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Original Paper

Assessment of root architecture traits in seedlings of Algerian maize populations grown under two nitrogen levels

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Despite the critical role played by roots for the acquisition of water and nutrients from the soil, especially in stressful conditions; root characteristics are rarely utilized as selection criteria to improve nutrient uptake efficiency in maize breeding programs. In this study, seedlings of twelve Algerian maize populations with two commercial maize hybrids were evaluated to: (i) study the phenotypic variation of nine root traits under low nitrogen (LN) and high nitrogen (HN) levels and (ii) determine root traits accounting for most of the phenotypic variation among the evaluated populations. High phenotypic diversity was observed for all studied traits under both N levels. Under LN conditions, seedlings adapt by rapidly increasing their root systems to exploit nitrogen (N) resources, resulting in significant increases in primary root length (PRL), seminal root length (SRL), seminal root number (SRN), and total root length (TRL). Conversely, shoot dry weight (SDW) decreased. The first two principal components explained 73.46 and 74.82% of the total variation among the maize populations under LN and HN conditions, respectively. Thus, root dry weight (RDW) and TRL were major contributors of the phenotypic variation. In addition, RDW and TRL were significantly correlated with most of the other traits under both N levels. Therefore, during the seedling stage, RDW and TRL can be used as favorable selection criteria. This diversity gives opportunities for enhancing N use efficiency, potentially leading to reducing N fertilizer requirements. As a result, this can contribute to reduce input costs and maintaining environmental quality.

Keywords: *Zea mays* L., algerian germplasm, seedling root traits, hydroponics, nitrogen

1 Introduction

Root system plays an important role in the acquisition of water and nutrient, plant anchorage (Li et al., 2019; Reddy et al., 2020), and interacting with both abiotic and biotic rhizosphere factors (Smith & De Smet, 2012). Root system, despite its obvious importance, had received less attention in selective breeding (Ma et al., 2020) due to its lower visibility compared to aboveground plant parts. Nitrogen (N) plays a major role in maize production (Abdel-Ghani et al., 2013), but it comes at a significant cost to both farmers and environment (Good & Beatty, 2011). It has been estimated that 50–75% of the N supplied to the soil is lost (Good & Beatty, 2011) through a combination of leaching, denitrification, and volatilization (Cameron & Moir, 2013). However, this situation can be improved by enhancing the N uptake efficiency. In maize, N uptake is strongly influenced by root system architecture (RSA) and its functioning (Mi et al., 2007; Foulkes et al., 2009), particularly under N-deficient conditions (Mi et al., 2007). Consequently, improved N acquisition through root morphology is a new strategy for increasing N use efficiency in maize (Torres et al., 2019). N is a mobile element that can be easily leached through the soil profile (Foulkes et al., 2009). Therefore, Maize cultivars having a larger root system may have a higher N uptake efficiency (Kumar et al., 2012). However, root characteristics have rarely been used as selection criteria to improve nutrient use efficiency in maize (Liu et al., 2017). Many studies have shown extensive genetic and phenotypic variation in root architecture (Pace et al., 2014; Torres et al., 2019), which gives opportunity for improving nutrient use efficiency (Abdel-Ghani et al., 2015; Reddy et al., 2020). Recently, plant breeders are turning their attention to select from natural resources to improve root traits in maize (Li et al., 2015). Maize has a complex root system

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that can be divided into embryonic and post-embryonic roots (Ju et al., 2018) formed during different stages of the development (Hochholdinger, 2009). Embryonic roots consist of one primary root and some seminal roots, which are important for early development at the seedling stage; while, post-embryonic roots are shoot-borne roots, including crown roots (formed above the soil surface), brace roots (formed below the soil surface) and lateral roots derived from the pericycles of other roots playing a major role in the soil resource acquisition and anchoring in later growth and developmental stages (Hochholdinger, 2004; Hochholdinger, 2009; Li et al., 2019). Post-embryogenic, shoot-borne roots make up the major portion of adult root biomass (Pace et al., 2014). However, young seedlings possess all the root types present in adult plants, except for brace roots (Hochholdinger & Tuberosa, 2009). Therefore, young seedlings are often used for root trait evaluation (Hochholdinger, 2004). In addition, investigating the root systems of young seedlings has been the prevalent method employed to circumvent challenges associated with time and labor constraints in collecting complete root systems from the soil (Li et al., 2015; Wang et al., 2019). Several laboratory-based approaches for root phenotyping have been adopted to enable an early screening of RSA traits in maize (Abdel-Ghani et al., 2016; Liu et al., 2017). However, paper roll culture under hydroponic conditions was discovered to be the best system to study root architecture at the seedling stage (Kumar et al., 2014; Wang et al., 2019). The use of paper roll tests offers a number of advantages, including rapid, inexpensive and accurate measurement at an early stage of growth (Abdel-Ghani et al., 2016; Liu et al., 2017). N availability in the soil has significant effects on maize RSA (Abdel-Ghani et al., 2013; Li et al., 2015). Nevertheless, contradictory results were discovered regarding the effect of N availability on RSA (Abdel-Ghani et al., 2013). Modifying RSA is a major adaptive strategy to optimize N acquisition (Mi et al., 2016), especially under N-deficient conditions (Li et al., 2016). Numerous studies have provided evidence of the extensive genetic diversity present in Algerian maize (Belalia et al., 2019), which offers a source of traits with an adaptive value for challenging environments due to its long-term adaptation over several centuries to extreme conditions.

Consequently, the objectives of this study were to:

1. characterize the phenotypic variation of Algerian maize populations at seedling stage under contrasting N levels;
2. determine the root traits that account for the majority of the variation in the tested populations.

2 Material and methods

2.1 Plant materials

The plant material used in this study was a collection of twelve open-pollinated Algerian maize populations (Table 1). These populations were collected on the basis of geographical origin in the oasis of Adrar and Timimoun in the southwest of the Algerian Sahara. We also used two hybrids (Torro, H1 and Kukuruza, H2), widely grown in Algeria, as checks.

Table 1 Origin and brief description of the twelve populations of maize from the Algerian Sahara

Code	Site	Region	Latitude	Longitude	Ear type	Kernel color
P1	Adrar	Adrar	28° 01′ 18″ N	0° 15′ 28″ W	flint	yellow
P2	OuledAhmed Tammi	Adrar	27° 50′ 25″ N	0° 17′ 02″ W	flint	orange
P3	Tsabit	Adrar	28° 24′ 00″ N	0° 14′ 08″ W	flint	yellow
P4	Aougrout	Timimoun	28° 42′ 00″ N	0° 20′ 30″ E	flint	orange
P5	Timimoun	Timimoun	29° 15′ 32″ N	0° 14′ 30″ E	flint	yellow-orange
P7	Zaouiet Kounta	Adrar	27° 13′ 27″ N	0° 11′ 23″ W	flint	yellow-violet
P9	Zaouiet Kounta	Adrar	27° 04′ 30″ N	0° 06′ 10″ W	flint/dent	yellow
P11	Fenoughil	Adrar	27° 39′ 27″ N	0° 18′ 35″ W	flint	yellow-violet
P12	Charouine	Timimoun	29° 11′ 23″ N	0° 16′ 10″ W	flint	orange
P13	Bouda	Adrar	28° 01′ 40″ N	0° 27′ 30″ W	flint/dent	yellow-orange
P14	Sebaa	Adrar	28° 12′ 14″ N	0° 10′ 26″ W	dent	yellow
P16	Tamantit	Adrar	27° 46′ 00″ N	0° 15′ 59″ W	dent	yellow

2.2 Hydroponics experiments for root phenotyping

RSA was evaluated according to the protocol described by Abdel-Ghani et al. (2016) in a paper roll system. For each genotype, 32 seeds of similar size were selected and surface sterilized for 15 min in a 6% sodium hypochlorite solution, then washed three times with distilled water. Thereafter, four sterilized seeds were placed on a double layer of brown germination papers pre-moistened with fungicide solution Captan® (2.5 g.l^{-1}).

All germination paper rolls were placed, in vertical orientation, in plastic containers (40 × 20 × 15 cm) containing 6 L Hoagland's nutrient solution with two N levels. The concentration of N in Hoagland's solution with high nitrogen (HN) is 15 mM of NO_3^-, whereas the concentration of N in Hoagland's solution with low nitrogen (LN) is 1.5 mM of NO_3^-. The composition of the solutions is described by Abdel-Ghani et al. (2016). The nutrient solution was aerated by an electric pump.

The experiments were conducted out in a growth chamber with controlled conditions: a photoperiod of 16/8 h (light/darkness) at 25/22 °C (day/night), and a relative humidity maintained at 65%. After 14 days, seedlings were preserved in 30% ethanol and stored in a cold room to prevent additional growth.

The experiment was conducted with a randomized block design with split-plot arrangement of treatments, with three replicates per N treatments (N treatments as main plots and genotypes as sub-plots). Each paper roll with three healthy seedlings of the four was considered as experimental unit.

At the crown root region, seedlings were divided into root systems and shoots. Nine seedling phenotypic traits were assessed at two N levels. The primary root length (PRL), crown root length (CRL) and seminal root length (SRL) were measured with a ruler. Total root length (TRL) was estimated by summing the lengths of PRL, CRL and SRL for each seedling. The numbers of seminal roots (SRN) and crown roots (CRN) were also counted. Seeds were removed from the roots to determine root dry weights (RDW), shoot dry weight (SDW) and root-shoot ratio (RDW/SDW). Roots and shoots were oven-dried at 65 °C until a constant weight. In addition, the percent reduction in response to N stress was estimated as follows (Abdel-Ghani et al., 2013):

$$\% \text{ of reduction} = (HN - LN)/HN \times 100$$

where: *HN* and *LN* – the mean of tested traits under high and low nitrogen levels, respectively

2.3 Statistical analysis

Data statistical analysis was carried out using the R software package. For each trait in both N treatments, descriptive statistics (means with their standard deviations, range and coefficient of variation) were calculated. Analysis of variance (ANOVA) was performed by "Doebioresearch" package to test for significance differences between N treatments (N), genotypes (G) and N × G interactions. Principal component analyses were performed using the "factoextra" package, while for correlation analysis the "corrplot" and "ggcorrplot" packages were used to calculate the coefficients and *p*-value matrices.

3 Results and discussion

3.1 Evaluation of RSA-related traits

Root characteristics have not been previously utilized as a selection criterion primarily due to the challenges associated with measuring within a field setting at the adult stage (Pace et al., 2014; Pace et al., 2015), especially in the case of larger plants like maize (Wang et al., 2019).

The ANOVA showed highly significant phenotypic differences between the genotypes for all investigated traits under both N levels (Table 2), with coefficients of variation (CV) ranging from 9.87 (SDW) to 39.15% (CRL) and from 11.66 (PRL) to 32.84% (R/S) under LN and HN levels, respectively, indicating the presence of substantial phenotypic variation (Table 3). The coefficients of variation were comparable to those obtained by Torres et al. (2019) with seedlings of 150 maize inbred lines. In addition, most of the traits exhibited higher CV values under LN than HN conditions. In general, traits had intermediate to low CV, with the exception of CRL, SRL and RDW/SDW, which had high CV values. Consequently, our results indicate good experimental precision.

This considerable phenotypic variation among the 12 populations for all measured traits evaluated under both LN and HN levels, provide an opportunity for improved N use efficiency under stressful conditions. Similarly, several studies have reported substantial genetic and phenotypic variations in root traits in response to N (Abdel-Ghani et al., 2013; Torres et al., 2019).

The results of this study showed that most root traits were significantly affected by N treatment, except for CRN, CRL, RDW and RDW/SDW. PRL (6.80%), SRN (11.95%), SRL (22.97%) and TRL (20.35%) were significantly higher in the LN level than in the HN level. On the other hand, SDW (15.88%) was significantly lower under LN level. Under LN treatment, average PRL, SRN, SRL, TRL, RDW and SDW were 17.04, 4.41, 54.14, 94.29, 47.83 and 58.99,

Table 2 Mean squares from the ANOVA for the measured traits under two nitrogen levels

Traits	ANOVA		
	genotypes (G)	N treatments (N)	interactions (G x N)
PRL	20.13 ***	24.70 *	8.73 *
CRN	2.98 ***	0.01 ns	1.78 ***
CRL	488.47 ***	158.45 ns	163.50 ***
SRN	2.76 ***	4.65 *	1.62 ***
SRL	523.10 **	2,147.57 *	337.88 *
TRL	768.30 **	5,134.50 *	753.10 **
SDW	856.11 ***	2,606.09 *	381.38 ***
RDW	766.24 ***	132.91 ns	277.82 ***
RDW/SDW	0.27 ***	0.54 ns	0.02 ns

PRL – primary root length, CRN – crown root number, CRL – crown root length, SRN – seminal root number, SRL – seminal root length, TRL – total root length, SDW – shoot dry weight, RDW – root dry weight, RDW/SDW – root-shoot ratio, ns – not significant at the 0.05 probability level; *, **, and *** – significant at the 0.05, 0.01, and 0.001 levels, respectively

Table 3 Descriptive statistics of the 09 tested traits under two nitrogen levels

Traits	N level	Min (G)	Max (G)	Mean ±SD	FC	CV%	% of reduction
PRL	LN	11.87 (H1)	22.50 (P5)	17.04 ±0.69	1.90	11.54	6.80
	HN	12.32 (P11)	18.60 (P7)	15.96 ±0.46	1.51	11.66	
CRN	LN	0.91 (P9)	4.67 (P7)	3.34 ±0.27	5.13	16.88	0.52
	HN	1.50 (P5)	4.33 (P7)	3.32 ±0.20	2.89	18.47	
CRL	LN	2.80 (P9)	41.92 (H2)	21.22 ±3.26	14.97	39.15	15.56
	HN	3.02 (P5)	35.25 (H2)	18.36 ±2.42	11.67	28.40	
SRN	LN	2.59 (P9)	5.67 (P5, H1)	4.41 ±0.22	2.19	12.65	11.95
	HN	2.83 (P4)	6.50 (H1)	3.94 ±0.23	2.30	18.08	
SRL	LN	22.11 (P9)	71.40 (H1)	54.14 ±3.18	3.23	20.92	22.97
	HN	20.22 (P11)	72.72 (H1)	44.03 ±3.23	3.60	31.07	
TRL	LN	40.60 (P9)	120.85 (H2)	94.29 ±4.86	2.98	15.01	20.35
	HN	47.12 (P11)	104.13 (H1)	78.35 ±4.67	2.21	20.77	
SDW	LN	30.59 (P1)	77.28 (P16)	58.99 ±3.76	2.53	9.87	-15.88
	HN	49.54 (P11)	106.17 (P7)	70.13 ±4.50	2.14	19.69	
RDW	LN	12.84 (P9)	70.77 (P16)	47.83 ±4.08	5.51	17.41	5.54
	HN	27.04 (P9)	58.87 (P7)	45.32 ±3.25	2.18	19.79	
RDW/SDW	LN	0.26 (P9)	1.31 (P1)	0.85 ±0.07	5.04	23.69	23.30
	HN	0.31 (P9)	1.05 (P1)	0.69 ±0.05	3.39	32.84	

G – genotype, N – nitrogen, LN – low nitrogen level, HN – high nitrogen level, SD – standard deviation, FC – fold change, CV% – coefficients of variation, PRL – primary root length (cm), CRN – crown root number, CRL – crown root length (cm), SRN – seminal root number, SRL – seminal root length (cm), TRL – total root length (cm), SDW – shoot dry weight (mg), RDW – root dry weight (mg), RDW/SDW – root-shoot ratio

respectively, whereas under HN level, average were 15.96, 3.94, 44.03, 78.35, 45.32 and 70.13, respectively.

In general, under LN level, genotypes demonstrated enhanced root growth than shoot growth, and vice versa. These results indicated that N resources were preferentially allocated to root development under LN level (Xu et al., 2020). Abdel-Ghani et al. (2013) have also indicated that the development of roots was more pronounced under LN than HN. However, contradictory results were found by Li et al. (2016) regarding the effects of LN on root growth. Several maize studies have shown that under LN conditions, the root system usually increases its size and weight to contact more soil volume and, as a result, increase nutrient uptake (Li et al., 2019).

Moreover, there was a significant interaction between genotype and N treatment for all investigated traits, with the exception of RDW/SDW, which indicates that genotypes responded differently at different N levels. Therefore, the best genotypes under HN are not necessarily the best ones under LN. This suggests that crop breeders should use different selection approaches at different N levels (Li et al., 2016). However, the presence of non-significant interactions for the ratio RDW/SDW indicates that, for this trait, the genotypes ranking under LN and HN conditions did not change.

All traits under both N treatments had a wide range of phenotypic values (Table 3). However, with the exception of SRN and SRL, ranges were larger under LN than under HN for other traits. In addition, all traits showed 2 to 5 fold difference between the minimum and maximum values and ranged from 1.51 to 14.97 among the genotypes under each N treatment, which is similar to the results obtained by Ma et al. (2020) from 74 maize lines. This large range among tested traits can be exploited to improve tolerance to drought or nutrient deficiency.

3.2 Seedling traits correlations

At each N level, significant correlations between different root traits were found, and the Pearson correlation coefficients ranged from 0.01 to 0.94 at LN and from –0.02 to 0.87 at HN level (Table 4). Out of 36 phenotypic correlation estimates, 15 coefficients were found to be significant under LN conditions, while 12 coefficients were significant under HN conditions. All correlations were positive except those between SDW and RDW/SDW under LN condition.

The same correlations have been found in both N levels between TRL and CRL, SRL and RDW; RDW and CRL, SRL and R/S; CRN and CRL; SRN and SRL. However, in the HN condition, the relationship between CRN and CRL; RDW and CRL; TRL and SRL were much smaller compared to the LN level. In addition, the correlations between RDW/SDW and CRN, SRN, SRL and TRL; between CRN and TRL and RDW and between RDW and SDW were significant under LN and not significant under HN conditions. Conversely, between PRL and TRL and RDW and between CRN and SDW correlations were significant under HN but not under LN conditions.

Similar to our results, other authors (Li et al., 2016; Torres et al., 2019) found a positive relationship between TRL and RDW, on the one hand, and other root traits, on the other hand. Therefore, selection based on RDW and TRL might be sufficient to use as an indicator of total root size and other root traits. Consequently, it would be easier, from a practical standpoint, to concentrate on RDW rather than time-consuming traits such as root length–related traits (Kumar et al., 2012).

In addition, no relation between Kernel size (TGW) and seedling traits was observed, which indicates that Kernel size has no effect on seedling performance. This suggests that the observed variations between genotypes in root length and RDW were attributed to genetic differences rather than being influenced by kernel size (Manavalan et al., 2011). This is similar to the results of Manavalan et al. (2011) and Abdel-Ghani et al. (2013) who didn't find any relation between seed size, root length, root and shoot dry weight. However, there are some contradicting

Table 4 Pearson correlation coefficients between various pairs of the seedling traits under low nitrogen (below diagonal) and high nitrogen (above diagonal) level

	PRL	CRN	CRL	SRN	SRL	TRL	SDW	RDW	RDW/SDW	TGW
PRL		0.17	0.41	-0.04	0.37	0.57*	0.47	0.65*	0.25	0.27
CRN	0.28		0.74**	-0.19	0.03	0.42	0.73**	0.28	-0.51	-0.02
CRL	0.17	0.63*		-0.19	0.22	0.71**	0.52	0.66*	0.05	-0.20
SRN	0.24	0.10	-0.15		0.86***	0.49	0.10	0.32	0.27	0.49
SRL	0.32	0.34	0.09	0.94***		0.84***	0.30	0.67*	0.40	0.51
TRL	0.44	0.66*	0.74**	0.52	0.73**		0.52	0.87***	0.32	0.27
SDW	0.25	0.20	0.43	-0.21	0.01	0.32		0.31	-0.54*	0.22
RDW	0.37	0.76**	0.71**	0.24	0.54*	0.86***	0.55*		0.59*	0.07
RDW/SDW	0.18	0.58*	0.32	0.56*	0.66*	0.64*	-0.41	0.59*		-0.10
TGW	-0.21	-0.28	-0.25	-0.26	-0.17	0.29	0.03	-0.047	-0.17	

PRL – primary root length, CRN – crown root number, CRL – crown root length, SRN – seminal root number, SRL – seminal root length, TRL – total root length, SDW – shoot dry weight, RDW – root dry weight, RDW/SDW – root-shoot ratio, TGW – thousand grain weight (Kernel size); *, **, and *** – significant at the 0.05, 0.01, and 0.001 levels, respectively

Table 5 Principle component analysis of tested traits under two nitrogen levels

Items	Traits	LN		HN	
		PC1	PC2	PC1	PC2
Eigen values		4.47	2.14	4.22	2.52
% variance		49.71	23.75	46.87	27.95
Cumulative % variance		49.71	73.46	46.87	74.82
Traits contribution	PRL	0.48	0.05	0.67	-0.04
	CRN	0.76	0.26	0.49	-0.79
	CRL	0.65	0.59	0.73	-0.46
	SRN	0.54	-0.76	0.41	0.60
	SRL	0.76	-0.55	0.78	0.48
	TRL	0.97	0.05	0.98	0.09
	SDW	0.28	0.73	0.61	-0.62
	RDW	0.90	0.32	0.90	0.22
	RDW/SDW	0.73	-0.44	0.28	0.80

LN – low nitrogen level, HN – high nitrogen level, PRL – primary root length, CRN – crown root number, CRL – crown root length, SRN – seminal root number, SRL – seminal root length, TRL – total root length, SDW – shoot dry weight, RDW – root dry weight, RDW/SDW – root-shoot ratio

results in the literature regarding the role of kernel size on root development that have been reported, indicating a modest influence of kernel size on root traits (Kumar et al., 2012).

3.3 Principal component analysis

A principal component analysis was performed to determine the most contributing traits at each N level. The first two principal components explained 73.46 and 74.82% of the total variation under LN and HN conditions, respectively (Figure 1).

Under HN conditions, the first principal component, which explained about 47% of the total variation, revealed that TRL, RDW and SRL, were the most important contributing traits (Table 5). For the second principal component, which contributed more than 27% of the total variation, the most predominant traits were RDW/SDW, CRN and SDW, respectively. Under LN conditions, the first principal component explained about 50% of the total variation, and revealed that TRL, RDW and CRN are the most important contributing traits. However, the most important contributing traits in the second principal component are SRN, SDW and CRL, which contributed more than 23% of the total variation.

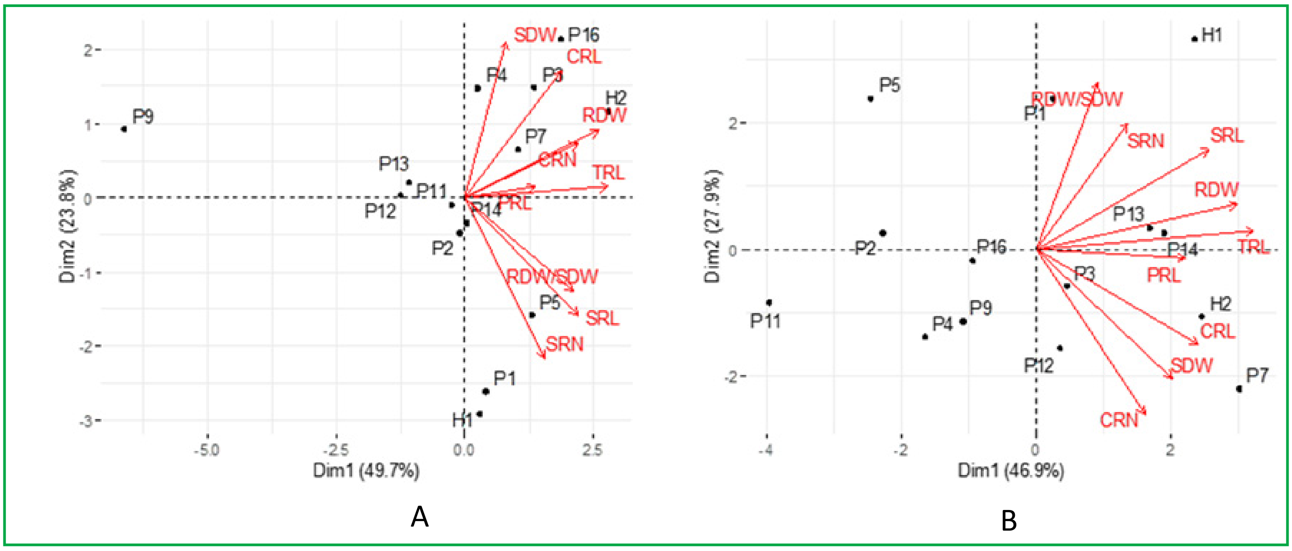

Figure 1 Principal components analysis of tested traits under low nitrogen (A) and high nitrogen (B) level
PRL – primary root length, CRN – crown root number, CRL – crown root length, SRN – seminal root number, SRL – seminal root length, TRL – total root length, SDW – shoot dry weight, RDW – root dry weight, RDW/SDW – root-shoot ratio

Thus, TRL, RDW and SRL, explained most of the phenotypic variation under both N levels; they can be utilized as seedling selection criteria for root morphology (Li et al., 2015). Similarly, Kumar et al. (2012) and Reddy et al. (2020) reported that TRL and RDW provide the most contribution to the total phenotypic variation and are sufficient to improve other root traits. In combination with correlation analysis, we observed that RDW and TRL were sufficient to explain most of the root variation and that they could be used as ideal traits for root morphology screening at the seedling stage.

4 Conclusions

There was a considerable phenotypic variation for all traits studied under both LN and HN levels. The results suggest a preferential allocation of N resources towards root development, indicating that genotypes under LN conditions prioritized the growth and development of their root systems. Consequently, this might be an adaptation strategy to increase N uptake efficiency under N-deficient conditions. Among the investigated root traits, RDW and TRL may be easier and more effective in estimating total root size and other RSA-related traits. In addition, some studied populations, such as P16, P3, and P5, showed higher performance under LN conditions, while P7, P13, and P14 showed higher performance under HN conditions, giving an opportunity for improved N use efficiency and/or N stress tolerance. Consequently, this can help reduce N fertilization rates and, hence, mitigate some economic and ecological problems, promoting the long-term sustainability of the cropping system.

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