REVISTA de la FACULTAD de AGRONOMIA

Morphological response of native maize (*Zea mays* L.) seedlings to contrasting nitrogen environments

Respuesta morfológica de plántulas de maíz nativo (*Zea mays* L.) a ambientes contrastantes de nitrógeno

Resposta morfológica de plântulas de milho nativo (*Zea mays* L.) a ambientes contrastantes de nitrogênio

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Rev. Fac. Agron. (LUZ). 2024, 41(4): e244134
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v41.n4.03

Crop production
Associate editor: Dr. Jorge Vilchez-Perozo
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

Received: 06-06-2024
Accepted: 11-09-2024
Published: 17-09-2024

Keywords:
Cereals
Nitrogen use efficiency
Root architecture
Native varieties

Abstract

Nitrogen plays a vital role in plant metabolism, influencing growth and development, particularly in crops like maize (*Zea mays* L.). This study aimed to evaluate the morphological response of maize seedlings to different nitrogen levels. The design was a completely randomized factorial arrangement of 4 x 2, involving four maize cultivars and two nitrogen levels.The variety Sb 302 Berentsen and three native varieties originating from Tecamachalco, Puebla, Mexico were studied. For a period of 14, 21, 28 and 35 days, seedlings were grown in nutrient solution with 10 % and 100 % nitrogen levels under hydroponic conditions. The results revealed significant variability in seedling morphology, particularly in root architecture and dry weight, between the 10 % and 100 % nitrogen treatments. High coefficients of variation were observed in the lengths of crown and seminal roots, alongside significant correlations between root and seedling dry weights at both nitrogen levels. Additionally, a strong correlation was found between root length and number under the 10 % nitrogen treatment. The results highlight the critical role of nitrogen in maize seedling development and the interaction between nitrogen concentration and maize variety, particularly in primary root length. The study improves understanding of nitrogen's role in optimizing maize growth and suggests strategies to enhance nitrogen use efficiency across different maize varieties.

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Resumen

El nitrógeno desempeña un papel vital en el metabolismo de las plantas, influyendo en su crecimiento y desarrollo, especialmente en cultivos como el maíz (*Zea mays* L.). El estudio tuvo como objetivo evaluar la respuesta morfológica de las plántulas de maíz a diferentes niveles de nitrógeno. El diseño fue un arreglo factorial completamente aleatorizado de 4 x 2, que incluía cuatro cultivares de maíz y dos niveles de nitrógeno.Se estudió la variedad Sb 302 Berentsen y tres variedades nativas originarias de Tecamachalco, Puebla, México. Por un periodo de 14, 21, 28 y 35 días, las plántulas se cultivaron en solución nutritiva con niveles de 10 % y 100 % de nitrógeno en condiciones hidropónicas.Los resultados revelaron una variabilidad significativa en la morfología de las plántulas, particularmente en la arquitectura de las raíces y el peso seco, entre los tratamientos con 10 % y 100 % de nitrógeno. Se observaron altos coeficientes de variación en las longitudes de las raíces adventicias y seminales, junto con correlaciones significativas entre los pesos secos de las raíces y las plántulas en ambos niveles de nitrógeno. También se encontró una correlación fuerte entre la longitud y el número de raíces en el tratamiento con 10 % de nitrógeno. Los resultados destacan el papel crítico del nitrógeno en el desarrollo de las plántulas de maíz y la interacción entre la concentración de nitrógeno y la variedad de maíz, particularmente en la longitud de la raíz primaria. El estudio mejora la comprensión del papel del nitrógeno en la optimización del crecimiento del maíz y sugiere estrategias para mejorar la eficiencia en el uso del nitrógeno en diferentes variedades de maíz.

Palabras clave: cereales, uso eficiente de nitrógeno, arquitectura radicular, variedades nativas.

Resumo

O nitrogênio desempenha um papel vital no metabolismo das plantas, influenciando o crescimento e o desenvolvimento, especialmente em culturas como o milho (*Zea mays* L.). O estudo teve como objetivo avaliar a resposta morfológica de plântulas de milho a diferentes níveis de nitrogênio. O delineamento foi um arranjo fatorial completamente casualizado de 4 x 2, envolvendo quatro cultivares de milho e dois níveis de nitrogênio. Foram estudadas a variedade Sb 302 Berentsen e três variedades nativas originárias de Tecamachalco, Puebla, México. Durante um período de 14, 21, 28 e 35 dias, as plântulas foram cultivadas em solução nutritiva com níveis de nitrogênio de 10 % e 100 % em condições hidropônicas. Os resultados revelaram uma variabilidade significativa na morfologia das plântulas, especialmente na arquitetura das raízes e no peso seco, entre os tratamentos com 10 % e 100 % de nitrogênio. Foram observados altos coeficientes de variação nas longitudes das raízes adventícias e seminal, juntamente com correlações significativas entre os pesos secos das raízes e das plântulas em ambos os níveis de nitrogênio. Além disso, foi detectada uma correlação mais forte entre o comprimento e o número de raízes seminal sob o tratamento com 10 % de nitrogênio. Também foi encontrada uma forte correlação entre o comprimento e o número de raízes no tratamento com 10 % de nitrogênio. Os resultados destacam o papel crucial do nitrogênio no desenvolvimento das plântulas de milho e a interação entre a concentração de nitrogênio e a variedade de milho, particularmente no comprimento da raiz primária. O estudo melhora a compreensão do papel do nitrogênio na otimização do crescimento do milho e sugere estratégias para melhorar a eficiência do uso do nitrogênio em diferentes variedades de milho.

Palabras-chave:cereais, eficiência do uso de nitrogênio, arquitetura radicular, variedades nativas.

Introduction

Nitrogen (N) is a crucial element for achieving satisfactory yields, as it is essential in plant metabolism and related to the production of stems and leaves, which absorb light to carry out photosynthesis, therefore nitrogen fertilizers have a significant impact on the growth and development of crops (Asibi *et al*., 2019); however, information on their efficient use in maize varieties is still limited (Zuffo *et al*., 2021). Additionally, excessive nitrogen use contributes to significant environmental issues such as atmospheric pollution, aquifer contamination, and aquatic ecosystem degradation through processes like volatilization, leaching, runoff, and denitrification (Martínez-Dalmau *et al*., 2021).

Maize is among the plants that strongly respond to nitrogen fertilization and is characterized by a specific nitrogen absorption dynamic (Barrios *et al*., 2012). Nitrogen deficiency in plants generally results in stunted growth and chlorotic leaves caused by poor assimilation leading to premature flowering and shortened growth cycles (Mu & Chen, 2021). Nitrogen excess promotes aboveground biomass development with abundant dark green tissues (high chlorophyll) of soft consistency and relatively poor root growth (Hokam *et al*., 2011). Maize plants form a complex root system that appears and modifies at different stages of their development; while the purpose is to extract water and mineral nutrients from the soil, each root type is structurally and functionally different from the others (Hochholdinger *et al*., 2018). Excessive nitrogen supply causes many environmental problems, such as greenhouse gas emissions and surface and groundwater contamination. Then, the efficient use of nitrogen is one of the factors for maintaining the productivity and sustainability of agroecosystems (Noor, 2017). In this context, the objective of the study was to evaluate the morphological response of native maize seedlings (*Zea mays* L.) seedlings under contrasting nitrogen conditions.

Materials and methods

The experiment was carried out at the facilities of the Agricultural Science Research Center (BUAP), located in the municipality of Puebla, Mexico, at an altitude of 2150 meters above sea level (19º13′48″ N, 98º19′42″ W). Four maize varieties were evaluated, involving one improved variety (Sb 302 Berentsen) and three native landraces (white, blue, and red) originating from the municipality of Tecamachalco in the state of Puebla, Mexico.

Seedlings were grown under hydroponic conditions using modified Hoagland and Arnon (1950) nutrient solution at 10 % and 100 % nitrogen levels. The seedling trials spanned periods of 14, 21, 28 and 35 days after sowing. A completely randomized design with a 4 x 2 factorial arrangement (four maize varieties and two nitrogen levels). Each trial was replicated three times.

Plant characterization was conducted using the paper roll method described by Woll *et al*. (2005). For seed preparation, they were disinfected with 6 % sodium hypochlorite for 5 min, followed by rinsing with water three times. Subsequently, four seeds were placed between wet filter paper sheets, which were rolled to form four rolls per variety and per nitrogen dose. The rolls were vertically arranged in cylindrical containers measuring 21.5 cm in height and 8 cm in diameter for the 14, 21, and 28-day trials, and 32 cm in height and 24 cm in diameter for the 35-day trial.

At each time point, for data analysis, six healthy seedlings were randomly selected for each variety and nitrogen level, totaling 18 observations per combination of factors each day. Each seedling was considered as an experimental unit. The evaluated parameters were as follows: primary root length (cm) (PRL), mesocotyl length (cm) (ML), seminal root number (SRN), total length of seminal roots (cm) (SRL), crown root number (CRN), total length of crown roots (cm) (CRL), plant length (cm) (PL), plant dry weight (g) (PDW) and root dry weight (g) (RDW).

The PL was measured from the node marked by the crown roots to the longest leaf of the seedling. The RDW and PDW were measured using a precision scale after drying the samples for 48 hours at 70 ºC.

Statistical model associated with the experimental design

For each variable, a descriptive statistical analysis was performed, calculating the minimum, maximum, mean, and standard deviation (*SD*) for each treatment (10 % and 100 % N) and for each trial (14, 21, 28, and 35 days). The coefficient of variation (CV) was estimated for the same database, defined as follows:

$$\text{CV}=\frac{SD}{Mean}\ x\ 100,$$

this coefficient multiplied by 100 expresses CV as a percentage. Additionally, the reduction in the response from one dose of N to another was estimated by the percentage of reduction of mean (% RM), according to:

$$\%\ \text{RM} = \left[\frac{(HN - LN)}{HN}\right] x\ 100,$$

Where *HN* and *LN* correspond to the higher and lower nitrogen levels, respectively. The percentage reduction in the response of one method compared to another, in this study based on the N dose.

After calculating the mean and standard deviation, the seedlings were classified into three yield categories: the first category includes varieties with low yield and undesirable root characteristics [≤ *mean-SD*], the second category includes varieties with medium yield with values between [≥ *mean-SD*] and [≤ *mean+SD*], and the third category includes varieties with high yield [≥ *mean+SD*]. This was based on the frequency percentage of seedlings in each interval for each variable or trait. Subsequently, these frequency data were used to calculate the Shannon-Weaver diversity index (*H'*), which is a value quantifying species biodiversity, given as follows:

$$H' = \sum_{i=1}^{n} P_i ln P_i$$

where *Pi* is the proportion of individuals in the *i*-th category and *n* is the number of phenotypic classes, in this case. In this study, the number of classes is three, as there are three categories in which the frequencies are found (low, medium, and high).

For each pair of variables in the treatments and trial days, correlation coefficients (*r*) were calculated between them, which help to determine the degree of relationship between each pair of variables.

Finally, in this study, an analysis of variance (two-way ANOVA) of the two factors studied was carried out with an interaction for each trial day, using the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon,$$

where Y_{ij} is the response of the variable at the *i*-th level of factor α (variety) and at the *j*-th level of factor β factor (nitrogen level), μ is the mean value of Y_{ij}, α_i represents the effect of the *i*-th level of factor α on the global mean μ, β_j represents the effect of the *j*-th level of factor β on the global mean μ, $\alpha\beta_{ij}$ represents the effect of the interaction (variety × nitrogen level) between the *i*-th level of factor α and the *j*-th level of factorβ, and ε is the random error of Y_{ij}. Through this model, it can be observed if there are differences between the N treatments or if there are significant differences in maize varieties, as well as in their interactions (variety x N level) (Zar, 2010).

Results and discussion

A descriptive statistical analysis was performed for each variable in both nitrogen treatments, analyzing data from the trials on days 14, 21, 28, and 35, the results in table 1 show the ranges of the measurements. For instance, the PRL variable exhibits consistent ranges for both the 100 % N treatment (5.7 to 28.2 cm) and the 10 % N treatment (9 to 25.4 cm). A similar pattern was observed for the ML variable, with values ranging from 0.6 to 2.5 cm for 10 % N and from 0.7 to 2.7 cm for 100 % N. The CRN and SRN variables showed a broader range, with the number of roots varying from 0 to 14 in both treatments and across all trials. Additionally, the SRL and CRL variables showed wide ranges, with SRL extending from 1.5 to 187.6 cm and CRL from 0 to 54.9 cm. The maximum and minimum values of dry weights of the seedlings and roots (PDW, and RDW) increased over time, although differences between nitrogen treatments were minimal. These results agree with those of Liu *et al*. (2017) and Schneider *et al*. (2021), who found that nitrogen supply significantly influences plant growth and root system roles in nitrogen acquisition.

The coefficient of variation showed that variability between measurement days for each parameter was relatively constant. PRL with 10 % N had the least dispersion, with a CV ranging from 14.3 % to 20.8 %. The PL registered a CV of 29.5 % on day 14 with 10 % N, decreasing to an average of 19 % on subsequent days. The CRN and SRN variables exhibited considerable data dispersion, with CVs for the SRN variable ranging from 30.9 % to 40.7 % for 10 % N and from 36.5 % to 52.4 % for 100 % N. For CRN, the CV ranged from 55.6 % to 63.5 % for the 10 % N treatment, except on day 21. Both SRL and CRL showed very high CV, most above 50 %, with CRL on day 14 having values of 111 % and 108.7 % for the 10 % and 100 % N doses, respectively. The dry weight variables (PDW, and RDW) also showed high variability, with CVs ranging from 30.6 % to 48.7 % for 10 % N and from 27.3 % to 43.8 % for 100 % N.

The last column of table 1 shows the percentage of mean reduction, calculated from the averages of both nitrogen treatments. The PRL variable had more favorable development at 14 and 21 days with 10 % N, with improvement percentages of 15 % and 27 %, respectively. For 28 and 35 days, the development was better with 100 % N, showing increases of 27 % and 14 %, respectively. A similar behavior was recorded for the ML variable, although with lower percentages. The SRN and SRL variables exhibited optimal development with the 10 % N dose, especially for SRN on day 35, where an increase of 113 % was observed. For crown roots, the 100 % N treatment showed a higher mean reduction percentage in the CRN variable, possibly because all seedlings developed at least one crown root, while with the lower dose some did not. However, the CRL had a higher percentage with 10 % N, indicating that those seedlings that developed crown roots had longer roots than in the 100 % N treatment. Maqbool *et al*. (2022) highlighted that a well-distributed root morphology is crucial for the efficient absorption of mobile nutrients.

Table 1. Mean, maximum value, minimum value, coefficient of variation, and percentage of reduction of mean.

Trait	Day	10 % nitrogen				100 % nitrogen				% reduction of mean
		Min.	Max.	Mean	CV %	Min.	Max.	Mean	CV %	
PRL	14	9	19.30	15.82	14.33	9.50	19.30	13.71	18.67	-15.37
	21	12.50	25	18.48	18.65	5.70	21.80	14.54	32.89	-27.07
	28	10.20	20.20	14.65	17.12	11.40	28.20	20.32	22.33	27.90
	35	10.40	25.40	15.64	20.82	5.70	27	18.34	30.18	14.71
ML	14	0.60	2	1.20	30.86	0.70	2	1.12	29.45	-7.40
	21	0.70	2.50	1.16	32.76	0.80	2.20	1.13	28.57	-2.44
	28	0.60	2.50	1.27	40.34	0.80	2.70	1.37	36.73	7.00
	35	0.70	2.20	1.24	32.80	0.60	2	1.33	32.70	6.56
SRN	14	1	7	4.04	36.83	2	6	3.41	36.53	-18.29
	21	1	6	3.66	40.74	0	7	3.39	52.40	-8.13
	28	3	10	6.95	30.99	1	8	3.62	48.68	-91.94
	35	3	14	7.29	32.97	2	6	3.41	39.47	-113.40
SRL	14	4.60	70.40	37.65	51.90	6.60	51.30	25.81	50.41	-45.87
	21	6.20	114.90	57.95	47.76	0	94.60	39.97	63.80	-44.98
	28	16.70	137.80	80.52	39.17	1.50	110.10	47.81	49.29	-68.41
	35	0.70	187.60	83.04	46.89	9	120.40	46.80	58.41	-77.43
CRN	14	0	7	3.12	56.86	1	7	3.62	41.33	13.79
	21	2	6	4.37	22.16	3	7	4.43	22.37	1.35
	28	0	7	3	55.60	2	10	4.87	47.75	38.46
	35	0	10	3.12	63.52	3	8	5.41	27.17	42.31
CRL	14	0	17	3.34	111.04	0.10	18.30	3.87	108.74	13.65
	21	1.30	54.90	26.79	54.30	0.90	39	14.42	69.90	-85.78
	28	0	48.30	14.57	87.15	0.50	18.60	6.41	82.49	-127.30
	35	0	38	10.60	89.80	1	34.20	7.85	98.70	-35.03
PL	14	0.80	19.30	11.78	29.57	9.60	19.30	14.04	21.30	16.08
	21	13.40	28.60	21.47	18.08	12.60	33.60	21.57	19.18	0.45
	28	19.10	40.90	27.26	19.59	19.50	45.10	27.09	23.12	-0.62
	35	19.70	45.40	32.60	18.22	15.50	41.40	26.97	23.28	-20.87
PDW	14	0.02	0.10	0.06	32.31	0.03	0.13	0.05	40.34	-5.72
	21	0.06	0.22	0.11	34.78	0.03	0.14	0.09	29.65	-21.33
	28	0.06	0.20	0.10	30.60	0.03	0.15	0.07	41.87	-41.21
	35	0.05	0.22	0.13	32.22	0.04	0.20	0.09	37.65	-37.71
RDW	14	0.01	0.09	0.05	39.88	0.03	0.08	0.04	38.02	-15.24
	21	0.03	0.16	0.08	41.68	0.02	0.12	0.06	36.10	51.40
	28	0.03	0.15	0.07	41.87	0.33	1	0.70	27.39	89.30
	35	0.03	0.24	0.11	48.77	0.03	0.15	0.07	43.89	-49.16

Legend: PRL: primary root length (cm), ML: mesocotyl length (cm), SRN: seminal root number, SRL: total length of seminal roots (cm), CRN: crown root number, CRL: total length of crown roots (cm), PL: plant length (cm), PDW: plant dry weight (g) and RDW: root dry weight (g).

Table 2 shows the frequency distributions of the seedlings. Most seedlings were classified at the medium level, with percentages ranging from 58 % to 87 % for 10 % N and from 41 % to 87 % for 100 % Ntreatments. The percentages of seedlings at the high level ranged from 4 % to 20 % for 10 % N and from 4 % to 29 % for 100 % N. Most biodiversity indices were high, ranging from 0.56 to 0.96 for 10 % N and from 0.61 to 0.99 for 100 % N, with a lower CRL index of 0.37 on day 14. Exceptions include CRL indices of 0.37 and 0.45 on days 14 and 35, and the RDW index of 0.37 on day 14. The SRN variable showed particularly high indices of 1 and 1.07 on days 14 and 35.

Table 3 shows the correlations between different pairs of variables for each day of the 10 % and 100 % N treatments. Medium correlations were observed between various combinations of variables. For example, PRL showed a medium positive correlation with ML (r=0.46 on day 35), SRN (r=0.64) and SRL (r=0.70 on day 21), and with PL, PDW, and RDW (r coefficients of 0.61, 0.52, 0.43, 0.54, and 0.50 on day 35) under the 10 % N treatment. For the 100 % N treatment, PRL correlated negatively with ML (r=-0.53 on day 35), and positively with SRL (r=0.55 on day 21 and r=0.57 on day 35), and PDW (r=-0.41, 0.45, and 0.42 on days 14, 21, and 35). Previous studies like those by Pace *et al*. (2014) have found that PRL

Table 2. Frequencies of the varieties divided into three categories and Shannon-Weaver index.

Trait	Day	Frequency 10 % nitrogen			H’	Frequency 100 % nitrogen			H’
		Low	Medium	High		Low	Medium	High	
PRL	14	0.08	0.75	0.16	0.72	0.16	0.62	0.20	0.91
	21	0.29	0.58	0.12	0.93	0.12	0.70	0.16	0.80
	28	0.16	0.70	0.12	0.80	0.25	0.66	0.08	0.82
	35	0.12	0.79	0.08	0.65	0.16	0.62	0.20	0.91
ML	14	0.08	0.70	0.20	0.77	0.04	0.70	0.25	0.72
	21	0.08	0.79	0.12	0.65	0.12	0.75	0.12	0.73
	28	0.08	0.70	0.20	0.77	0.08	0.70	0.20	0.77
	35	0.08	0.75	0.16	0.72	0.16	0.54	0.29	0.99
SRN	14	0.12	0.75	0.12	0.73	0.20	0.54	0.25	1.00
	21	0.20	0.66	0.12	0.85	0.16	0.70	0.12	0.80
	28	0.16	0.79	0.04	0.61	0.12	0.70	0.16	0.80
	35	0.08	0.75	0.16	0.72	0.33	0.41	0.25	1.07
SRL	14	0.20	0.58	0.20	0.96	0.29	0.58	0.12	0.93
	21	0.08	0.75	0.16	0.72	0.12	0.75	0.12	0.73
	28	0.16	0.66	0.16	0.86	0.12	0.75	0.12	0.73
	35	0.08	0.83	0.08	0.56	0.08	0.79	0.12	0.65
CRN	14	0.25	0.62	0.12	0.90	0.20	0.75	0.04	0.67
	21	0.12	0.75	0.12	0.73	0.20	0.70	0.08	0.77
	28	0.20	0.66	0.12	0.85	0.08	0.70	0.20	0.77
	35	0.16	0.75	0.08	0.72	0.08	0.66	0.25	0.82
CRL	14	0.00	0.87	0.12	0.37	0.00	0.87	0.12	0.37
	21	0.20	0.62	0.16	0.91	0.20	0.62	0.16	0.91
	28	0.20	0.62	0.16	0.91	0.16	0.70	0.12	0.80
	35	0.12	0.70	0.16	0.80	0.00	0.83	0.16	0.45
PL	14	0.04	0.79	0.16	0.61	0.12	0.66	0.20	0.85
	21	0.20	0.66	0.12	0.85	0.12	0.79	0.08	0.65
	28	0.20	0.66	0.12	0.85	0.08	0.75	0.16	0.72
	35	0.16	0.70	0.12	0.80	0.08	0.79	0.12	0.65
PDW	14	0.16	0.66	0.16	0.86	0.04	0.79	0.16	0.61
	21	0.16	0.70	0.12	0.80	0.12	0.75	0.12	0.73
	28	0.12	0.70	0.16	0.80	0.16	0.66	0.16	0.86
	35	0.16	0.62	0.20	0.91	0.16	0.70	0.12	0.80
RDW	14	0.29	0.62	0.08	0.86	0.00	0.87	0.12	0.37
	21	0.16	0.66	0.16	0.86	0.12	0.79	0.08	0.65
	28	0.16	0.66	0.16	0.86	0.12	0.66	0.20	0.85
	35	0.08	0.75	0.16	0.72	0.20	0.62	0.16	0.91

Legend: PRL: primary root length (cm), ML: mesocotyl length (cm), SRN: seminal root number, SRL: total length of seminal roots (cm), CRN: crown root number, CRL: total length of crown roots (cm), PL: plant length (cm), PDW: plant dry weight (g) and RDW: root dry weight (g).

is closely related to RDW. Similarly, Kumar *et al*. (2012) and Abdel-Ghani *et al*. (2013) suggest that positive correlations between PRL, SRL, CRL, and RDW, and other root characteristics indicate cultivars with well-developed roots at the seedling stage. Also, ML showed a medium correlation with CRL (r=0.53 on day 14), PL (r=0.57 on day 21 and r=0.41 on day 35), and with weights PDW, and RDW, with r coefficients of 0.54, 0.58, 0.56; 0.70, 0.62, 0.65; and 0.46, 0.60, 0.56; and 0.57, 0.60, 0.66 respectively for days 14, 21, and 35 under the 10 % N treatment.

In the 100 % N treatment, ML shows various correlations: it is negatively correlated with SRL (r=-0.74 on day 14) and positively with CRN (r=0.55 on day 28) and CRL (r=-0.44 and r=0.42 on days 21 and 28). It also correlates with PL (r=0.53 on day 28 and r=0.45 on day 35), and PDW (r=0.68 on the same day). The correlation with RDW is notable (r=0.48 and r=0.54 on days 14 and 28). Overall, SRN, SRL, CRN, CRL, PL, PDW, and RDW show moderate correlations on various measurement days with both nitrogen treatments. This pattern supports Wang *et al*. (2005), who reported the absence of consistent correlations between different levels of nitrogen supply.

In the 10 % N treatment, there is a high correlation between SRL and SRN, with coefficients of 0.72, 0.93, 0.83, and 0.79 for days 14, 21, 28, and 35, significant at $p<0.01$. For 100 % N, these correlations are lower, with values of 0.52, 0.66, and 0.64 for the same days and significantly lower on day 35.

Table 3. Data correlations. The upper triangular matrix shows the correlations between variables for each assay with 10 % nitrogen treatment. The lower triangular matrix shows the correlations between variables for each assay with 100 % nitrogen treatment.

Trait	Day	PRL	ML	SRN	SRL	CRN	CRL	PL	PDW	RDW
PRL	14		-0.28	-0.02	0.22	0.18	-0.17	-0.09	-0.05	0.00.
	21		-0.07	0.64**	0.70**	0.36	0.19	0.22	0.17	0.27
	28		0.30	0.01	0.05	0.01	-0.07	0.35	-0.23	-0.22
	35		0.46*	0.07	0.09	-0.27	-0.19	0.61**	0.54**	0.50*
ML	14	-0.53**		0.06	0.17	0.35	0.53**	0.35	0.46*	0.57**
	21	-0.40		0.06	0.16	0.27	0.24	0.57**	0.60**	0.60**
	28	0.20		-0.31	-0.11	0.15	0.15	0.26	0.25	0.34
	35	-0.09		0.18	0.37	0.18	0.13	0.41*	0.56**	0.66**
SRN	14	-0.04	-0.25		0.72**	0.27	0.26	0.13	0.05	0.21
	21	-0.16	0.03		0.93**	0.21	0.29	0.39	0.41*	0.48*
	28	0.26	0.25		0.83**	-0.26	-0.12	0.46*	-0.53**	-0.41*
	35	0.00	-0.09		0.79**	-0.33	-0.07	0.31	0.11	0.43*
SRL	14	0.55**	-0.74**	0.52**		0.44*	0.33	0.10	0.12	0.47*
	21	0.31	-0.18	0.66**		0.35	0.31	0.40	0.48*	0.59**
	28	0.37	0.18	0.64**		-0.18	-0.19	0.50*	-0.28	-0.19
	35	0.57**	0.13	0.24		-0.13	-0.03	0.30	0.18	0.43*
CRN	14	-0.35	0.16	0.15	-0.10		0.71**	0.21	0.54**	0.66**
	21	0.02	-0.38	0.49*	0.64**		0.37	0.18	0.37	0.50*
	28	-0.02	0.55**	0.31	0.16		0.51*	0.02	0.10	0.34
	35	0.17	0.24	0.06	0.41*		0.73**	-0.06	0.16	0.07
CRL	14	0.01	-0.35	0.13	0.27	0.53**		0.45*	0.55**	0.57**
	21	0.26	-0.44*	0.31	0.42*	0.52		0.53**	0.64**	0.74**
	28	0.16	0.42*	0.26	0.50*	0.56**		-0.12	0.14	0.26
	35	0.28	-0.12	0.19	0.46*	0.58**		0.20	0.45*	0.37
PL	14	-0.32	0.28	0.16	0.01	0.37	0.18		0.44*	0.34
	21	0.14	0.24	0.22	0.33	0.32	0.43*		0.76**	0.73**
	28	0.13	0.53**	0.23	0.36	0.59**	0.52**		-0.25	-0.12
	35	0.26	0.45*	0.02	0.18	0.43*	0.11		0.77**	0.63**
PDW	14	-0.41*	0.37	0.11	-0.17	0.42*	0.19	0.64**		0.79**
	21	0.45*	-0.03	0.12	0.33	0.28	0.21	0.56**		0.91**
	28	0.26	0.68**	0.44*	0.46*	0.63**	0.51*	0.67**		0.74**
	35	0.42*	0.39	0.20	0.45*	0.58**	0.52**	0.69**		0.82**
RDW	14	-0.22	0.48*	0.15	-0.21	0.47*	-0.00	0.56**	0.77**	
	21	0.34	-0.00	0.33	0.57**	0.45*	0.44*	0.58**	0.87**	
	28	0.13	0.54**	0.21	0.26	0.25	0.11	0.17	0.62**	
	35	0.36	0.37	0.39	0.52**	0.50*	0.59**	0.54**	0.74**	

*Significant at $p<0.05$. **Significant at $p<0.01$. Legend: PRL: primary root length (cm), ML: mesocotyl length (cm), SRN: seminal root number, SRL: total length of seminal roots (cm), CRN: crown root number, CRL: total length of crown roots (cm), PL: plant length (cm), PDW: plant dry weight (g) and RDW: root dry weight (g).

The analysis of variance in Table 4 reveals significant differences. The average ML value varies significantly between maize varieties over the four trial days ($p<0.001$). Similarly, PRL shows significant differences on days 14 and 21, but not on days 28 and 35. Meanwhile, the SRN and CRL variables do not exhibit significant differences between varieties on any measurement day. In nitrogen treatments, the PRL variable differs on all days ($p<0.01$), but no consistent differences are observed in ML on the trial days. Other parameters vary with nitrogen levels.

Blanco *et al*. (2004) found that dry matter production does not increase with higher nitrogen doses, consistent with the lack of differences in PDW in this study. The interaction between maize varieties and nitrogen levels is not significant, except on specific days for PRL, ML, and SRL($p<0.05$), and for CRL ($p<0.01$). Li *et al*. (2017) emphasize the importance of an efficient root system in maize hybrids, reducing the need for nitrogen fertilizers.

Conclusions

Native maize seedlings show distinct morphological responses to different nitrogen levels. Under the 10 % nitrogen treatment, some seedlings did not develop crown roots, while all seedlings in the 100 % nitrogen treatment formed at least one.

Table 4. P-values of the model's analysis of variance.

Trait	Day	ANOVA		
		Variety	Nitrogen level	Variety × Nitrogen level
PRL	14	0.000739 ***	0.000962 ***	0.354250
	21	8.59e-05 ***	0.00162 **	0.03070 *
	28	0.9600	2.29e-06 ***	0.0522
	35	0.270	0.038 *	0.135
ML	14	1.22e-12 ***	0.118	0.175
	21	6.2e-07 ***	0.610	0.594
	28	6.89e-09 ***	0.315	0.579
	35	1.06e-08 ***	0.2611	0.0487 *
SRN	14	0.214	0.117	0.439
	21	0.231	1	0.323
	28	0.814	8.92e-07 ***	0.260
	35	0.880	6.11e-08 ***	0.576
SRL	14	0.06113	0.00821 **	0.02575 *
	21	0.00796 **	0.11386	0.35454
	28	0.424991	0.000285 ***	0.786802
	35	0.956508	0.000761 ***	0.322807
CRN	14	0.0202 *	0.2427	0.0800
	21	0.260	1	0.124
	28	0.002682 **	0.000556 ***	0.120126
	35	0.659	7.54e-05 ***	0.625
CRL	14	0.3794	0.6124	0.0092 **
	21	0.63787	0.00127 **	0.38105
	28	0.67955	0.00808 **	0.85496
	35	0.789	0.292	0.521
PL	14	0.00444 **	0.00991 **	0.42681
	21	0.132	0.647	0.704
	28	0.00533 **	0.91130	0.27702
	35	0.000111 ***	0.000387 ***	0.849149
PDW	14	0.0954	0.5906	0.6994
	21	0.0218 *	0.1601	0.0954
	28	0.006686 **	0.000713 ***	0.832823
	35	0.000431 ***	0.000497 ***	0.386704
RDW	14	0.00144 **	0.14523	0.12282
	21	0.0190 *	0.0176 *	0.1280
	28	0.0695	$<$2e-16 ***	0.3129
	35	0.000643 ***	0.001620 **	0.249387

*Significant at $p<0.05$. **Significant at $p<0.01$. ***Significant at $p<0.001$. Legend: PRL: primary root length (cm), ML: mesocotyl length (cm), SRN: seminal root number, SRL: total length of seminal roots (cm), CRN: crown root number, CRL: total length of crown roots (cm), PL: plant length (cm), PDW: plant dry weight (g) and RDW: root dry weight (g).

The analysis revealed significant variation in seedling lengths, root development, and dry weights under both nitrogen levels, with the 10 % nitrogen treatment resulting in greater variability, particularly in crown and seminal root traits. Despite this variability, significant differences were detected among maize varieties, particularly in PRL and ML, on specific days. The nitrogen trials significantly affected PRL across all days, highlighting the influence of nitrogen availability on root development. Additionally, a significant interaction between maize varieties and nitrogen levels was observed, particularly for PRL, ML, and SRL on certain days.

The biodiversity index was generally high across the nitrogen treatments, with the highest correlations observed among the dry weight variables (PDW and RDW). However, the correlation between SRL and SRN was stronger in the 10% nitrogen treatment compared to the 100 % nitrogen treatment.

The importance of nitrogen in shaping the morphological traits of native maize seedlings and highlight the significant role of variety-specific responses and their interaction with nitrogen levels. The native maize varieties adapt to different nitrogen conditions, with implications for optimizing nitrogen use in maize cultivation.

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