Response of yield, root traits and plasticity of nitrogen-efficient cultivars of drip-irrigated rice to a nitrogen environment

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Abstract The response of drip-irrigated rice physiological traits to water and fertilisers has been widely studied. However, the responses of yield, root traits and their plasticity to the nitrogen environment in different nitrogen-efficient cultivars are not fully understood. An experiment was conducted from 2020-2022 with high nitrogen efficient (high-NUE) cultivars (T-43) and low-NUE cultivars (LX-3), and four nitrogen levels (0, 150, 300, and 450 kg ha^{-1}) under drip irrigation in large fields were used. The aim was to study the relationship between root morphology, conformation, biomass, and endogenous hormone content and yield and nitrogen use efficiency. The results showed the following: 1) Under the same N application rate, compared with LX-3, T-43's yield, N partial factor productivity (PFP), fine root length density (FRLD), shoot dry weight (SDW), root indole-3-acetic acid (IAA), and root zeatin and zeatin riboside (Z+ZR) were significantly increased by 11.4-18.9%, 11.3-13.5%, 11.6-15.7%, 9.9-31.1%, 6.1-48.1%, and 22.8-73.6%, respectively, while the root-shoot ratio (RSR) and root abscisic acid (ABA) were significantly decreased (P<0.05). 2) Nitrogen significantly increased rice root morphological indexes and endogenous hormone contents (P<0.05). Compared to N0, yield, root length density (RLD), surface area density (SAD), Root volume density (RVD), and root endogenous hormones (IAA, Z+ZR) were significantly increased in the 2 cultivars under N2 by 61.6-71.6%, 64.2-74.0%, 69.9-105.6%, 6.67-9.91%, 54.0-67.8%, and 51.4-58.9%, respectively. Compared with N3, the PFP and N agronomic efficiency (NAE) of nitrogen fertiliser under N2 increased by 52.3-62.4% and 39.2-63.0%, respectively. 3) The response of root trait plasticity to the N environment significantly differed (P<0.05). Compared with LX-3, T-43 showed a longer root length and larger specific surface area, which is a strategy for adapting to changes in the nutrient environment. For rice cultivars with high-NUE, the root-shoot ratio was optimised by increasing the FRLD, root distribution in upper soil layers, and root endogenous hormones (IAA, Z+ZR) under suitable nitrogen conditions (N2). An efficient nutrient acquisition strategy can occur through root plasticity, leading to increased yield and NUE.

Keywords: drip irrigation rice, nitrogen environment, root traits, plasticity, yield, nitrogen use efficiency[1]

1. Introduction

Rice accounts for 18.1% of the total global food crop production and is one of the most important food crops in the world, providing almost 60% of dietary calories for more than 3 billion people (Zhang *et al*. 2021). China is the largest rice producer in the world, with 65% of the population eating rice as the staple food. In 2022, China's rice planting area reached 29.45 million ha, with a total production of 208 million tons, accounting for 40.9% of the world's total rice production (National Bureau of Statistics of China 2022). Traditional rice cultivation consumes a large amount of water, resulting in severe fertiliser leaching and leakage (Yang *et al*. 2017). Furthermore, the N fertiliser utilisation rate is approximately only 30% (Ju and Gu 2014), which is one reason for limiting rice yield and improving water and fertiliser utilisation efficiency. Xinjiang has abundant land and sufficient sunshine and is thus suitable for large-scale commercial crop production. However, due to the lack of water resources, rice production in Xinjiang has been severely restricted (Pandey and Shukla 2015). As the main cultivation method in this area, mulched drip irrigation can effectively reduce water evaporation and adjust the ground temperature so that water and nutrients are evenly distributed in the soil, water and fertiliser are quickly absorbed by the root system, and the utilisation efficiency of water and fertiliser is improved, thus enhancing water and fertiliser integration for crops such as rice (He *et al*. 2013). Compared with traditional flooded rice production, drip irrigation can save 65% of water and increase fertiliser utilisation by 10% (Guo and Chen 2012). Therefore, it is necessary to

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continuously explore and enrich the theory and technology for rice cultivation that uses mulched drip irrigation, as these factors are important for increasing grain production in the Xinjiang region and ensuring national food security.

Nitrogen (N) is one of the essential elements for crop growth and development and is a factor in most crop physiological and biochemical processes, such as root system establishment (Zhang *et al*. 2018), hormone synthesis (Xu *et al*. 2020), and dry matter accumulation and distribution (Walch-Liu *et al*. 2006). Increases in soil N content can promote the proliferation of root branching in rice (Walch-Liu *et al*. 2006). When N is sufficient, the root biomass and root vitality of rice increase significantly in the later stage of growth (Chu *et al*. 2021), thereby improving nitrogen use efficiency (NUE); however, when N is excessive, soil nutrients are too high, root biomass and endogenous root hormone content decline, aboveground growth is negatively affected, and NUE decreases (Liu *et al*. 2020). In addition to environmental factors, cultivars with other characteristics are closely related to rice NUE. Studies have shown that resource acquisition strategy genotypes have higher root tissue density and longer total root length; in contrast, resource conservation strategy genotypes have higher root construction costs (Wen *et al*. 2020). The variation in root traits between genotypes is the focus of research on nutrient acquisition strategies in rice (Wen *et al*. 2019). Compared with the resource conservation strategy genotypes, the resource acquisition strategy genotypes increased environmental N concentrations via increased root trait plasticity (Roucou *et al*. 2018). Therefore, screening rice cultivars with higher plasticity and NUE, and clarifying the response of different cultivars to the N environment under drip irrigation are sustainable solutions for increasing rice yield under drip irrigation and reducing planting costs.

The root system is an important organ for absorbing water and nutrients (Xu *et al*. 2018) and its morphology and physiological characteristics are closely related to the synthesis and transport of photosynthetic products and leaf senescence in crops (Liu *et al*. 2023). Nitrogen can greatly regulate root morphological construction and growth and development processes. Studies have shown that low N can reduce root diameter and root tissue density (Freschet *et al*. 2018), excessive N application can lead to excess soil nutrients and inhibit the increase in root biomass and root physiological activity, and appropriate N application can increase root dry weight (RDW) and root length density (RLD) (Liu *et al*. 2023). Root hormones, as signal molecules, regulate the physiological functions of crops by transporting tissues and are important in crop growth (Ha *et al*. 2012; Talla *et al*. 2016). Studies have shown that hormone synthesis in roots is regulated by N levels (Takei *et al*. 2002); with an increase in N application, the growth-promoting hormones in plants increase significantly, but the ratios of zeatin riboside (ZR)/gibberellin (GA) and abscisic acid (ABA)/GA gradually decrease, and the NUE decreases significantly (Li *et al*. 2015). Therefore, it is essential to explore the response mechanism of rice root morphology and physiological characteristics to N and to clarify the morphophysiological mechanism of rice yield enhancement under drip irrigation in different N environments.

Current research on drip irrigation cultivation systems focuses on physiological characteristics and yield responses to water and fertiliser, and research on changes in root morphology and plasticity of cultivars with different nitrogen levels is insufficient. Therefore, in this paper, rice cultivars with high-NUE (T-43) and low-NUE (LX-3) were selected as the test materials, and the response mechanisms of rice yield, biomass, root morphology and physiological characteristics to N levels under drip irrigation in large field conditions were studied to achieve the following objectives: 1) to clarify the changes in root traits, endogenous hormones and their relationships with yield and NUE, and 2) to determine whether there are differences in root plasticity changes among rice cultivars with different NUE and whether such differences lead to various nutrient acquisition strategies. This study can provide a theoretical basis and practical guidance for the screening of high-yield cultivation of rice cultivars with high-NUE under drip irrigation in Xinjiang.

2. Materials and methods

2.1. Experimental site and weather conditions

This experiment was carried out at the Xinjiang Academy of Agricultural and Reclamation Sciences (XJAARS) (Shihezi, Xinjiang, 44°18´N, 86°03´E) from 2020 to 2022. The study area has a typical arid and semiarid continental climate, with scarce precipitation and concentrated light and heat. The average annual temperature is 6.5-8.1°C, the average annual precipitation is 180 mm, and the average annual evaporation is approximately 1200 mm. The soil tested was Calcaric Fluvisals, with pH 8.37, organic matter content (OMC) 1.07%, total N 0.68 g kg^{-1}, available phosphorus (P) 36 mg kg^{-1}, available potassium (K) 204 mg kg^{-1}, and medium fertile soil. In 2020, 2021, and 2022, the precipitation during the crops' growth period was 73.1, 84.6 and 72.2 mm, respectively; the number of effective precipitation events >5 mm was 3, 5 and 5, respectively; the daily average maximum temperature was 27.8, 28.5 and 28.5°C, respectively; and the minimum air temperature was 12.3, 12.8 and 12.9°C, respectively (Fig. 1).

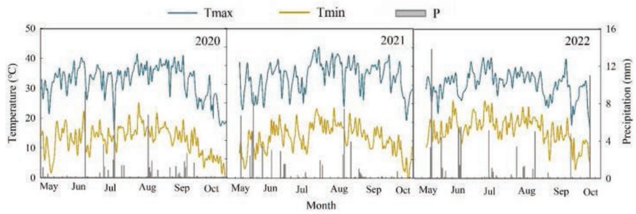

Fig. 1 Maximum temperature (Tmax), minimum temperature (Tmin) and precipitation in 2020-2022.

2.2. Experimental design and treatments

Rice cultivars with high-NUE (T-43) and low-NUE (Liangxiang No. 3, LX-3) were used as the test subjects, and 4 N application rates were set, i.e., N0 (0 kg ha^{-1}, CK), N1 (150 kg ha^{-1}), N2 (300 kg ha^{-1}) and N3 (450 kg ha^{-1}). The experiment had a split-plot design, with nitrogen application rate as the main area and cultivars as the subarea, with a plot area of 60 m^2 and three replicates. Mulched drip irrigation was adopted, and the planting mode comprised 1 film with 2 tubes and 8 rows, the plant spacing was 10 cm, and the row spacing was 10 cm+26 cm+10 cm (Fig. 2). Tube laying, film mulching, seeding and soil covering were performed simultaneously. Inlaid patch drip irrigation tape was applied, the distance between the drippers was 30 cm, and the flow rate of the drippers was 2.1 L h^{-1}. Well water was used for drip irrigation, with a salinity of 2.53 g L^{-1}, pH 7.2, and an irrigation volume of 10200 m^3 ha^{-1} throughout the growth period. The fertilisers were urea (N 46%), monoammonium phosphate (N 12%, P_2O_5 60%) and potassium sulfate (K_2O 50%). Fertigation (integrated management of water and fertiliser) was adopted (Table 1), and other management practices were consistent with field production. Seeds were sown on April 28 in 2020 and May 1 in 2021 and 2022. The sowing depth was 1.5-2.0 cm, the covering soil thickness was 1.0-1.5 cm, with 8-10 grains per hole, and the crop was harvested on September 30.

Table 1 Irrigation amount, fertilisation amount and distribution ratio during the growth period

Index		Seeding stage	Seeding-Panicle initiation stage	Panicle initiation -heading stage	Heading-15 d before maturity	15 d before maturation-Harvest	Total
Irrigation times		1	6	14	16	0	37
Irrigation frequency		1 irrigation	Every 5 days	Every 4 days	Every 2 days	0	
Irrigation quantity(m^3 ha^{-1})		450	300	300	240	0	10200
Nitrogen application rate (kg/ha^2)	N0	0	0	0	0	0	0
	N1	0	66.3	41.8	41.8	0	150
	N2	0	140.8	79.6	79.6	0	300
	N3	0	198.8	125.6	125.6	0	450
	fertilisation times	0	3	2	2	0	7

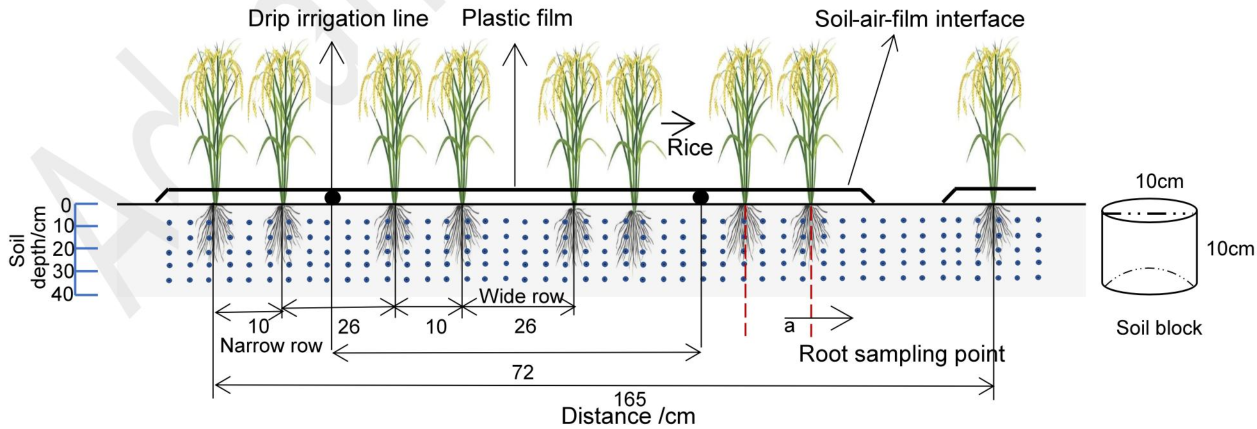

Fig. 2 Schematic for planting mode of mulched-drip irrigation and location of root sampling.

2.3. Measurement items and methods

Morphological characteristics of the root system At the heading stage, 4 representative rice holes were selected, and the soil coring method (10 cm in height, 10 cm in diameter) was used in the middle position to obtain 4 layers of the rice root system (0-10, 10-20, 20-30, and 30-40 cm). Roots were scanned with an EpsonV800 scanner (Suwa Inc., Japan) into images (300 dpi) and then dried at 85°C to a constant weight to determine the RDW. WinRHIZO Pro software (Regent Instruments Inc., Canada) was used to analyse morphological parameters, such as adventitious roots (AR) (RD>0.9 mm), coarse branch roots (CBR) (0.3 mm<RD≤0.9 mm), and fine branch roots (FBR) (RD≤0.3 mm) (Li *et al*. 2017). RLD, root surface area density (SAD) and root volume density (RVD) were calculated using the following formulae:

$$RLD = \frac{RL}{SV} \#(1)$$

$$SAD = \frac{RSA}{SV} \#(2)\#$$

$$RVD = \frac{RV}{SV} \#(3)$$

In the formulae: RLD, root length density (m m^{-3}); RL, root length (cm); SV, soil volume (m^3); SAD, root surface area density (m^2 m^{-3}); RSA, root surface area (cm^2); RVD, root volume density (m^3 m^{-3}); RV, root volume (cm^3). Root length, root surface area and root volume were directly measured by software. The soil volume of each depth was 0.785 dm^3.

Root System Architectural Parameters Using the asymptotic equation model proposed by M.R. Gale and D.F. Grigal (Gale and Grigal 1987), the vertical spatial distribution model of RLD, SAD, and RVD was established as:

$$Y = 1 - \beta^D \#(4)$$

where D is the soil depth (cm); Y is the proportion of the morphological indices from the surface to the soil layer D in the total; and β is the depth coefficient, where the smaller β is, the closer the root distribution is to the soil surface, and the larger β is, the deeper the root distribution. The β value was calculated when D was 20 cm.

Dry matter weight The shoots were obtained at the heading stage and 20 days after heading, divided into stems, leaves and panicles, and dried at 85°C in an oven. The mass of each organ was weighed with a 1/10000 electronic balance.

Root endogenous hormones When collecting a root system, other root systems in the 0-10 cm and 10-20 cm soil layers were taken, placed on a 20-mesh sieve, rinsed with tap water, and then rinsed with distilled water. The root systems were frozen with liquid N and sent to the College of Agriculture, China Agricultural University for testing. The contents of ABA, zeatin and zeatin riboside (Z+ZR), and indole-3-acetic acid (IAA) in these rice roots were determined by enzyme-linked immunosorbent assay (ELISA), with 3 repeats.

Test species and production Thirty holes of rice were taken in each plot at the maturity stage, and the panicle numbers, 1000-grain weight (10^3-GW), total number of grains per spike, actual number of grains per spike, filled grain rate, N partial factor productivity (PFP) and N agronomic efficiency (NAE) were measured. The calculation formulae are as follows:

$$\text{PFP(Partial factor productivity of applied N)} = \frac{Y}{NAR} \#(5)$$

$$\text{NAE(N agronomic efficiency)} = \frac{NAY - 0\,NY}{NAR} \#(6)$$

Y, yield; NAR, nitrogen application rate; NAY, nitrogen application yield; 0 NY, 0 nitrogen yield

2.4. Statistical analysis

Microsoft 365 was used for data processing and analysis, and all results were expressed as the mean±standard deviation (SD) of the results of three repeated measurements. One-way analysis of variance (ANOVA) was performed using SPSS 20.0 software (SPSS Inc., USA), and the least significant difference (LSD 0.05) was used to compare the mean ($P < 0.05$). SigmaPlot 12.5 software (SYSTAT Inc., USA) was used to plot figures. To compare the plastic responses of cultivars' different root traits with different NUEs to the N environment, the response ratio (RR) of root traits was calculated under N0 and N1, N0 and N2, and N0 and N3.

$$RR_k = \frac{\Sigma\left(\frac{R_{i'j'} - R_{ij}}{R_{i'j'} + R_{ij}}\right)}{n} \quad (k = 1,2,3)\#(7)$$

where RR_k is the root trait RP under N0 and N_k, $R_{i'j'}$ and R_{ij} are root traits under N0 and N_k, respectively; for a given cultivar, i' and i represent N0 and N_k treatments, and j' and j are two randomly selected individuals from three replicates of the same species under N0 and N_k, respectively; and n is the number of $R_{i'j'} - R_{ij}$.

3. Results

3.1. Rice yield, components and NUE

The yield, components and NUE of different rice cultivars had significantly different responses to the N environment (P<0.05), and the trends were basically the same among years (Table 2). When the N application rate was 0-300 kg ha^{-1}, the yields of the two cultivars increased with increasing N application rate. When the N application rate increased to 450 kg ha^{-1}, the yields of the two cultivars decreased, and the differences between the treatments were significant. Compared with N0, the yields of T-43 under N1, N2, and N3 increased significantly by 25.5, 61.6, and 43.8%, respectively, while the yields of LX-3 increased by 30.8, 71.6, and 50.2%, respectively. The yield of T-43 significantly increased by 11.4-18.9% compared with that of LX-3. The PFP of the 2 cultivars showed a decreasing trend with increasing N application rate, while the NAE increased first and then decreased and reached a maximum (9.67-11.87 kg kg^{-1}) for N2. Compared with LX-3, the PFP of T-43 increased by 11.3-13.5%, with no significant difference in the NAE.
The panicle number and spikelet per panicle of the two cultivars first increased and then decreased with increasing N application rate; compared with N0, the panicle number and spikelet per panicle of T-43 increased by 13.3 and 31.8%, respectively, and those of LX-3 increased by 14.5 and 33.9% under N2, respectively, with no significant difference in the filled grain rate and 10^3-GW (P<0.05). Compared to LX-3, the panicle number, spikelet per panicle and filled-grain rate of T-43 were significantly higher by 2.7-3.8%, 12.3-16.3% and 2.2-6.3%, respectively, while 10^3-GW was significantly lower by 5.4-8.7%. ANOVA showed that there were no significant differences in the indicators between years, the indicators showed high significant differences (P<0.01) between nitrogen applications, and there were no significant differences in the interactions between years, cultivars, and nitrogen applications.

Table 2 Responses of rice yield, yield components and nitrogen use efficiency to nitrogen environment

Year	Cultivar	Nitrogen application rate	Panicle numbers (×10^4 ha^{-1})	Spikelet per panicle (particle/spike)	Filled grain rate (%)	1000-grain weight (g)	Yield (t ha^{-1})	PFP (kg kg^{-1})	NAE (kg kg^{-1})
2020	T-43	N0	371.74±22.86 b	79.67±0.47c	82.83±3.36a	22.10±0.64a	5.41±0.22c	-	
		N1	392.26±18.67 ab	86.33±2.49b	83.40±0.36a	22.67±0.09a	6.35±0.44b	42.31±2.95a	6.27±1.70b
		N2	410.52±9.96a	106.00±1.41 a	84.30±2.14a	22.47±0.52a	8.31±0.15a	27.69±0.51b	9.67±0.70a
		N3	408.91±20.32 a	105.33±2.49 a	84.14±3.18a	22.53±0.23a	8.17±0.54a	18.17±1.20b	6.13±1.05c
	LX-3	N0	355.57±45.71 c	69.00±0.82c	77.77±0.73a	23.03±0.47b	4.39±0.53c	-	-
		N1	382.73±11.67 b	81.00±2.16b	82.26±2.00a	23.63±0.72ab	6.03±0.49b	40.24±3.24a	10.85±3.77 a
		N2	408.10±5.96a	95.00±2.45a	80.54±7.28a	24.46±0.08a	7.64±0.79a	25.48±2.65b	10.99±4.40 a
		N3	396.97±9.94ab	92.67±3.09ab	79.21±3.30a	24.43±0.23a	7.18±0.65ab	15.89±1.44c	7.89±1.07b
2021	T-43	N0	371.74±30.24 b	72.00±0.82c	80.08±5.61b	22.51±0.83a	4.81±0.39c	-	-
		N1	396.84±10.1b	85.33±1.89b	82.45±2.31ab	22.43±0.21a	6.34±0.09b	42.27±0.60a	10.20±3.05 ab
		N2	417.53±10.08 a	99.67±1.25a	88.29±4.64a	22.83±1.06a	8.37±0.36a	27.91±1.22b	11.87±0.99 a
		N3	409.54±16.96 a	95.33±1.25a	87.09±3.63ab	22.90±0.82a	7.88±0.34ab	17.18±0.76c	5.49±1.58b
	LX-3	N0	360.91±12.34 c	72.33±2.87c	74.53±4.68b	23.50±0.10a	4.59±0.58c	-	-
		N1	382.73±4.55b	79.33±2.05b	78.58±1.93ab	23.83±0.33a	5.68±0.22b	37.91±1.49a	9.26±2.45b
		N2	406.75±26.67 a	92.00±0.82a	81.91±3.60a	24.80±1.43a	7.60±0.83a	25.36±2.76b	10.04±3.14 a
		N3	402.88±14.32 a	84.67±2.49b	80.29±1.33a	25.06±0.91a	7.56±0.91a	16.38±2.03c	6.17±1.53c
2022	T-43	N0	365.27±11.2c	84.00±1.00b	78.15±3.26a	22.41±0.44a	5.37±0.26c	-	-
		N1	387.90±13.	95.33±1.7	81.84±1.5	22.61±0.0	6.84±0.24	45.59±1.5	9.78±1.83

71 b	0a	2a	8a	b	9a	b	

Table 3 Response of rice root morphology to nitrogen environment

Year	cultivars	Nitrogen Application rate	Fine branch root (D≤0.3 mm)			Coarse branch root (0.3 mm<D≤ 0.9 mm)			Adventitious root (D>0.9 mm)		
			RLD 10^3 m m^{-3}	SAD m^2 m^{-3}	RVD m^3 m^{-3}	RLD 10^3m m^{-3}	SAD m^2 m^{-3}	RVD m^3 m^{-3}	RLD 10^3 m m^{-3}	SAD m^2 m^{-3}	RVD m^3 m^{-3}
2020	T-43	N0	7.3±0.4 d	10.8±0.3c	0.7±0.1 c	6.1±0.1 c	15.6±0.0 c	5.4±0.1 b	2.3±0.3d	6.5±0.2 c	0.8±0.0 d
		N1	15.3±0.1 c	10.4±0.5c	0.9±0.0 b	16.2±0.1 b	20.7±0.5 c	5.4±0.0 b	2.7±0.1c	14.5±1.1 c	1.9±0.6 c
		N2	24.4±0.4 a	17.3±0.2a	1.3±0.0 a	17.0±0.9 a	26.5±0.8 a	7.1±0.3 a	5.1±0.2a	18.1±0.4 a	5.6±0.0 a
		N3	22.4±1.1 b	13.8±0.3b	0.9±0.2 b	16.9±0.6 b	23.3±0.2 b	5.4±0.2 b	2.6±0.2b	14.3±0.0 b	2.6±0.0 b
	LX-3	N0	10.9±0.	6.4±0.1	0.4±0.	8.5±0.2	23.6±0.1	3.6±0.2	2.7±0.	6.2±0.1	5.1±0.6
		N2	427.33±13.13 a	104.00±3.56 a	83.08±3.56a	22.85±0.34a	8.43±0.46 a	28.10±1.52b	10.20±0.85a		
		N3	419.49±15.29 a	99.00±5.35a	82.58±2.55a	22.70±0.35a	8.12±0.28 a	18.94±0.62c	7.89±1.09 c		
		N0	351.37±23.97 c	65.00±3.56c	77.44±1.47a	23.61±0.29b	4.18±0.49 c	-	-		
	LX-3	N1	380.14±15.27 b	74.00±1.41bc	81.50±1.98a	23.93±0.26ab	5.48±0.26 b	36.78±2.03a	8.88±1.36 ab		
		N2	407.78±16.86 a	89.00±7.48a	81.80±2.44a	24.81±0.60a	7.32±0.16 a	24.43±0.53b	10.48±1.15 a		
		N3	398.79±8.78ab	80.67±6.65ab	79.53±3.20a	24.34±0.32ab	6.97±0.20 a	15.71±0.44c	7.41±1.34 b		
Analysis of variance (ANOVA)											
Year (Y)			ns	5.48^{*}	ns	ns	ns	ns	ns		
Cultivar (C)			ns	130.04^{**}	9.97^{**}	93.24^{**}	77.44^{**}	67.64^{**}	ns		
Nitrogen (N)			10.52^{**}	102.91^{**}	56.3^{**}	12.6^{**}	107.42^{**}	287.23^{**}	24.89^{**}		
Y×C			ns	22.00^{**}	ns	ns	4.48^{*}	8.04^{**}	ns		
Y×N			ns	ns	ns	ns	ns	ns	ns		
N×C			ns	ns	ns	ns	ns	4.14^{*}	ns		
Y×C×N			ns	ns	ns	ns	ns	ns	ns		

T-43, high-NUE cultivars; LX-3, low-NUE cultivars; RLD, root length density; SAD, surface area density; RVD, root volume density; In the data marked with letters in the table, different lowercase letters in the same column indicate that different nitrogen application rates under the same cultivars reach a significant level of 5%; * means significant (P<0.05), ** means much significant, ns means no significant difference (P<0.01); PFP, nitrogen partial factor productivity; NAE, N agronomic efficiency; The same as below.

3.2. Root morphology

The responses of the root morphology of different rice cultivars to the N environment were significantly different (P<0.05), and the trend was basically the same among years (Table 3). For fine lateral roots, the RLD, SAD, and RVD accounted for 46.4-61.3%, 27.1-28.2%, and 6.9-9.5% of the total root length, total surface area and total volume, respectively. Compared with N0, N1, N2, and N3 treatments significantly increased the RLD by 45.3, 33.4, and 37.2%, respectively, and the SAD by 12.4, 71.5, and 42.3%, respectively. Compared with LX-3, the RLD, SAD, and RVD of T-43 were significantly increased by 11.6-15.7%, 8.0-39.9%, and 30.2-65.0%, respectively. For coarse lateral roots, the RLD, SAD and RVD accounted for 37.9-39.8%, 43.6-58.5%, and 40.5-56.3% of the total root system, respectively; compared with N0, the average increases in RLD, SAD, and RVD in the N2 treatment were 112.4, 105.2, and 98.5%, respectively; compared with LX-3, the RLD of T-43 was significantly increased by 2.7-19.0%, while the SAD and RVD were not significantly different. For adventitious roots, the RLD, SAD and RVD accounted for 13.6-16.0%, 21.2-26.3% and 34.6-52.6% of the total root system of rice, respectively; compared with N0, the RLD, SAD and RVD of the N2 treatment were significantly increased by 121.4, 178.5 and 232.2%, respectively; compared with LX-3, the RLD and RVD of T-43 were significantly decreased by 6.2-47.5% and 47.8-71.8%, respectively, while the SAD was significantly increased by 10.8-30.8%. ANOVA showed that the differences between year and cultivar, and the interaction between year, cultivar, and nitrogen application were significant (P<0.05) for each indicator.

			0 d	c	0 d	d	c	c	4d	c	d
		N1	17.0±0.7 c	9.8±0.1 b	0.5±0.1 c	10.7±0.0 c	24.4±0.4 b	5.3±0.1 b	5.3±0.2c	8.3±0.4 b	10.4±0.3 c
		N2	19.8±0.0 a	12.2±0.9a	0.8±0.0 a	18.7±0.0 a	28.1±0.5 a	7.3±0.2 a	7.3±0.1a	10.0±0.1 a	17.4±0.1 a
		N3	16.1±0.3 b	10.2±0.3b	0.7±0.1 b	15.4±0.2 b	25.4±0.3 b	5.4±0.3 b	5.6±0.3b	8.1±0.1 b	13.2±0.1 b
2021	T-43	N0	12.3±0.8 b	10.3±0.7c	0.6±0.0 c	9.6±0.5 b	24.4±0.9 c	5.3±0.1 a	2.0±0.1b	6.6±0.2 c	0.8±0.0 d
		N1	16.8±0.2 a	11.2±1.4c	0.9±0.1 b	12.6±0.0 a	24.9±0.2 c	5.4±0.0 a	2.8±0.0a	8.2±0.2 c	1.5±0.3 c
		N2	16.9±0.6 a	18.6±0.6a	1.2±0.0 a	13.7±0.3 a	28.3±1.5 a	5.8±0.1 a	2.8±0.1a	9.2±0.2 a	4.9±0.5 a
		N3	12.8±0.7 b	14.1±0.5b	0.9±0.0 b	13.4±0.3 b	26.3±0.9 b	5.4±0.2 a	2.9±0.0a	8.6±0.5 b	2.6±0.0 b
	LX-3	N0	8.21±0.8 c	5.8±0.4 c	0.3±0.0 d	7.6±0.1 c	15.7±0.7 d	3.1±0.2 c	2.3±0.0c	6.0±0.3 d	4.1±0.4 d
		N1	11.1±0.3 b	9.5±0.8 b	0.6±0.1 c	10.0±0.2 b	20.9±0.6 c	4.8±0.6 b	5.4±0.4b	14.2±0.4 c	9.2±0.8 b
		N2	15.2±0.0 a	12.6±0.0a	0.8±0.0 a	14.1±1.5 a	29.0±0.2 a	7.3±0.2 a	7.4±0.1a	18.8±0.1 a	18.5±0.9 a
		N3	13.4±0.9 b	11.0±0.2 ab	0.7±0.0 b	9.8±0.7 b	24.2±0.1 b	4.9±0.1 b	5.2±0.1b	14.6±0.0 b	13.7±0.5 b
2022	T-43	N0	9.8±0.2 c	9.7±0.1 d	0.6±0.0 d	7.7±0.1 c	17.8±1.1 c	1.8±0.5 c	3.5±1.4b	8.8±0.1 d	1.5±0.4 b
		N1	11.0±0.1 c	14.7±1.1 c	0.7±0.1 c	8.9±0.7 c	17.7±0.0 c	2.5±0.0 b	2.9±0.2b	11.7±0.6 c	1.3±0.3 b
		N2	20.1±0.7 a	23.4±0.6 a	0.8±0.1 a	15.4±1.0 a	29.6±1.2 a	4.2±0.0 a	9.0±0.8a	22.4±1.4 a	4.6±0.7 a
		N3	16.7±1.6 b	17.2±0.1 b	0.8±0.1 b	12.8±0.3 b	22.3±0.1 b	3.1±0.1 b	7.9±0.1a	15.7±1.7 b	1.8±0.6 b
	LX-3	N0	8.3±1.3 c	13.2±0.8 c	0.5±0.0 c	7.0±0.3 c	16.1±0.9 b	2.3±0.0 c	3.2±0.4c	11.6±0.6 b	1.5±0.5 c
		N1	10.4±1.0 c	14.7±0.1 b	0.6±0.0 b	11.2±1.0 b	13.5±1.9 b	1.9±0.2 c	5.3±0.5b	11.7±0.3 b	2.6±0.4 b
		N2	18.0±0.4 a	18.4±0.4 a	0.7±0.1 a	14.9±0.7 a	25.4±2.7 a	4.2±0.2 a	8.4±0.1a	13.8±1.5 a	4.7±0.4 a
		N3	15.3±0.9 b	19.3±1.0 a	0.6±0.0 b	14.0±1.1 a	27.4±3.3 a	3.4±0.0 b	5.5±1.1b	15.4±0.7 a	2.6±0.0 b
Analysis of variance (ANOVA)											
Year (Y)				72.13**	148.49**	377.43**	50.74**	35.87**	63.07**	312.42**	529.42**
Cultivar（C）				750.41**	246.24**	831.37**	549.57**	ns	463.91**	496.14**	536.37**
Nitrogen（N）				ns	ns	ns	ns	216.05**	396.80**	ns	36.49**
Y×C				352.15**	13.78**	8.18*	69.24**	122.27**	ns	39.81**	64.19**
Y×N				15.21**	29.15**	ns	140.57**	ns	10.43**	48.90**	8.37*
N×C				297.55**	212.09**	174.07**	69.24**	ns	35.05**	270.09**	879.87**
Y×C×N				13.98**	ns	7.32*	7.78*	48.14**	6.77**	25.56**	114.52**

RLD, root length density; SAD, surface area density; RVD, root volume density.

3.3. Root architecture

Vertical distribution of roots The root morphological index gradually decreased with increasing soil depth (Fig. 3). The roots were mainly distributed in the 0-10 cm soil layer, and the distribution ratios of root RLD, SAD and RVD in this soil layer were 34.4-65.9%, 28.9-48.3%, and 7.2-9.6%, respectively. Among them, compared with N0, the RLD of the N2 treatment significantly increased by 51.5%. The root distribution in soil layers > 10 cm was less. In the 10-20 cm and 20-30 cm soil layers, compared with N0, the root RLD, SAD and RVD of the N2 treatment increased significantly by 34.3, 53.4 and 64.2%, respectively. In the 30-40 cm soil layer, the distribution ratios of root RLD, SAD, and RVD were 8.9-10.2%, 7.3-9.1%, and 6.4-8.9%, respectively, with N0 and N1 being significantly higher than N2 and N3 (P<0.05). Under the middle and high N application rates, the rice roots tended to be distributed to the upper soil layer, and under the low N application rate, the roots tended to be distributed to the deep layers, which was beneficial to the absorption and utilisation of nutrients. The root system ratio of T-43 with high-NUE was significantly higher than that of LX-3 with low-NUE in the 0-40 cm soil layer (P<0.05); the root system of the rice cultivars with high-NUE can expand in the soil, facilitating water and fertiliser uptake and utilisation.

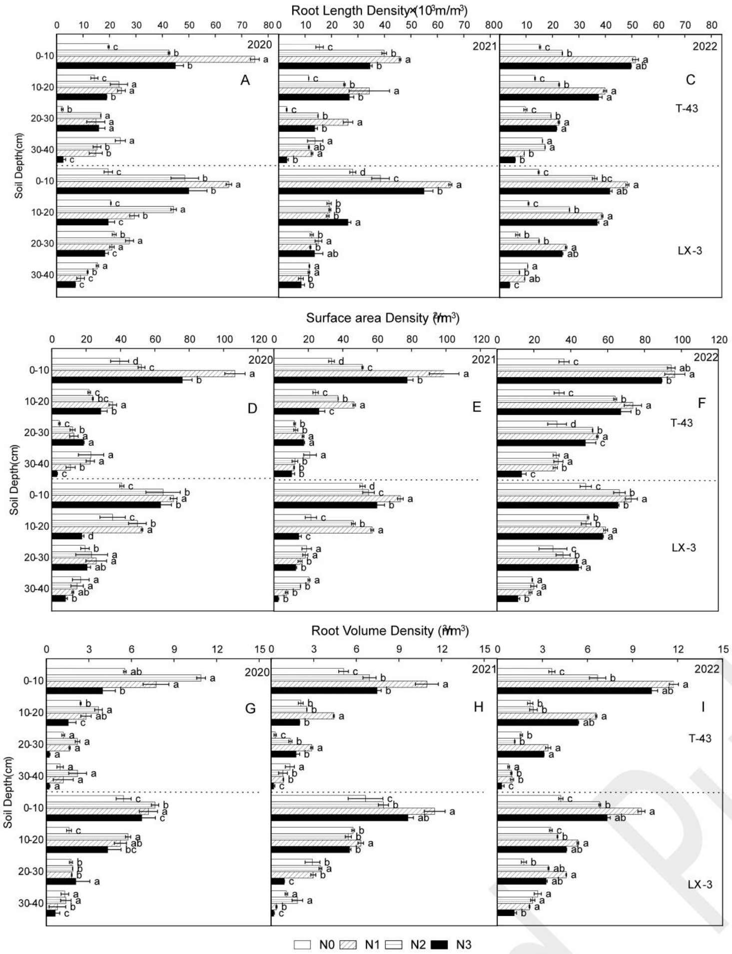

Fig. 3 Response of the rice roots' vertical distribution to the nitrogen environment. T-43, high-NUE cultivars; LX-3; low-NUE cultivars. A-C, the root length density in 2020-2022. D-F, the surface area density in 2020-2022. G-I, the root volume density in 2020-2022. The lowercase letters in the figure represent the significant differences between nitrogen application rates at the P<0.05 level.

Root system vertical distribution characteristic parameters The responses of the root β value to the N environment were significantly different (P<0.05). At 0-300 kg ha^{-1}, the β values of rice RLD, SAD and RVD showed a decreasing trend with increasing N application rate. When the N application rate increased to 450 kg ha^{-1}, the β value showed an increasing trend. For the two cultivars, the β values of RLD, SAD and RVD under N2 were the lowest, ranging from 0.840 to 0.944, 0.895 to 0.948, and 0.870 to 0.948, respectively (Table 4). Compared with N0, the RLD β, SAD β, and RVD β of T-43 under N2 decreased by 5.9, 6.5, and 8.7%, respectively, and LX-3 under N2 decreased by 2.6, 3.0, and 4.6%, respectively. Compared with LX-3, the β values of RLD, SAD and RVD of T-43 were not significantly different. ANOVA showed that year, cultivar, and nitrogen application for RVD β reached significant levels (P<0.05), and the interaction between year, cultivar, and nitrogen application was not significantly different for each indicator.

Table 4 Response of rice root vertical distribution characteristic parameters to nitrogen environment

Year	Cultivars	Nitrogen application rate	RLD β (m m^{-3})	SAD β (m^2 m^{-3})	RVD β (m^3 m^{-3})
2020	T-43	N0	0.953±0.002 a	0.939±0.001 a	0.930±0.005 a
		N1	0.929±0.008 b	0.930±0.007 b	0.925±0.012 a
		N2	0.900±0.003 c	0.895±0.003 c	0.877±0.011 b
		N3	0.946±0.00 a	0.923±0.011 b	0.934±0.001 a
	LX-3	N0	0.958±0.002 a	0.942±0.001 a	0.942±0.000 a
		N1	0.939±0.002 c	0.936±0.002 b	0.922±0.013 b
		N2	0.934±0.001 d	0.930±0.011 c	0.916±0.005 b
		N3	0.946±0.003 b	0.934±0.001 c	0.921±0.003 b
2021	T-43	N0	0.947±0.007 a	0.938±0.002 a	0.926±0.001 a

		N1	0.941±0.001 a	0.928±0.000 b	0.920±0.003 a
		N2	0.920±0.006 b	0.919±0.006 c	0.870±0.000 b
		N3	0.940±0.002 a	0.926±0.002 b	0.926±0.00 a
	LX-3	N0	0.943±0.003 a	0.936±0.002 a	0.926±0.006 a
		N1	0.927±0.00 b	0.933±0.002 a	0.926±0.000 a
		N2	0.944±0.004 a	0.915±0.001 c	0.875±0.001 b
		N3	0.926±0.002 b	0.926±0.00 b	0.925±0.002 a
2022	T-43	N0	0.909±0.008 b	0.941±0.004 b	0.940±0.005 b
		N1	0.918±0.003 a	0.945±0.003 a	0.941±0.000 b
		N2	0.886±0.001 c	0.945±0.001 ab	0.948±0.001 a
		N3	0.920±0.002 a	0.947±0.001 a	0.946±0.002 a
	LX-3	N0	0.891±0.002 a	0.954±0.003 a	0.949±0.001 a
		N1	0.869±0.002 b	0.948±0.001 b	0.895±0.004 d
		N2	0.840±0.004 c	0.948±0.001 b	0.916±0.005 c
		N3	0.866±0.001 b	0.947±0.003 b	0.925±0.004 b
Analysis of variance (ANOVA)					
Year(Y)			ns	ns	16.89^{**}
Cultivar（C）			ns	ns	10.60^{**}
Nitrogen（N）			ns	8.85^{**}	7.17^{*}
Y×C			8.13^{*}	ns	ns
Y×N			ns	6.02^{*}	ns
N×C			14.13^{**}	ns	20.09^{**}
Y×C×N			ns	ns	ns

3.4. Root dry weight (RDW), shoot dry weight (SDW), and root-shoot ratio (RSR)

When the N application rate was 0-300 kg ha^{-1}, the RDW and SDW of the two cultivars increased with increasing N application rate (Table 5). When the N application rate was 300-450 kg/ha^{2}, there was no significant difference in RDW between the two cultivars at the heading stage, but there was a significant difference at 20 days after heading (P<0.05); the SDW showed no significant differences in the 2 stages. Compared with LX-3, the RDW of T-43 decreased significantly by 32.2-77.1% at the heading stage and decreased by 9.0-44.4% at 20 days after heading; the SDW increased significantly by 9.9-31.1% for the 2 stages. The root-shoot ratio of the two cultivars first increased and then decreased with increasing N application rate in the two stages, with the lowest value occurring under N2 (0.07-0.14). The root-shoot ratio of T-43 was significantly lower than that of LX-3 (P<0.05). ANOVA showed that year, cultivar, and the interaction with nitrogen application highly significantly (P<0.01) affected root dry weight and root-crown ratios at the tasselling stage, and the interaction between cultivar, year and nitrogen were significant (P<0.05) for all indicators.

Table 5 Response of root dry weight, shoot dry weight and root-shoot ratio to nitrogen environment

Year	Cultivars	Nitrogen application rate	Root dry weight (t ha^{-1})		Shoot dry weight (t ha^{-1})		Root shoot ration	
			HS	20 DAH	HS	20 DAH	HS	20 DAH
2020	T-43	N0	0.75±0.06 c	0.59±0.05 b	10.56±0.59 b	11.18±0.16 c	0.07±0.003 c	0.05±0.004 b
		N1	0.90±0.01 b	0.63±0.09 b	12.66±0.11 ab	14.95±0.00 b	0.07±0.001 c	0.04±0.006 c
		N2	1.40±0.07 a	1.08±0.10 a	12.94±0.67 a	16.90±0.00 a	0.10±0.011 a	0.06±0.006 a
		N3	1.05±0.03 ab	0.91±0.04 a	12.05±0.02 ab	16.51±0.18 a	0.09±0.002 b	0.06±0.003 ab
	LX-3	N0	1.54±0.01 a	0.94±0.10 c	8.25±0.02 b	9.75±0.52 c	0.19±0.001 a	0.10±0.005 a
		N1	1.57±0.16 a	1.05±0.05 c	9.61±0.20 b	11.76±0.90 b	0.16±0.014 b	0.09±0.011 a
		N2	1.57±0.06 a	1.31±0.01 a	11.50±0.23 a	13.38±0.11 a	0.14±0.002 c	0.10±0.002 a
		N3	1.70±0.08 a	1.19±0.01 b	11.62±0.14 a	13.05±0.34 a	0.15±0.005 c	0.09±0.001 a
2021	T-43	N0	0.90±0.00 a	0.69±0.04 b	10.23±0.44 c	14.37±1.24 a	0.09±0.004 a	0.05±0.001 b
		N1	0.94±0.08 a	0.78±0.02 b	10.97±0.28 bc	14.04±0.41 ab	0.09±0.010 a	0.06±0.000 b
		N2	0.90±0.07 a	0.85±0.05 b	12.11±0.53 ab	15.40±0.32 a	0.07±0.009 ab	0.06±0.005 b
		N3	0.75±0.07 b	1.06±0.14 a	12.27±0.93 a	14.32±0.79 b	0.06±0.010 b	0.08±0.006 a
	LX-3	N0	1.66±0.12 a	1.40±0.38 a	10.47±0.30 c	11.74±0.91 b	0.16±0.007 ab	0.12±0.042 a

		N1	1.57±0.16 a	1.00±0.25 a	10.68±0.27 bc	13.94±0.41 b	0.15±0.012 bc	0.07±0.020 a
		N2	1.57±0.06 a	1.25±0.02 a	11.81±0.09 a	16.56±0.44 a	0.13±0.004 c	0.08±0.001 a
		N3	1.84±0.06 a	1.01±0.10 a	11.17±0.38 ab	15.14±1.86 ab	0.16±0.000 a	0.08±0.003 a
2022	T-43	N0	0.99±0.10 b	0.93±0.00 c	10.08±0.54 c	13.4±0.05 c	0.10±0.016 ab	0.07±0.000 b
		N1	0.94±0.01 b	0.97±0.03 bc	11.22±0.01 b	13.91±0.12 c	0.08±0.001 b	0.07±0.001 b
		N2	1.40±0.07 a	1.29±0.06 a	12.24±0.12 a	15.95±0.17 a	0.11±0.005 a	0.08±0.003 a
		N3	1.20±0.03 ab	1.03±0.05 b	12.20±0.15 a	15.24±0.56 ab	0.10±0.001 b	0.07±0.006 b
	LX-3	N0	0.80±0.00 c	0.66±0.01 d	9.85±0.27 c	11.38±0.04 c	0.08±0.002 c	0.06±0.001 c
		N1	1.24±0.09 b	0.79±0.01 c	10.07±0.05 c	13.87±0.66b	0.12±0.009 b	0.06±0.004 c
		N2	1.53±0.01 a	1.21±0.01 a	10.84±0.15 b	14.95±0.72 a	0.14±0.001 a	0.08±0.003 a
		N3	1.49±0.06 a	1.04±0.01 b	11.44±0.08 a	15.17±0.06 a	0.11±0.006 b	0.07±0.001 b
Analysis of variance (ANOVA)								
Year (Y)			49.42**	ns	ns	8.55*	9.43**	13.06**
Cultivar (C)			156.96**	84.31**	110.23**	55.84**	177.4**	339.78**
Nitrogen (N)			56.03**	ns	92.05**	261.83**	ns	ns
Y×C			202.68**	67.69**	28.91**	17.58**	220.07**	149.03**
Y×N			19.32**	ns	6.34*	30.23**	30.31**	ns
N×C			7.93*	ns	ns	ns	ns	ns
Y×C×N			9.78**	ns	10.53**	ns	15.89**	5.16*

HS, heading stage; 20 DAH, 20 days after heading.

3.5. Root endogenous hormones and the ratio

There were significant differences between the responses of IAA, Z+ZR and ABA contents in the roots of the two drip-irrigated rice cultivars and the N environment (P<0.05), and the trends were basically the same among years (Fig. 4). IAA gradually decreased with increasing depth, while Z+ZR and ABA gradually increased. Compared with N0, IAA of T-43 significantly increased by 54.0% under N2 and that of LX-3 increased by 67.8% under the N2. Compared with LX-3, the IAA of T-43 roots significantly increased by 6.1-48.1% in the 0-10 cm soil layer but significantly decreased by 8.0-33.2% in the 10-20 cm soil layer; the Z+ZR of T-43 roots significantly increased in the 0-10 cm and 10-20 cm soil layers, with increases of 27.8-71.3% and 17.8-75.9%, respectively. Compared with N0, in the 0-10 cm soil layer, root ABA increased by 21.0, 41.9, and 26.2% in N1, N2, and N3, respectively, and in the 10-20 cm soil layer, root ABA increased by 13.3, 224.5, and 14.6%, respectively; among cultivars, compared with that of LX-3 roots, the ABA of T-43 roots decreased by 12.9-39.8% in the 0-10 cm soil layer and decreased by 35.1-55.5% in the 10-20 cm soil layer. The ZR/ABA ratios of the two cultivars were the largest under N0, followed by N1 and N2, and the smallest under N3. The IAA/ABA ratio and ZR/ABA ratio showed similar trends, with significant differences between treatments. The ratios of growth-promoting hormones (Z+ZR, IAA) to the growth-inhibiting hormone (ABA) were the largest under N3 and were significantly higher than those under N1 and N2. The Z+ZR/ABA, IAA/ABA and ((Z+ZR)+IAA)/ABA of the rice cultivars with high-NUE were all significantly greater than those of the rice cultivars with low-NUE.

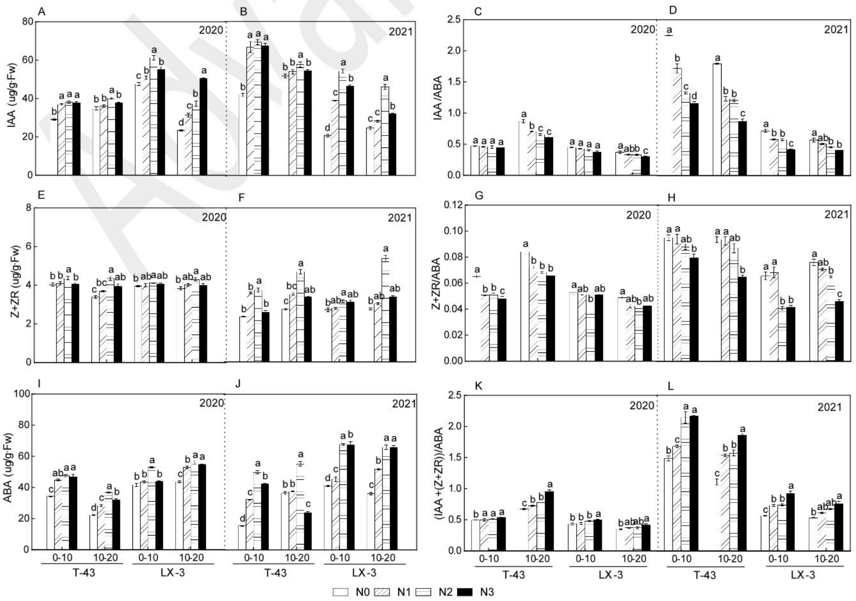

Fig. 4 Response of auxin and zeatin riboside abscisic acid contents in roots to nitrogen. T-43, high-NUE cultivars; LX-3,

low-NUE cultivars. A and B, the auxin of 2020-2021. C and D, the ratio of auxin to abscisic acid of 2020-2021. E and F, the zeatin and zeatin riboside of 2020-2021. G and H, the ratio of zeatin, zeatin riboside to abscisic acid. I and J, the content of abscisic acid. K and L, the ratio of auxin, zeatin, zeatin riboside to abscisic acid. The 0-10 and 10-20 are soil depth.

3.6. Plastic responses of root morphology and endogenous hormones to the N environment

The plastic changes in the root morphology, endogenous hormones, and dry matter mass of drip-irrigated rice to N environments were mainly reflected in the RR of N. The FBR (RLD, SAD), CBR (SAD, RVD), adventitious roots (SAD and RVD), RLD at 0-10 cm and 30-40 cm, ZR at 0-10 cm and 10-20 cm, and SDW were significantly lower in T-43 than in LX-3. The FSAD and RSR of T-43 showed positive responses under low N treatment and negative responses under the medium N and high N treatments; the 30-40 cm RLD of LX-3 showed a positive response under low N treatment and negative responses under the medium N and high N treatments; and SDW showed a positive response. For root traits, the response ratios of CRVD, 0-10 cm ABA and 10-20 cm ZR under different N environments were close to 0, showing low plasticity to N deficiency.

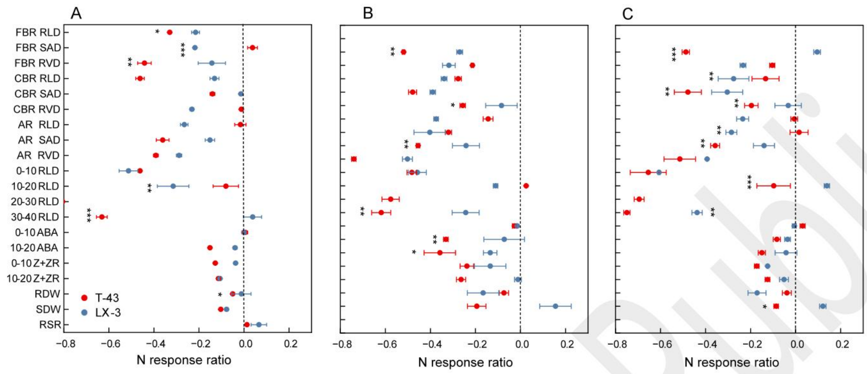

Fig. 5 Response of root morphology and endogenous hormones to nitrogen plasticity. A, nitrogen response ratio under N1 and N0; B: nitrogen response ratio under N2 and N0; C: nitrogen response ratio under N3 and N0. FBR RLD, root length density of fine branch roots; CBR SAD, surface area density of coarse branch roots; AR RVD, adventitious root volume density; 0-10RLD: root length density of the 0-10 cm soil layer; RDW, root dry weight; SDW, shoot dry weight; RSR, root shoot ratio. The asterisk represents that the trait significantly differed between the high- and low-nitrogen-sensitive genotypes. *, P<0.05; **, P<0.01, ***, P<0.001.

3.7. Correlations between root morphology and physiology and yield and NUE

The results of principal component analysis (PCA) (Fig. 6) showed that the explained variance rate for the first axis of the T-43 principal components was 67.7%, which could be explained by most root traits, while the explained variance rate for the second axis was 17.8%, which could be mainly explained by the 10-20 cm RLD. The first axis and second-axis explained that the variance rates of the LX-3 principal components were 52.8 and 27.5%, respectively, but the distributions were more dispersed. For T-43, CSAD was very significantly positively correlated with yield; FRLD, 0-10 cm SAD and RVD significantly affected yield and NAE; and 0-10 cm RLD was very significantly positively correlated with NPFP. For LX-3, yield was significantly positively correlated with 0-10 cm SAD and CSAD and significantly negatively correlated with 20-30 cm SAD, 30-40 cm RLD and RSR; FSAD was significantly positively correlated with NPFP and NAE.

The correlation network diagram shows (Fig. 7) that the yield of T-43 was significantly positively correlated with 0-10 cm IAA and ZR/ABA (0.79**-0.87**) and significantly negatively correlated with 10-20 cm IAA/ABA (-0.92***); for LX-3, the yield was significantly positively correlated with 0-10 cm IAA and ABA (0.59*-0.78**) and significantly negatively correlated with 0-10 cm Z+ZR/ABA and 10-20 Z+ZR/ABA (- 0.59*-0.64*).

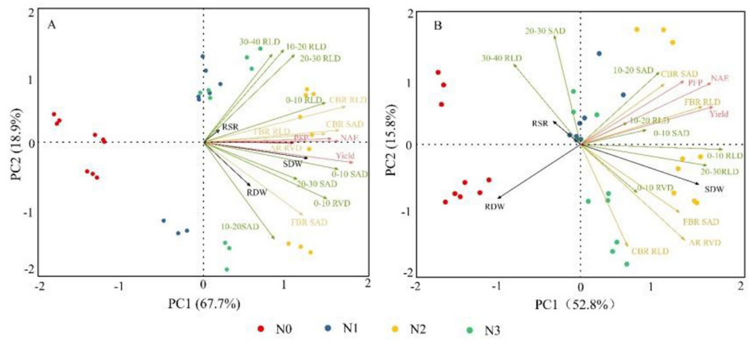

Fig. 6 Correlations between root traits and yield, yield components and nitrogen use efficiency. FBR RLD, fine branch root length density; FBR SAD, fine branch root surface area density; CBR RLD, coarse branch root length density; AR RVD, adventitious root volume density; 0-10RLD, root length density of 0-10 cm soil layer; RDW, root dry weight; SDW, stem dry weight; RSR, root-shoot ratio.

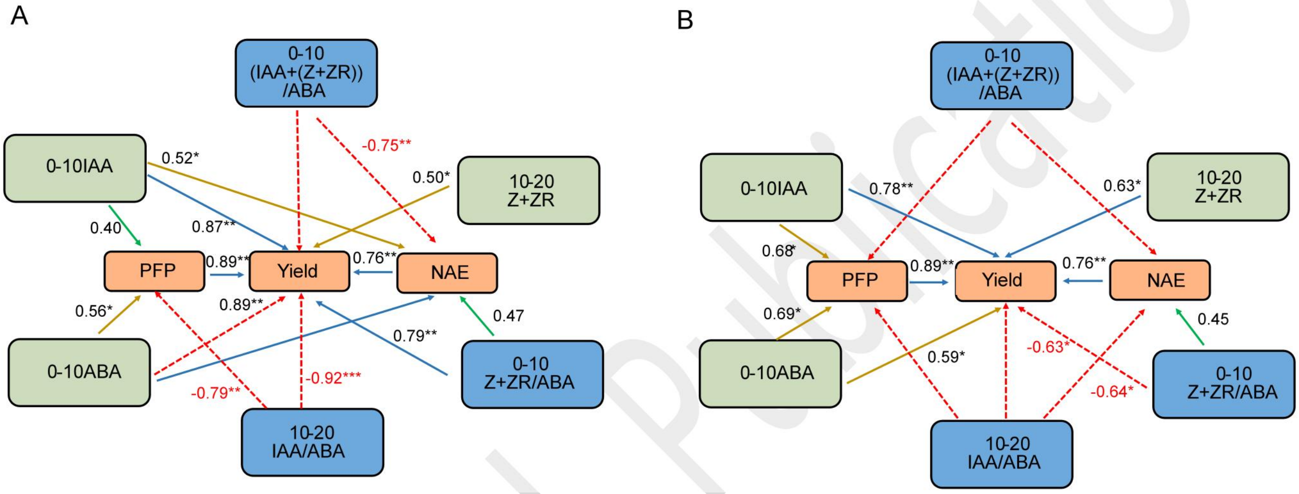

Fig. 7 Correlation analysis of endogenous hormones with yield, partial factor productivity of nitrogen fertiliser and agronomic utilisation efficiency. A, T-43, B, LX-3; PFP, nitrogen partial factor productivity; NAE, N agronomic efficiency; * represents the correlation of different levels; *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; value is the path coefficient; the red dotted line represents a significant negative correlation, the blue line represents a very significant correlation, the yellow line represents a significant correlation, and the green line represents no significant correlation.

4. Discussion

4.1. Relationships between root morphology and architecture with yield and NUE of different drip-irrigated rice cultivars

The root system is the main organ through which rice absorbs N, and root length and root surface area are important role rice N uptake (Zhang *et al*. 2020). The location, length and function of different types of rice roots are important manifestations of root adaptation to the external N environment (Ji *et al*. 2012). We found that T-43 had obvious advantages in RLD, SAD and RVD in FBR, CBR and adventitious roots, and the differences in genotype were responsible for the differences in yield and NUE. Under different N application rates, the FRLD of T-43 was significantly greater than that of LX-3. For T-43, the specific surface area of the root system was expanded by increasing the FRLD, thereby increasing the contact of the root system with water and nutrients. During plant growth, adventitious roots mostly transport nutrients and fix plants. Under the appropriate N application rate (N2), the RVD of adventitious roots of T-43 was high, so T-43 had a strong rooting ability and could absorb and utilise the N in soil more effectively, thus promoting the accumulation of dry matter in the shoots (Table 5). The distribution of soil nutrients could affect the growth of plant roots. Studies have shown that excessively high nutrient concentrations in the upper soil often cause a shallower root system, which is not conducive to plant lodging resistance (Doussan *et al*. 2003). We found that the roots of the two cultivars tended to grow in the upper soil layer (0-10 cm) under drip irrigation, and the root system in this soil layer accounted for more than 60% of the total root system. Using the vertical distribution model ($Y=1-\beta^{D}$) to further scrutinise root morphological attributes in relation to soil depth, we found that the parameter β of the root characteristics (RLD, SAD, and RVD) initially decreased and subsequently increased with increasing nitrogen application rate. This trend was likely due to the limited water supply delivered during each drip irrigation cycle, concentrating nutrients primarily in the upper layers of the soil. Correlation analysis showed that the RLD of FBR and CBR and the RLD and SAD of the 0-10 cm soil layer were significantly positively correlated with yield and NUE, which was different from the significant negative correlation between the root system and yield in the 0-10 cm soil layer suggested by Deng *et al*. (2022). The possible reason for this was that drip-irrigated rice relies more on the upper root system to absorb water and nutrients to achieve high rice yields.

4.2. Relationships between root physiological function and yield and NUE of different drip-irrigated rice cultivars

The absorption of water and nutrients by the root system is the result of the combined effect of morphology and physiology. The effect of N application on the growth and development of crops can be affected by hormone content changes in the plant. When crops are under nutrient stress, the root system can quickly sense nutrient stress, synthesise chemical signals, and send them to the shoots, thereby affecting the growth and development of the shoots and the absorption and utilisation of nutrients (Ha *et al*. 2012; Xu *et al*. 2018). In this study, under a medium N application rate (N2), the contents of Z+ZR and IAA in the roots of T-43 were increased by 6.1-48.1% and 22.8-73.6%, respectively, compared with those of LX-3, while the content of ABA was decreased by 24.0-47.6%. This finding indicated that after drip irrigation, the soil oxygen concentration increases, reducing the toxic effects of reductive toxic substances such as H_2S on the roots. As a result, the vitality of rice roots is enhanced (Zhou *et al*. 2014; Xu *et al*. 2017), which is conducive to Z+ZR and IAA synthesis in the roots of high-NUE cultivars. The increase in Z+ZR can counteract the synthesis of ABA in the roots (Ha *et al*. 2012), thereby reducing the ABA content in the roots. Under low N (N1) conditions, the contents of Z+ZR and IAA in the root system decreased, affecting the growth of the root system and causing premature senescence of the shoot (Xu *et al*. 2017). Under high N (N3) conditions, the root system was in a high nutrient-rich environment, which limited the transport of photosynthetic products to the root system, thereby reducing the contents of Z+ZR and IAA in the root system. The growth and development of the root system is related not only to hormone content but also, more importantly, to the interaction between hormones, especially the ratio and balance between growth-promoting hormones and growth-inhibiting hormones (Li *et al*. 2015). In this study, T-43 yield and NPFP were significantly positively correlated with 0-10 cm Z+ZR/ABA; and LX-3 yield was significantly negatively correlated with 0-10 cm IAA/ABA and (IAA+(Z+ZR))/ABA. This showed that the synthesis of promoting hormones can delay the ageing and death rate of T-43 and ensure that the root system can fully absorb nutrients to meet the shoot's growth requirements.

The coordinated development between the root and the shoot not only strengthens the absorption capacity of the root system to fertiliser and water resources in the soil but also helps to enhance the physiological activity of the plant and delay the senescence of the plant; this promotes high rice yield formation and efficient utilisation of resources (Yang *et al*. 2012). The results showed that the RDW of LX-3 was significantly greater than that of T-43, while the SDW of LX-3 was lower than that of T-43, indicating that an excessively large root system is not conducive to enhancing the physiological activity of the rice root system. Rice cultivars with high-NUE have a better coordinated root-shoot ratio, superior aboveground traits, and a strong root system, thus accelerating the absorption and utilisation of N by plants. Further increases in N fertiliser (N3) caused the RDW of the 2 cultivars to decrease significantly; the root-shoot ratio was significantly higher than that of the N2 treatment, indicating that excessive fertilisation weakened the roots' abilities to absorb nutrients, hindered dry matter accumulation and was not conducive to yield formation, resulting in low-NUE. There may be a compensatory effect between root mass and root physiology (Yang *et al*. 2012). In summary, the enhancement in crop yield and NUE is accompanied by the coordinated growth of the aboveground and underground parts.

4.3. Response of root plasticity of different drip-irrigated cultivars to N resource acquisition strategies

Crop roots explore, acquire and utilise nutrient resources through a series of morphological and physiological changes in plasticity (the ability of roots to adjust their form and function under different N environments) (Zhang *et al*. 2020). Studies have shown that due to the input of many chemicals (chemical fertilisers, pesticides, plant growth regulators, etc.) in modern agriculture, people are more inclined to select and cultivate resource-acquiring crop cultivars (Milla *et al*. 2015). Such cultivars tend to have small root diameters and high root trait plasticity (Wen *et al*. 2019) and can easily acquire key growth resources, such as water and nutrients, which are also important strategies for plants to adapt to changes in the nutrient environment (Kramer-Walter *et al*. 2016; Wang *et al*. 2020). In this study, regardless of the N environmental changes, the rice cultivars with high-NUE had strong root system plasticity, could actively adjust root morphology according to the nutrient distribution in the soil, and produced more fine roots (Table 3) to increase the surface area for nutrient absorption. By adjusting the growth direction of the root system, the root system could be better distributed in the nutrient-rich upper soil layer (Fig. 3), which is a strategy for efficiently acquiring nutrient resources; rice cultivars with low-NUE have low root system plasticity, have difficulty to actively adapt to different nutrient distributions, and exhibit low root traits and nutrient acquisition capacity. Rice has evolved from swamp plants and has long been adapted to the growth environment of NH_4^+; however, due to the aerobic cultivation conditions in drip irrigation, NO_3^- is dominant in the soil, which limits the absorption of N by rice cultivars with low-NUE. Milla (Milla *et al*. 2015) believed that the wild genotypes of undomesticated crops can better adapt to natural environments with greater nutrient stress, and the N acquisition strategy of rice cultivars with low-NUE may be more consistent with that of their ancestors in the natural environment. In summary, high-NUE cultivars can enhance nitrogen utilisation efficiency by adjusting root morphology and distribution for better access to soil nutrient resources (Mofijul Islam *et al*. 2018). Modern agriculture emphasises the reduction in fertiliser use and environmental pollution but not yield (Zörb *et al*. 2018). Furthermore, this study can provide ideas for selecting high-NUE rice with plastic root traits for drip irrigation rice cultivation to achieve a synergistic increase in yield and nitrogen rate, which is important for sustainable agroecosystems. A limitation of this study is the limited number of rice genotypes, and many different types of cultivars should be selected to compare the differences between genotypes.

5. Conclusion

Compared with low-NUE cultivars (LX-3), high-NUE cultivars (T-43) under a suitable N environment (N2) increased the ratios of FRLD and CSAD, enhanced the RLD, SAD, and RVD of the surface root system, promoted the synthesis of root endogenous hormones (IAA, Z+ZR), and optimised the root-shoot ratio, all of which contributed to a superior grain yield and PFP, exhibiting an effective nutrient acquisition strategy (the yield and PFP of the rice cultivars with high-NUE (T-43) increased by 11.4-18.9% and 11.3-13.5%, respectively). For rice cultivars with low-NUE (LX-3), the plasticity of root traits was low, the root-shoot ratio was excessive, and the ABA content in the root system was high, exhibiting a conservative nutrient acquisition strategy. Therefore, rice cultivars with high-NUE (T-43) and strong adaptability can better utilise nutrient resources, thus achieving efficient growth and yield enhancement of drip-irrigated rice.

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