# EFFECTS OF DIFFERENT SEEDLING SUBSTRATES ON SEEDLING QUALITY AND YIELD OF MACHINE-TRANSPLANTED RICE

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**Abstract.** To study the influences of the physical and chemical properties of different seedling cultivation substrates on the quality and yield of machine-transplanted seedlings in China, seeds of the rice (Oryza sativa L.) variety Yangjing 805 were sown in topsoil (TS), commercial seedling substrate (CS), straw (ST), rice husk (RH) and mushroom residue (MR). Changes in the seedling quality, nutrient content, and grain yield were analyzed. The results showed that the morphological indices and nitrogen metabolism-related enzymatic activities of seedlings treated with TS, CS, and MR were significantly higher than of those treated with ST and RH. The reduction in total N, alkali-hydrolyzed N, and available P was lower under TS and MR than under any other treatment. The highest rice yield was recorded for the MR treatment, with a 28.93% higher yield compared with the ST treatment. Among the seedling morphological indices, stem thickness ($R^2 = 0.9604$) and shoot dry weight ($R^2 = 0.8835$) showed the highest correlations with yield, while root length ($R^2 = 0.8919$) and root surface area ($R^2 = 0.9125$) were the root indices with maximum values. The variables that showed the closest relationship with seedling quality were bulk density, conductivity, and pH of the growing medium.

**Keywords:** *Oryza sativa L., straw, rice husk, mushroom residue, circular planting system, nutrient utilization*

## Introduction

Rice (*Oryza sativa* L.) is a major grain crop grown in China, it constitutes up to 44% of the total grain produced nationally and feeds more than 65% of the population of China as a staple food (Kargbo et al., 2016; Zheng et al., 2022). Transplanting seedlings provides numerous advantages over direct seeding, and using a machine to transplant seedlings is more efficient and labor saving compared to manual transplanting; it also reduces production costs and enables large-scale planting (Rashid et al., 2018), all of which are consistent with China’s current agricultural production needs and rice production-developmental trends.

Regarding production of quality rice, the seedling stage is one of the most important growth stages, as it directly influences the final plant performance, both from the nutritional and the productive perspective. Healthy seedlings and rice productivity have a direct relationship, and seedling quality has a considerable influence on the rooting, greening, and tillering stages of growth, as well as on the panicle and grain numbers at harvest. Healthy and well-nourished seedlings with uniform growth are prerequisites for

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uniform transplanting in the field. In addition, these seedlings need to meet certain technical standards to comply with the mechanical rice seedling-transplanting system (Huang et al., 2020).

The cultivation of rice seedlings mainly involves the use of fertilized soil and a commercial substrate (Monaco et al., 2020). Topsoil is nutritious, highly permeable, has a high water-retention capacity, and it is collected from the field using simple technology at a low cost. However, the large-scale use and standardization of topsoil are associated with various problems; for example, it is labor intensive and time consuming to prepare, it can be damaged by extraction, transportation costs are high, and a heavy mechanical operation load is required. In contrast, commercial substrates are light and durable, are mainly derived from natural resources, and their use saves time and labor. Nevertheless, inherent problems associated with the use of commercial substrate as well; for example, commercial substrates are rather unstable, their sources are not always entirely safe, they are expensive, their fertility is typically low, and they have a low water-retention capacity (Peyvast et al., 2010). Furthermore, the seedlings produced using commercial substrates are of low quality with poorly developed root systems. Therefore, investigating these limitations is necessary to enhance the use of commercial substrates for rice seedling cultivation.

Rice straw (ST), rice husk (RH), and mushroom residue (MR) are common waste materials derived from agricultural production (Jiang et al., 2022; Unglaube et al., 2021; Wiafe-Kwagyan et al., 2022), and improper disposal of these wastes can result in serious environmental problems that contribute to the release of greenhouse gases, the proliferation of pests, and N immobilization in the soil (Dinardo-Miranda et al., 2013; Ferreira et al., 2016). Yamanuch et al. (1997) and Khatun et al. (2002) reported that rice seedling growth can be enhanced if cultivated in these substrates, when the medium is supplemented with a reasonable fertilizer formula. Using these materials is thus an effective way of recycling agricultural waste, reducing agricultural production costs, and improving productivity (Hong et al., 2016).

Previous studies in this regard have mainly focused on the selection of substrate materials, their proportions, and the addition of nutrients (Aung et al., 2019; Trevisan et al., 2010; Wang et al., 2021), whereas, the relationship between the physicochemical properties of the substrate and the associated seedling quality and rice yield have been mostly ignored. Therefore, this study aimed to show that these abundant substrates (rice straw, rice husk, and mushroom residue) can be effectively used in rice seedling cultivation. Additionally, we aimed to clarify the effects of using different substrates on the morphological indices, nutrient absorption characteristics, and enzyme activities of rice seedlings. Our results provide a practical reference and a solid theoretical foundation for the recycling of agronomic wastes and improving the overall production capacity of machine-transplanted rice.

## Materials and methods

### *Experimental site*

The experiments were conducted in 2020, in the experimental field of the Agricultural College of Yangzhou University (32°39’18” N, 119°41’97” E), in Jiangsu Province, China during the rice growing season (May to October). This area lies within the northern subtropical humid climate zone and has sufficient sunshine and rainfall.

The annual average temperature is 14.8°C, and annual sunshine hours amount to 2140, annual average precipitation is 1024.8 mm, and the frost-free period extends over 220 days per year, the daily average temperature and precipitation during the experimental period are shown in *Figure 1*. Wheat stubble was at the front of the study site. The soil type in the experimental field is sandy loam with the following basic physical and chemical indicators in the 20-cm topsoil layer: 1280 mg/kg total nitrogen (N), 87.3 mg/kg alkaline N, 32.5 mg/kg available phosphorus (P), and 88.5 mg/kg available potassium (K).

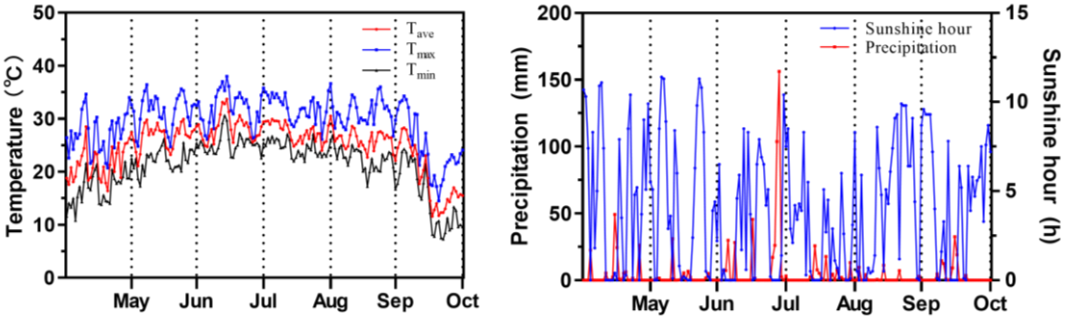


***Figure 1.*** *Daily temperature, sunshine hour, and precipitation during the rice growing seasons in the experimental fields*

### *Materials and experimental design*

For the experiments reported herein, we used the high-quality, late-maturing, medium-japonica rice variety, Yangjing 805 for the experiments reported herein. Yangjing 805 has a seed setting rate of 93.8%, a full growth period of 152 days, and a 1000-kernel weight of 27.2 g. We used a single-factor randomized complete-block design with five treatments: TS was a fertilized soil treatment, where the plow-layer soil was collected, dried, and pulverized by passing through a 10-mesh sieve for later use; CS was a commercial substrate that was selected in the market and publicized as being a highly stable substrate for raising seedlings over a large-area, its main ingredients are decomposed wood chips (60%), cow dung (20%), vinegar grains (10%), and humic acid vermiculite and other ingredients. It is produced by Yangzhou Runjiang Ecological Agriculture Co., Ltd.. A hard disk (inner dimension length, width, and height of 580 mm, 280 mm, and 25 mm, respectively), commonly used for seedling machine-transplanting was selected as the test seedling tray, and 15 g of compound fertilizer (13%, 7%, and 5% effective N, P, and K components, respectively) was added to each tray before sowing the seeds (*Table 1*).

Seeds were sown on May 25, 2020. Dry seeds (120 g) were evenly sown in each tray, and 40 trays of each treatment were sown. A hydroponic seedling bed that had been ploughed, harrowed, and leveled was used to provide a bed surface with a width of 1.5 m, in which the height difference throughout did not exceed 5 mm. The plates were arranged, fertilized soil or substate was spread evenly thereon and watered thoroughly, and the seeds were sown and then covered in 0.9 kg of topsoil to ensure they were covered. The bed soil was then thoroughly soaked in flat ditch water, and covered for five days with plastic film and a black sunshade net. The film was removed when the seedlings were seen to be aligned, and flat ditch water was again applied to ensure that the seedling board, soil, and substrate in the seedling tray were kept fully moist. After

22 days, seedlings were transplanted into a paddy field at a planting density of 30 cm × 12 cm, with four seedlings per hole and 74,000 seedlings. Each experimental plot in the field was 8 m × 10 m, and the plots for each treatment were established in triplicate. After transplanting, management practices were the same for all treatments; fertilizer and water were controlled as required to avoid yield loss, and weed, disease, and insect pest control were strictly exercised throughout the crop cycle.

***Table 1.*** *Chemical composition of the experimental growth substrates*

| Treatments | Total-N (g/Kg) | Available-N (mg/Kg) | Available-P (mg/Kg) | Available-K (mg/Kg) |
|---|---|---|---|---|
| TS | 1.56 ± 0.02b | 519.02 ± 4.27b | 58.72 ± 0.41b | 121.97 ± 0.22b |
| CS | 1.74 ± 0.03a | 568.09 ± 1.79a | 64.37 ± 0.14a | 126.30 ± 1.27a |
| ST | 1.30 ± 0.06c | 424.81 ± 2.23e | 44.65 ± 0.85d | 114.58 ± 1.7d |
| RH | 1.28 ± 0.05c | 444.74 ± 2.09d | 50.10 ± 1.69c | 118.03 ± 0.27c |
| MR | 1.49 ± 0.02b | 504.51 ± 1.16c | 57.23 ± 1.7b | 120.23 ± 0.35bc |

TS, topsoil; CS, commercial seedling; ST, substrate straw; RH, rice husk; MR, mushroom residue. Different letters indicate statistical significance at the P = 0.05 level within the same column. The same as below

## *Sample collection*

Evenly mixed fertilized soil or substrate (500 g) for each treatment was placed in a sealed bag and stored for testing on the first day of sowing. Subsequently, six seedlings (10 cm × 10 cm) were harvested from each treatment plot on days 7, 14, and 21 after sowing, and stored separately from the subsoil (substrate). The seedling growth medium was later used to determine their nutrient contents, and dried powder samples were stored for analysis after the termination of the seedling test. The following procedure was used to determine rice yield: The following procedure was used to determine rice yield: Grain yield was determined from a harvest area of 5.0 $m^2$ (five sampling points were selected and each sampling point was 1 $m^2$) in each plot and adjusted to 14% moisture. And 10 hills from each point (50 hills in total) were selected to determine the number of panicles. According to the average number of panicles, 10 hills were selected in each plot, the number of grains per panicle and the seed setting rate were measured, and 1000 solid grain samples (dry seeds) were weighed three times (the error was lower than 0.05 g).

## *Sample analysis*

### *Quality measurements for seedlings*

A total of 30 seedlings per treatment plot were sampled to measure plant height, SPAD (Minolta SPAD-502 chloro-phyll meter (SPAD 502 plus, Konika Minolta Inc., Japan) were used to obtain SPAD readings. Its accuracy is ± 1.0 SPAD units, which can display a range of -9.9 to 199.9 SPAD units.), and stem base width. Additionally, 100 seedlings were collected and divided into shoots and roots, placed in an oven at 105°C for 30 min, then dried at 80°C to a constant weight, and dry weight was then measured. Seedling roots were placed in a glass dish covered with a thin layer of water; then, an image was obtained using a scanner (Epson Expression 1680 Scanner), The WinRHIZO root system analysis system was subsequently used to determine the total length, surface area, average diameter, and system diameter of the roots.

### *Determination of seedling nutrient contents*

The nutrient contents of the dried and pulverized seedlings sampled were determined as follows: After $H_2SO_4$-$H_2O_2$ digestion, an automatic Kjeldahl N analyzer (FOSS company) was used to measure the N content. In addition, the vanadium-silver yellow colorimetric method and the flame photometer method were used to measure P and K contents, respectively.

### *Determination of nitrogen metabolism-related enzymatic activity of seedlings*

The activities of glutamine synthase (GS), glutamic acid synthase (GOGAT), and transaminase were measured according to the methods of Zou et al. (2015), Chen et al. (2008) and Zhao et al. (2008), and Wu et al. (1998), respectively. Enzyme activity was expressed by the number of micromoles (μmol) of pyruvate catalyzed per gram of sample within 1 h. The following procedure was used to determine GS and GOGAT activities: 0.1 g of fresh sample was weighed and 1 mL of extraction buffer was added to it for centrifugation for 25 min at 4℃ and 13,000 rpm. The supernatant represented the crude enzyme extract. The GS activity was calculated as the complex formed by γ-glutamyl hydroxamic acid and iron per unit time; in turn, GOGAT activity was calculated by measuring the absorbance of the coupled Gln and NADH oxidation reaction. Alanine aminotransferase (Glutamic Pyruvic Transaminase, GPT) activity was determined using the following procedure: 0.1 g of fresh sample was weighed and 1 mL of extraction buffer added and then centrifuged at for 25 min 4℃ and 13,000 rpm. The supernatant represented the crude enzyme liquid extract. The pyruvate content was subsequently measured to calculate GPT activity.

### *Determination of nutrients in the experimental growth substrates*

The seedling-raising media (fertilized soil, substrate) were uniformly measured to determine the contents of total N, alkaline N, available P, and available K using the Kelvin digestion method, the alkaline solution diffusion method, the sodium bicarbonate method, and the ammonium acetate extraction method, respectively.

### ***Statistical analysis***

Basic data processing and statistical analysis were performed using Excel 2019 and SPSS 19.0. Data from each sampling date were analyzed separately, one-way ANOVA was used to determine differences between treatments. Means were tested by least significant difference at $P = 0.05$ ($LSD_{0.05}$). A Spearman correlation was used to assess the associations between physicochemical properties of the substrates (i.e. Bulk density, Conductivity, pH) and seedling shoot parameters (i.e. Plant height, Stem thick, Shoot dry weight) and seedling root system (i.e. Root length, Surface area, Root volume) in SPSS 19.0. Figures were drawn using GraphPad Prism.

## Results

### ***Physical and chemical properties and changes in N, P, and K contents of the growth substrates tested***

Significant differences in physical and chemical properties were found among the different seedling substrates tested (*Table 2*), each providing a different environment for rice seedling growth. Among the various indices, the largest difference among treatments was recorded for conductivity, which ranked in the order of ST > MR > RH > CS > TS), and was 325.68% higher in ST than in TS. Similarly, there were significant differences in bulk density among the different treatments, and the values followed the order of TS > MR > CS > RH > ST. Further, the CS and MR treatments were weakly acidic, and their pH values were significantly lower than those of TS, ST, and RH. Furthermore, there were significant differences in N, P, and K contents among the different growth substrates (*Fig. 2*). The N, P, and K contents followed the order of CS > TS > MR > RH > ST on the day of sowing, and then TS > MR > CS > RH > ST on days 7, 14, and 21 after sowing. Compared with sowing day, a decrease in total N, alkaline N, available P, and available K in TS, CS, ST, RH, and MR on day 21 after sowing. Specifically, total N decreased by 27.32%, 51.86%, 57.54%, 52.89%, and 32.95%, respectively, alkaline N by 68.65%, 77.89%, 81.98%, 81%, 70.81%, respectively, available P by 45.04%, 66.61%, 61.03%, 68.26%, 59.60%, respectively, and available K by 72.81%, 79.84%, 79.04%, 79.98%, 79.30%, respectively. The reduction in nutrient contents in the growth substrates was related to two factors: seedling absorption and nutrient loss.

***Table 2.*** *Physicochemical properties of the experimental growth substrates*

| Treatments | Bulk density (g/cm³) | Aeration porosity (%) | Water-holding pore (%) | Conductivity (mS/cm) | pH |
|---|---|---|---|---|---|
| TS | 1.16 ± 0.02a | 15.73 ± 0.02d | 31.30 ± 0.66d | 0.74 ± 0.05e | 7.30 ± 0.10b |
| CS | 0.95 ± 0.02c | 18.29 ± 0.13bc | 39.78 ± 1.06c | 1.04 ± 0.07d | 6.57 ± 0.31c |
| ST | 0.78 ± 0.02e | 20.88 ± 0.40a | 53.25 ± 0.76a | 2.41 ± 0.06a | 7.30 ± 0.17b |
| RH | 0.86 ± 0.03d | 18.91 ± 0.11b | 47.18 ± 1.14b | 1.78 ± 0.06c | 7.87 ± 0.21a |
| MR | 1.07 ± 0.02b | 17.37 ± 0.16c | 34.41 ± 0.48d | 1.46 ± 0.06b | 6.37 ± 0.25c |

TS, topsoil; CS, commercial seedling; ST, substrate straw; RH, rice husk; MR, mushroom residue. Different letters indicate statistical significance at the P = 0.05 level within the same column. The same as below

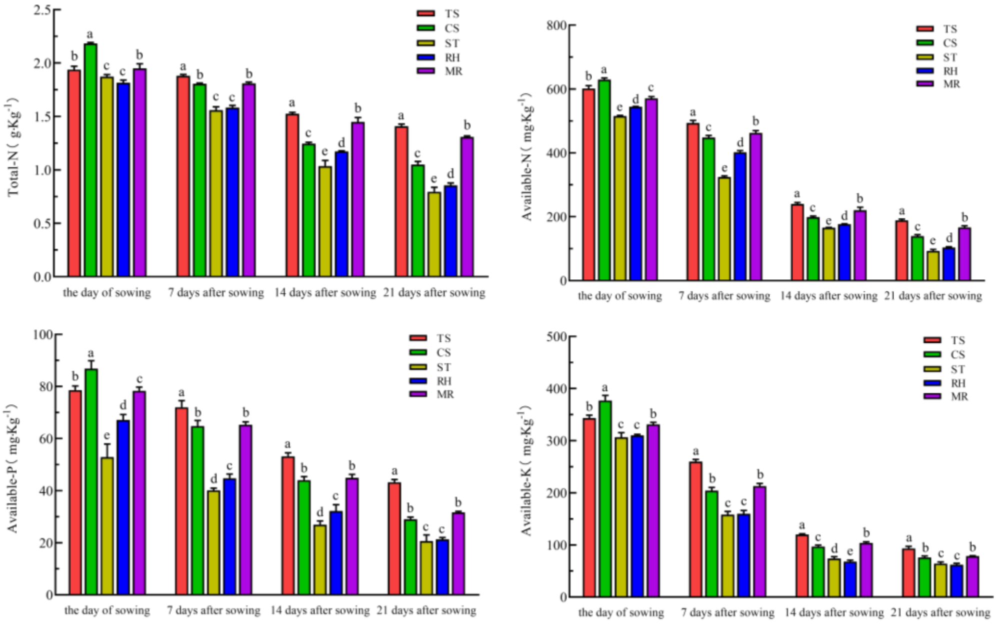


***Figure 2.*** *Changes in N, P, and K contents in the seedling growth-media tested. (Vertical bars represent means ± SD. Different letters above the column indicate statistical significance at the P = 0.05 level within the same measurement stage. The same as below)*

### *Changes in seedling shoots under different growth substrate treatments*

Seedling shoot physiological indices were directly related to the quality of the seedlings, and the corresponding morphological indices differed significantly among the different treatments (*Table 3*). The indices values recorded in the TS, CS, and MR treatments were significantly higher than those recorded in the ST and RH treatments. Plant height, stem thickness, and shoot dry weight all showed an increasing trend with time after sowing, with different increases at different stages, while SPAD showed a slight decreasing trend. Seven days after sowing, SPAD, stem thickness, and shoot dry weight all ranked as follows: TS > MR > CS > RH > ST, while plant height ranked as follows: CS > MR > TS > RH > ST. Of all indices measured, plant height differed the most among treatments, and the greatest plant height was observed in the CS treatment, which was 34.73% greater than that observed in the ST treatment. The smallest difference among treatments was observed in relation to shoot dry weight, the highest value was recorded in the TS treatment plots, where it was 8.43% higher than that in the ST treatment, where the smallest dry weight was recorded. The highest plant height, SPAD, and shoot dry weight values in the TS treatment were observed 14 days after sowing, the ST treatment showed the lowest values, and the differences between the TS and ST treatments gradually increased, such that the TS treatment showed the highest values for SPAD, dry weight, and plant height, which were 37.20%, 36.43%, and 33.39% higher, respectively, than the corresponding values in the ST treatment, where the lowest values were recorded. At 21 days after sowing, all of the indicators in the MR treatment were at a relatively high level, and SPAD and stem thickness values ranked as follows: MR > TS > CS > RH > ST. No significant differences were found between the shoot dry weights in the TS, CS, and MR treatments; however, their values were significantly higher than those in the ST and RH treatment plots. Plant height in the CS treatment was significantly higher than in any other treatment. Specifically, it was 7.74%, 10.79%, 25.23%, and 32.52% greater in the CS treatment than in TS, MR, RH, and CS, respectively.

***Table 3.*** *Changes in seedling shoot parameters at different times after sowing*

| Sampling time | Treatments | Plant height (cm) | SPAD | Stem thick (mm) | Shoot dry weight (g/100plants) |
|---|---|---|---|---|---|
| 7 days after sowing | TS | 10.79 ± 0.26b | 30.27 ± 0.72a | 1.42 ± 0.07a | 2.83 ± 0.02a |
| | CS | 12.26 ± 0.63a | 27.41 ± 0.06b | 1.33 ± 0.01b | 2.76 ± 0.03a |
| | ST | 9.10 ± 0.34c | 24.38 ± 0.79d | 1.17 ± 0.08c | 2.61 ± 0.06b |
| | RH | 10.38 ± 0.19b | 25.73 ± 0.35c | 1.26 ± 0.04c | 2.66 ± 0.04b |
| | MR | 10.99 ± 0.34b | 29.38 ± 0.63a | 1.39 ± 0.02a | 2.77 ± 0.03a |
| 14 days after sowing | TS | 15.54 ± 0.37a | 28.73 ± 0.82a | 1.71 ± 0.04ab | 4.98 ± 0.16a |
| | CS | 14.82 ± 0.21ab | 25.55 ± 0.88b | 1.78 ± 0.03a | 4.74 ± 0.09a |
| | ST | 11.65 ± 0.57d | 20.94 ± 0.90c | 1.52 ± 0.06c | 3.65 ± 0.13c |
| | RH | 12.98 ± 0.32c | 25.84 ± 0.80b | 1.62 ± 0.04b | 3.92 ± 0.06b |
| | MR | 14.45 ± 0.32b | 28.05 ± 0.83a | 1.75 ± 0.03a | 4.91 ± 0.06a |
| 21 days after sowing | TS | 16.68 ± 0.65b | 27.16 ± 0.44ab | 1.90 ± 0.01ab | 7.04 ± 0.01a |
| | CS | 17.97 ± 0.15a | 26.63 ± 0.16b | 1.86 ± 0.03b | 6.82 ± 0.04a |
| | ST | 13.56 ± 0.58c | 21.24 ± 0.56d | 1.63 ± 0.04d | 5.50 ± 0.23c |
| | RH | 14.35 ± 0.43c | 23.72 ± 0.45c | 1.72 ± 0.02c | 6.06 ± 0.09b |
| | MR | 16.22 ± 0.30b | 27.71 ± 0.36a | 1.94 ± 0.03a | 6.95 ± 0.03a |

### *Changes in seedling root system under different growth substrate treatments*

Root morphology indicators of the different treatments all showed increasing trends with time after sowing (*Table 4*). At each sampling time point, the indicators in ST and RH treatments were significantly lower than those in TS, CS and MR. Thus, at 7 days after sowing, root length ranked as follows: TS > CS > MR > RH > ST, with the differences among treatments reaching a significant level. Additionally, surface area, average diameter, and root dry weight in the MR treatment were all significantly higher than those in the other treatments. Among these variables, surface area in the MR treatment was 45.20% higher than that in the ST treatment, where the lowest value for surface area was recorded. In turn, root volume in the CS treatment was the largest, and it was 47.82% higher than that in the RH treatment, where the lowest value was registered. The trends followed by the various root system indicators at 14 days after sowing were consistent with those observed at 7 days after sowing. Moreover, 21 days after sowing, the largest length, surface area, and average diameter in the MR treatment plots were 20.47%, 37.44%, and 15.43% higher, respectively, than those in the CS treatment, where the lowest values for the same variables were recorded. In addition, the largest root volume in the CS treatment was 28.26% higher than the smallest root volume in the RH treatment. Furthermore, the highest root dry weight was observed for the TS treatment and was 21.14% higher than the weight recorded for the RH treatment, for which the lowest value was registered.

***Table 4.*** *Root system changes of different treatments in different periods*

| Sampling time | Treatments | Root length (cm) | Surface area (cm$^2$) | Average diameter (mm) | Root volume (cm$^3$) | Root dry weight (g/100plants) |
|---|---|---|---|---|---|---|
| 7 days after sowing | TS | 39.110 ± 0.378a | 6.371 ± 0.160a | 0.416 ± 0.008ab | 0.085 ± 0.002b | 2.05 ± 0.02b |
| | CS | 37.780 ± 0.474b | 6.242 ± 0.094a | 0.405 ± 0.007b | 0.102 ± 0.004a | 1.88 ± 0.02c |
| | ST | 33.745 ± 0.482e | 4.502 ± 0.057c | 0.366 ± 0.009d | 0.076 ± 0.002c | 1.36 ± 0.04d |
| | RH | 35.155 ± 0.278d | 5.292 ± 0.206b | 0.383 ± 0.005c | 0.069 ± 0.003c | 1.38 ± 0.04d |
| | MR | 36.664 ± 0.304c | 6.537 ± 0.189a | 0.425 ± 0.009a | 0.091 ± 0.004b | 2.12 ± 0.03a |
| 14 days after sowing | TS | 51.636 ± 0.676a | 8.323 ± 0.085b | 0.479 ± 0.005ab | 0.098 ± 0.001c | 3.11 ± 0.03a |
| | CS | 47.561 ± 0.636c | 7.902 ± 0.216c | 0.464 ± 0.006b | 0.110 ± 0.002a | 2.88 ± 0.04b |
| | ST | 45.939 ± 0.48d | 6.716 ± 0.065e | 0.426 ± 0.015c | 0.091 ± 0.003d | 2.40 ± 0.05c |
| | RH | 46.135 ± 0.407d | 7.343 ± 0.024d | 0.458 ± 0.003b | 0.079 ± 0.002e | 2.48 ± 0.04c |
| | MR | 49.033 ± 0.323b | 8.754 ± 0.203a | 0.494 ± 0.008a | 0.102 ± 0.002b | 3.13 ± 0.03a |
| 21 days after sowing | TS | 61.218 ± 0.466b | 11.980 ± 0.359a | 0.570 ± 0.007a | 0.107 ± 0.003b | 3.61 ± 0.05a |
| | CS | 58.696 ± 0.276c | 11.375 ± 0.147b | 0.547 ± 0.004b | 0.118 ± 0.003a | 3.48 ± 0.03a |
| | ST | 52.286 ± 0.344e | 8.787 ± 0.131d | 0.499 ± 0.005d | 0.101 ± 0.002c | 3.07 ± 0.09b |
| | RH | 53.975 ± 0.69d | 10.485 ± 0.124c | 0.512 ± 0.007c | 0.092 ± 0.004d | 2.98 ± 0.07b |
| | MR | 62.980 ± 0.820a | 12.077 ± 0.107a | 0.576 ± 0.002a | 0.113 ± 0.002a | 3.51 ± 0.05a |

### *Changes in the N, P, and K contents of seedlings grown under different growth substrate treatments*

The N, P, and K contents of the seedling shoot and root systems differed significantly among treatments (*Fig. 3*). However, all of these showed a general decreasing trend with growth, and the nutrient content of the shoots was higher than those of the root system. At different sampling time points, the N and K content of the shoot and root of seedlings in the TS, CS, and MR treatments was significantly higher than those for seedlings grown in the ST and RH treatment plots. At 7 days after sowing, there was no significant difference in root P content among treatments, whereas shoot and root P contents in TS-, CS-, and MR-grown seedlings was significantly higher than that of ST- and RH-grown seedlings at other sampling time points. The largest difference in shoot and root N content was observed at 7 days after sowing. The shoots of CS-grown seedlings showed the highest N content, which was 157.48% higher than that of ST-grown seedlings, and the roots of TS-grown seedlings showed the highest N content, which was 158.14% higher than that in ST-grown seedlings. The difference in shoot and root P contents was largest at 21 days after sowing. The shoots of TS-grown seedlings showed the highest P content, which was 141.03% higher than that of the RH-grown seedlings. In turn, TS-grown seedlings showed the highest root N content, which was 163.30% higher than that of ST-grown seedlings. Meanwhile, shoot and root K contents differed the most at 21 days after sowing. The shoot P content was highest in TS-grown seedlings, and was 171.84% higher than that of ST-grown seedlings, and the roots of TS-grown seedlings also showed the highest P content, which was 194.01% higher than that of the RH-grown seedlings.

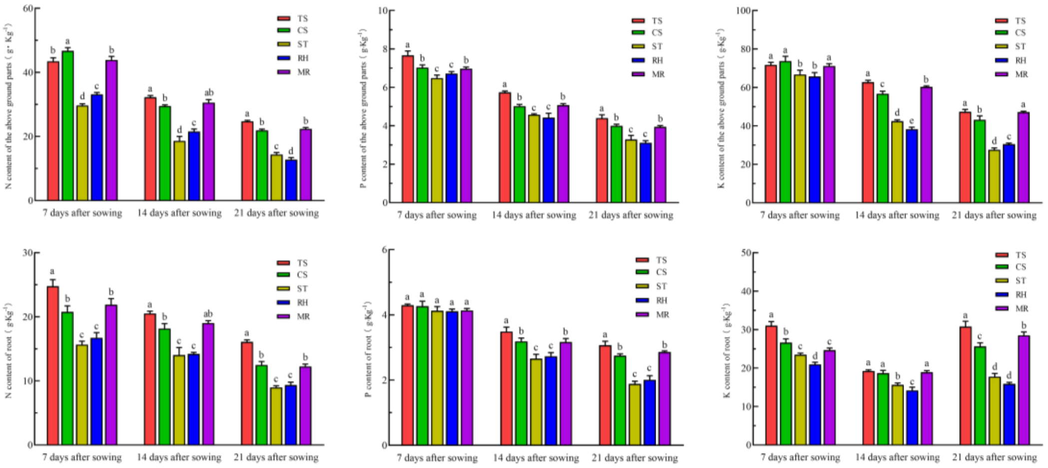


*Figure 3.* *Changes in seedling N, P, and K nutrient contents in different substrate treatments*

## *Changes in N, P, and K accumulation in seedlings under different growth substrate treatments*

The amount of N, P, and K accumulated in seedlings gradually increased with growth (*Fig. 4*), but they tended to stabilize between days 14 and 21. However, the amounts of N, P, and K accumulated in the shoots and roots in different substrate treatments differed significantly, and N, P, and K contents in the shoots and roots in TS-, CS-, and MR-grown seedlings were significantly higher than those in ST- and RH-grown seedlings. Further, the amounts of N, P, and K accumulated in the shoots and roots in the different treatments differed the most at 21 days after sowing. Among these, the TS treatment had the highest amounts of shoot N, P, and K accumulated, which were 219.79%, 171.67%, and 220.26% higher, respectively, than those in the ST treatment, where the lowest amounts accumulated. Further, the highest amount of root N was accumulated in TS-treated seedlings, and the amount was 210.77% higher than that accumulated in ST-grown seedlings. In addition, the highest amount of P was accumulated in the TS treatment, and this amount was 191.87% higher than that in the ST-grown seedlings. Furthermore, the highest amount of K accumulated was observed in TS-grown seedlings, and this amount was 235.07% higher than that accumulated in the RH-grown seedlings.

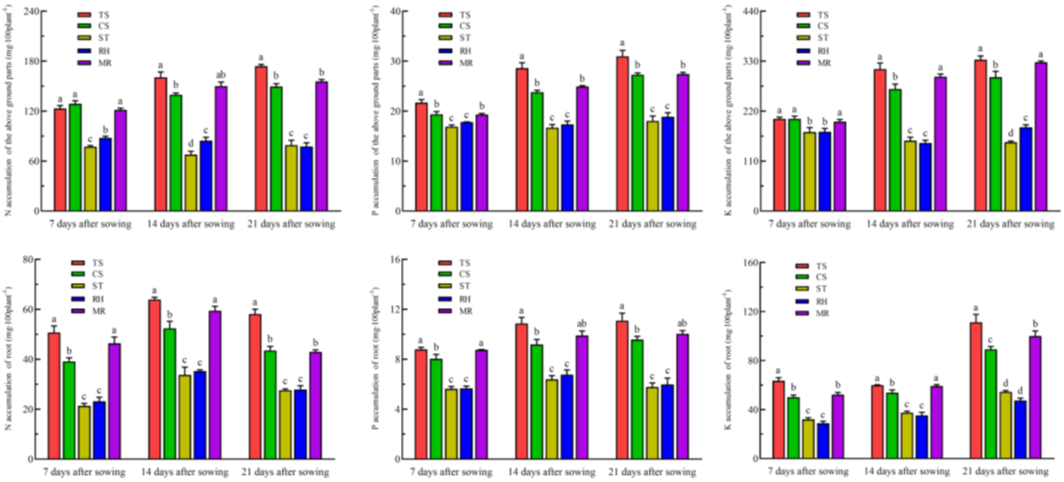


*Figure 4. Changes in N, P, and K accumulation in seedlings under different substrate treatments*

### *Changes in the N metabolism-related enzymatic activity under different growth substrate treatments*

The N metabolism-related enzyme activities in seedlings differed significantly among treatments (*Fig. 5*). Further, GS, GOGAT, GPT, and GOT activities showed decreasing trends with the progression of growth. Thus, at different sampling time points, N metabolizing enzyme-activity levels in TS-, CS-, and MR-grown seedlings were significantly higher than those in ST- and RH-grown seedlings, while there was no significant difference between ST and RH. Particularly, GS and GOT activities followed the order of TS > MR > CS > RH > ST. The difference in enzyme activity in seedlings under different treatments peaked at 21 days after sowing. Specifically, TS-grown seedlings showed the largest GS, GOGAT, and GPT activities, which were 370.79%, 192.30%, and 193.38% higher than those in ST-grown seedlings, respectively, where these activities were lowest. In addition, TS-grown seedlings showed the greatest GOT activity, which was 205.05% higher than that of RH-treated seedlings (which showed the lowest levels).

### *Effects of different growth substrate treatments on rice yield*

The different seedling-raising substrates affected seedling quality, which in turn affected rice yield (*Table 5*). Thus, yield in TS, MR, CS treatment plots was significantly higher than those in RH and ST treatment plots, but there was no significant difference among TS, MR, and CS treatments, among which the yield in the MR treatment was the highest, and was 28.93% higher than that of the ST treatment, which registered the lowest yield. As for yield components, there were only small non-significant differences in seed setting rate and 1000-kernel weight among treatments. Seed setting rates in TS, MR, and CS treatment plots were significantly higher than those in CS and ST treatment plots. The highest seed setting rate under TS treatment was 1.41% larger than that of the ST treatment, which showed the lowest seed setting rate. The change trends in effective panicle and grain number per panicle were consistent with yield, and the largest percentage increases of 11.68% and 13.76%, respectively, compared with the lowest values (ST treatment), were observed under the

MR treatment. The slow seedling growth period was short owing to the vigor of the seedlings transplanted to the field, which was conducive to early tillering, which in turn increased the effective number of panicles, the number of grains per panicle, and, ultimately, yield. Among seedling physiological indices, the correlation coefficients between grain yield and stem thickness, shoot dry weight, root length, and root surface area were found to be the highest (*Fig. 6*), with $R^2$ values of 0.9604, 0.8835, 0.8919, and 0.9125, respectively, with all reaching significant positive correlations.

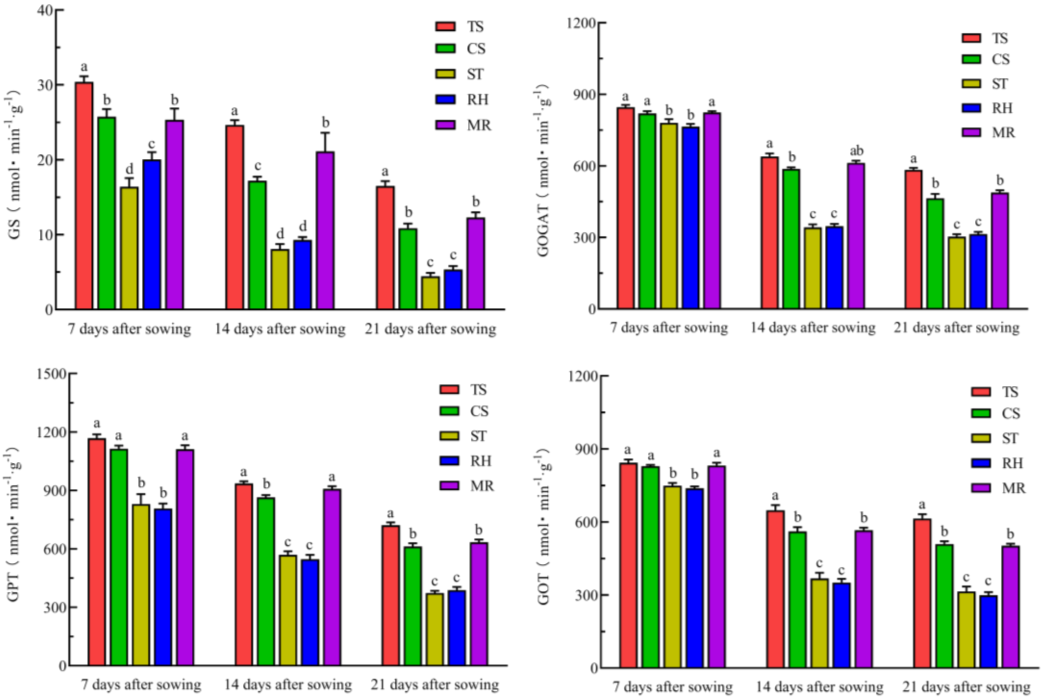


***Figure 5.*** *Effects of different seedling media on GS, GOGAT, GPT, GOT activities in seedling leaves*

***Table 5.*** *Rice grain yield and yield components*

| Treatments | Panicles per $m^2$ | Spikelets per panicle | Filled grains (%) | 1000-grain weight (g) | Grain yield (t/ha) |
|---|---|---|---|---|---|
| TS | 322 ± 3a | 122 ± 2a | 93.4 ± 0.5a | 27.2 ± 0.2a | 9.97 ± 0.06a |
| CS | 316 ± 4a | 121 ± 4a | 92.9 ± 0.3ab | 27.2 ± 0.2a | 9.71 ± 0.36a |
| ST | 291 ± 4c | 109 ± 3b | 92.1 ± 0.3c | 27.3 ± 0.3a | 7.95 ± 0.1c |
| RH | 306 ± 4b | 114 ± 3b | 92.6 ± 0.2bc | 27.3 ± 0.1a | 8.81 ± 0.16b |
| MR | 325 ± 4a | 124 ± 3a | 93.1 ± 0.3ab | 27.3 ± 0.2a | 10.25 ± 0.38a |

## Discussion

Growth substrates provide water, gases and nutrients for seedling growth; additionally, they provide anchorage, cushioning, and support for the plant. Under the same temperature and water conditions, the physicochemical properties and nutrient contents of the substrates affect seedling growth (Schäfer et al., 2008). For example, Lam et al. (2019) showed that a substrate with a high carbon content and a large surface area provides a greater water and nutrient retention potential and a larger number of adsorption sites for plant growth, thereby promoting plant growth and improving yield.

In addition, the porosity and bulk density of the substrate affects its water storage and aeration capacity, which subsequently affect its fertility. Furthermore, a previous study found that the addition of organic manure material to the growing media at rice nurseries improved the morpho-physiological features of the rice seedlings, which improved stand establishment in the paddy fields (Cheng et al., 2018). The nutrient content of substrates directly affects the absorption of nutrients by the seedlings, and further affects seedling quality (Zárate-Salazar et al., 2020). An appropriate nutrient content is crucial for the formation of strong machine-transplanted seedlings, and Lampayan et al. (2015) reported that improving fertilization in the seedbed is important for reducing rice development delays, increasing yield, and enhancing total water productivity. In this research, the fastest substrate-nutrient decline rate in the different treatments occurred 7–14 days after sowing, which coincides with the period (out of the entire experimental period) during which the seedlings grew faster and absorbed the largest proportion of nutrients. However, the reductions in the total N, alkaline N, and available P content were significantly lower in the TS and MR treatment plots than in the other three treatment plots, and this phenomenon was conducive to the efficient cultivation of strong seedlings.

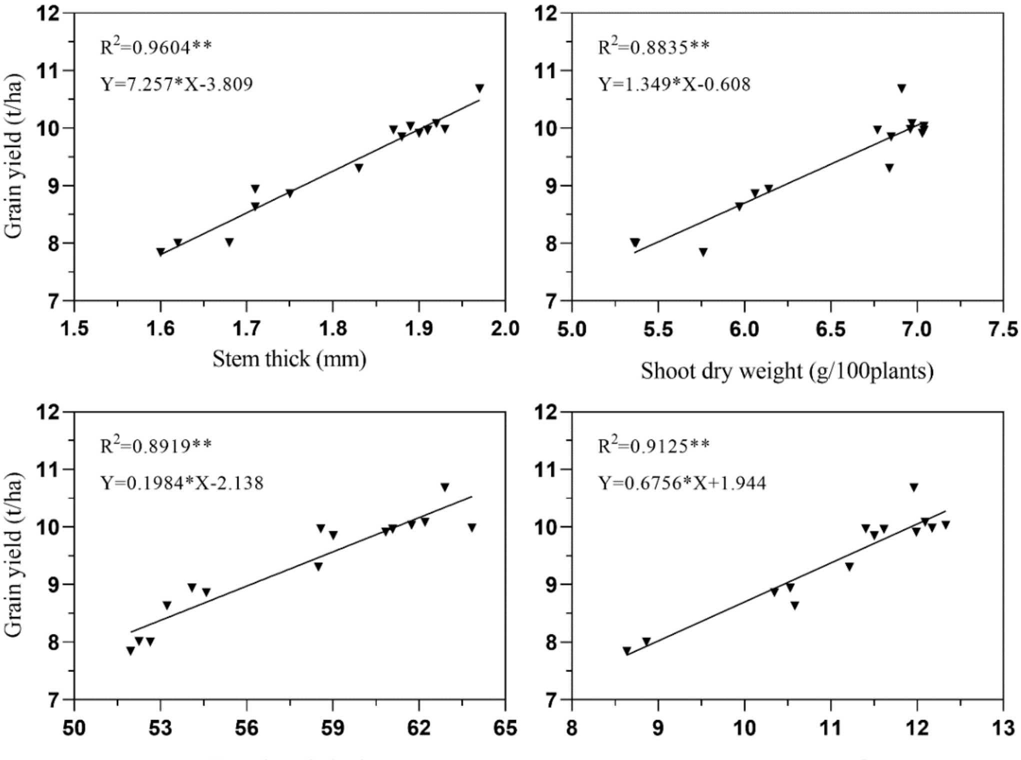


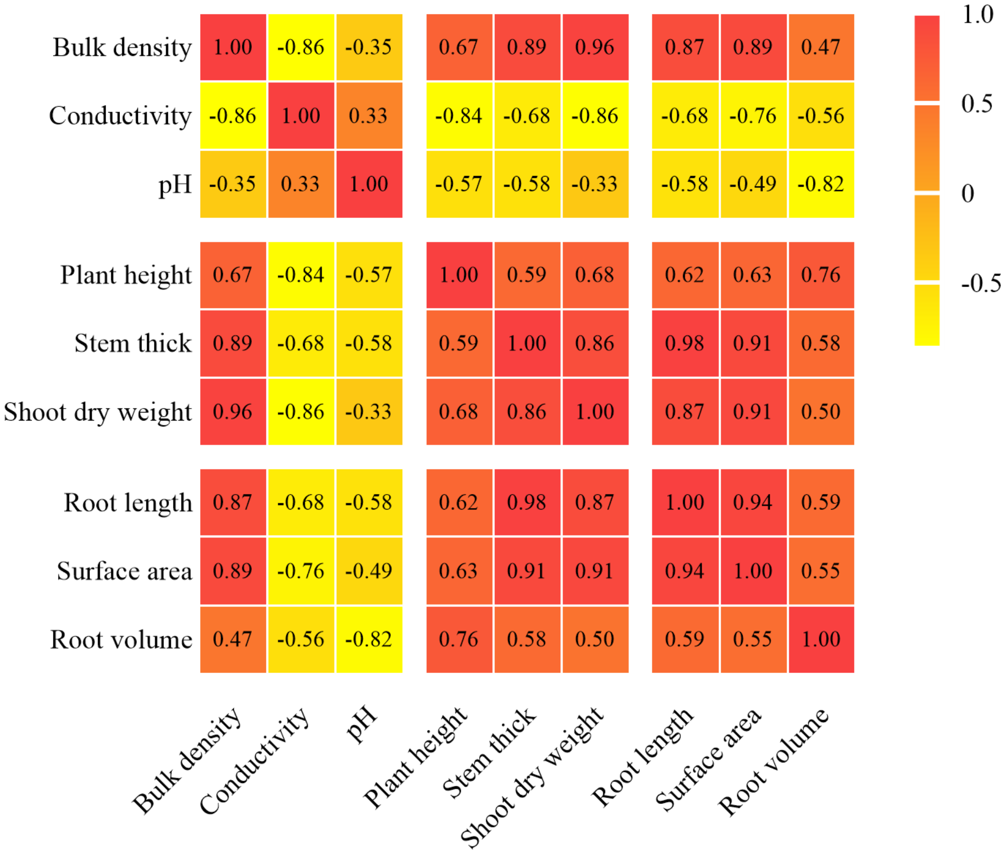
***Figure 6.*** *Correlation between seedling physiological indices and yield*

Nitrogen is the most sensitive factor affecting the growth of rice plants, and a highly efficient N uptake and accumulation are usually indicators of a high rice yield. Most previous studies have shown that low N accumulation during the early phase of rice plant growth and higher N accumulation during the middle and late rice plant growth phases are important N-accumulation characteristics associated with a high rice yield

(Huang et al., 2019; Zhu et al., 2020). Therefore, the amount of N accumulated in the early phases of rice growth is related to rice yield (Xing et al., 2023). The N content, the amount of N accumulated, and the N metabolism-related enzymatic activities at the seedling stage were high for the TS, CS, and MR treatments, and the corresponding rice yields were significantly higher than those for the ST and RH treatments. Zhou et al. (2022) found that the effective control and regulation of N uptake and transfer in rice during the transplanting period may effectively guarantee high N accumulation during the whole rice growth period, but there was a high N requirement to produce 100 kg of grain at a high yield. Conversely, a shortage of fertilizer during the seedling stage causes reduction in plant height, SPAD, stem thickness, and dry matter of seedlings, while a balanced fertilization obviously improves seedling quality and root vitality, and thus the growth status of seedlings after transplanting (Chew et al., 2022).

The nutrients required for seedling growth are mainly derived from the seedling substrate itself and the nutrients added to the medium at the seedling stage. In this study, an equal amount of fertilizer was applied to the different treatments. Therefore, the significant differences in the N, P, and K accumulation capacities of the seedlings under different treatments can be directly attributed to the differences between the physical and chemical properties of the seedling growth substrates. In this respect, nutrient loss was the lowest and nutrient utilization rate was the highest in the TS, CS, and MR treatments. In addition, the physical and chemical properties of the seedling growth substrates were significantly related to their water and fertilizer retention abilities, which further affected seedling growth and development (Liu et al., 2016). The bulk density of the seedling growth substrate was positively correlated with seedling quality, and the coefficients of conductivity and pH of the seedling growth substrates were the highest, as well as those of shoot dry weight and root surface area, while conductivity and pH of the seedling substrate were negatively correlated with seedling quality. The correlation coefficients between pH and root volume were -0.86 and -0.82, respectively (*Fig. 7*). Therefore, selecting substrates for seedling cultivation which possess appropriate physical and chemical properties is conducive for improving the nutrient utilization rate, and it also results in vigorous seedling growth, which in turn improves rice yield.

Seedling quality during transplanting is closely related to rice yield (Takahashi et al., 2018). In this study, the theoretical yields under TS, CS and MR treatments, which rendered higher seedling quality, were significantly higher than those under the other treatments, and the differences in the theoretical yields were mainly due to differences in the effective panicle number and in the number of spikelets per panicle. Previous reports (Chen et al., 2022) have suggested that fast and vigorous seedling growth after transplanting leads to an increase in the number of productive tillers and to the formation of large rice panicles, while weak seedlings result in slow growing plants with poor tillering which in turn leads to a decline in yield. Grain yield was significantly and positively correlated with seedling root length, root surface area, stem thickness, and shoot dry weight. Seedlings with strong roots and strong stems exhibited faster root development and greater accumulation of shoot dry matter after transplanting, which improved the tillering rate in the field, shortened the rice growth period, increased the number of effective panicles, and thus improved yield. These results were similar to those reported by Li et al. (2020), who showed that the transplantation of young seedlings resulted in an appropriate amount of accumulated dry matter, a high photosynthetic production capacity, and an efficient and sustainable output of the photosynthetic systems, which thus resulted in high yields. Transplanting of young vigorous seedlings results in a strong growth advantage, which may also be related to the absence of premature senescence, strong root activity, long-lasting photosynthetic function of the leaves, and high physiological and biochemical activity rates. All of which warrant further study.



| | Bulk density | Conductivity | pH | Plant height | Stem thick | Shoot dry weight | Root length | Surface area | Root volume |
|---|---|---|---|---|---|---|---|---|---|
| Bulk density | 1.00 | -0.86 | -0.35 | 0.67 | 0.89 | 0.96 | 0.87 | 0.89 | 0.47 |
| Conductivity | -0.86 | 1.00 | 0.33 | -0.84 | -0.68 | -0.86 | -0.68 | -0.76 | -0.56 |
| pH | -0.35 | 0.33 | 1.00 | -0.57 | -0.58 | -0.33 | -0.58 | -0.49 | -0.82 |
| Plant height | 0.67 | -0.84 | -0.57 | 1.00 | 0.59 | 0.68 | 0.62 | 0.63 | 0.76 |
| Stem thick | 0.89 | -0.68 | -0.58 | 0.59 | 1.00 | 0.86 | 0.98 | 0.91 | 0.58 |
| Shoot dry weight | 0.96 | -0.86 | -0.33 | 0.68 | 0.86 | 1.00 | 0.87 | 0.91 | 0.50 |
| Root length | 0.87 | -0.68 | -0.58 | 0.62 | 0.98 | 0.87 | 1.00 | 0.94 | 0.59 |
| Surface area | 0.89 | -0.76 | -0.49 | 0.63 | 0.91 | 0.91 | 0.94 | 1.00 | 0.55 |
| Root volume | 0.47 | -0.56 | -0.82 | 0.76 | 0.58 | 0.50 | 0.59 | 0.55 | 1.00 |

***Figure 7.*** *Correlation heat map between physical properties of substrates and seedling shoot and root systems*

## Conclusions

The results of this study showed that decomposed rice husk, straw, and mushroom residue can be effectively used as substrates to successfully cultivate rice seedlings. The physicochemical properties and nutrient contents of the substrates tested were found to directly affect rice seedling growth. Bulk density, conductivity, pH, a high nutrient content, and a high water-retention capacity all closely associated with the growth of good quality seedlings. Therefore, these are important indices that can be used to evaluate the quality of substrates used to cultivate seedlings. Stem thickness, shoot dry weight, root length, and root surface area of rice seedlings significantly and positively correlated with rice yield in the later stage. These are also important indices that can be used to evaluate the potential of rice seedlings. The seedlings cultivated from mushroom residue were robust, with large amounts of accumulated dry matter and nutrients, and a strong enzyme activity in relation to N metabolism. The seedlings cultivated from mushroom residue also grew faster following transplanting in the field, which was conducive for increasing effective tillers, forming large panicles, and subsequently providing high grain yields.

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