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Differential response of maize genotypes to low and normal nitrogen supply at seedling stage

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ABSTRACT

High nitrogen (N) efficiency crop genotypes utilize resources rationally and reduce N losses. To evaluate the extent of genotypic variation in response to N supply, twenty-four maize genotypes were grown in Soilrite (peat mass, vermiculite and pearlite mix) supplied with low N level (2mM) or normal N level (25mM) in Hoagland solution. At 21 DAS the average shoot height (SH), leaf area (LA), stem dry weight (SDW), leaf dry weight (LDW), above ground dry weight (ADW), root dry weight (RDW) and total plant dry weight (PDW) were 38.1, 257.5, 67.3, 121.5, 96.9, 49.4, 76.9 and 140.6% higher respectively in seedlings exposed to normal N treatment than those grown under low N level. The shoot N content (SNC), root N content (RNC), above ground N accumulation (ANA), root N (RNA) and total plant N accumulation (PNA) were found to be 1.79, 2.23, 3.51, 3.30 and 3.44-fold increased at normal N supply over low N. Coefficients of variation (CV) varied greatly and ranged from 10.9-37.8 and 9.4-36.7 under low-N and normal-N treatment, respectively. LA, SDW, LDW, ADW, TDW, SNC, RNC, ANA, RNA and TNA showed significant positive correlation both under low N and normal N regime (p< 0.05). SDW, LDW, ADW, PDW, SNC, RNC, ANA, RNA and PNA were significantly correlated and had high CVs reflecting the difference of N in different maize genotypes and these parameters were selected for calculation of N efficiency comprehensive index. According to the N efficiency comprehensive index scatter map under low and normal N conditions, genotypes HKI-164-D4, HKI-325-17 AN, HKI-1332, DTL-3, DTL-2, DHM-117 and DTL-1 were efficient under both low and normal-N conditions and genotypes DTL-10, SNJ-2011-96, PSRJ-13099, NSJ-211 and Z59-17were inefficient under both low and normal-N conditions.

Keywords: Maize, genotypes, seedling stage, N efficiency comprehensive index.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal and multifunctional crop cultivated all over the world, mainly in developing countries, with a varied range of consumed forms and utilizations. This cereal crop mainly used in human food, animal and poultry feed. Moreover, in industry there is a huge demand to produce variety of purposes including maize starch, dextrose, maize syrup, and maize flakes (Gul *et al.,* 2021). Maize grows well in a wide range of soil and climatic conditions. However, it extracts more nutrients than other crops such as tiny grain cereals and grain legumes. In financial year 2021, India's production volume of maize was over 31 million metric tons. Since last decades, the production volume for this food grain is marginally increased (Statista Search Department, 2021).

Maize is a plant with high nutrient demands because of its ability to form abundant vegetative mass and a high quantity of seeds at the unit area. It has a high requirement for nitrogen, phosphorus, potassium, magnesium, calcium, and micro-elements. Among the nutrients,nitrogen plays a significant role in the growth and development of maize plants. This is an essential component of various biological chemicals that play critical roles in photosynthetic activity and agricultural productivity. N is also responsible for wide range of physiological and metabolic functions (Vijayalakshmi et al., 2013). Available soil nitrogen shows direct impact on maize plant growth and grain yield. As a result, several researchers have discovered that increasing nitrogen availability improves maize yield (Gheith *et al.,* 2018; Bashir *et al.,* 2021; Shah *et al.,* 2021b). The impact of nitrogen availability on maize grain yield can be assessed by examining physiological factors like the interception and efficient utilization of radiation, along with the allocation of nitrogen to reproductive organs (Sandhu *et al.,* 2021). Maize dry matter production is influenced by nitrogen fertilizer,

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affecting the development of leaf area, its maintenance, and photosynthetic efficiency (Kaur *et al.,* 2012; Shah *et al.,* 2021a). Nitrogen plays a crucial role in enhancing soil productivity and crop efficiency (Habtegebrial *et al.,* 2007).

The present environmental scenario of the globe and the concurrent need for adequate food productivity makes it essential to consider an approach that sustains the environment and the food economy at the same time. The application of fertilizers has been a crucial factor in enhancing crop productivity. However, the excessive use of fertilizers has led to significant environmental issues. Therefore, it is imperative to reduce fertilizer usage without compromising crop productivity (Hakeem *et al.,* 2013). In agricultural crop production, nitrogen (N) is commonly applied through various nitrogenous fertilizers (Prasad, 1998). Globally, the consumption of nitrogenous fertilizers is reported to be 113.06 million tons (FAO, 2011). In India alone, fertilizer consumption amounts to 14 million tons annually, with a continuous annual growth of 5% (FAI, 2014; Pathak *et al.,* 2008). However, it raises concern that agricultural crops, particularly wheat, rice, and maize, utilize only 30–40% of the applied nitrogen. The remaining 60–70% remains unused in the agricultural fields, posing significant environmental and health risks (Cassman *et al.,* 2002; Hakeem *et al.,* 2011). Annually, approximately 100 teragrams of nitrogen are released into the environment in the form of nitrous oxide from agricultural fields. Nitrous oxide has a global warming effect 300 times greater than carbon dioxide (EPA, 2010). Enhancing agricultural production can be achieved through various means, including the adoption of advanced farming techniques, integration of technology, and the cultivation of new, high-yielding maize hybrids known for their nitrogen efficiency and responsiveness to increased nitrogen fertilizer rates, resulting in higher grain yields. While the substantial increase in maize yield has historically relied on the application of significant amounts of inorganic fertilizer, this practice has posed challenges for both intensive arable farming and the environment. To mitigate nitrogen pollution and maintain profitability, farmers and breeders must curtail nitrogen fertilizer usage and opt for plant varieties exhibiting superior nitrogen use efficiency (NUE). The enhancement of NUE is vital, involving improvements in crops capacity to uptake and assimilates nitrogen.

NUE is commonly measured as fresh weight or yield per unit of available nitrogen in the soil. Calculated as the ratio between the fertilizer nitrogen removed with the crop and the applied amount, NUE in plants is intricate and influenced by various internal and external factors, including soil nitrogen availability, uptake and assimilation, photosynthetic carbon, nitrate signaling, and regulation. Achieving sustained reductions in fertilizer input while improving or stabilizing yield necessitates an enhanced understanding of NUE. Approaches to comprehend NUE involve exploring plant responses to nitrogen deficiency stress to identify affected processes and utilizing natural or induced genetic variation. Therefore, an increased understanding of mechanisms governing plant growth and development under nitrogen stress conditions is crucial for improving NUE and reducing excessive fertilizer input.In order to evaluate the extent of genetic variation in response to N supply in maize during early stage of growth, the current study involved the quantification of differences in plant growth, uptake and utilization capacity for a diverse range of maize inbred genotypes. Screening high N efficiency cultivars of maize should be less fertilizer, less waste with higher N use efficiency and higher yield. In this study, twenty-four different maize genotypes were investigated, and N efficiency types of maize genotypes were divided. It is expected that high N use efficiency, low N loss, and precision N fertilization can be achieved for maize management through this development.

MATERIALS AND METHODS

Growth conditions for raisingthe maize seedlings

The experiment was conducted at ICAR-CRIDA (Central Research Institute for Dryland Agriculture), Hyderabad, India, during June-July 2017-18. Seed material of twenty-four maize genotypes of diverse origin were used for the study. Out of these, eight genotypes (SNJ-2011-37, NSJ-366, NSJ-211, NSJ-189, NSJ-221, SNJ-2011-96, RJR-270, PSRJ-13099) were obtained from National Bureau of Plant Genetic Resources (NBPGR), Hyderabad, five (HKI-

1332, HKI-164-D4, HKI-325 -17AN, LM-6, HKI-164-7-4) from Directorate of Maize Research (DMR), Hyderabad, three (Z59-17, Z60-72, Z93-170) from CIMMYT, Regional Centre, Hyderabad, five (DTL-1, DTL-2, DTL-3, DTL-4-1, DTL-10) from Central Research Institute for Dry land Agriculture (CRIDA), Hyderabad and three (composite -VARUN and ASHWINI and hybrid, DHM-117) Maize Research Centre, Professor Jaya Shankar Telangana State Agriculture University (PJTSAU), Hyderabad.

Experiment was laid out in a randomized complete block design with three replications. Seeds were grown in 500 ml capacity plastic pots. Plastic pots were filled with Soilrite (mixture of vermiculite, Irish peat mass and perlite) and watered until dripping from the bottom. It doesn't contain any source of nutrients to plants. To study the N response in plants, each genotype was grown at low-N level (2mM) and normal N-level is (25 mM) supplemented with Hoagland solution added to the growing medium in pot, based on preliminary experiments. In the preliminary experiment, five N application rates (2, 8, 12.5, 25 mM and 40 mM nitrogen) were tested to confirm the low-N and normal-N level. Significant differences were observed for growth, biomass and N traits among genotypes under 2mM compared to 8 and 12.5mM rates. Plant growth was normal under 25mM but inhibited under N excessive rate (40 mM). Therefore, it was confirmed that 2mM as the low-N level and 25mM as the normal-N level. Plants in both N treatments were supplied with a Hoagland nutrient solution. Two seeds were sown per pot and three pots for each N level for a genotype. Full strength Hoagland's medium (Hoagland and Arnon., 1950) consisted of macronutrient K_2SO_4 (0.75mM), KCl (0.1 mM), KH_2PO_4 (0.25mM), $MgSO_4.7H_2O$ (2 mM), Fe-EDTA (45µM) and micronutrients were H_3BO_3 (46µM), $MnCl_2.4H_2O$ (9.1 µM), $ZnSO_4.7H_2O$ (0.76 µM), $CuSO_4.5H_2O$ (0.17 µM) and $H_2MoO_4.H_2O$ (0.5 µM). All the macro and micronutrients were supplied at constant concentration for every treatment to overcome their deficiency and nullify their effect. Nitrogen was applied as potassium nitrate ([KNO_3 and Ca $(NO_3)_2.4H_2O$]) and ammoniacal (NH_4 NO_3) form. Initially, half strength of Hoagland solution was applied 4 days after sowing and rest half was given during initial vegetative growth of the plant (10 and 17days after sowing). The pots were placed on raised platform in the field. Plant pots were shuffled randomly to avoid border effect. All genotypes with the respective N treatment were harvested in triplicates at 21 days after sowing.The two plants from each pot were harvested and the average of two plants per pot was taken as one replication. After harvesting of plants, growth observations of maize seedlings were recorded.

Analysis of Crop growth associated parameters

The plants from each genotype and each treatment were fractionated into leaves and stems. Roots were harvested from respective pots. Shoot height was measured using portable tape from the stem base. Root length was measured after gently washing the roots. The leaves were excised and leaf area (cm^2) was measured by using LI-3100 Area Meter (LICOR-Lincoln, Nebraska, USA). Further, plant parts were kept in paper bags and dried in hot-air oven at a temperature of 65^oC till constant weight was attained. After drying, dry weight of leaf, stem and root was taken, and the above ground dry weight and total plant weight was calculated. Root to shoot ratio was calculated on the basis of dry weights of root and shoot.

Chlorophyll Content and **N%**

The chlorophyll levels in the leaves were assessed utilizing an *in vivo* chlorophyll meter (SPAD-502 from Japan) to ascertain the SPAD value. Plant fractions' samples were ground and sieved (<0.25mm) before undergoing total nitrogen analysis. The Kjeldahl digestion procedure (Bremner and Mulvaney, 1982) was employed for the analysis of total nitrogen concentration in both above-ground biomass (stem-leaf) and roots.

Measurement of N uptake

The uptake of N (mg N plant^{-1}) was calculated using the following formula (Wang *et al.,* 2018). Uptake N (mg N plant^{-1}) = Dry matter (mg/plant^{-1}) x N content (%) / 100
SNA (mg plant $^{-1}$) = SNC (%) x SDW (mg plant^{-1})
RNA (mg plant^{-1}) = RNC (%) x RDW (mg plant^{-1})
PNA (%) = SNA+RNA
Nuse efficiency = SDW/PNA

In this experiment, shoot height (SH), leaf area (LA), stem dry weight (SDW), leaf dry weight (LDW), root dry weight (RDW), whole plant dry weight (PDW), stem-leaf N content (SNC), root N content (RNC), stem-leaf N accumulation (SNA), root N accumulation (RNA) and whole plant N accumulation (PNA) as the variables were used. The coefficient of variation (CV) was figured out as follows:

CV = (SD/Mean) x 100

Where, SD is the standard deviation of a given parameter value, and the mean is the average value; the coefficient represents the extent of variation in the parameter among the 24 genotypes.

N efficiency comprehensive index (NEI):

N Efficiency comprehensive index (NEI) indicates the N nutrition status of various genotypes under different N supplies. The membership function was used to comprehensively evaluate the N efficiency of maize (Zadeh, 1965) as follows:

$$B_{mn}=(A_{mn}-A_{nmin})/(A_{nmax}-A_{nmin}).$$

Where, B_{mn} is the membership function value of m genotype with n index; A_{mn} is the measured value of m genotype with n index; A_{nmin} is the minimum value of all genotype with n index; A_{nmax} is the maximum value of all genotype with n index; m is genotype; n is index.

The weight is measured by objective weighting formula: $D_n = C_n / \sum C_n$. Where D_n is the weight of index; Cn is the coefficient of variation.

N efficiency Comprehensive Index (NEI) = $\sum(Bmn \times D_n)$.

RESULTS AND DISCUSSION

Growth, biomass and N%

Growth, biomass and N% of maize seedling plants grown under low (2 mM N) and normal N (25 mM N) regime were measured at 21 DAS to determine the variability in twenty-four maize genotypes in their response to external N supply. SDW, PDW, RL, SNA, RNA and PNA of maize under low N (2 mM) and normal N (25mM) in seedling stage had a different response. The average shoot height (SH), stem dry weight (SDW), leaf dry weight (LDW), aboveground dry weight (ADW), root dry weight (RDW) and total plant dry weight (PDW) were 38.1, 67.3, 121.5, 96.9, 49.4, 76.9 and 140.6% higher respectively in seedlings exposed to normal N treatment than those grown under low N level. Moreover, shoot N content (SNC), root N content (RNC), aboveground N accumulation (ANA), root N(RNA) and total plant N accumulation (PNA) were found to be 1.79, 2.23, 3.51, 3.30 and 3.44 - fold increased at normal N supply over low N.

Table 1: SH, RL, LA, SPAD, SDW, LDW, ADW, RDW, PDW, SNC, RNC, ANA, RNA and PNA ranges, mean and Coefficient of variation (% CV) of maize genotypes at the seedling stage under different nitrogen levels

Index	Low Nitrogen level			Normal Nitrogen level		
	Range	Mean	CV	Range	Mean	CV
SH (cm plant^{-1})	16.45-29.45	23.05	15.8	23.45-36.79	31.84	10.3
RL (cm plant^{-1})	17.13-30.73	24.16	14.4	13.93-29.03	20.36	18.6
LA (cm^2 plant^{-1})	12.38-50.80	33.46	33.3	51.19-234.34	119.55	36.9
SPAD	19.57-31.95	23.50	13.3	30.15-42.30	35.78	9.4
SDW (g plant^{-1})	0.16-0.33	0.25	17.3	0.34-0.57	0.42	15.7
LDW (g plant^{-1})	0.18-0.40	0.30	19.5	0.45-0.84	0.66	17.3
ADW (g plant^{-1})	0.34-0.73	0.55	17.7	0.79-1.39	1.08	15.3
RDW (g plant^{-1})	0.21-0.72	0.40	28.0	0.44-0.84	0.59	16.9
PDW(g plant^{-1})	0.66-1.32	0.94	18.7	1.37-2.03	1.67	11.9
SNC (%)	1.08-1.60	1.32	10.9	1.74-2.86	2.37	12.8
RNC (%)	0.61-1.18	0.86	17.8	1.28-2.42	1.90	16.4
ANA (mg plant^{-1})	3.8-10.9	7.22	23.1	13.74-39.75	25.45	19.9
RNA (mg plant^{-1})	1.90-6.70	3.39	37.8	5.63-20.33	11.27	24.6
PNA (mg plant^{-1})	6.0-15.9	10.61	25.4	19.38-60.08	36.72	18.3

Note: SH, RL, LA, SPAD, SDW, LDW, ADW, RDW,PDW, SNC, RNC, ANA, RNA and PNA represents shoot height, root length, leaf area, chlorophyll content (SPAD), stem and leaf dry weight, aboveground dry weight, root dry weight, total plant dry weight, shoot and root nitrogen content, above ground nitrogen accumulation, root nitrogen accumulation and total plant nitrogen accumulation

The coefficient of variations (CVs) can be used to measure variation in growth, biomass and N-related among the maize genotypes. Under low-N conditions, the CV ranking was RNA> LA > RDW > TNA >ANA > LDW > TDW > RNC > ADW > SDW > SH > RL> SPAD > SNC showing that these indicators are sensitive to genotypic differences. Almost a similar pattern was observed under normal N conditions and CV ranking was > RNA >ANA > RL > TNA >LDW > RDW > RNC > SDW > ADW > SNC > TDW > SH > SPAD. The CVs for SDW, LDW, RDW, ADW, TDW, SNC, RNC, ANA, RNA and TNA parameters were more than 10 % under low and normal N supplies (Table 3). A similar coefficient of variations for growth and N- related parameters was reported by Liu *et al.,* (2022) for 28 alfalfa genotypes grown under N21 (21 mg/L Nitrogen) and N210 (210 mg/l nitrogen) in the seedling stage and in cotton (Zhang *et al.,* 2018).

Table 2: Correlation of different parameters under low nitrogen level (2 mM N) in 21days old maize plants

N0	SH	RL	LA	SPAD	SDW	LDW	ADW	RDW	TDW	SNC	RNC	ANA	RNA	TNA
SH	1													
RL	0.188	1												
LA	0.026	460*	1											
SPAD	0.08	-0.042	-0.138	1										
SDW	-0.054	0.372	0.769**	-0.059	1									
LDW	0.176	0.286	0.696**	-0.148	0.834**	1								
ADW	0.079	0.345	0.760**	-0.100	0.941**	0.969**	1							
RDW	-0.027	0.047	0.256	0.255	0.499*	0.381	0.447*	1						
TDW	0.026	0.219	0.576**	0.105	0.828**	0.769**	0.827**	0.872**	1					
SNC	-0.269	0.120	0.251	0.042	0.311	0.254	0.287	0.225	0.299	1				
RNC	-0.233	0.124	0.293	-0.017	0.329	0.267	0.302	0.181	0.279	0.979**	1			
ANA	-0.074	0.293	0.664**	-0.037	0.854**	0.839**	0.882**	0.452*	0.766**	0.697**	0.694**	1		
RNA	-0.138	0.093	0.323	0.218	0.541**	0.414*	0.485*	0.891**	0.824**	0.626**	0.597**	0.674**	1	
TNA	-0.111	0.226	0.564**	0.080	0.785**	0.716**	0.775**	0.700**	0.863**	0.727**	0.712**	0.938**	0.889**	1

Notes: see the note to Table-1, Asterisks (* and **) represent significance at the 0.05 and 0.01 two tailed level

Correlations among agronomic traits under low (2 mM N) and normal N (25 mM N) levels are shown in Tables 2 and 3. Under low N supply there was a significant positive correlation between LA, SDW, RDW, LDW, ADW, TDW, SNC, RNC, ANA, RNA and TNA ($p< 0.005$) and the range correlation coefficient was from 0.414 to 0.979 (Table 2).

Table 3: Correlation of different parameters under normal nitrogen level (25 mM N) in 21days old maize plants

N3	SH	RL	LA	SPAD	SDW	LDW	ADW	RDW	TDW	SNC	RNC	ANA	RNA	TNA
SH	1													
RL	0.175	1												
LA	0.315	0.323	1											
SPAD	-0.074	-0.122	-0.338	1										
SDW	0.101	-0.11	0.129	0.421*	1									
LDW	0.306	0.32	0.647**	0.214	0.625**	1								
ADW	0.257	0.173	0.503**	0.314	0.842**	0.947**	1							
RDW	0.104	-0.062	0.25	-0.471*	0.028	0.056	0.063	1						
TDW	0.266	0.112	0.544**	0.022	0.713**	0.815**	0.863**	0.559**	1					
SNC	-0.178	-0.368	-0.484	0.447*	0.205	-0.198	-0.061	-0.022	-0.062	1				
RNC	-0.185	-0.368	-0.473	0.432	0.193	-0.188	-0.059	-0.017	-0.057	0.995**	1			
ANA	0.077	-0.117	0.05	0.563**	0.810**	0.608**	0.747**	0.024	0.633**	0.612**	0.609**	1		
RNA	-0.045	-0.276	-0.13	-0.053	0.132	-0.093	-0.006	0.722**	0.36	0.659**	0.667**	0.420*	1	
TNA	0.04	-0.202	-0.016	0.402*	0.665**	0.420*	0.560**	0.316	0.626**	0.734**	0.735**	0.927**	0.729**	1

Notes: see the note to Table-1, Asterisks (and **) represent significance at the 0.05 and 0.01 two tailed level*

Under normal N supply, the coefficient correlation was from 0.503 to 0.995 for similar traits except RDW (Table 3). Under normal N supply RDW was correlated with TDW and RNA only. Comprehensively, LA, SDW, LDW, ADW, TDW, SNC, RNC, ANA, RNA and TNA showed significant positive correlation both under low N and Normal N regime ($p<$ 0.05). Under different N levels, parameters that were significantly correlated and having high CVs could reflect the difference of N in different maize cultivars. LA, SDW, LDW, ADW, TDW, SNC, RNC, ANA, RNA and TNA could reflect the difference among the genotypes of maize and these parameters can be used for screening maize genotypes at the seedling stage for response to low and normal nitrogen levels.

Screening of cultivars for different N efficiency at the seedling stage was not exclusive to cereal crops, like rice (Feng *et al.,* 2014), maize (Li *et al.,* 2014) and foxtail millet (Chen *et al.,* 2016), but also in harvesting vegetable crops, such as rapeseed (Zou *et al.,* 2017), spinach (Liu *et al.,* 2012), cucumber (Zhao *et al.,* 2015), alfalfa (Liu *et al.,* 2022) and cotton (Zhang *et al.,* 2018). Various indices exist for assessing low-nitrogen tolerance capacities, including SPAD, crop yield, nitrogen content, dry weight, nitrate reductase activity, and glutamine synthase activity. However, consensus regarding their universal adoption has not been reached thus far (Robinson *et al.,* 2007; Wang *et al.,* 2011; Zhang *et al.,* 2015). In the present study, the N efficiency screening parameters at the seedling stage were SDW, LDW, ADW, PDW, SNC, RNC, ANA, RNA and PNA which were screened from growth-related and N-related parameters (their CVs were more than 10% and they were significantly positively correlated). High CV value for parameters reflects the genetic variation of the screening index and the parameters were significantly correlated under low-N and normal-N conditions, suggesting that the selection indexes are feasible.

Table 4: Weight values of SDW, LDW, ADW, PDW, SNC, RNC, ANA, RNA and PNA for 24 maize genotypes grown under different levels of nitrogen at seedling stage (21 DAS)

Weight Value	SDW	LDW	ADW	TDW	SNC	RNC	ANA	RNA	TNA
	(g plant^{-1})	(g plant^{-1})	(g plant^{-1})	(%)	(%)	(%)	(mg plant^{-1})	(mg plant^{-1})	(mg plant^{-1})
2 mM N	0.092	0.104	0.094	0.100	0.058	0.095	0.123	0.199	0.135
25 mM N	0.103	0.114	0.100	0.078	0.084	0.108	0.131	0.162	0.120

Note: see the note to Table 1

Simultaneous selection in both low-N and high-N environments increases the probability of identifying genotypes with the potential to perform well under optimum as well as N-limiting conditions. Genetic variation of agronomic traits and NUE under low-N and high-N conditions have been reported in cotton (Zhang *et al.,* 2018) and alfalfa (Liu *et al.,* 2022). In the present study maize genotypes showed a large variation in response to N levels (Table 1), with the N supply significantly affecting plant height, leaf area, biomass and N content. The seedlings exposed to normal nitrogen treatment exhibited higher average shoot height (SH), stem dry weight (SDW), leaf dry weight (LDW), aboveground dry weight (ADW), root dry weight (RDW), and total plant dry weight (PDW) compared to those grown under low nitrogen levels, with increases of 38.1%, 67.3%, 121.5%, 96.9%, 49.4%, and 76.9%, respectively. Maize nitrogen efficiency was also found to be associated with the root system's capacity to extract available nitrogen from the soil profile (Worku *et al.*, 2012). The root dry weight was 76.9% higher under normal nitrogen compared to low nitrogen levels. Nitrogen accumulation parameters such as shoot nitrogen content (SNC), root nitrogen content (RNC), above-ground nitrogen accumulation (ANA), root nitrogen (RNA), and total plant nitrogen accumulation (PNA) were 1.79, 2.23, 3.51, 3.30, and 3.44 times higher, respectively, under normal nitrogen supply compared to low nitrogen. Differences in nitrogen efficiency, including total nitrogen accumulation, nitrogen uptake, and nitrogen translocation, were observed (Xu *et al.*, 2012). Parameters such as plant biomass, nitrogen uptake, and root growth have been employed to assess nitrogen efficiency in various crops, such as oilseed rape (Wang *et al.*, 2014). Additionally, Chun *et al.* (2005) demonstrated the utility of root growth, nitrogen uptake, and yield in screening different nitrogen efficiency levels in maize cultivars. Therefore, biomass, root parameters, and nitrogen accumulation are commonly utilized as evaluation parameters for nitrogen efficiency.

N efficiency at seedling stage

Under the 2 mM and 25 mM N, the weight value of root nitrogen accumulation (RNA) was the highest (0.199 and 0.162 respectively), and that of shoot nitrogen content (SNC) was the lowest (0.058 and 0.084 for low and normal N supply) (Table 4). At 2 mM supply of N, the maximum comprehensive parameter of N efficiency was 0.844 (NSJ-189) and minimum was observed 0.021 (Z60-72). The comprehensive parameters of N efficiency of three genotypes were more than 0.8, 10 genotypes were between 0.4 and 0.8 while 11 genotypes were below 0.4 value (Table 5). Under 25 mM N supply of N, the highest comprehensive parameters of N efficiency was 0.854 (HKI-164-D4) and lowest was 0.152 (SNJ-2011-37). There were two genotypes with comprehensive parameters of N efficiency more than 0.8, 11 genotypes between 0.4 and 0.8 while 10 genotypes bellow 0.4 value. According to the N efficiency comprehensive index scatter map under low and normal N conditions, maize genotypes were classified into four groups (Fig.1). Less effective under low-nitrogen conditions but effective under normal-nitrogen conditions (Class I), effective under both low and normal-nitrogen conditions (Class II), ineffective under both low and normal-nitrogen conditions (Class III), and effective under low-nitrogen conditions; however, ineffective under normal-nitrogen conditions (Class IV).

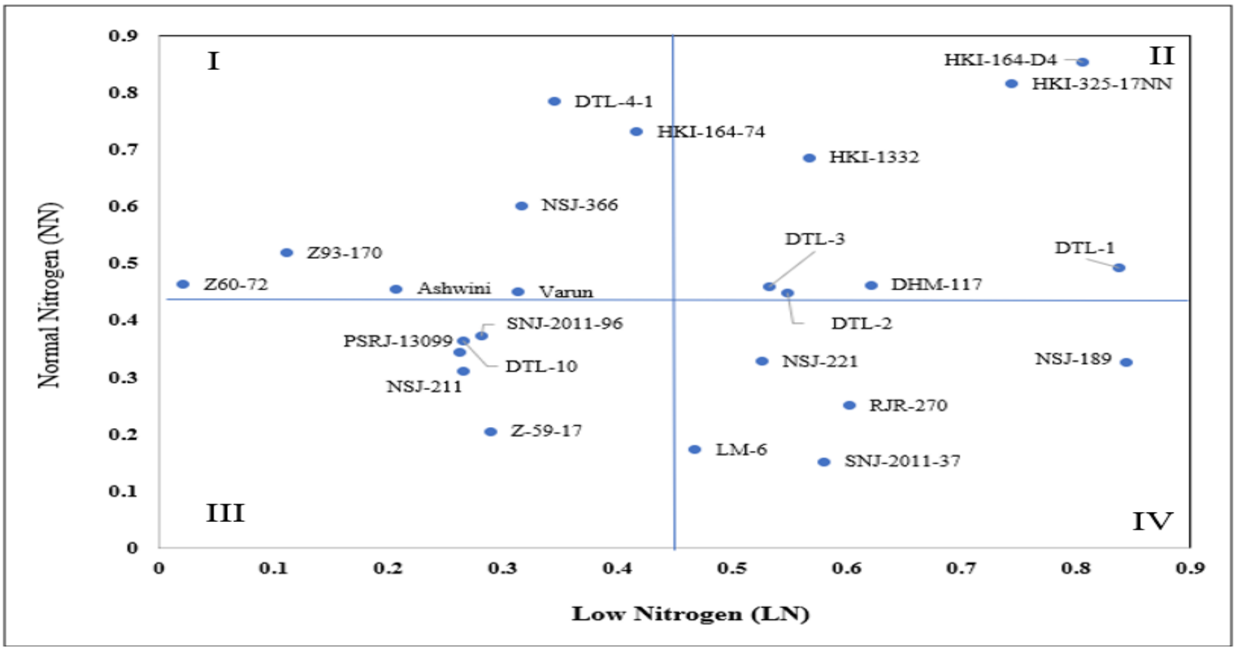

Figure 1: Scatter map of the nitrogen efficiency comprehensive index under low and normal N level

The distributions of 24 maize genotypes among classes were 29.17, 29.17, 20.83, and 20.83% for classes 1–4, respectively. The comprehensive value of DTL-4-1, HKI-164-74, Varun, Ashwini, Z-93-170, Z60-72 and NSJ-366 were lesser than 0.45 under 2 mM N supply, but were greater than 0.45 under 25 mM N (Class I).The comprehensive value of HKI-164-D4, HKI-325-17 AN, HKI-1332, DTL-3, DTL-2, DHM-117 and DTL-1were greater than 0.45 under low and normal N (Class II). The comprehensive value of DTL-10, SNJ-2011-96, PSRJ-13099, NSJ-211 and Z59-17 were lesser than 0.45 under bothlow and normal N (Class III).The comprehensive value of NSJ-221, NSJ-189, RJR-270, LM-6 and SNJ-2011-37 were greater than 0.45 under low N supply but were lesser than 0.45 under normal N supply (Class IV). Therefore, by combining tolerance to low-N with N efficiency, HKI-164-D4, HKI-325-17 AN, HKI-1332, DTL-3, DTL-2, DHM-117 and DTL-1 were identified as N-efficient and low-N tolerant genotype, and DTL-10, SNJ-2011-96, PSRJ-13099, NSJ-211 and Z59-17 as N-inefficient and low-N sensitive genotype.

Tsai *et al.* (1983) categorized maize into three N efficiency types, namely Sensitive, Intermediate, and Non-sensitive, where sensitivity represented the crop's responsiveness to environmental nitrogen rather than its overall nitrogen efficiency. Another classification by Kumar *et al.* (2013) outlined four N efficiency types for maize: Efficient and Responsive, Non-efficient and Responsive, Non-efficient and Non-responsive, and Efficient and Non-responsive. He *et al.* (2017) extended this classification to oilseed rape cultivars, dividing them into N-responder, N-non-responder, N-efficient, and N-inefficient, with the standards being N-responder and N-efficient. In the present study, N-efficiency served as the sole classification criterion. Chen *et al.* (2013) classified maize into four types: Efficient-efficient, High-N efficient, Low-N efficient, and Nonefficient-nonefficient. This classification by Chen *et al.* (2013) elucidated the relationship between environmental nitrogen and crops. Notably, the alfalfa classification directly reflected the N efficiency of alfalfa. Beyond cereal crops, N efficiency classifications for harvesting vegetative crops have been reported. For instance, Liu *et al.* (2012) categorized spinach into Efficient-efficient, High-N efficient, Low-N efficient, and Nonefficient-nonefficient. In our study, the N efficiency classification directly mirrors the N efficiency of maize, making it a suitable screening system for maize N efficiency.

Table 5: Nitrogen efficiency comprehensive value for 24 maize genotypes grow under different nitrogen levels at seedling stage

Genotypes	N efficiency comprehensive value	
	Low N	Normal N
	(2 mM N)	(25 mM N)
SNJ-2011-37	0.58	0.152
Z60-72	0.021	0.463
Z93-170	0.111	0.519
HKI-164-74	0.417	0.731
HKI-164-D4	0.805	0.854
HKI-325-17AN	0.744	0.816
LM-6	0.468	0.173
NSJ-366	0.316	0.602
NSJ-211	0.266	0.31
NSJ-189	0.844	0.327
DTL-1	0.838	0.492
DTL-2	0.548	0.447
DTL-3	0.533	0.46
DTL-4-1	0.345	0.784
DTL-10	0.266	0.363
NSJ-221	0.526	0.328
HKI-1332	0.567	0.686
SNJ-2011-96	0.281	0.373
Z59-17	0.289	0.205
RJR-270	0.602	0.251
PSRJ-13099	0.263	0.343
VARUN	0.313	0.45
ASHWINI	0.207	0.455
DHM-117	0.621	0.462

Chen *et al.* (2013) discovered that the average yield of maize cultivars classified as "Very efficient" exceeded that of less nitrogen-efficient cultivars. Maize cultivars demonstrating nitrogen efficiency could be well-suited for both high and low input farming systems, thereby reducing management costs associated with nitrogenous fertilizer application (Kumar *et al.,* 2013). The findings indicated that nitrogen-efficient maize cultivars generally exhibited a significantly high yield potential under conditions of high nitrogen. Consequently, these nitrogen-efficient cultivars could prove suitable for both high and low input farming systems, contributing to a reduction in management costs related to nitrogenous fertilizers. Chen *et al.* (2013) demonstrated that utilizing "Efficiency" cultivars in North and Northeast China could increase maize yield by 10%–15% while reducing nitrogen fertilizer input by 10%–20%. In summary, the classification of nitrogen efficiency serves not only to identify high-efficiency maize germplasm resources but also to provide guidance for the judicious application of nitrogen fertilizer.

CONCLUSIONS

Screening in low and high N environments simultaneously is critical to determining N-efficient genotypes in this study. Based on our results, genotypic variations were recorded in maize growth, biomass and N -related parameters under low-N and normal-N conditions. The precise evaluation index system is urgently needed to evaluate low-N tolerance capacities. SDW, LDW, ADW, PDW, SNC, RNC, ANA, RNA and PNA were established as the screening indexes for predicting maize N-efficient genotypes. From the N efficiency comprehensive index scatter map under low and normal N conditions, genotypes HKI-164-D4, HKI-325-17 AN, HKI-1332, DTL-3, DTL-2, DHM-117 and DTL-1 were efficient under both low and normal-N conditions and genotypes DTL-10, SNJ-2011-96, PSRJ-13099, NSJ-211 and Z59-17 were inefficient under both low and normal-N conditions. These genotypes would need to be verified for their yield and heritability effects in open field conditions.

REFERENCES

Bashir, S., Bashir, S., Gulshan, A.B., Khan, M. J., Iqbal, J. and Sherani, J. (2021) The role of different organic amendments to improve maize growth in wastewater irrigated soil. *Journal of King Saud University Science,***33**: 101583.

Bremner, J.M. and Mulvaney, C.S. (1982) Nitrogen-Total. In: Methods of soil analysis. Part 2. Chemical and microbiological properties, Page, A.L., Miller, R.H. and Keeney, D.R. Eds., *American Society of Agronomy, Soil Science Society of America*, Madison, Wisconsin, 595-624.

Cassman, K.G., Dobermann, A. and Walters, D.T. (2002) Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio*,**31**: 132–140.

Chen, EY., Yang, YB., Qin, L., Zhang, HW., Liu, B., Wan, HL., Chen, GL., Yu, ST. and Guan, YA. (2016) Evaluation of nitrogen efficient cultivars of foxtail millet and analysis of the related characters at seedling stage. *Scientia. Agricultura Sinica*, **49**: 3287–329.

Chen, FJ. Fang, ZG. Gao, Q., Ye, YL. Jia, LL. Yuan, LX. Mi, GH. and Zhang, FS. (2013) Evaluation of the yield and nitrogen use efficiency of the dominant maize hybrids grown in North and Northeast China. *Science China Life Sciences*,**56**: 552–560.

Chun, L., Chen, F. J., Zhang, F. S., and Mi, G. H. (2005) Root growth, nitrogen uptake and yield formation of hybrid maize with different N efficiency. *Plant Nutrition and Fertilizer Science*, **11**, 615–619**.**

EPA (2010) Methane and Nitrous Oxide Emissions from Natural Sources. U.S. *Environmental Protection Agency. Washington*, DC: EPA.

Feng, Y., Chen, HF., Hu, XM. Zhao, W., Fang, S. and Cai, HM. (2014) Nitrogen efficiency screening of rice cultivars popularized in south China. *Plant Nutrition and Fertilizer Science*, **20**: 1051–1062.

Food Agriculture Organization of the United Nations (2011) Current World Fertilizer Trends and Outlook to 2015.

Gheith, E.M.S., Shafik, M.M., El-Badry, O.Z. and Kareem, B.M.A. (2018) Growth and productivity of maize (*Zea mays L.*) as affected by nitrogen and zinc fertilizer levels: 1. Growth analysis. *Bioscience Research*, **15:** 54–59**.**

Gul, H., Rahman, S., Shahzad, A., Gul, S. and Qian, M., Xiao, Q. (2021) Maize (*Zea mays* L.) productivity in response to nitrogen management in Pakistan**.** *American Journal of Plant Sciences*, **12:** 1173–1179.

Habtegebrial, K., Singh, B.R. and Haile, M. (2007) Impact of tillage and nitrogen fertilization on yield, nitrogen use efficiency of tef (*Eragrostis tef* (Zucc.) Trotter) and soil properties. *Soil* and *Tillage Research*, **94**: 55–63.

Hakeem K.R., Ahmad A., Iqbal M., Gucel S. and Ozturk M. (2011) Nitrogen-efficient rice cultivars can reduce nitrate pollution. *Environmental Sci.* and *Pollution Research,* **18**: 1184–1193.

Hakeem K.R., Mir B.A., Qureshi M. I., Ahmad A., Iqbal M. (2013) Physiological studies and proteomic analysis for differentially expressed proteins and their possible role in the root of N-efficient rice (*Oryza sativa* L.). *Molecular Breeding,* **32**: 785–798**.**

He, HY. Yang, R., Li, YJ., Ma, AS., Cao, LQ., Wu, XM., Chen, BY., Tian, H. and Gao, TJ. (2017) Genotypic variation in nitrogen utilization efficiency of oilseed rape (*Brassica napus*) under contrasting N supply in pot and field experiments. *Frontiers in Plant Scienc,***8**: 1–15.

Hoagland D.R. and Arnon D.I. (1950) The water-culture methodf or growing plants without soil. *California Agricultural Experiment Station, circular***-347:** 1910–1994.

Kaur, A., Bedi, S., Gill, G.K. and Kumar, M. (2012) Effect of nitrogen fertilizers on radiation use efficiency, crop growth and yield in some maize (Zea mays L) genotypes. *Maydica,* **57**: 75–82.

Kumar, N., Joshi, VN. and Dagla M.C. (2013) Multivariate analysis for yield and its component traits in maize (*Zea mays* L.) under high and low N levels. Bioscan **8**:959–964.

Li, Q., Luo, YH., Tan, J., Kong, FL., Yang, SM. and Yuan, JC. (2014) Indexes screening and comprehensive evaluation of low nitrogen tolerance of hybrid maize cultivars at seedling stage. *Chinese Jouranl of Eco-Agriculture*, **22**: 1190–1199.

Liu, MN., Liu, XX., Ding, W.Y., Chen, QH. and Liu XY. (2012) Variation in nitrogen uptake and utilization efficiency in spinach genotypes and its evaluation. *Journal of Zhejiang University,*38: 599–607.

Liu, X., Zhao, Y. and Hao, F. (2022) Development of nitrogen efficiency screening system in alfalfa (*Medicago sativa* L.) and analysis of alfalfa nitrogen efficiency types. *PeerJ*, **10**: e13343.

Pathak R. R., Ahmad A., Lochab S. and Raghuram N. (2008) Molecular physiology of plant nitrogen use efficiency and biotechnological options for its enhancement. *Current Sci.*, **94**: 1394–1403.

Prasad R. (1998) Fertilizer urea, food security and the environment. *Current Science*, **75**: 677–683.

Robinson, N., Fletcher, A. and Whan A. (2007) Sugarcane genotypes differ in internal nitrogen use efficiency. *Functional Plant Biology,* **34**(12):1122–9.

Sandhu, N., Sethi, M., Kumar, A., Dang, D., Singh, J. and Chhuneja, P. (2021) Biochemical and genetic approaches improving nitrogen use efficiency in cereal crops: a review. *Frontiers in Plant Science*, **12**: 657629.

Shah, A. N., Tanveer, M., Abbas, A., Yildirim, M., Shah, A. A. and Ahmad, M. I. (2021a) Combating dual challenges in maize under high planting density: stem lodging and kernel abortion. *Frontiers in Plant Science*, **12**: 699085.

Shah, A.N., Wu, Y., Iqbal, J., Tanveer, M., Bashir, S. and Rahman, S. U. (2021b) Nitrogen and plant density effects on growth, yield performance of two different cotton cultivars from different origin *Journal of King Saud University Science,* **33**: 101512.

Statista Search Department (2021, September 13th) *Leading software as a service (SaaS) countries worldwide in 2021, by number of companies* [Infographic]. Statista. https://www.statista.com

Tian, X.L., Wang, G.W. and Zhu, R. (2008) Conditions and indicators for screening cotton (*Gossypium hirsutum*L.) varieties tolerant to low potassium. *Acta Agronomica Sinica*, **34**(8): 1435–43.

Tsai, C.Y., Huber, DM. and Glover, DV., (1984) Relationship of N deposition to grain yield and N response of three maize hybrids. *Crop Science,* **24**(2):277 –81.

Vijayalakshmi, P., Kiran, T. V., Rao, Y. V., Srikanth, B., Rao, I. S.and Sailaja, B. (2013) Physiological approaches for increasing nitrogen use efficiency in rice**.** *Indian Journal of Plant Physiology,* **18:** 208–222**.**

Wang, RF., An, DG. and Hu, CS. (2011) Relationship between nitrogen uptake and use efficiency of winter wheat grown in the North China Plain. *Crop and Pasture Science*, **62**(6):504 –14.

Wang, Y., Liu, B., Ren, T., Li, X., Cong, R., Zhang, M., Yousaf, M. and Lu, J. (2014) Establishment method affects oilseed rape yield and the response to nitrogen fertilizer. *Agronomy Jour.*, ***106***(1): .131-142.

Wang, Yin., Xu, Zhuo., Li, B., Gao, Q., Feng, G., Li, C., Yan, L. and Wang, S. (2018) Effects of urea ammonium nitrate solution on grain yield and nitrogen uptake of spring maize in black soil region. *Scientia Agricultura Sinica,* **51**: 718-72.

Worku, M., Bänziger, M., Erley GS., Friesen, D., Diallo AO. and Horst, WJ. (2012) Nitrogen efficiency as related to dry matter partitioning and root system size in tropical midaltitude maize hybrids under different levels of nitrogen stress. *Field Crops Research,* **130**: 57–67.

Xu, G., Fan, X. and Miller AJ. (2012) Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, **63**: 153-82.

Zadeh L. (1965) Fuzzy Sets. *Information and Control*, **8:338**-353.

Zhang, FF., Gao, S. and Zhao, YY. (2015) Growth traits and nitrogen assimilation-associated physiological parameters of wheat (*Triticum aestivum* L.) under low and high N conditions. *Journal of Integrative* Agriculture, **14**(7):1295 –308.

Zhang, H., Fu, X., Wang, X., Gui, H., Dong, Q., Pang, N., Wang, Z., Zhang, X. and Song, M. (2018) Identification and screening of nitrogen efficient cotton genotypes under low and normal nitrogen environments at the seedling stage. *Journal of Cotton Research,***1**: 6.

Zhao, C.B., Song, S.Y., Zhao, J., Zhang, X.M., Zhang, Y. and Zhang, ST. (2015) Variation in nitrogen uptake and utilization efficiency of different Cucumber varieties in Northern China. *Acta Agrestia Sinica*, **48**: 1569–1578.

Zou, XY., Liu, BL., Song, LQ. and Guan, CY. (2017) Identification and evaluation of nitrogen nutrition efficiency in rapeseed germplasm at seedling stage. *Chinese Journal of Oil Crop Sciences,* **39**: 069–077.