis and silking dates. For dry matter accumulation three plant samples were oven-dried at 60-65°C for 48 hours. It was measured in gram per plant. The yield associated traits as total number of ear per plant, ear length, ear girth, number of rows per ear, number of grains per row, ear height, total ear weight, total grain weight, and 100-grain weight were also recorded under nitrogen sufficient and limiting conditions.

Statistical analysis

Three biological replicates were used for morpho-physiological analyses at different growth stages. The analyses of variance of different parameters were calculated using the one-way ANOVA for statistically significant differences (*p*-value >0.05). The coefficient of correlation among all the traits was done using SPSS ver.19.0 software. The relation between morpho-physiological traits was analyzed using Jaccard's similarity index and average taxonomic distance which was calculated by NTSYS-pc v2.1 software (Rohlf 2002). Duncan's Multiple Range Test (DMRT) ($p= 0.05$) was used to evaluate differences among clusters for significance by using SPSS.

Results and discussion

Phenotyping under nitrogen sufficient and limiting conditions

Initially, forty inbred lines were screened under nitrogen sufficient and limiting conditions. Significant differences were found in morpho-physiological traits at different growth stages. The effect of nitrogen availability was first visibly recorded at the seedling stage where retarded growth and development were observed under N-limiting conditions. Similar, results have been reported by Barraclough et al. (2010) in wheat. Reduced rate of growth and development in plant canopy and vigor was visible in few inbred lines under N- limiting conditions while other lines performed well. Identification of maize inbred lines with low nitrogen requirements and high utilization is essential to develop nitrogen use efficient hybrids (Le et al. 2000; Mansour et al. 2017). For that purpose, a set of morphological traits were used for preliminary screening of 40 inbred lines based on similarity indices which classified them into two groups GI and GII. The group GI consisted of inbred lines that performed well under nitrogen limiting conditions whereas GII inbred lines had stunted growth and development. The inbred line, DMI47 was distinct and not included in these groups. Based on economic yield, a total of fifteen contrasting lines including ten high yielding and five low yielding lines were selected for further screening.Screening techniques relying on variation in growth and development of morphological traits are reliable in identifying contrasting maize germplasm lines in abiotic stress tolerance (Kumar et al. 2020).

Fifteen contrasting inbred lines from group GI and GII were then evaluated in the *kharif* 2017 and 2018. These inbred lines again formed two clusters CI and CII based upon varying response under N-limiting conditions. The cluster CI included a total of 5 inbred lines, *viz*., DMI 4, DMI 5, DMI 22, DMI 27 and DMI 56. These inbred lines performed well under N-limiting conditions compared to cluster CII lines, *viz*., DMI 2, DMI8, DMI 13, DMI 26, DMI 64, DMI 81, DMI 96, DMI 97, DMI 98 and DMI 102. Cluster CI consisted of inbred lines that were derived from the drought or thermal tolerant parent HKI 335 and LM 17. Similarly, cluster CII possessed inbred lines that were derived from the drought and thermal sensitive genotypes MGUD 22 and HKI 1015wg8. Considering the fact that, these lines possess the genetic region contributed by the tolerant and sensitive parent for abiotic stress conditions,*viz*., drought and thermal, inbred lines responded similarly under N-limiting conditions also. These lines in cluster CI and CII were classified as nitrogen efficient and inefficient in response to N limiting conditions, respectively.

The nitrogen efficiency percent for each trait is indicated by the relative increase or decrease of CI to CII, which was calculated as (CI – CII)/CII × 100. Traits such as NR and TSP showed a signiûcant difference under N-sufficient and limiting conditions ($p < 0.05$) (Fig. 1). Hirel et al. (2007) also reported that the enzymatic activity of nitrate reductase controls the leaf nitrogen content at the initial stage so it can be used as selection criteria for high nitrogen utilization. The inbred lines of the CI cluster had higher growth and development than cluster CII under N-limiting conditions. Higher economic yield signifies high dry matter accumulation and partitioning and helps in the identification of true nitrogen efficient lines (Echarte et al. 2004). Significant (p = 0.05) differences for all the traits were observed among both the clusters. The differences in the growth parameters and physiological traits indicated that inbred lines of cluster CI can yield higher under N-limiting conditions.

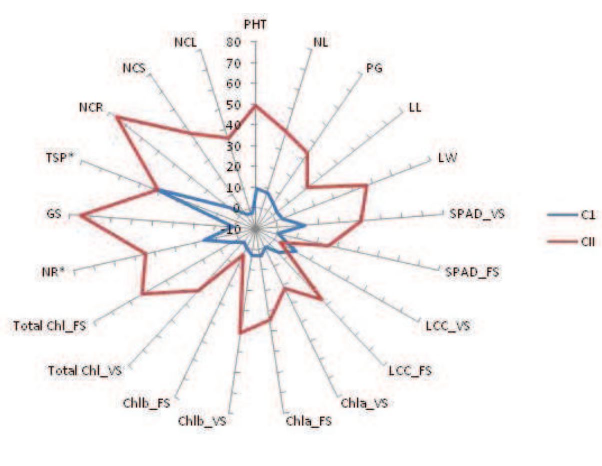

Fig. 1. The phenotypic difference between the two clusters *i.e.* CI and CII under sufficient and deficit nitrogen levels. The difference for each trait is indicated by the relative increase or decrease of CI to CII, which was calculated as (CI – CII)/CII × 100%. The red line and blue line represent the percentages under deficit and sufficient levels, respectively. Traits that showed a signiûcant difference between two N levels are labeled by an asterisk (*P < 0.05)

Morphological, physiological and biochemical trait analysis

Plant height is an important trait to measure the growth and development of any crop. It influences yield and yield associated traits in crops. The essential supply of nitrogen element in the required quantity is necessary for proper plant height (Karasu 2009). In the present study, various inbred lines responded differently to nitrogen stress leading to reduced plant growth (Fig. 2). Chlorophyll content represents the photosynthetic efficiency of leaves which affects economic yield (Schlemmer et al. 2013). Leaf area and total Chl content decreased from seedling to grain filling stage (Fig. 2). A similar reduction was observed

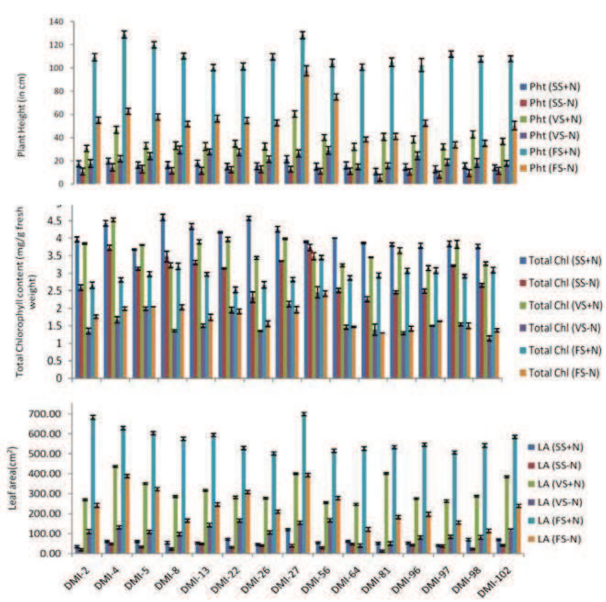

Fig. 2. Plant height, leaf area and total Chlorophyll morpho-physiological traits to the nitrogen (N) availability (N-deficit and -sufficient conditions) at seedling, vegetative, and flowering stages of fifteen maize inbred lines (represented on X-axis)

with N metabolism enzymes *i.e*., nitrate reductase (NR) activity, glutamine synthetase (GS) activity, and total soluble protein (TSP) (Fig. 3). For the development of nitrogen efficient inbred lines, NR (Nitrate Reductase), GS (Glutamine Synthetase) and TSP (Total Soluble Protein) are key parameters as they play prominent role in nitrogen metabolism (Forde and Lea 2007). In the initial growth stages, there were no visible effects of nitrogen deficit as plants get sufficient nitrogen from the residual soil N but immense effects were visible in later stages i.e. flowering and post-flowering.

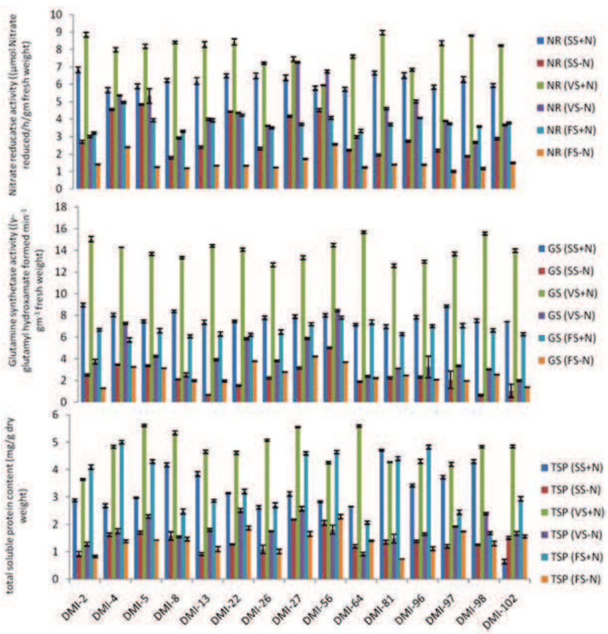

Fig. 3. Nitrate reductase (NR) activity, glutamine synthetase (GS) activity and total soluble protein (TSP) to the nitrogen (N) availability (N-deficit and -sufficient conditions) at seedling, vegetative, and flowering stages of fifteen maize inbred lines (represented on X-axis)

The maize yield increased linearly with increasing N input. A total of 12% increase in grain yield was reported by Li et al. (2019) and Li et al. (2020). Similarly, in the present study grain yield-related traits, *viz*., ear length (CL), ear girth (CG), rows per ear (RC), no of grains per row (GR), the numberof grains per ear(GN), grain weight per ear (GW), hundred-grain weight (HGW), nitrogen harvest index(NHI), and dry matter accumulation (DMA) of all fifteen lines decreased by 4.5-53.6% under N-deficit conditions (Table 2a and 2b). Aboveground biomass production is highly affected by N uptake potential (Peng et al. 2010). The excess nitrogen accumulated in maize biomass split into grains, leaves and stalks (Byers 2005; Hirel et al. 2007). However, the overall gain in maize biomass depends on factors like soil moisture, temperature, structure, and bulk density in addition to adequateN supply and uptake by maize (Masclaux-Daubresse et al. 2010; Hammadet al. 2017). The relative differences were highly significant for ear length, ear girth, ear weight, nitrogen harvest index, and dry matter accumulation in the study.

Nitrogen content estimation

The nitrogen content in above-ground maize largely depends on the availability of soil nitrogen (Worku et al. 2007). The accumulation of N in the above ground biomass could directly represent the availability and nitrogen uptake efficiency of the plants. Higher soil N can lead to a high proportion of N in plant biomass and *vice versa* (Kiniry et al. 2001). Total nitrogen accumulation in the root, shoot, and leaves under N limiting and sufficient conditions were analyzed at regular intervals of seedling, vegetative, and flowering stages. Among all fifteen lines, nitrogen content in root was found higher in DMI 4, DMI 5, and DMI 56 lines under N sufficient conditions at the vegetative stage (Fig. 4). Further, a huge difference was observed in N content in roots of lines namely, DMI 8, DMI 26, DMI 64, DMI 81, DMI 96, DMI 98, and DMI 102 under

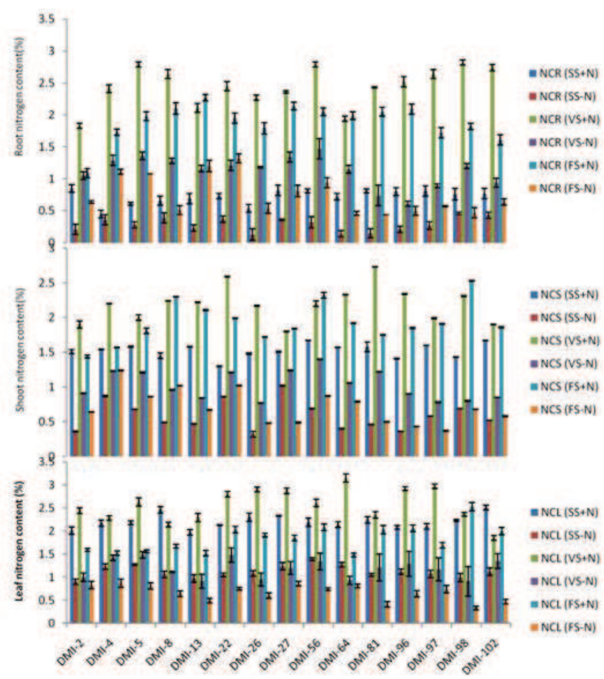

Fig. 4. Nitrogen content in the root, shoot, and leaves to the nitrogen (N) availability (N-deficit and -sufficient conditions) at seedling, vegetative, and flowering stages of fifteen maize inbred lines (represented on X-axis)

Table 2a. Mean yield component parameters of 15 inbred lines screened in the year 2017 and 2018

Genotypes	Cob length (cm)			Cob girth (cm)			No. of rows/ear			No. of grains/row		
	+N	-N	Mean	+N	-N	Mean	+N	-N	Mean	+N	-N	Mean
DMI 2	12.97	5.57	9.27	12.07	4.90	8.48	13.00	6.33	9.67	20.67	10.00	15.33
DMI 3	13.27	7.37	10.32	11.17	6.20	8.68	14.67	6.00	10.33	22.33	12.67	17.50
DMI 4	13.20	9.57	11.38	11.23	8.03	9.63	11.67	9.67	10.67	25.33	17.67	21.50
DMI 5	11.93	9.13	10.53	11.53	9.43	10.48	11.00	8.67	9.83	18.67	15.67	17.17
DMI 6	11.47	6.83	9.15	11.27	8.40	9.83	12.67	6.33	9.50	19.33	13.00	16.17
DMI 8	11.97	6.37	9.17	11.07	6.50	8.78	13.00	8.00	10.5	20.00	14.33	17.17
DMI 13	12.73	6.80	9.77	12.60	6.20	9.40	10.33	5.67	8.00	21.67	11.67	16.67
DMI 17	11.23	3.67	7.45	10.60	5.37	7.98	12.00	6.67	9.33	21.33	7.33	14.33
DMI 19	14.80	7.57	11.18	10.7	5.53	8.12	12.00	9.00	10.5	24.67	13.67	19.17
DMI 22	11.50	8.63	10.07	11.07	7.83	9.45	11.67	8.33	10.00	22.00	12.67	17.33
DMI 26	11.53	6.23	8.88	11.00	5.47	8.23	11.33	8.33	9.83	21.33	11.33	16.33
DMI 27	14.20	9.17	11.68	12.4	7.73	10.07	12.00	10.00	11.00	25.67	15.00	20.33
DMI 31	11.83	9.20	10.52	10.87	8.23	9.55	11.00	8.67	9.83	22.00	16.33	19.17
DMI 41	12.83	9.03	10.93	10.5	8.80	9.65	11.33	8.67	10.00	22.67	14.00	18.33
DMI 56	13.27	9.40	11.33	10.63	7.90	9.27	11.00	8.33	9.67	20.33	16.67	18.50
DMI 64	12.77	5.20	8.98	10.00	4.80	7.40	12.00	7.00	9.50	22.67	8.67	15.67
DMI 81	11.30	5.97	8.63	11.33	5.70	8.52	12.67	6.33	9.50	24.67	12.33	18.50
DMI 96	10.77	5.80	8.28	11.97	4.53	8.25	12.33	6.33	9.33	23.00	12.67	17.83
DMI 97	11.20	4.57	7.88	10.33	5.07	7.70	14.00.	7.00	10.5	21.67	10.33	16.00
DMI 98	12.63	5.53	9.08	9.80	5.23	7.52	12.33	5.67	9.00	22.00	15.00	18.50
DMI 102	11.87	6.77	9.32	10.77	5.40	8.08	11.00	6.00	8.50	21.33	11.33	16.33
Mean	12.35	7.07		11.09	6.54		12.05	7.48		22.06	12.97	
Factors	SE(m)	C.D.		SE(m)	C.D.		SE(m)	C.D.		SE(m)	C.D.	
Genotypes	1.24	0.99		1.08	0.67		1.45	0.88		1.94	1.02	
Nitrogen	0.58	1.08		0.53	0.94		0.85	1.26		0.76	1.68	
G x N	1.75	N/A		1.53	N/A		2.04	N/A		2.74	N/A	

N limiting and sufficient conditions at the flowering stage. Similar results were obtained for N content in leaves and shoot for these inbred lines suggesting their higher sensitivity towards N deficiency compared to other lines (Fig. 4). However, these inbred lines were extensively diverse and considerable phenotypic variation existed as revealed by the standard deviation and standard error mean value. Besides genotype, significant effects were observed for N levels, environment and their corresponding interactions, indicating strong G × E interaction (Table 3). In the present study it was observed that inbred lines having high leaf nitrogen content (Fig. 4) had better grain yield as compared to inbred line with lower leaf nitrogen content (Table 2b). It was reported that leaf nitrogen serve as mineral source under nitrogen deficient in soil (Crawford and Glass 1998) particularly during grain filling stage (Below et al. 2000). Teyker et al. (1989) and Plenet and Lemaire (1999) also concluded that the plant absorbs and stores the excess of mineral nitrogen for use in later stages i.e. through translocation to kernels. So, the study suggests that leaf nitrogen content at early plant stages can be important criteria for screening of germplasm under nitrogen deficit conditions. Reed et al. (1980) on maize hybrids stated that yield and its components were positively correlated with N assimilation and N remobilization. The comprehension of the metabolic

Table 2(b). Mean yield component parameters of 15 inbred lines screened in the year 2017 and 2018

Genotypes	Total grain weight(g)			100 grain weight(g)			Nitrogen harvest index(%)			Dry matter accumulation (g)		
	+N	-N	Mean	+N	-N	Mean	+N	-N	Mean	+N	-N	**Mean**
DMI 2	73.67	10.97	42.32	24.18	10.63	17.41	0.71	0.27	0.49	15.15	7.74	11.45
DMI 3	69.77	11.93	40.85	22.85	13.29	18.07	1.01	0.17	0.59	12.56	4.72	8.64
DMI 4	68.63	17.88	43.26	21.92	15.3	18.61	0.79	0.69	0.74	20.94	8.48	14.71
DMI 5	72.7	16.32	44.51	24.19	16.97	20.58	0.86	0.3	0.58	13.58	9	11.29
DMI 6	69.3	12.46	40.88	22.41	11.78	17.1	0.79	0.26	0.53	18.61	5.25	11.93
DMI 8	71.6	10.06	40.83	23.37	12.74	18.05	0.68	0.24	0.46	16.33	3.42	9.88
DMI 13	67.8	11.18	39.49	23.58	11.03	17.31	0.84	0.42	0.63	21	7.23	14.12
DMI 17	62.73	9.29	36.01	24.88	11.62	18.25	0.51	0.2	0.36	17.98	6.89	12.43
DMI 19	68.46	11.8	40.13	25.17	10.69	17.93	0.95	0.45	0.7	19.98	9.52	14.75
DMI 22	75.05	17.72	46.38	24.91	14.22	19.57	0.77	0.45	0.61	18.19	8.42	13.3
DMI 26	75.21	9.71	42.46	23.35	10.74	17.05	0.62	0.21	0.42	17.69	5.47	11.58
DMI 27	68.42	19.27	43.85	25.08	17.09	21.09	0.76	0.49	0.63	24.8	10.7	17.75
DMI 31	68.27	17.42	42.85	23.67	15.16	19.41	0.67	0.26	0.47	22.48	9.7	16.09
DMI 41	69.26	18.48	43.87	22.82	18.15	20.48	0.73	0.28	0.5	20.92	7.98	14.45
DMI 56	69.53	19.55	44.54	23.21	18.65	20.93	0.63	0.28	0.46	18.34	8.46	13.4
DMI 64	65.66	11.34	38.5	23.66	12.74	18.2	0.7	0.3	0.5	17.07	5.85	11.46
DMI 81	64.04	11.08	37.56	23.62	8.84	16.23	0.56	0.23	0.4	21.05	15.25	18.15
DMI 96	63.82	9.26	36.54	22.83	9.44	16.14	0.46	0.21	0.34	19.09	6.08	12.59
DMI 97	73.75	10.19	41.97	21.96	11.02	16.49	0.66	0.14	0.4	27.66	7.68	17.67
DMI 98	67.42	10.82	39.12	23.04	12.11	17.58	0.69	0.21	0.45	21.02	4.73	12.88
DMI 102	68.22	11.48	39.85	23.37	9.97	16.67	0.51	0.28	0.4	25.48	11.51	18.5
Mean	69.21	13.25		23.53	12.96		0.71	0.3		19.52	7.81	
Factors	SE(m)	C.D.		SE(m)	C.D.		SE(m)	C.D.		SE(m)	C.D.	
Genotypes	2.29	N/A		1	2.81		0.1	N/A		2.59	N/A	
Nitrogen	0.71	1.99		0.31	0.87		0.03	0.09		0.8	2.25	
G x N	3.23	N/A		1.41	3.97		0.15	N/A		3.66	N/A	

pathway and genetic dominion of nitrogen possession and remobilization during growth stages i.e. vegetative and reproductive phases plays a crucial role in maize improvement (Aziiba et al. 2019). Maintaining the synchronous relation with demand and supply of N at growth stages are key factors in maize productivity (Qiu et al. 2015).

Correlation of enzymatic activity with morphological traits

The correlation between eachenzymatic activity under N sufficient and limiting conditions was assessed with other morphological traits. The significant correlation was observed between leaf nitrate reductase (NR) and TSP (r=0.59) while negatively correlated to CW (r=-0.33), GW (r=-0.10), HGW (r=-0.31), Chla (r=-0.11) and GS (r=-0.28) under N-sufficient conditions (Table 4). Hirel et al. (2001) also reported that nitrate reductase showed a negative correlation with thousand kernel weight under high N input. The coefficients were higher under N-deficit condition with CL (r =0.71), CW (r =0.73), GW (r =0.71), HGW (r =0.59), NHI (r =0.58) (Table 4). A significant and positive correlation between leaf NR activity and thousand-grain weight, grain yield was also observed in young vegetative plants under N deficit conditions by Hirel et al. (2001). It suggests that during grain filling the capacity of the plant to reduce leaf nitrate is low. Reed et al. (1980) reported

Table 3. Morpho-physiological traits used to evaluate the effect of stage and environment on the genotype with respect to SE mean and CD Value

Factors	PH		NL		PG		LA		Total Chl		NR		GS		TSP		NCR		NCS		NCL	
	SE (m)	C.D.	SE	C.D.	SE	C.D.	SE	C.D.	SE (m)±	C.D. at 5%	SE (m)±	C.D. at 5%	SE (m)±	C.D. at 5%	SE (m)±	C.D. at 5%	SE (m)±	C.D. at 5%	SE (m)±	C.D. at 5%	SE (m)±	C.D. at 5%
Stage	0.39	1.09	0.05	0.13	0.04	0.12	0.41	1.14	0.023	0.064	0.06	0.16	0.18	0.5	0.13	0.35	0.039	0.108	0.058	0.161	0.065	0.182
Genotypes	1.04	2.89	0.12	0.34	0.11	0.31	1.08	3.02	0.053	0.147	0.16	0.43	0.48	1.33	0.33	NS	0.102	NS	0.153	NS	0.172	NS
S x G	1.8	5.01	0.21	0.6	0.2	0.54	1.88	5.22	0.106	0.294	0.27	0.75	0.83	NS	0.57	NS	0.177	NS	0.264	NS	0.299	NS
Nitrogen	0.32	0.89	0.04	0.11	0.04	0.1	0.33	0.93	0.016	0.045	0.05	0.13	0.15	0.41	0.1	0.28	0.032	0.088	0.047	0.131	0.053	0.148
N x S	0.55	1.54	0.07	0.18	0.06	0.17	0.58	1.61	0.033	0.091	0.08	0.23	0.26	0.71	0.18	0.49	0.055	0.153	0.082	NS	0.092	0.257
N x G	1.47	4.09	0.18	0.49	0.16	0.44	1.53	4.27	0.075	0.208	0.22	0.61	0.68	1.88	0.47	NS	0.145	NS	0.216	NS	0.244	NS
N x S x G	2.54	7.08	0.3	0.84	0.28	0.77	2.65	7.39	0.15	0.416	0.38	1.06	1.17	NS	0.81	NS	0.251	NS	0.374	NS	0.422	NS

The abbreviation follows as PH=Plant height, NL=Number of leaves, PG=Plant girth, LA=Leaf area, Total Chl=Total chlorophyll, NR=Nitrate reductase, GS=Glutamine synthetase, TSP=Total soluble protein, NCR=Nitrogen content in root, NCS=Nitrogen content in shoot, NCL=Nitrogen content in leaves. Also S=Stage, N=Nitrogen, G=Genotype

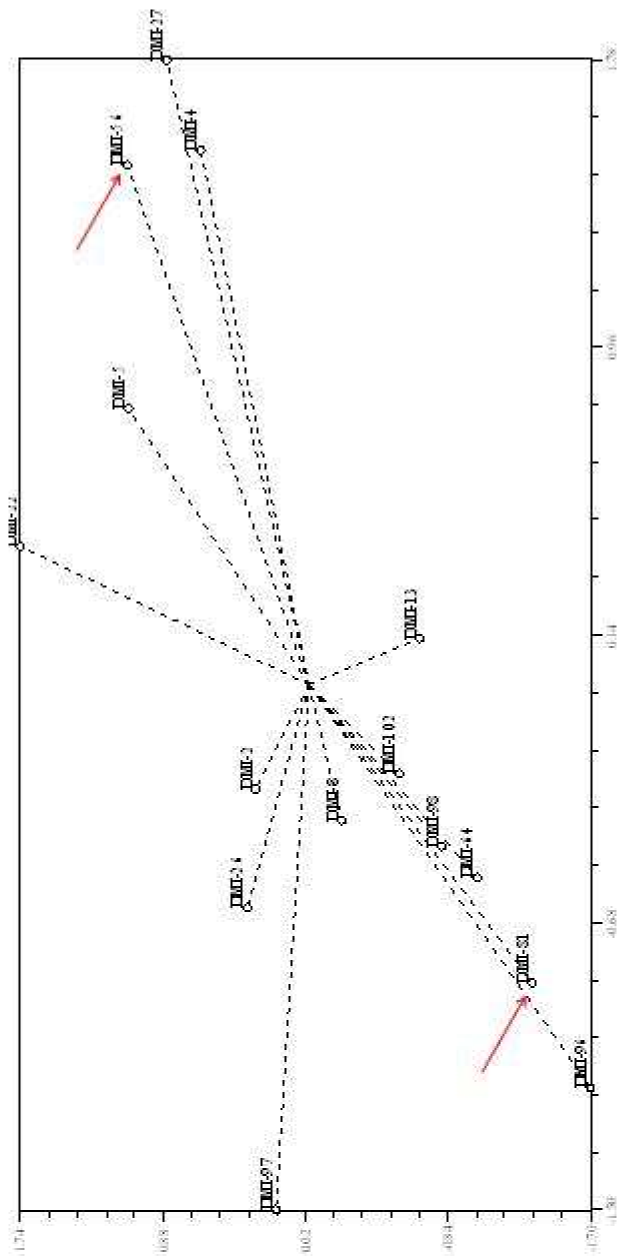

Fig. 5. Two-dimensional plot depicting the relationship between 15 maize inbred lines based on grain yield and ear length recorded in the year 2017 and 2018

that genotypes exhibiting low NR activity yield higher. The assimilation and recycling of mineral N are mainly governed by GS (EC 6.3.1.2), involved in catalyzing the ATP-dependent conversion of Gln into Glu, with ammonia as a substrate (Cren and Hirel 1999). The Glutamine synthetase (GS) activity is considered a highly significant trait for detecting a correlation between physiological and agronomic traits. In our study, GS had a significant correlation with yield governing traits under low-N levels (r =0.51-0.85) and negatively correlated with ASI (r =-0.59) whereas a very low or non-significant relation was observed under sufficient nitrogen conditions.Hirel et al. (2001) also reported that total leaf GS activity was positively correlated with GY, KN, and grain metabolic efficiency (GME) under low-

Table 4. Phenotypic correlations between agronomic and physiological traits,where N+correspond to plants grown in the field under sufficient nitrogen input and N- to plants grown in the field with no nitrogen fertilization

	CL	CG	CW	GW	HGW	NHI	ASI	SPAD	Chla	Chlb	TotalChl	NR	GS	TSP
NR-N+	0.043	0.136	-0.33	-0.104	-0.31	0.194	0.041	0.031	-0.112	0.06	0.398	1		
NR-N-	.711**	0.484	.728**	.711**	.593*	.580*	-0.345	0.308	0.482	-0.007	0.462	1		
GS-N+	0.237	-0.182	0.399	-0.155	0.096	-0.2	-0.277	-0.207	0.096	-0.092	-0.231	-0.277	1	
GS-N-	.780**	.745**	.834**	.846**	.787**	0.511	-.587*	0.359	.615*	0.085	.787**	0.489	1	
TSP-N+	0.187	.581*	-0.318	-0.197	0.092	-0.03	0.102	-0.121	-0.116	0.353	0.05	.538*	0.063	1
TSP-N-	0.501	0.488	.650**	.639*	.704**	0.163	-.587*	0.155	.752**	-0.057	.612*	0.412	.518*	1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

CL=Cob length, CG=Cob girth, CW=Cob weight, GW=Grain weight, HGW=Hundred grain weight, NHI=Nitrogen harvest index,ASI=Anthesis silking indexing, Chla=Chlorphyll a, Chlb=Chlorphyll b, Total Chl= Chlorphyll total, NR=Nitrate reductase, GS=Glutamine synthetase and TSP=Total soluble proteins

N input. The role of plastidic isoenzyme (GS2) in the process of primary N assimilation and cytosolic GS isoenzyme (GS1) during the recycling of organic N during lower amounts of nitrate is very well understood (Masclaux et al. 2000). However, the positive correlation found between GS activity and grain number suggests that a high GS activity is required to avoid embryo abortion just after fertilization (Below 1995). As expected, TSP was highly related to plant yield (CW, GW, and HGW, r =0.65, 0.64 and 0.70 respectively), while highly positively correlated with Chla, Total Chl and GS (r =0.75, 0.61, 0.52 respectively) and negatively correlated with ASI (r =0.59) under N-deficit conditions while positively correlated with CG (r =0.58) and NR (r =0.54) under sufficient N conditions.

Principal Co-ordinate analysis

Principal Co-ordinate analysis (PCA) based on pedigree formed two major population groups. Group, I included inbred lines DMI 4, DMI 5, DMI 22, DMI 27 and DMI 56 performing well under N-deficit conditions. Group II included inbred linesthat performed well under N sufficient conditions (Fig. 5). PCA differentiated the inbred lines based on the average reduction value of morphological traits under N sufficient and limiting conditions indicating that lines are more likely to be genetically associated with these traits. The diverse genetic nature of maize inbreds is very well illustrated by principal coordinate analysis (Pal et al. 2020). The contrasting inbred lines, *viz*., DMI 4, DMI 5, DMI 22, DMI 27 and DMI 56 were derived from the drought or thermal tolerant parent with reduced effect on yield and yield associated traits under nitrogen deficit conditions**.**The role of enzymatic activities *viz*., nitrate reductase (NR) and glutamine synthetase (GS) were found to play a significant role and their assessment at the early growth stages is critical in screening of maize breeding lines for nitrogen use efficiency at later stages. The identified inbred lines have the potential for developing maize hybrids and to study molecular mechanisms playing a role in low nitrate stress tolerance. These lines with improved nitrogen use efficiency characteristics will be used in future breeding programs.

Authors' Contribution

Conceptualization of research (IS); Designing of the experiments (IS, PS, SR); Contribution of experimental materials (IS, KK, BK, SR); Execution of field/lab experiments and data collection (PS); Analysis of data and interpretation (PS, RST); Preparation of the manuscript (PS, IS, SR, RST, KK, BK).

Declaration

The authors declare no conflict of interest.

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