Genetic Diversity of Maize (*Zea mays* L.) Genotypes under Low Level of Nitrogen

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Maize (*Zea mays* L.) is India's second most significant crop, behind rice. As a cross-pollinated crop, hybrid vigour is commonly used to achieve the best production potential. The selection of various genotypes is a primary goal in the maize hybrid breeding programme. Maize is a voracious feeder and requires a lot of fertiliser. The biggest limits on maize production in India are low soil nitrogen and high fertiliser costs. Jharkhand consumes only 82.5 kg of total fertiliser nutrients per acre. In order to discover genetically varied maize genotypes for nitrogen usage efficiency, the current study

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evaluated 121 maize genotypes in a randomised block design with two replications at low nitrogen levels. Plant height, days to 50% pollen shed, days to 50% silk, anthesis-silking interval (ASI), days to 75% maturity, number of leaves per plant, root length, stover yield per plant, grain yield per plant, and harvest index were all recorded. Statistical analysis was performed using the morphological trait data. Analysis of variance revealed substantial ($p \leq 0.05$) variations in morphological features between maize genotypes. For statistical analysis, a clustering method was used to locate various genotypes with greater genetic distance in order to develop good heterotic combinations. One hundred twenty one genotypes were divided into six clusters using the dendogram-by-ward minimum variance approach. Cluster III and V had the greatest inter-cluster distance (79.26), followed by cluster III and VI (74.77) and cluster II and III (73.80). The many heterotic combinations developed from these clusters can be employed in subsequent breeding programmes to produce low nitrogen-requiring hybrids with a low yield penalty.

Keywords: Ward minimum variance method; Anthesis-Silking Interval (ASI); heterotic combinations; Zea mays.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most significant cereal crops due to its tolerance to a wide range of agro-climatic conditions. It is used to make human food, cattle feed, alcohol and non-alcohol drinks, building materials, fuel, and medical and decorative plants (Bekric and Radosavljevic, 2008). Nitrogen, along with phosphorus, is one of the macronutrients that most limits maize grain yield globally (D'Andrea *et al.,* 2006). Nitrogen fertilisation remains an important agronomic method for maize production, either to achieve high yield under low nitrogen (LN) conditions or to efficiently convert N fertiliser into yield under high nitrogen (HN) conditions (Sattelmacher *et al.,* 1994). Nitrogen is a vital element in maize production because it increases vegetative growth, maximises kernel initiation and kernel set, and aids in filling the kernel sink. Santos *et al.* (1997) showed a 65.8% yield reduction when an open pollinated variety bred in fertile soils was cultivated in low N circumstances. Lafitte *et al.* (1995) proposed that low N settings can be improved by selecting N efficient genotypes. Increased varietal tolerance to low soil N stress provides an efficient partial solution for improving maize productivity and food security among resource-constrained small-scale farmers. Plants can resist nitrogen deficit by partitioning more N and carbohydrates to the ear. A suitable breeding approach can be utilised to create genotypes that can withstand stress and provide high grain yield in both low-nitrogen and optimum environments (Miti *et al.,* 2010). Since maize grain yield is quantitative and polygenically regulated, effective yield improvement and simultaneous improvement in yield components are required (Bello and Olaoye, 2009). However, hybrid maize breeding programmes around the world have focused their efforts in recent decades on generating high-yielding hybrids under high soil-N conditions, also known as hybrids with 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 (Al-Naggar *et al.,* 2010, Al-Naggar *et al.*, 2011, Al-Naggar *et al.,* 2017). At the present time, it needs to improve the productivity of maize under such conditions, i.e. develop drought and/or Low-N tolerant maize cultivars. At the moment, it is necessary to increase maize production under such conditions, i.e., produce drought and/or Low-N resistant. To begin a successful breeding programme for improving low N and/or drought tolerance, available maize germplasm should be screened for productivity, agronomic and physiological performance under such stressed and non-stress conditions in order to identify the best genotypes that could be used directly or indirectly as suitable sources for developing drought-tolerant hybrids. The effectiveness of developing new maize cultivars that are resistant to these pressures is dependent on the availability of genetic variability (Mustafa *et al.,* 2014). To accomplish this, available maize germplasm should be tested for genetic diversity. The larger the genetic diversity, the better the prospects of creating new superior cultivars. Measuring available genetic diversity is critical for effectively evaluating and utilising germplasm. Breeders must use various material in their breeding programmes to develop crossovers and choose heterotic inbred lines and pools (Kumari

et al., 2017). Genetic diversity studies in maize have been thoroughly recorded by several authors, offering a reason for their value (Dao *et al.*, 2014, Legesse *et al.,* 2007, Cholastova *et al.,* 2011). According to Dao *et al.* (2014), genetic diversity in diverse populations promotes and enhances adaptability to changing circumstances and market requirements. Furthermore, Legesse *et al.* (2007) emphasised the significance of genetic variety in the creation of heterotic groupings for use as breeding materials. Genetic development in yield and other economic variables in any breeding programme is heavily dependent on and impacted by genetic variability within the breeding population (Cholastova *et al.,* 2011). As a result, the availability of genetic variety influences the selection of enhanced breeding material. The current work aims to uncover genetically diverse genotypes in low nitrogen conditions.

2. MATERIALS AND METHODS

The experiment was carried out at Birsa Agricultural University's research farm in Kanke, Ranchi, during the kharif season in 2018.Ranchi falls inside the Central and North Eastern Plateau zones. It is located at 23^0 17' N latitude and 85^0 10' E longitude. Soil samples were collected from depths ranging from 0 to 20 cm and tested for chemical and physical parameters at the UG laboratory, Department of Soil Science, Faculty of Agriculture, Kanke, Ranchi. The soil is characterised by PH 5.15 (1:2.5 soil water suspension by using a glass electrode pH metre (Jackson1973), and available N: 141.74 Kg N/ha (Alkaline permanganate technique ,Subbiah and Asija,1956)P: 16 Kg N/ha Olsen's method (Olsen *et al.*, 1954); K: 160 Kg N/ha Flame photometer method (Jackson,1973).During the trial period, rainfall was 676.2 mm, while minimum temperatures ranged from 15.2^0 to 22.7^0C and maximum temperatures ranged from 25.9^0 to 31.6^0C. The present study included 121 maize genotypes for screening at low nitrogen levels having different source of origin. Seeds of all genotypes were collected from Birsa Agricultural University's maize research project in Kanke, Ranchi. The experiment was carried out using a randomised block design with two replicates. Sowing was done by placing two seeds in a hole and then thinned to one plant per hill to keep row to row and plant to plant spacing of 65cm and 20 cm, respectively. Prior to sowing, the NPK 0:60:40 Kg ha^{-1} nutrient was administered. During the crop period, three manual weedings were done. The

Department of Genetics and Plant Breeding at Birsa Agricultural University in Kanke, Ranchi, provided all kinds of facilities needed for the effective growing of crops, including workers, irrigation facilities, inputs, and field preparation.

3. DATA COLLECTION AND ANALYSIS

Observations were made on five selected plants for each entry .Data were collected on the nine characters viz., plant height(Average height of plants in centimeters measured from soil surface to the point on stem tassel branching begins), Days to 50%pollen shed (number of days from sowing to the date when 50% of the plants in a row have pollen shed, days to 50% silk (number of days from sowing to the date when 50% of the plants in a row have visible silk), anthesis-silking interval(number of days to 50% silk minus number of days to 50%pollen shed),Days to maturity(number of days taken from the date of sowing to the date when husk turned brown in 75% of the plants in each plot), number of leaves per plant(average number of leaves obtained from selected plants in each row), root length(average length of roots of selected plants), stover yield per yield, grain yield per plant and harvest index. Data were subjected to diversity analysis using ward minimum variance method (1963).

4. RESULTS

The present study investigated 121 maize genotypes by 10 characters under low nitrogen level. Morphological traits depicted significant ($p \leq 0.05$) differences among maize genotypes. Variation for all traits under study was observed. Coefficient of variation was low (<10%) for most of the studied traits indicating good accuracy of the experiment. The exceptions were anthesis-silking interval and leaf number where CV was 14.908 %and 10.204 % respectively (Table-1). The result of Cluster analysis based on all the studied traits, divided the genotypes into 6 clusters (Table-2).Among the 6 clusters, cluster III consisted of maximum number of genotypes (35) followed by cluster II (34), cluster V (30), cluster IV (16) and cluster I & VI (8each).The clustering pattern reflected the presence of considerable amount of genetic diversity in the genetic material taken under study. A comparison of the mean values of six clusters for 10 characters has been presented in (Table-4). Considerable differences in cluster mean values were evident for all the characters studied. The present study revealed that among all VII
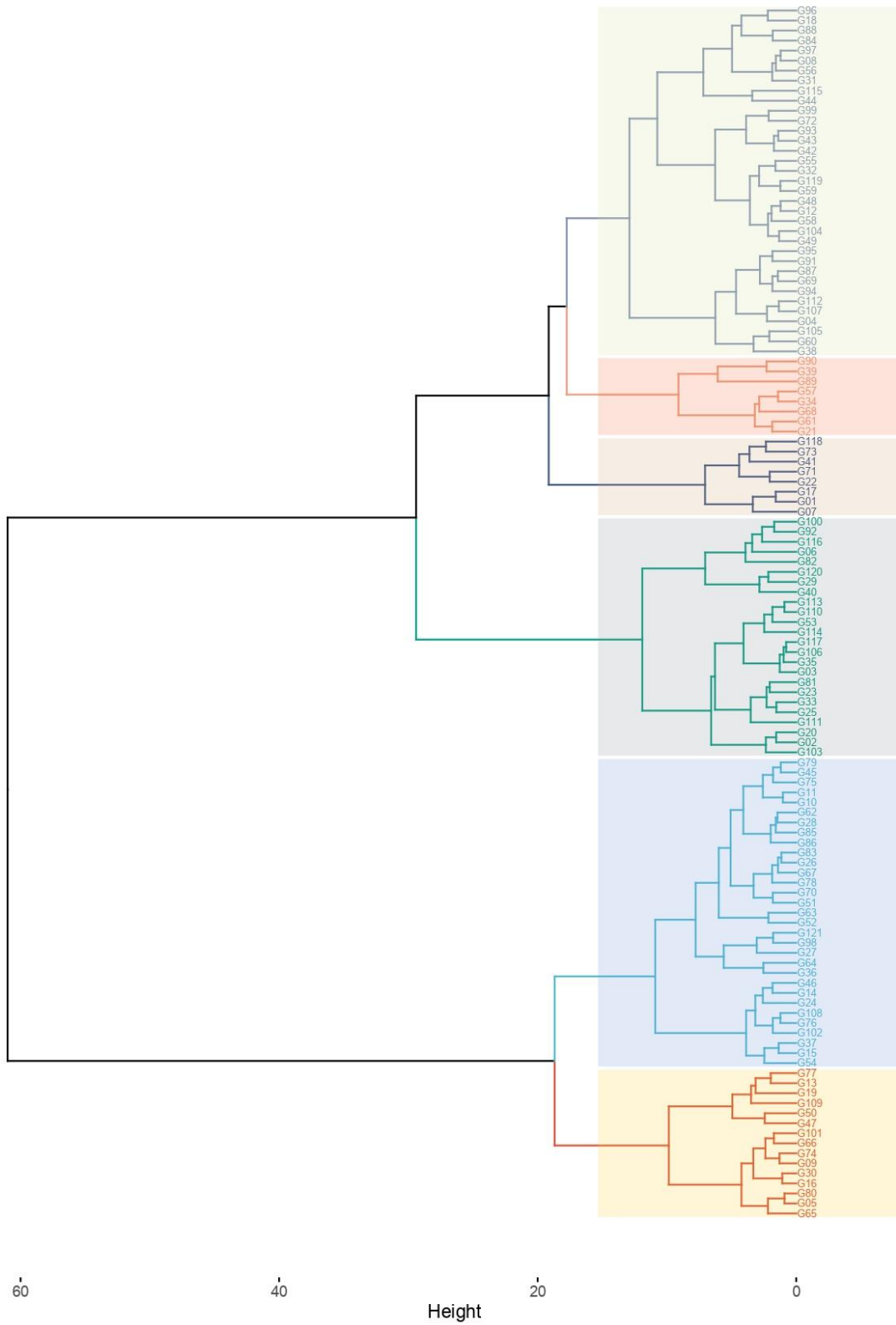
Cluster Dendrogram

Fig. 1. Dendogram based on ward's minimum variance method (1963)

Table 1. Summary statistics of 121 maize genotypes for ten characters evaluated under low nitrogen condition

Characters	Minimum	Maximum	Mean	CD	CV (%)	MS(genotypes)
PH	53.05	130.40	107.68	13.078	6.134	489.710*
DTP	45.50	61.00	53.17	3.686	3.501	35.862*
DTS	49.00	65.00	56.91	3.714	3.296	35.675*
ASI	2.00	5.50	3.74	1.104	14.908	0.692*
DTM	79.00	102.50	90.56	4.600	2.566	45.370*
LN	8.70	14.00	11.28	2.279	10.204	2.099*
RL	10.30	21.30	15.66	1.649	5.319	11.183*
GYPP	11.20	18.70	15.25	2.147	7.113	4.023*
SYPP	26.20	45.60	34.80	5.834	8.466	36.185*
HI	24.37	36.88	30.64	4.563	7.523	17.065*

PH-Plant height, DTP-days to 50% pollen shed, DTS-days to 50% silk, ASI-anthesis-silking interval, DTM- days to75% maturity, LNPP-leaf number per plant, RL-root length, GYPP-grain yield per plant, SYPP-stover yield per plant, HI-harvest index, CD-critical difference, CV- coefficient of variation, MS-mean sum of squares from ANOVA,*-5%level of significance

Table 2. Distributing Pattern of 121 maize genotypes into 6 clusters based on D^2 statistic

Clusters	Number of maize genotypes	Name of maize genotypes
I	08	ZH17260, ZH17256, ZH17455, ZH17450, ZH17259,VH131167, ZH15563, WNCDMR11R6429
II	34	ZH17261,CAH153, ZH17336, ZH137723, ZH161315, ZH17448, ZH161349, ZH17504, ZH138312, ZH17322, ZH17237, ZH138278, ZH17231, ZH138278, ZH17231, BAUIM-5, B1105TE, G26C32HS#54-1-2-12-1-B-CML-193, POP61CQPMTEYF-51-2-1-2-2-B-1-B/CML-193, IC-283412, IC-724720,APMF-22, CLQRCYQ60-B-B, WNCDMR11R1248, WNCDMR11R4093, WNCDMRSCY18R695-1
III	35	ZH17258, ZH17398, ZH17347, ZH17432, ZH17485, ZH17430, ZH17246, ZH17308, ZH17320, ZH17340, ZH17353, ZH17475, ZH17476, ZH17489, ZH17456, ZH14331, ZH17457, ZH17486, ZH1374, ZH17378, ZH17353, ZH17373, CML600, CML425, HKI193-1, HKI577, HKI335, HKI1532, HKI1105, S99TLYQ(HG-AB)BBB-36-BBB/CML-193, G22C12HS#214-1-3-2-1-B/CML-193, (Vera193 XG18) X G18)HS#167-2-4-1-1-13/CML-193,APH-4, WLS-F2S7-1-2-1-B-1-B-B, WNCDMR10RYFWS8481
IV	16	ZH17267, ZH17381, ZH17363, CAH153, ZH17477, ZH17475, ZH17355, ZH17450, ZH17479, ZH14404, ZH17496, ZH17503, ZH138294, RHORYO, WNCDMR19RYSFWS1813
V	30	ZH17390, ZH17374, ZH17394, ZH161348, ZH14331, ZH17484,VH112857, ZH17463,CAH153, ZH15563,ZH17257, ZH17352, ZH17491, ZH17478, ZH161361, ZH17477, ZH17471, ZH15574, ZH17434, ZH17499, ZH15561, ZH17497, ZH17509, ZH17508, ZH17357, ZH17345, BAUIM-2, RQOOZA, WNCDMR11R27290,BAU-15-95
VI	08	ZH161359,VH11128, ZH17446, ZH17435, ZH17432, ZH17436, ZH17465,BAUIM-3, BAUIM-1

Table 3. Average intra and inter cluster distance among 6 Clusters for 121 maize genotypes

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	38.21	44.10	68.87	55.80	50.19	46.57
Cluster II	44.10	41.56	73.80	61.08	49.79	46.60
Cluster III	68.87	73.80	76.01	65.49	79.26	74.77
Cluster IV	55.80	61.08	65.49	41.65	63.88	62.67
Cluster V	50.19	49.79	79.26	63.88	48.01	46.15
Cluster VI	46.57	46.60	74.77	62.67	46.15	24.91

Table 4. Mean Values of clusters of different characters taken under study in 121 maize genotypes

Clusters	PH	DTP	DTS	ASI	DTM	NLPP	RL	SYPP	GYPP	HI
Cluster I	105.56	49.75	54.69	4.94	86.56	11.18	17.01	32.15	12.34	27.84
Cluster II	110.59	50.42	54.00	3.58	87.58	11.40	16.23	31.43	16.21	34.09
Cluster III	106.81	50.24	54.03	3.79	87.26	11.16	14.43	37.43	15.23	29.02
Cluster IV	88.19	56.66	60.31	3.66	94.47	10.40	14.76	32.14	15.49	32.56
Cluster V	112.16	58.05	61.75	3.70	95.93	11.44	15.80	35.24	15.25	30.37
Cluster VI	120.14	52.31	55.50	3.19	89.81	12.76	19.28	39.76	14.88	27.30

clusters, cluster VI had highest mean values for plant height (120.14), followed by cluster V(112.16),cluster II (110.59), cluster III (106.81),cluster I (105.56) and cluster IV(88.19).The highest mean value for the character Days to 50% pollen shed was found in cluster V (58.05) followed by cluster IV(56.66),cluster VI (52.31),cluster II (50.42), cluster III (50.24) and cluster I (49.75).For Days to 50%silking,ClusterV(61.75) had highest mean value, followed by cluster IV (60.31),cluster VI (55.50),cluster I (54.69),cluster III(54.03)and cluster II (54.00).The Anthesis-silking interval was found highest in the cluster I(4.94) followed by cluster III (3.79), cluster V (3.70), cluster IV (3.66),cluster II (3.58) and cluster VI (3.19). Furthermore, the highest mean value for days to 75% maturity was found in cluster V(95.93) followed by cluster IV(94.47),cluster VI (89.81),cluster II (87.58),cluster III (87.26) and cluster I (86.56).The number of leaves per plant was found highest in cluster VI (12.76) followed by cluster V (11.44),cluster II (11.40),cluster I (11.18), cluster III (11.16) and cluster IV (10.40).The root length was found highest in cluster VI (19.28) followed by cluster I (17.01),cluster II (16.23),Cluster V (15.80),cluster IV (14.76) and cluster III (14.43).The highest mean value for stover yield per plant was observed in cluster VI (39.76) followed by cluster III (37.43), cluster V (35.24), cluster IV (32.15),cluster IV (32.14) and cluster II (31.43).The grain yield per plant was found highest in cluster II (16.21) followed by cluster IV (15.49),cluster V (15.25),cluster III (15.23),cluster VI (14.88) and cluster I (12.34).The highest mean

value for harvest index was found in cluster II (34.09) followed by cluster IV (32.56),cluster V (30.37),cluster III (29.02),cluster I (27.84) and cluster VI (27.30).

The intra and inter cluster average distances among 6 clusters were variable, the result represented in Table-3. The highest intra-cluster was recorded for cluster III (76.01) followed by cluster V (48.01) and cluster IV (41.65).Lowest intra-cluster distance was observed for cluster I (38.21).The highest inter cluster distance was observed between cluster III &V (79.26) followed by cluster III &VI (74.77) and cluster II & III (73.80).

5. DISCUSSION

Although morphological analysis for genetic diversity assessment presents many limitations as low polymorphism and influence of environment on phenotypic expression (Beyene *et al.*,2005), phenotypic traits were helpful as a preliminary evaluation of maize genetic diversity and provided practical and critical information required to characterize genetic resources (Ignjatovic *et al.*,2015,Belalia *et al.*,2019). Morphological traits are very important for grouping corn genetic resources, and also are essential and useful for plant breeders seeking to improve existing germplasm by introducing novel genetic variation for certain traits into breeding populations (Asare *et al.*, 2016, Twumasi *et al.*, 2017, Salami *et al.*, 2017, Nelimor *et al.*, 2019). The results showed considerable amount of differences among all 6 clusters. None of the

cluster contained genotypes with all the desirable traits for improved nitrogen use efficiency which could be directly selected and utilized. The hybridization between genotypes of different clusters is necessary for the development of desirable genotypes. Selection of genotype with higher mean value for particular character may be used for adaptation or may be used as parent in future breeding programme .The points to be considered while selecting the genotypes during crossing programme for improving any particular trait, the genotypes should belong to more divergent clusters, and it should show desirable mean performance for various characters. Parents with high yield potential and belonging to distant clusters are likely to yield superior segregants within a short period. Considering the genetic divergence, clustering pattern and mean performance of genotypes for grain yield per plant and anthesis –silking interval and other characters, genotypes from cluster III (HKI193-1, HKI335, HKI1532, HKI577) & V (BAUIM-2 ,RQOOZA) and III (HKI193-1, HKI335, HKI1532, HKI577) & VI (BAUIM-3, BAUIM-1) can be selected as better genotypes under low N level for further hybridization programme.(Mounika *et al.*,2018,Singh *et al.*,2005).

6. CONCLUSION

Genetic diversity was studied to find out the more diverse inbred lines in maize under low nitrogen condition which might be used in hybridization programme. One hundred twenty one genotypes were grouped into six different clusters. The crosses involving parents/inbred lines from most divergent clusters are expected to manifest maximum heterosis and generate wide variability in genetic architecture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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