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RESEARCH ARTICLE

Plant growth regulators improve root growth of rice seedlings after mechanical transplanting and increase grain yield

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Summary

Enhancing seedling quality and promoting root growth post-transplantation are crucial for improving mechanically transplanted rice productivity. Here we investigated the impact of various plant growth regulators on hybrid and conventional rice varieties. Treatments, including two-diethylaminoethyl hexanoate (DA-6, 10 mg L^{-1}), a combination of potassium 3-indole-butyrate + potassium 1-naphthylacetate + 6-benzylaminopurine (C3, 50 + 50 + 10 mg L^{-1}), potassium 3-indole-butyrate + potassium 1-naphthylacetate + 1-triacontanol (C4, 50 + 50 + 50 mg L^{-1}), potassium 3-indole-butyrate + potassium 1-naphthylacetate + 1.8% sodium nitrophenolate (C5, 50 + 50 + 1 mg L^{-1}), and a combination of potassium 3-indole-butyrate + potassium 1-naphthylacetate + 1.8% sodium nitrophenolate + DA-6 (C6, 50 + 50 + 1 + 10 mg L^{-1}), were sprayed either 3 or 10 days before transplanting. Seedlings sprayed 10 days before transplanting exhibited a higher number of white roots and total roots at the returning green stage, along with increased grain yield, irrespective of the plant growth regulator used. The C6 combination emerged as the most effective treatment, enhancing the growth of both hybrid and conventional rice seedlings, accelerating the growth rate of white roots and total roots, and increasing the length of the longest white root during the greening period, ultimately resulting in higher grain yield. Our findings demonstrate that pre-transplantation application of a combination of plant growth regulators positively influences rice seedling growth.

Keywords: Grain yield; plant growth regulator; root growth; transplanting

Introduction

The surge in large-scale farming has led to the widespread adoption of mechanical transplanting for rice in China, covering over 30% of the total rice planting area (Huang and Zou, 2018). Despite its advantages, this method poses challenges, requiring high-quality rice seedlings (Biswas *et al.*, 2000) and causing substantial root damage, extending the recovery period (Singh and Singh, 2014; Tang *et al.*, 2020), limiting productivity gains.

Agrochemicals, such as plant growth regulators (PGRs), offer potential solutions by enhancing rice seedling root growth (Li *et al.*, 2016). Rice crop growth faces various stresses (Dobermann and Fairhurst, 2000; Paul *et al.*, 2014), and PGRs have been employed to boost stress resistance and increase yields (Pan *et al.*, 2013). Various PGRs, including brassinolide (Kaur *et al.*, 2015),

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gibberellic acid (GA3) (Steffens and Sauter, 2006), ethephon (Watanabe *et al.*, 2015), triacontanol (Ahmed, 1990), uniconazole (Kazuo *et al.*, 1988), multi-effect triazole (Shen and Wu, 1993), abscisic acid (ABA), and benzyladenine (BA) (Gurmani *et al.*, 2006), have shown effects on rice plant growth. Studies indicate that treatments with GA3, ethephon, or their combination effectively enhance seedling growth (Watanabe *et al.*, 2015), while soaking seeds in uniconazole increases tiller number but decreases height (Shankun, 1993). Soaking seeds in brassinolide or triacontanol solutions has been shown to increase root length, volume, the number of roots, and both shoot and root dry weight (Ahmed, 1990; Kaur *et al.*, 2015). Multi-effect triazole at 50 ppm enhances seedling activity, root growth, and early tillering (Shen and Wu, 1993). ABA and BA improve both root system dry weight and aboveground dry matter accumulation (Gurmani *et al.*, 2006). While existing research suggests PGRs can enhance seedling growth, it remains unknown whether they can improve seedling root growth during the recovery period after mechanical transplanting. Broad-spectrum PGRs like 2-diethylaminoethyl hexanoate (DA-6) and sodium nitrophenolate, known for promoting crop growth and stress resistance (Górnik and Grzesik, 2002; Qi *et al.*, 2013), hold promise for addressing slow growth due to mechanical transplanting-induced injuries.

In this study, hybrid and conventional rice varieties received various PGR treatments before transplanting, aiming to investigate the impact of PGRs and application timing on rice seedling growth during the returning green stage and optimize seedling cultivation.

Materials and Methods

Plant material and chemicals

The rice varieties used in this study, Quanliangyou 681 (hybrid) and Fenghuazhan (conventional), were provided by Hubei Quanyin High-tech Seed Industry Co., Ltd. and Hunan Changde Fengyu Seed Co., Ltd., respectively. These varieties were selected for their suitability for local cultivation. Dry hybrid (70 g seeds) and conventional (90 g seeds) rice seedlings were raised in hard plastic seedling trays (58 × 28 cm) on May 4, 2016, and May 6, 2017. Before sowing, the seeds underwent a 12-hour disinfection soak in a strong chlorine solution. The substrate in the seedling trays consisted of a soil and substrate mixture (1:3 vermiculite:peat, v/v) with a ratio of 4:6.

Plant growth regulators (PGRs), including 2-diethylaminoethyl hexanoate (DA-6), potassium 3-indole-butyrate, potassium 1-naphthylacetate (NAA), 1-triacontanol, and 6-benzylaminopurine, were purchased from Shanghai Yuanye Bio-Technology Co., Ltd., and 1.8% sodium nitrophenolate was obtained from Asahi Chemical Industry Co., Ltd. These PGRs were all of analytical grade.

Field site and experimental design

The experiments were conducted from 2016 to 2017 in greenhouse facilities for seedling cultivation and at the Agricultural Science and Technology Industrial Park of Yangtze University, Huazhong Agricultural High−Tech Industrial Development Zone, Jingzhou City, Hubei Province, China (30°22'N, 112°40'E, 34 m a.s.l.). This region, situated in the Jianghan Plain along the middle reaches of the Yangtze River, experiences a subtropical monsoon climate with an average annual sunshine duration of approximately 2000 hours and a total annual solar radiation ranging from 4600 to 4800 MJ m^{-2}. The average annual precipitation varies between 1100 and 1300 mm, with around 70% occurring from April to September.

Plant growth regulators (PGRs) were sprayed onto the leaves of rice seedlings either 10 days (D1) or 3 days (D2) before transplanting. Each treatment with PGRs (Table 1) utilized five seedling trays. Mechanical transplanting of seedlings into a medium-fertility paddy field occurred on May 27, 2016, and May 29, 2017, with harvest dates on September 18, 2016, and September 20, 2017. Fertilization included the application of 650 kg ha^{-1} compound fertilizer (NPK, 15:15:15) during transplanting, 127.2 kg ha^{-1} and 84.8 kg ha^{-1} urea (46% N) at the tillering and panicle initiation stages, respectively, and 24 kg ha^{-1} potassium chloride (60% K2O) at the panicle initiation stage. A random block experimental design with three replicates per treatment was

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Table 1. Treatments and plant growth regulators used in this study

Code	Treatment	Dosage (mg L^{-1})
CK	Deionized water	–
DA-6	2-diethylaminoethyl hexanoate	10
C3	Potassium 3-indole-butyrate + potassium 1-naphthylacetate + 6-benzylaminopurine	50 + 50 + 10
C4	Potassium 3-indole-butyrate + potassium 1-naphthylacetate + 1-triacontanol	50 + 50 + 50
C5	Potassium 3-indole-butyrate + potassium 1-naphthylacetate + 1.8% sodium nitrophenolate (1000× dilution)	50 + 50 + 1
C6	C5 + DA-6	50 + 50 + 1 + 10

Note: Spraying about 150 mL of PGR for each seedling tray.

employed, with rice plants spaced 30 × 16 cm within each 45 m^2 plot across all 72 plots. Additional management practices, such as disease and pest control, followed local routine procedures, including the application of 1.5 L ha^{-1} Pretilachlor 24 hours after rice seeding, 2 kg ha^{-1} validamycin for rice sheath blight control from June to July, and 0.75 L ha^{-1} flubendiamide for rice borer (Cnaphalocrocis medinalis) control in August.

Rice seedling growth

Various plant characteristics were assessed one day prior to transplanting seedlings, including plant height, leaf area per plant, leaf age, the total number of roots per plant, and the 100-plant dry weight for both aboveground and belowground components. The dry weight measurements were conducted after oven-drying at 80 °C until a constant weight was achieved. Each treatment had three replications. Individual leaf area was determined using ImageJ 1.51j8 (NIH, Bethesda MA, USA). Leaf age was quantified based on the number of leaves on the main stem, considering partial leaf extraction from the sheath. The root-shoot ratio was calculated by comparing the dry weights of aboveground and belowground components.

Root growth during the returning green stage

To evaluate root growth during the returning green stage, rice plants were carefully uprooted from the field 3 and 7 days after transplanting, considering the softness of the surface soil. After uprooting, the collected plants were washed with clean water, and various root parameters were recorded, including the number of white roots per plant (roots ≥5 mm in length that were white from base to tip, indicating indefinite roots), the length of the longest white root on each plant, and the total number of roots per plant. Each treatment had ten replications.

Yield and its components

Yield and its components were assessed after the maturation of rice plants, when 90% of the rice husks had turned yellow. In each plot, a 5 m^2 area at the central part was chosen. The assessment involved selecting ten rice plants to determine the average effective panicle number. Additionally, five randomly chosen plants were used to assess the number of grains per year, seed setting rate, and 1000-grain weight.

Economic analysis

Economic benefits were quantified by calculating the ratio of total output (in USD per kilogram) to total agricultural input (in USD). Total output comprised the value of grain yield, while total agricultural input encompassed the costs of PGRs, seeds, fertilizers, herbicides, pesticides, and labor.

The exchange rate was 6.75 RMB to 1 USD in 2017. The economic benefit associated with the costs of seed, fertilizers, herbicide, pesticide, and labor was consistent across all treatments.

Statistical analysis

The data were analyzed using SPSS 17.0 (SPSS Inc., Chicago IL, USA), and Origin 2019 (OriginLab Corp., Northampton MA, USA) software was employed for plotting. Two-way ANOVA was conducted, and treatment means were compared using the least significant difference (LSD) test at a significance level of $p \leq 0.05$. All data are presented as mean ± LSD. The growth rate of rice roots after transplantation was computed following the method described by Islam *et al.* (2021): Increase in white root number per day = [(white root number per plant at 7 days after transplanting) – (white root number per plant at 3 days after transplanting)]/4 days; Increase in total root number per day = [(total root number per plant at 7 days after transplanting) – (total root number per plant at 3 days after transplanting)]/4 days; Increase in the length of the longest white root per day = [(the length of the longest white root at 7 days after transplanting) – (the length of the longest white root at 3 days after transplanting)]/4 days.

Results

The response of rice growth characteristics, yield, and its components to hybrid rice and conventional rice under different PGRs

The comparative performance of hybrid rice and conventional rice under various PGR application methods is summarized in Table S1. In seedlings treated 10 days before transplanting, hybrid rice exhibited significantly increased seedling height, leaf area (D1CK, D1B1), and 100-plant dry weight (D1C6) compared to conventional rice. However, leaf age (D1C5), root number (D1B1, D1C3, D1C4), and root-shoot ratio (D1C4) were reduced in hybrid rice. In seedlings treated 3 days before transplanting, hybrid rice showed improved seedling height, leaf age, 100-plant dry weight, and root–shoot ratio in several PGR methods. Leaf area increased in all treatments, while root number decreased only in D2B1. Table 8 reveals that, compared to conventional rice, hybrid rice exhibited significantly lower effective panicle number (CK, C3) but increased spikelet number per panicle, seed setting rate (several PGR methods), 1000-grain weight, and grain yield (all PGR methods except D1C6 in 1000-grain weight).

Transplanted rice growth as affected by PGRs

For each PGR and considering hybrid rice, seedling height under DA-6 and C3 treatments 10 days before transplanting was significantly higher than plants subjected to the same treatments 3 days before transplanting (Table 2). Additionally, the number of roots per plant sprayed 10 days before transplanting was 11.6–25.6% higher than those sprayed 3 days before transplanting (Table 3). The application of C6 caused the highest 100-plant dry weight in both hybrid and conventional rice seedlings when sprayed 10 days before transplanting and only in conventional seedlings when sprayed 3 days before transplanting (Table 3). The application time of the DA-6, C3, C4, C5, and C6 treatments did not significantly affect the leaf area of hybrid rice seedlings (Table 2). Among all combinations of growth regulators and application times, the C6 treatment 10 days before transplanting had the strongest promoting effect on the number of roots per plant and 100-plant dry weight (Table 3). Additionally, the C6 treatment 3 days before transplanting had a moderate promoting effect on the root–shoot ratio in hybrid rice seedlings.

Overall and for each regulator solution sprayed, treatments 10 days before transplanting tended to increase the leaf area, leaf age, and root number (per plant) of conventional rice by 31.0–62.9%, 2.7–12.8%, and 1.9–34.6%, respectively, compared with spraying 3 days before transplanting (Tables 2 and 3). Some growth traits of plants treated with C6 both 10 and 3 days before transplanting were

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Table 2. Quality of hybrid (Hyb) and conventional (Conv) rice seedlings as affected by plant growth regulators (PGRs): height (H), leaf area (LA), and leaf age (LAge)

Days before transplanting	PGRs+	H (cm)		LA (cm^2)		LAge	
		Hyb	Conv	Hyb	Conv	Hyb	Conv
10 (D1)	CK	16.00 ± 0.26b	13.50 ± 1.37d	2.83 ± 0.04d	2.24 ± 0.11d	2.42 ± 0.06c	2.50 ± 0.16b
	DA-6	17.70 ± 0.31a*	14.20 ± 1.58cd	3.95 ± 0.50a	3.16 ± 0.05bc*	2.90 ± 0.08a*	2.82 ± 0.21a*
	C3	17.30 ± 0.93ab*	16.90 ± 0.59ab	3.69 ± 0.35ab	3.60 ± 0.44a*	2.58 ± 0.18b	2.82 ± 0.23a
	C4	17.30 ± 1.59ab	16.30 ± 1.14ab	3.30 ± 0.04bc	3.41 ± 0.18ab*	2.69 ± 0.09b	2.74 ± 0.10ab
	C5	16.70 ± 0.26ab	15.70 ± 1.72bc	2.97 ± 0.02cd	3.05 ± 0.16c*	2.42 ± 0.12c	2.70 ± 0.08ab
	C6	17.40 ± 0.43a	17.50 ± 2.06a*	3.45 ± 0.39b	3.68 ± 0.10a*	2.86 ± 0.07a	2.74 ± 0.15ab
3 (D2)	CK	16.00 ± 0.26b	13.50 ± 1.37b	2.83 ± 0.04c	2.24 ± 0.11a	2.42 ± 0.06c	2.50 ± 0.16a
	DA-6	16.28 ± 0.85b	15.60 ± 0.78a	3.60 ± 0.24ab	2.41 ± 0.08a	2.74 ± 0.17ab	2.50 ± 0.07a
	C3	16.16 ± 1.25b	15.80 ± 0.53a	3.66 ± 0.21a	2.21 ± 0.16a	2.78 ± 0.01a*	2.56 ± 0.15a
	C4	15.70 ± 0.49b	15.40 ± 0.41a	3.51 ± 0.19ab	2.30 ± 0.34a	2.62 ± 0.11b	2.58 ± 0.21a
	C5	16.46 ± 1.04ab	15.00 ± 0.22ab	3.26 ± 0.08b	2.13 ± 0.17a	2.78 ± 0.03a*	2.63 ± 0.16a
	C6	17.64 ± 0.85a	15.80 ± 1.07a	3.79 ± 0.11a	2.42 ± 0.11a	2.86 ± 0.02a	2.66 ± 0.21a

+See Table 1 for details. For each rice type (hybrid or conventional), different letters (a, b, c) indicate significant differences among treatments for a given spraying time based on LSD's multiple range tests ($p < 0.05$, $n = 3$).
Star (*) represents statistical significance between the two spraying times under given PGR treatment based on independent samples *t* test ($p < 0.05$, $n = 3$). The values in table are two-year averages.

Table 3. Quality of hybrid (Hyb) and conventional (Conv) rice seedlings as affected by plant growth regulators (PGRs): number of roots (NR), 100-plant dry weight (PDW), and root-shoot ratio (RSR)

Days before transplanting	PGRs+	NR (units $plant^{-1}$)		PDW (g)		RSR	
		Hyb	Conv	Hyb	Conv	Hyb	Conv
10 (D1)	CK	8.40 ± 0.52d	9.00 ± 0.60d	2.04 ± 0.36b	1.70 ± 0.16c	0.27 ± 0.03bc	0.22 ± 0.07b
	DA-6	10.80 ± 0.63c*	12.30 ± 0.38b*	2.22 ± 0.44b	2.00 ± 0.22bc	0.35 ± 0.02a	0.34 ± 0.04a*
	C3	11.50 ± 0.53b*	14.00 ± 0.36a*	2.01 ± 0.03b	1.85 ± 0.10bc	0.20 ± 0.02c	0.18 ± 0.05b
	C4	12.60 ± 0.52a*	14.00 ± 0.73a*	2.07 ± 0.57b	2.15 ± 0.09ab	0.22 ± 0.05bc	0.34 ± 0.06a*
	C5	12.30 ± 0.82a*	11.00 ± 0.74c	2.01 ± 0.38b	1.83 ± 0.06c	0.28 ± 0.03ab	0.31 ± 0.03a*
	C6	12.50 ± 0.53a*	13.20 ± 0.58a*	2.74 ± 0.07a*	2.38 ± 0.05a	0.22 ± 0.01bc	0.24 ± 0.06b*
3 (D2)	CK	8.40 ± 0.52c	9.00 ± 0.60d	2.04 ± 0.36a	1.70 ± 0.16b	0.27 ± 0.03b	0.22 ± 0.06ab
	DA-6	8.60 ± 0.70c	10.20 ± 0.38c	1.98 ± 0.23a	1.98 ± 0.42ab	0.37 ± 0.04a	0.17 ± 0.05ab
	C3	10.20 ± 0.63b	10.40 ± 0.15c	2.18 ± 0.09a	1.80 ± 0.09b	0.24 ± 0.05b	0.23 ± 0.09a
	C4	11.00 ± 1.05a	11.60 ± 0.21ab	2.07 ± 0.24a	1.94 ± 0.28ab	0.26 ± 0.04b	0.23 ± 0.10a
	C5	10.40 ± 0.70b	10.80 ± 0.14bc	2.19 ± 0.08a	1.88 ± 0.08b	0.27 ± 0.09b	0.15 ± 0.04b
	C6	11.20 ± 0.63a	12.00 ± 1.33a	2.27 ± 0.09a	2.24 ± 0.08a	0.31 ± 0.02ab*	0.17 ± 0.02ab

+See Table 1 for details. For each rice type (hybrid or conventional), different letters (a, b, c) indicate significant differences among treatments for a given spraying time based on LSD's multiple range tests ($p < 0.05$, $n = 3$).
Star (*) represents statistical significance between the two spraying times under given PGR treatment based on independent samples *t* test ($p < 0.05$, $n = 3$). The values in table are two-year averages.

higher than those with the other growth regulators and the control treatment. For example, the height, leaf area, and 100-plant dry weight of seedlings treated with C6 at 10 days before transplanting were significantly higher than those of control plants by 29.6%, 64.3%, and 40.0%, respectively. When sprayed 3 days before transplanting, C6 application caused higher height (+17.0%), leaf area (+7.8%), leaf age (+6.4%), and the number of roots (+33.3%) than the control treatment.

Root growth of transplanted rice seedlings as affected by PGRs

Under the same growth regulator solution application, the increases in white root number per plant and total root number per plant sprayed 10 days before transplanting were significantly

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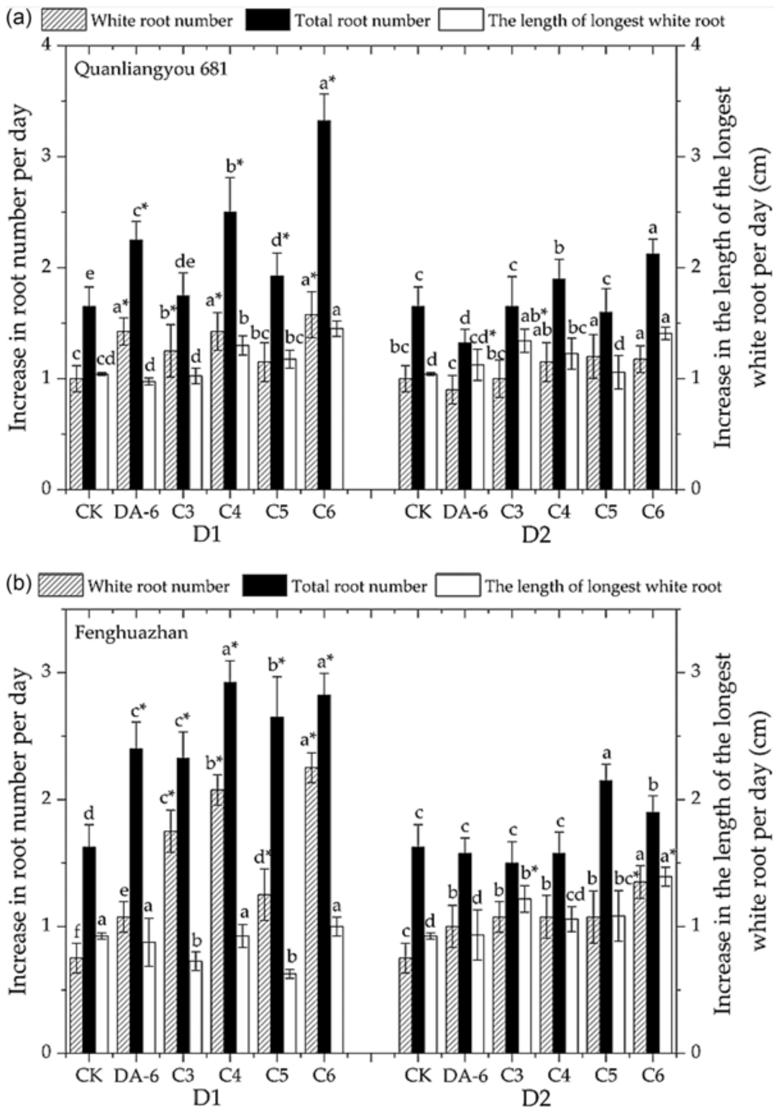

Figure 1. Root growth rate of hybrid (a) and conventional (b) rice as affected by PGRs applied 3 (D2) or 10 (D1) days after transplanting. See Table 1 for details about the treatments. Different letters (a, b, c) indicate significant differences among PGR solutions for a given spraying time based on LSD's multiple range tests ($p < 0.05$, $n = 10$). Star (*) represents statistical significance between the two spraying times under a given PGR solution based on independent samples *t* test ($p < 0.05$, $n = 10$). The values in table are two-year averages.

higher than those sprayed 3 days before transplanting (Fig. 1a). However, the DA-6 and C3 treatments better promoted white root growth when applied 3 days before transplanting than 10 days before transplanting. When considering treatments 10 days before transplanting (D1 time) and the hybrid rice seedlings, the increase (+25.0% to +57.5%) in white root number per plant under DA-6, C3, C4, and C6 was significantly higher than that under the control condition (Fig. 1a). The same was noticed for the total root number per plant when seedlings were sprayed with DA-6, C4, C5, and C6 solutions, with increments between 16.7% and 101.5% compared to the control treatment. The length of the longest white root was also increased under C4 and C6 treatments at 10 days before transplanting. When compared to the control condition,

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there was an increase in white root number per plant under the C5 and C6 treatments 3 days before transplanting, whereas the total root number per plant was increased by C4 and C6 treatments (Fig. 1a). The growth rate of the longest white root under C3, C4, and C6 treatments was significantly lower than in the control treatment.

For each growth regulator treatment, and considering conventional rice, plants treated 10 days before transplanting had 7.5% to 93.0% more white roots and 23.3% to 85.7% more total roots than those treated 3 days before transplanting (Fig. 1b). However, the increase in white root length was lower in plants sprayed 10 vs. 3 days before transplanting. The increase in white root number per plant and total root number per plant of seedlings sprayed with growth regulators 10 before transplanting was significantly higher than that of seedlings under the control treatment. The increase in the length of the longest white root under C3 and C5 treatments was significantly lower than that under the control treatment when regulators were sprayed 10 days before transplanting (Fig. 1b). Considering treatments done 3 days before transplanting, plant growth regulators increased the number of white roots by 33.3% to 80.0% compared to the control condition. In relation to the control condition, there were increases in total root number per plant under C5 and C6, and increases in the length of the longest white root under C3, C5, and C6 when solutions were sprayed 3 days before transplanting.

Rice yield and its components as affected by PGRs

For each growth regulator applied, the yield of the treatments sprayed 10 days before transplanting was 1.4% to 9.2% higher than those sprayed 3 days before transplanting (Table 4). The yield in C4 and C6 treatments applied 10 days before transplanting was significantly higher than that of the control treatment by 8.0% and 11.2%, respectively. When considering treatments done 3 days before transplanting, the yield in C6 was significantly higher than that of the other treatments. As shown in Table 4, the grain yield of hybrid rice treated 10 days before transplanting outperformed that of plants treated 3 days before transplanting, mainly owing to increases in effective panicle number (+1.2% to +10.3%) and the number of grains per panicle (+1.1% to +10.2%). For the two spraying times, the effective panicle number under the C6 treatment was the highest (Table 4).

The grain yield of conventional rice was 1.7% to 11.0% higher when plants were sprayed 10 days before transplanting compared to those sprayed 3 days before transplanting (Table 4). The yield in C4 and C6 treatments was significantly higher than that in the control treatment when plants were treated 10 days before transplanting. The yield in the C6 treatment sprayed 3 days before transplanting was significantly higher than that of the control treatment (+6.0%). When comparing spraying times, the yield increase of conventional rice due to growth regulators noticed in plants sprayed 10 days before transplanting was caused by increases in effective panicle number (+0.3% to +12.9%). For both spraying times, the effective panicle number under the C6 treatment was the highest.

Economic benefit as affected by PGRs

Overall, the net economic benefit (NEB) of the PGR treatments sprayed 10 days before transplanting was higher (+1.4% to +9.3%) than those sprayed 3 days before transplanting (Table 5). When considering only treatments done 10 days before transplanting, NEB in C4 and C6 treatments was significantly higher than in the control treatment. The NEB in the C6 treatment was the highest when compared to the other treatments sprayed 3 days before transplanting (Table 5).

Discussion

Our study reveals that seedlings sprayed 10 days before transplanting exhibited a higher number of white roots and total roots at the returning green stage, resulting in a higher grain yield

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Table 4. Yield and yield components of hybrid (Hyb) and conventional (Conv) rice as affected by plant growth regulators (PGRs): effective panicle number (EPN), spikelet number per panicle (SNP), seed setting rate (SS), 1000-grain weight (TGW), and grain yield (GY)

		EPN (10^4 ha^{-1})		SNP (units)		SS (%)		TGW (g)		GY (Mg ha^{-1})	
DBT#	PGRs+	Hyb	Conv	Hyb	Conv	Hyb	Conv	Hyb	Conv	Hyb	Conv
10 (D1)	CK	213 ± 6c	230 ± 5b	200 ± 11a	180 ± 6bc	83 ± 1ab	81 ± 1a	27.8 ± 0.9a	25.4 ± 0.3bc	9.6 ± 0.5bc	8.4 ± 0.5c
	DA-6	254 ± 7a*	232 ± 9b	172 ± 7b	190 ± 13ab	84 ± 2a	79 ± 3a	26.9 ± 0.2bc	25.4 ± 0.5bc	9.6 ± 0.1c	8.5 ± 0.1bc
	C3	239 ± 10b*	271 ± 5a*	212 ± 5a*	169 ± 9c	78 ± 2b	80 ± 8a	27.0 ± 0.3bc	24.2 ± 0.1d	10.0 ± 0.5b*	8.8 ± 0.2bc
	C4	267 ± 6a*	266 ± 7a	178 ± 5b	165 ± 8c	82 ± 6ab	81 ± 1a	27.7 ± 0.3ab	25.8 ± 0.4b	10.4 ± 0.1a*	8.9 ± 0.1ab*
	C5	241 ± 3c	239 ± 8b	180 ± 10b	197 ± 6a*	84 ± 3a	80 ± 2a	28.0 ± 0.4a	24.9 ± 0.8cd	9.5 ± 0.1c	8.8 ± 0.2bc
	C6	268 ± 6a	272 ± 9a	208 ± 4a*	174 ± 10c	80 ± 1ab	76 ± 5a	26.5 ± 0.6c	27.2 ± 0.3a*	10.7 ± 0.1a	9.4 ± 0.2a*
3 (D2)	CK	213 ± 4c	230 ± 5c	200 ± 11a	180 ± 11ab	83 ± 3ab	81 ± 1a	27.8 ± 0.9b	25.4 ± 0.5a	9.6 ± 0.5b	8.4 ± 0.5b
	DA-6	238 ± 15b	231 ± 9c	170 ± 7b	183 ± 10a	85 ± 2ab	80 ± 1a	27.3 ± 0.4b	25.0 ± 0.4a	9.3 ± 0.2b	8.4 ± 0.2b
	C3	219 ± 10c	240 ± 8bc	192 ± 7a	179 ± 3ab	81 ± 6bc	81 ± 3a	28.9 ± 0.6a*	24.8 ± 0.9a	9.4 ± 0.1b	8.5 ± 0.3ab
	C4	242 ± 12b	252 ± 11ab	171 ± 12b	166 ± 5b	86 ± 2ab	78 ± 2a	27.3 ± 0.4b	25.4 ± 0.3a	9.5 ± 0.1b	8.1 ± 0.5b
	C5	235 ± 11b	237 ± 19bc	174 ± 9b	177 ± 8ab	87 ± 3a	79 ± 2a	27.9 ± 0.1b	25.4 ± 0.4a	9.4 ± 0.1b	8.3 ± 0.1b
	C6	265 ± 8a	268 ± 8a	191 ± 12a	183 ± 9a	77 ± 3c	81 ± 4a	28.0 ± 0.4b*	25.0 ± 0.3a	10.5 ± 0.1a	8.9 ± 0.1a

#Days after transplanting;
+See Table 1 for details. For each rice type (hybrid or conventional), different letters (a, b, c) indicate significant differences among treatments for a given spraying time based on LSD's multiple range tests ($p < 0.05$, $n = 3$).
Star (*) represents statistical significance between the two spraying times under given PGR treatment based on independent samples *t* test ($p < 0.05$, $n = 3$). The values in table are two-year averages.

Table 5. Economic benefit of rice as affected by plant growth regulators (PGRs): economic benefit of cost of PGRs ($EB_{PGR\ cost}$); economic benefit of rice yield (EB_{yield}); and net economic benefit ($NEB = EB_{yield} - EB_{PGR\ cost}$)

		Hybrid rice			Conventional rice		
Days before transplanting	PGRs+	$EB_{PGR\ cost}$ (RMB ha^{-1})	EB_{yield} (RMB ha^{-1})	NEB (RMB ha^{-1})	$EB_{PGR\ cost}$ (RMB ha^{-1})	EB_{yield} (RMB ha^{-1})	NEB (RMB ha^{-1})
10 (D1)	CK	0	3999bc	3999bc	0	3476c	3476c
	DA-6	0.07	3986c	3986c	0.67	3526bc	3525bc
	C3	0.52	4136b*	4135b*	0.36	3642bc	3642bc
	C4	6.28	4318a*	4312a*	4.25	3708ab*	3704ab*
	C5	0.46	3957c	3957c	0.31	3630bc	3629bc
	C6	0.53	4447a	4446a	0.36	38876a*	3887a*
3 (D2)	CK	0	3999b	3999b	0	3476b	3476b
	DA-6	0.07	3874b	3874b	0.67	3468b	3467b
	C3	0.52	3916b	3915b	0.36	3530ab	3530ab
	C4	6.28	3953b	3947b	4.25	3339b	3335b
	C5	0.46	3903b	3903b	0.31	3451b	3451b
	C6	0.53	4372a	4372a	0.36	3684a	3683a

+See Table 1 for details. The currency USD/RMB was 6.75/1 in 2017. The prices of 2-diethylaminoethyl hexanoate, potassium 3-indole-butyrate, potassium 1-naphthylacetate, 1-triacontanol, 6-benzylaminopurine, and 1.8% sodium nitrophenolate were 0.62, 0.53, 0.19, 0.69, and 0.71 USD g^{-1}, respectively. Rice grain price was 0.41 USD kg^{-1}. Different letters (a, b, c) indicate significant differences among different PGR solutions for a given spraying time based on LSD's multiple range tests ($p < 0.05$, $n = 3$).
Star (*) represents statistical significance between the two spraying times under a given PGR solution based on independent samples *t* test ($p < 0.05$, $n = 3$). The values in table are two-year averages.

compared to those sprayed 3 days before transplanting. Furthermore, a solution composed of potassium 3-indole-butyrate (50 mg L^{-1}), potassium 1-naphthylacetate (50 mg L^{-1}), 2-diethylaminoethyl hexanoate (10 mg L^{-1}), and 1.8% sodium nitrophenolate (1 mg L^{-1}) significantly increased the growth rate of white roots, total roots, and the length of the longest white root during the greening period. This enhancement led to an increased grain yield and net economic benefit in both hybrid and conventional rice. The results highlight that the growth of rice seedlings raised in substrate trays can be substantially improved after transplanting by using a combination of plant growth regulators.

Plant growth regulators (PGRs) exhibit diverse effects on plant growth due to their mechanisms of action, and the growth regulators used in this study were predominantly growth-promoting and broad-spectrum. For example, potassium 3-indole-butyrate promotes cell division and induces the formation of adventitious roots in plants, while potassium 1-naphthylacetate, a broad-spectrum PGR, stimulates cell division, expansion, and the formation of adventitious roots. DA-6 enhances protein, chlorophyll, nucleic acid content, and photosynthetic rate, promoting root growth and improving stress resistance. Sodium nitrophenolate acts as a potent cell activator, quickly absorbed by cells to enhance viability and promote rooting. 6-benzylaminopurine, when absorbed by leaves, inhibits chlorophyll, nucleic acid, and protein degradation, delays plant senescence, and improves nutrient transport. 1-Triacontanol, another broad-spectrum PGR, promotes cell division, increases plant weight, enhances enzyme activity, and has root growth-promoting abilities.

The proper development of seedlings and roots serves as the cornerstone for achieving higher yields in any crop. In previous studies, PGRs, including DA-6, potassium 3-indole-butyrate, potassium 1-naphthylacetate, sodium nitrophenolate, triacontanol, and 6-benzylaminopurine, have been reported to improve the quality of rice seedlings and increase yield. In this study, some growth regulators did not consistently promote rice growth in certain conditions (Tables 2 and 3). Height, leaf area, leaf age, and root number per plant after a single application of DA-6 tended to be higher than those in the control treatment in both hybrid and conventional rice. At the

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returning green stage, application of DA-6 at 10 days before transplanting increased the growth of white root and total root number per plant in both hybrid and conventional rice, a positive effect not observed in grain yield (Fig. 1, Table 4). Additionally, spraying growth regulators (C3 to C6) 10 days before transplanting proved more conducive to rice growth compared to control and DA-6 treatments, with the C6 treatment providing the greatest benefit for increasing yield. This result aligns with previous reports that mixed PGR solutions exert a greater growth-promoting effect than single PGRs (Watanabe *et al.*, 2015; Xiao *et al.*, 2008). This may be attributed to the synergistic effects of multiple growth regulators, enhancing various underlying processes linked to plant growth. Moreover, yield components were balanced, and the ultimate grain yield of plants treated with C6 was the highest among all treatments.

PGRs can be applied throughout most of the rice growth period, yet limited studies have explored the influence of PGR spraying time on rice growth (Pal *et al.*, 2009; Rezaeieh *et al.*, 2014; Watanabe *et al.*, 2015). Our current study contributes evidence demonstrating that the timing of growth regulator application significantly affects rice growth, influencing the root number per plant in hybrid rice seedlings, as well as both leaf area and root number per plant in conventional rice seedlings (Fig. 1, Table 2). The 7-day period post-transplanting emerges as a crucial phase for the resumption of normal rice root growth. Notably, white roots, indicative of newly grown roots, exhibit heightened vigor and play a pivotal role in nutrient absorption (Jiang *et al.*, 2014). Compared to spraying 3 days before transplanting, applying PGRs 10 days before transplanting provides an additional week to stimulate root development. When comparing the two spraying times, PGR application 10 days before transplanting led to an increase in the number of white roots and total roots in both hybrid and conventional rice seedlings after transplanting. However, there was no significant difference in the growth rate of white roots between the two spraying times (Fig. 1). Additionally, the spraying time exerted a more pronounced impact on the yield of conventional rice than on hybrid rice (Table 4). Such disparity may be caused by the differences in growth characteristics.

This study observed a differential response between hybrid rice and conventional rice to PGRs (Supplementary Material Table S1). In plants treated 10 days before transplanting, the leaf area in all treatments was higher in hybrid rice than in conventional rice (Table 2). Regardless of the spraying time and treatment, the 1000-grain weight and grain yield of hybrid rice were higher than those of conventional rice, with the exception of the C6 treatment at 10 days before transplanting (Table 4). The strong heterosis in biomass production and the high yield potential of hybrid crops have been widely recognized (Cheng *et al.*, 2007; Yuan, 1997). Numerous studies have indicated that hybrid rice exhibits higher total N, P, and K accumulation, a 'darker' leaf color (higher leaf N content), and a longer leaf greenness than conventional rice. As a consequence, hybrid rice demonstrates an increased seedling index, leaf area, chlorophyll level, high activity of protective enzymes in leaves, as well as high root/shoot biomass (Chen *et al.*, 2021; Mahajan *et al.*, 2014; Wei *et al.*, 2017).

Conclusions

When compared to non-sprayed plants and other treatments with plant growth regulators sprayed alone or in combination, the solution composed of potassium 3-indole-butyrate (50 mg L^{-1}), potassium 1-naphthylacetate (50 mg L^{-1}), 2-diethylaminoethyl hexanoate (10 mg L^{-1}), and 1.8% sodium nitrophenolate (1 mg L^{-1}) applied 10 days before transplanting resulted in the highest grain yield and net economic benefit in both hybrid and conventional rice, while also improving root growth at the returning green stage.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0014479724000048

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