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Uniconazole and Adaptability of Transplantations by Enhancing the Competition Tolerance in a High Sowing Density of Rapeseed Blanket Seedlings

Xiangyu Zhou [1], Yu Zhang [1], Suohu Leng [1,2,*], Zeyu Wang [1], Chenhu Gong [1], Qingsong Zuo [1,2] and Guang Yang [1,2]

[1] Jiangsu Key Laboratory of Crop Genetics and Physiology, Agricultural College, Yangzhou University, Yangzhou 225009, China
[2] Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China
* Correspondence: oilseed@yzu.edu.cn

Abstract: Having nursery rapeseed (*Brassica napus* L.) seedlings at a high density in a tray is an indispensable step to realizing mechanized transplanting for rapeseed. The reduction in seedling quality caused by high sowing density is one of the key factors affecting transplanting quality and yield. Uniconazole has been considered as a potential plant growth regulator to improve plant growth under diverse unfavorable circumstances. In two sowing densities (400 and 800 seeds per tray), an experiment was carried out between 2021 and 2022 to investigate the effects of uniconazole seed-coating treatments on pre-transplant and post-transplant seedling characteristics. The results demonstrate that uniconazole treatment can effectively reduce the high-density-induced reduction in seedling dry matter and leaf area, stem thinness, and stem and petiole overgrowth. Further evidence that uniconazole can improve seedling quality, enhance yield, and lessen yield loss due to high-density sowing was provided by yield at maturity. However, because of the uncontrolled growth during the late stage in the tray, the relative growth rate of seedlings after transplant in the transplant shock stage revealed that lower doses of uniconazole treatment have a negative effect on the seedling recovery. The results of principal coordinate analysis and partial correlation analysis proved that the yield and net assimilation rate were related to the improvement of seedling high-density tolerance by uniconazole treatment. Consequently, 500–750 mg L^{-1} uniconazole coating per 100 g of seeds in 5 mL is recommended by this study, considering the potential risk of seedling emergence and growth caused by an overdose of uniconazole treatment.

Keywords: rapeseed blanket seedling; high density; uniconazole; mechanized transplanting

Citation: Zhou, X.; Zhang, Y.; Leng, S.; Wang, Z.; Gong, C.; Zuo, Q.; Yang, G. Uniconazole and Adaptability of Transplantations by Enhancing the Competition Tolerance in a High Sowing Density of Rapeseed Blanket Seedlings. *Agronomy* **2022**, *12*, 2637. https://doi.org/10.3390/agronomy12112637

Received: 19 September 2022
Accepted: 24 October 2022
Published: 26 October 2022

1. Introduction

Rapeseed is a major source of vegetable oil in the world, as well as an important oil crop in China [1,2]. Winter rapeseed accounts for more than 90% of the total rapeseed cultivated area in China, and the Yangtze River Basin is the largest cultivated area for winter rapeseed cultivation [3]. In this region, direct seeding and transplanting are common rapeseed cultivation methods, with double cropping systems where rapeseed is typically cultivated after rice [4,5]. However, direct seeding requires more time to occupy the cropland than transplanting seedlings, which leads to cropland conflict because of the late rice harvest. The sowing date is usually influenced by the harvest time of the preceding crops. A delayed rice harvest time seriously affected the yield of direct seeding rapeseed in the Yangtze River Basin [6,7]. Moreover, abnormal weather also made direct seeding of rapeseed difficult and led to reduced yields. The shortened vegetative growth stage of rapeseed is the primary reason that leads to the linear yield loss with the delayed sowing time [8]. Transplant as an efficient approach was used to lessen the contradiction of cropland use in double cropping systems, allowing implementation of multiple cropping systems on the limited cropland to increase total crop yield [9]. Rapeseed seedlings are initially raised in the seedbed on the cropland using the conventional transplanting technique, which takes about a month, before they are artificially transplanted [10]. Artificial transplantation as a labor-intensive cultivation practice that requires a large amount of agricultural labor. Due to China's increasing urbanization, the rural population is still moving towards cities, creating a manpower shortage in tillage agriculture [11]. The cost of agricultural labor is so expensive that transplanted rapeseed is not often cost-effective and attractive for agricultural laborers, which leads to a decrease in planted areas of rapeseed [12]. Thus, the expansion of rapeseed cultivation in the Yangtze River Basin depends on high-efficiency mechanized transplanting.

Since 2010, the key technological issues pertaining to the mechanized transplanting of rapeseed seedlings have been developed collaboratively by Yangzhou University and Nanjing Research Institute of Agricultural Mechanization, Ministry of Agriculture [13,14]. It was a kind of soilless method of culturing rapeseed seedlings with a high density in a plastic shallow tray filled with substrate. Because of the high sowing density of raising seedlings, the roots of the seedlings are knotted together like a blanket, they were called "rapeseed blanket seedlings" as well. The shape of the blanket makes it incredibly efficient and practical for mechanized transplanting and standardized production such as rice. The improvement of transplanting efficiency makes it easier to increase transplanting density, which is conducive to the formation of high-yield populations. Although this method appears to have many advantages, it actually has some problems in practice. The most obvious issue is that weak and thin seedlings result from the increased competition because of the excessive increase in sowing density. In general, nutrients, water, and light are the main resources that limit plant growth at a high density. Nutrients, water, and light each have unique properties that lead to unique ways that plants compete for these resources [15]. In agricultural practice, nutrients and water, as resources derived from the substrate, can be managed properly to reduce the pressure generated by competition. In the previous study, at the one-leaf stage, the application of N at rates of 1.0 to 1.5 g N in the seedling trays for rapeseed can effectively improve seedling quality and survival rate [16]. Other studies have also shown that a low blue photon flux ratio light provided by LEDs showed a higher efficiency than utilizing low photon flux and enhanced the net photosynthetic rate [17]. However, due to its expensive price and complex technological requirements, it is challenging to promote it in vast rural areas. Hence, in large-scale farming, it is therefore difficult for growers to directly regulate light. A lack of light usually causes abnormal plant growth, as well as shade avoidance syndrome (SAS). The main characteristics of SAS are enhanced stem elongation and reduced leaf expansion [18]. Furthermore, various plant hormones such as auxins, brassinosteroids, ethylene, and gibberellins are potentially involved in SAS elongating responses [19,20]. The production of rapeseed will be substantially impacted by the quality of the seedlings. Rapeseed seedlings' declining dry weight, green leaf area, and stem strength have a significant negative impact on yield [21]. Hence, controlling hormone balance with plant growth regulators is a potentially effective strategy to lessen the competitive strain in high-density growth.

Uniconazole [(E)-(RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pent-1-en-3-ol] inhibits P450 ent-kaurene oxidase, an enzyme that catalyzes the oxidation of ent-kaurene to ent-kaurenoic acid in the gibberellin acid (GA) biosynthesis pathway [22], which, as a well-known GAs inhibitor, can effectively regulate plant growth. In addition, uniconazole can also interfere with the isoprenoid biosynthetic pathway, which leads to enhanced abscisic acid (ABA) and cytokinin (CK) contents [23,24]. It has various effects on plant growth and development, such as enhancing plant dry matter under stress [25], inhibiting overgrowth [26], increasing carotenoid and chlorophyll contents [27], and regulating endogenous hormone levels [28]. These intricate influences show great potential in agricultural production applications. To produce compact transplants and extend the marketability of tomato transplants, use of uniconazole changes tomato seedling height [29].

Applying uniconazole can improve the lodging resistance of hybrid indica rice under low-light conditions [30]. In order to increase productivity, maize at a high population density can benefit from the use of uniconazole and nitrogen fertilization to delay leaf senescence and improve lodging resistance [31,32]. In agricultural practice, uniconazole is frequently applied through seed coating, seed soaking, and foliar spraying [33]. The flexible method of use and diversity of regulation functions make it a viable candidate for wide use in future agricultural production.

In the present study, we cultured rapeseed seedlings at various sowing densities, and then two sowing densities were selected for further uniconazole treatments. We hypothesized that uniconazole could effectively inhibit overgrowth at high sowing density and that this increased tolerance to high sowing density would effectively improve seedling transplanting suitability and have beneficial effects on yield at maturity. Through the determination of the seedling dry accumulation, leaf area extension, and other individual and colony indicators, we aimed to find how uniconazole altered these indicators and how they related to assisting the seedling through the transplant shock stage and influenced yield. The findings can help us gain a deeper understanding of how seedlings at high sowing densities develop and how uniconazole can lessen the detrimental impacts of high density sowing while also assisting seedlings in recovering from transplant injury safely and increasing yield.

2. Materials and Methods

2.1. Study Site and Design

The experiment was established at Yangzhou University (32.30° N, 119.43° E), Jiangsu province, China in 2021 and 2022. Ningza118, a kind of hybrid rapeseed, was used in this experiment. The substrate (Jiangsu Xingnong Substrate Technology Co., Ltd., Zhenjiang, China) used for nursery was commercially purchased, which has total N, P, and K nutrients of >3%, total organic matter of ≥35%, and a pH of 5.8–7.0. First, in order to determine the suitable sowing densities for future research, we conducted an experiment on the impact of sowing densities using 200, 400, 600, 800, and 1000 seeds per plate. The impact of uniconazole concentration on seedling growth at two sowing densities was then studied in another experiment. As a test variable, we selected 400 (sparse) and 800 (dense) seeds per plate. Uniconazole application levels were 0, 250, 500, and 750 mg L^{-1}.

2.2. Seeding and Pre-Seeding Processes

Prior to sowing in the tray, the seeds were treated with uniconazole (5% wettable powder, (S)-uniconazole, Sichuan Runer Technology Co. Ltd., Chengdu, China), which was dissolved in 0.75% polyvinyl alcohol. For every 100 g of seeds, a five milliliter uniconazole solution was employed. The seeds were evenly spaced and placed in sturdy trays (length × width × height = 580 mm × 280 mm × 25 mm). These trays were piled after sowing and left in the dark for roughly 72 h. When the seedlings broke through the surface, the trays were placed in sunlight and irrigated regularly. A 0.5% urea foliar nitrogen solution was applied weekly in 1.2 L per square meter after two weeks.

2.3. Pot Experiment

The soil used for the pot experiment was collected from 0 to 25 cm depth at Yangzhou University agricultural experimental field. The soil was air-dried and sieved through a screen (4 × 4 mm mesh) before being thoroughly mixed with basal fertilizer. Then, 18 kg portions of soil were placed in black plastic pots (height × diameter = 250 mm × 250 mm). Urea, superphosphate, and potassium chloride were used at basal fertilizer rates of 800 mg kg^{-1} N, 500 mg kg^{-1} P_2O_5, and 300 mg kg^{-1} K_2O for all pots. Pots were arranged in blocks randomly, then the 30-day-old seedlings from each treatment were transplanted into the pots. Before transplant, the roots of the seedlings were cut to a 2 cm length to simulate transplanting root damage uniformly and exclude the influence of root damage on growth after transplanting. Each pot received one transplanted seedling.

2.4. Sample Collection and Measurement

At 30 days following sowing, measurements of the plastic responses to treatments were obtained and a total of 30 seedlings were randomly chosen from three trays. These measurements were taken using electronic vernier calipers and included seedling height, seedling width, stem length, and stem diameter. Vertical inclination in response to competition was evaluated as the ratio between seedling height and seedling width, i.e., the maximum distance between the two furthermost leaf tips. In addition, leaf area, leaf length, and petiole length were taken with a digital camera and measured using ImageJ (v.1.53o, National Institutes of Health, USA). For each organ, samples were then taken separately and placed in an oven to dry at 70 °C until they attained a constant weight. The dry matter was measured by analytical balance. The samples in the pot experiment were collected at the "transplant shock stage" and the mature stage. The "transplant shock stage" was the period of 30 days after transplant. At 1 d, 5 d, 10 d, 15 d, 20 d, 25 d, and 30 d, we measured the seedlings' shoot and root dry matter as well as their leaf area. At the mature stage, we assessed the yield of 10 individual plants, along with each of their constituent parts, for each treatment. The yield components included effective branches, main effective pods, branch effective pods, seeds per silique, and 1000-grain weight.

2.5. Data analysis

Seedling growth analysis parameters, including stem/leaf ratio (Equation (1)), root/shoot ratio (Equation (2)), specific leaf area (SLA, Equation (3)), relative growth rate (RGR, Equation (4)), and net assimilation rate (NAR, Equation (5)), were calculated with the following equations.

$$\text{Stem/Leaf ratio}(\text{g g}^{-1}) = \frac{\text{stem dry matter}}{\text{leaf dry matter}} \tag{1}$$

$$\text{Root/Shoot ratio}(\text{g g}^{-1}) = \frac{\text{root biomass}}{\text{shoot biomass}} \tag{2}$$

$$\text{SLA}(\text{m}^2\ \text{g}^{-1}) = \frac{\text{leaf area}}{\text{leaf dry matter}} \tag{3}$$

$$\text{RGR}(\text{g g}^{-1}\ \text{day}^{-1}) = \frac{\ln W_1 - \ln W_0}{t_1 - t_0} \tag{4}$$

$$\text{NAR}(\text{g m}^{-2}\ \text{day}^{-1}) = \frac{W_1 - W_0}{t_1 - t_0} \times \frac{\ln L_1 - \ln L_0}{L_1 - L_0} \tag{5}$$

where W_0 and W_1 are the initial and final dry matter; t_0 and t_1 are the initial and final growing period (days); and L_0 and L_1 are the initial and final leaf area.

The data was collected and sorted using Microsoft Excel 2019. Analysis of variance (ANOVA) was performed with R (v.4.2.1, R Foundation for Statistical Computing) using the LSD for multiple comparisons. All the figures were plotted with the package "ggplot2" (v.3.3.6) in R. To better estimate the treatment effects on the allometric relationships between plant height and leaf length as well as between petiole length and leaf length, these relationships and effects were analyzed using standardized major axis (SMA) regression. SMA regression was used to test the effects of sowing density and uniconazole treatments on shifts along with a common slope (β) and on intercept (logα) with the equation: $\log Y = \log \alpha + \beta \log X$. The equation assumes that there are certain exponential functions among the biogenic elements of plant growth: $Y = \alpha X^{\beta}$ and transformed into natural logarithms. Prior to the analysis, the data were log transformed, and the SMA regression was performed using the "Smatr" (v.3.4-8) package in R [34]. A multivariate method was used to evaluate the complete array of seedling responses to the various treatments. Principal coordinate analysis (PCoA) was performed with the seedling traits as response variables explained by the sowing density and uniconazole treatments. To account for different measuring units, traits were centered and standardized before analysis. The packages "ade4" (v.1.7-19) and "vegan" (v.2.6-2) in R were used to carry out the PCoA [35].

3. Results

3.1. Sowing Density Effect on Seedling Performance

Sowing density has a remarkable influence on seedling growth. The performance of seedlings in response to increasing sowing density, as evaluated by the dry matter and the seedling architecture size, was continuously declining, indicating that seedlings were negatively affected by competition because of the sowing density (Table 1). Additionally, the shoot and root biomass exhibited a greater increase under a higher sowing density. Due to competition, seedling growth was more readily transferred to the root and stem, as shown by the result that both the root/shoot ratio and the stem/leaf ratio increased with increasing density (Table 2). Instead of thickening, stem growth tends to elongate. In terms of leaf traits, the leaf area exhibited a shape decline, as did the leaf length and petiole (Table 1). Because of the limited amount of available living space, seedlings' vertical inclination was measured as height per diameter ratio (Table 2) and increased in response to sowing density.

Table 1. The effect of sowing density on seedling performance.

SowingDensity	Seedling Height (mm)	Seedling Width (mm)	Height per Diameter	Stem Length (mm)	Stem Diameter (mm)	Leaf Area (cm^2)	Leaf Length (cm)	Petiole Length (cm)
200	80.29 a	83.50 a	0.97 c	31.84 c	2.11 a	25.96 a	3.67 a	4.37 a
400	71.09 b	69.86 b	1.03 abc	33.20 bc	1.73 b	15.08 b	2.83 b	4.16 ab
600	70.60 b	63.66 c	1.11 ab	34.40 b	1.71 b	12.43 c	2.75 b	4.03 b
800	63.89 c	62.63 c	1.01 bc	34.50 b	1.59 c	9.59 d	2.39 c	3.55 c
1000	62.71 c	57.01 d	1.12 a	42.24 a	1.37 d	7.61 e	2.27 c	3.15 d

Here, 200, 400, 600, 800, and 1000 represent sowing densities of 200, 400, 600, 800, and 1000 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters within a column.

Table 2. The effect of sowing density on dry matter and biomass.

Sowing Density	Leaf Dry Matter (mg plant^{-1})	Stem Dry Matter (mg plant^{-1})	Stem/Leaf Ratio	Shoot Biomass (g/m^2)	Root Biomass (g/m^2)	Root/Shoot Ratio
200	109.34 a	24.32 a	0.23 d	133.13 c	12.01 d	0.09 c
400	68.90 b	18.91 b	0.28 c	132.33 c	21.32 c	0.16 b
600	60.43 b	18.79 b	0.33 b	182.74 a	30.36 b	0.17 b
800	41.46 c	13.30 c	0.33 b	165.63 ab	41.07 a	0.25 a
1000	30.35 d	11.69 d	0.39 a	157.43 b	37.52 ab	0.24 a

Here, 200, 400, 600, 800, and 1000 represent sowing densities of 200, 400, 600, 800, and 1000 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters within a column.

3.2. Uniconazole Effect on Seedling Architecture

Architecture construction and seedling growth are negatively impacted by intensive sowing. For the following uniconazole test, we selected 400 and 800 seeds per tray based on the sowing density experiment. Uniconazole can improve the inhibition of plant growth caused by sowing density. The seedling height, seedling width, and stem diameter were increased by uniconazole treatment. Furthermore, the overgrowth of stem length was effectively relieved (Figure 1a,b,d,e). Nevertheless, on seedling height and width, the different doses of uniconazole showed a various degree of inhibitory effect. The height per width represented the vertical convergence of seedlings, and it was significantly increased at 250 mg L^{-1} uniconazole treatment (Figure 1c).

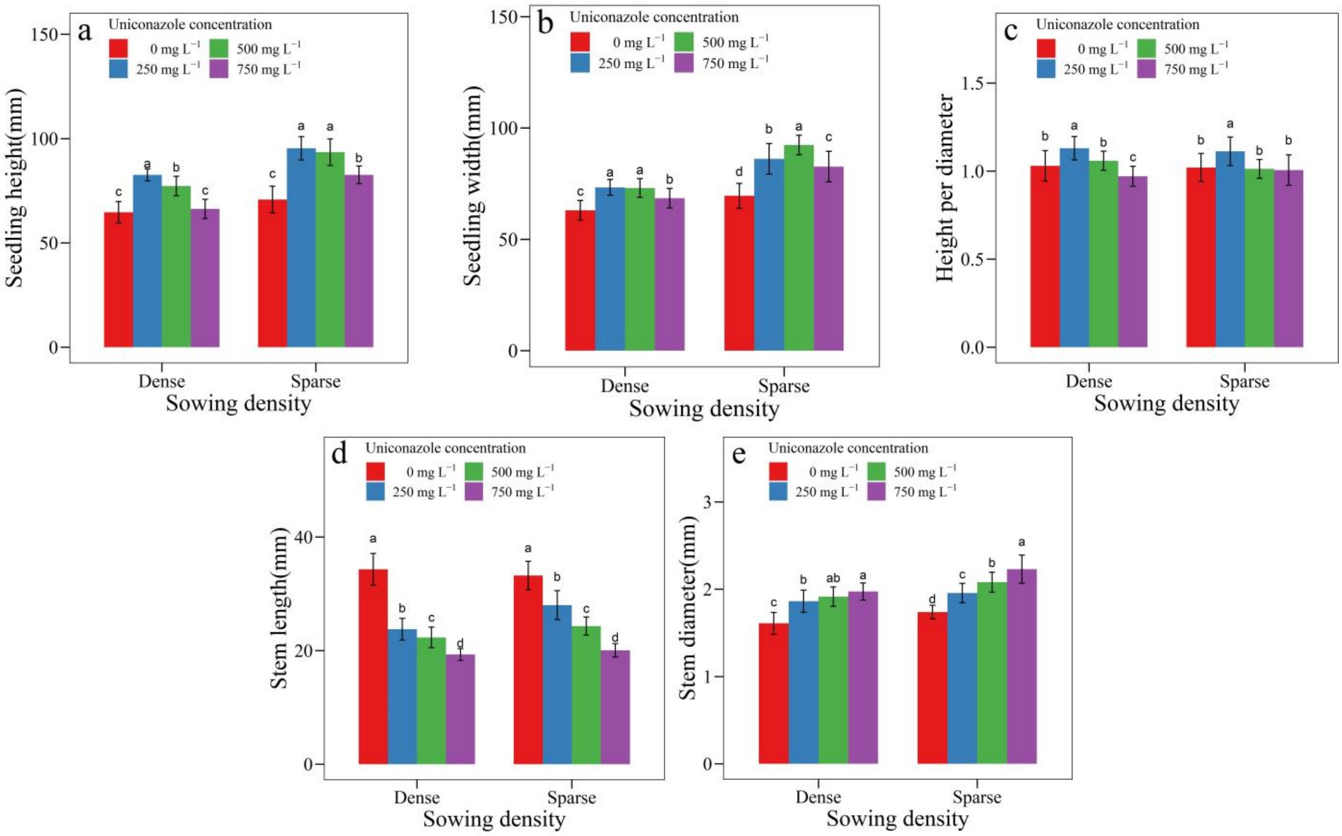

Figure 1. The effect of uniconazole on seedling architecture in two sowing densities. Values are the seedling architecture measured at 30d of seedling height (**a**), seedling width (**b**), height per diameter (**c**), stem length (**d**), and stem diameter (**e**). Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters in the same sowing density.

Leaves are the photosynthetic organs of plants, providing energy for growth. Uniconazole have impressive effects on leaf development. The application of uniconazole greatly enhanced the leaf area, although the higher doses of uniconazole had a small inhibitory effect (Figure 2a). Additionally, leaf length and petiole length were increased using 250 mg L^{-1} and 500 mg L^{-1} uniconazole treatments. Nevertheless, the 750 mg L^{-1} uniconazole treatment eliminated the increase in leaf elongation (Figure 2b,c). The smaller specific leaf area shows the higher shade tolerance of the seedlings. Specific leaf area was shown to be diminished by treatment with higher doses of uniconazole in all sowing densities (Figure 2d).

3.3. Uniconazole Effect on Seedling Growth and Allocation

Regardless of whether there were 400 or 800 seeds per tray, uniconazole greatly prevented the loss of leaf dry matter and shoot biomass caused by high seeding densities (Figure 3a,d). However, stem dry matter and root biomass showed different responses to uniconazole treatment at different sowing densities. In the dense sowing density, the stem dry matter and root biomass tended to be stable, in contrast to the sparse density, where the stem dry matter and root biomass tended to increase (Figure 3b,e). The distribution of dry matter was shown by the root/shoot and stem/leaf ratios. Treatment with uniconazole considerably decreased the stem/leaf ratio in all seeding densities (Figure 3c). Another phenomenon was shown by the root/shoot ratio being reduced by treatment with 250 mg L^{-1} uniconazole before increasing with higher dose treatments (Figure 3f).

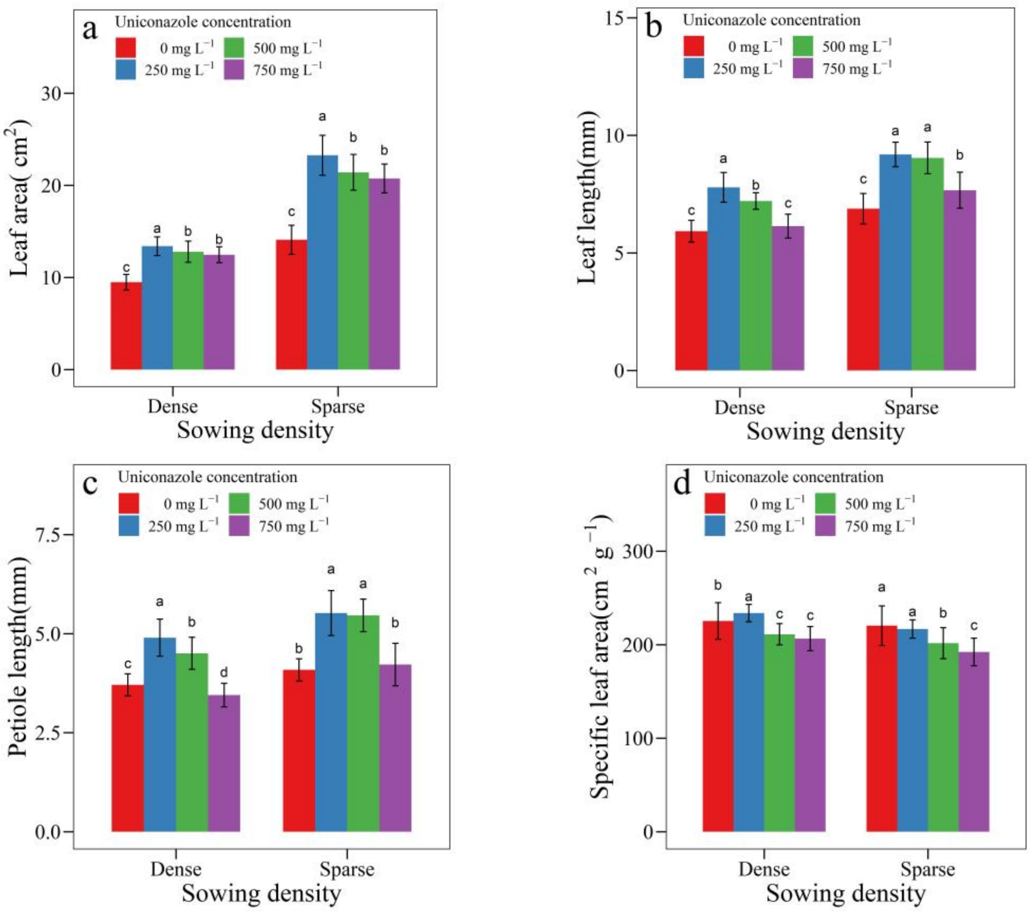

Figure 2. The effect of uniconazole on leaf traits in two sowing densities. Values are the leaf traits measured at 30 d of leaf area (**a**), leaf length (**b**), petiole length (**c**), and specific leaf area (**d**). Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters in the same sowing density.

At the same time, the allometric relationship of the seedlings showed the distribution conditions of growth. The result of the SMA test showed the allometric relationships between vertical and lateral growth and the internal lateral growth (Figure 4, Table 3). The decreased seedling height in the treatment with uniconazole is also shown by the shift in the seedling height–leaf length and petiole length–leaf length relationship in the SMA test. In conditions of sparse or dense densities, the seedling height–leaf length relationship showed an obvious decrease in slope. Moreover, the allometric relationship between petiole length and leaf length in response to 250 mg L^{-1} uniconazole treatment was shown by greater shifts along the slope. These results suggest that the uniconazole treatment shifts in leaf length in this case were associated with shorter seedling height. In the relationship between leaf length and petiole length, the low-dose uniconazole treatment shifts in leaf length in this case were associated with a longer petiole length.

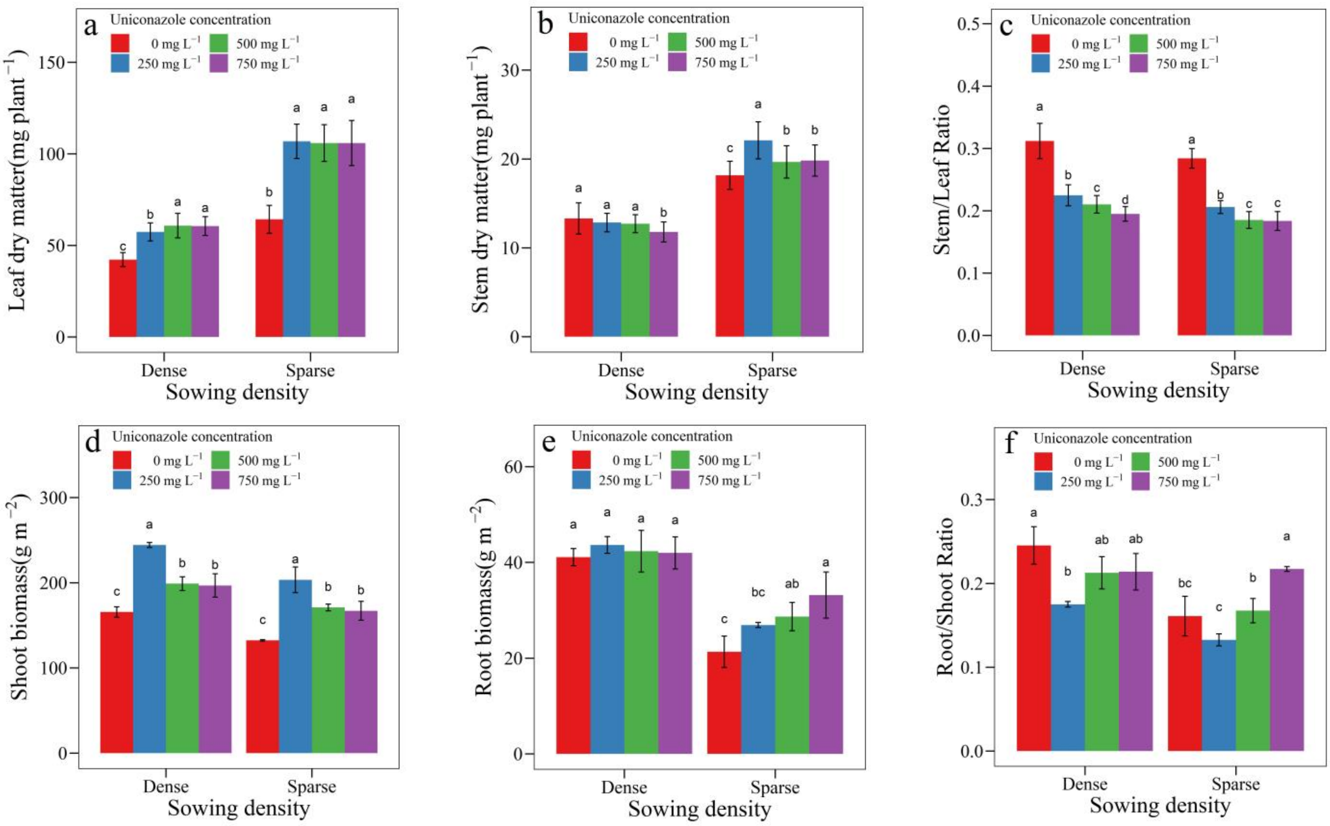

Figure 3. The effect of uniconazole on seedling dry matter, biomass, and allocation in two sowing densities. Values are the seedling dry matter and biomass measured at 30 d of leaf dry matter (**a**), stem dry matter (**b**), stem/leaf ratio (**c**), shoot biomass (**d**), root biomass (**e**), and root/shoot ratio (**f**). Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters in the same sowing density.

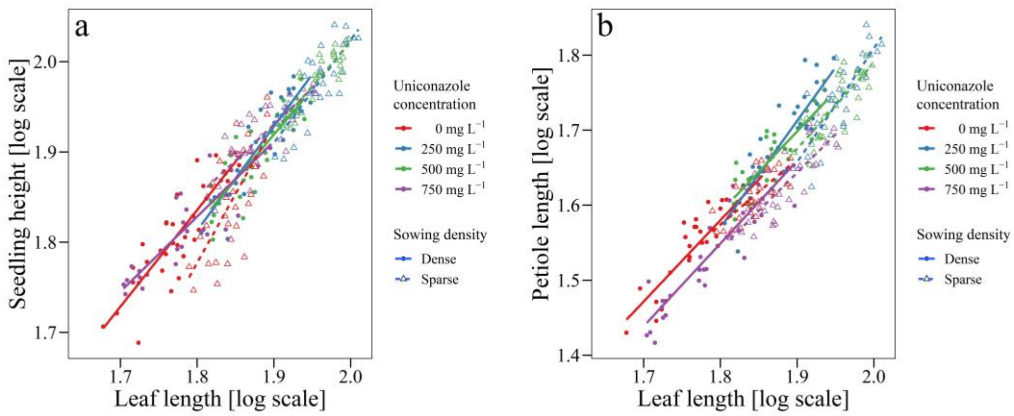

Figure 4. Treatment effect on the allometric relationship between seedling traits. Standardized major axis (SMA) relationship between seedling height and leaf length (**a**) and between petiole length and leaf length (**b**) within two sowing densities and various uniconazole concentrations. (See Table 3 for further information regarding SMA regression results).

Table 3. Standardized major axis (SMA) regression results for the effect of treatments on the allometric relationships between plant traits.

Sowing Density	Concentration	Intercept (logα)	Slope (β)	Significance of Slope	95% Confidence Intervals of Slope
	Seedling height vs. Leaf length				
Dense	0 mg L^{-1}	−0.4401	1.2674	a	1.0323–1.5560
	250 mg L^{-1}	−0.5447	1.3029	a	1.0898–1.5577
	500 mg L^{-1}	−0.3226	1.1831	ab	0.9651–1.4503
	750 mg L^{-1}	0.1528	0.9314	b	0.7838–1.1068
	Petiole height vs. Leaf length				
	0 mg L^{-1}	−0.6297	1.2298	b	1.0257–1.4745
	250 mg L^{-1}	−1.4075	1.644	a	1.3561–1.9930
	500 mg L^{-1}	−0.6597	1.244	b	1.0253–1.5094
	750 mg L^{-1}	0.6366	1.2148	b	1.0399–1.4273
	Seedling height vs. Leaf length				
Sparse	0 mg L^{-1}	−2.1771	2.1804	a	1.6606–2.8629
	250 mg L^{-1}	−0.7763	1.4059	b	1.1234–1.7595
	500 mg L^{-1}	−0.1709	1.0991	bc	0.9257–1.3049
	750 mg L^{-1}	0.9749	0.975	c	0.7817–1.2160
	Petiole height vs. Leaf length				
	0 mg L^{-1}	−0.6118	1.206	b	0.9264–1.5700
	250 mg L^{-1}	−1.8916	1.8577	a	1.4888–2.3179
	500 mg L^{-1}	−1.096	1.4499	ab	1.1470–1.8329
	750 mg L^{-1}	−0.6749	1.2219	b	0.9888–1.5101

Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. "Significance of Slope" represents tests for shifts along a common slope. Significance ($p \leq 0.05$) is shown by different letters in the same sowing density.

3.4. Principal Coordinate Analysis

Sowing density has a significant negative influence on seedling growth. However, in some organs, such as stems or petioles, sowing density provides an extremely stimulating environment for overgrowth. In order to identify the major sources of variation and trade-offs among seedling traits within uniconazole treatments in two sowing densities, we performed a preliminary principal coordinate analysis on the seedling traits before transplant. In the results, the first two axes explained more than 70% of the total variation for treatments (Figure 5a). We refer to the first axis as the "absolute size" of seedlings. The second axis was associated with the "competition tolerance" of the seedlings. At the same time, we produced boxplots to show how uniconazole treatments affected seedling absolute size (PCoA1) and competition tolerance (PCoA2) in two sowing densities (Figure 5b,c). These results demonstrate the competition tolerance of seedlings and show a noticeable increase with uniconazole treatment. The absolute size did not show a linear change. The seedling absolute size was increased at lower doses of uniconazole and decreased with higher doses. Regarding the impact of sowing density, whichever uniconazole treatment was used the absolute size of the seedlings was significantly decreased in dense sowing density compared with sparse sowing density. However, compared with sparse sowing density, the uniconazole treatment demonstrated a more pronounced increase in competition tolerance.

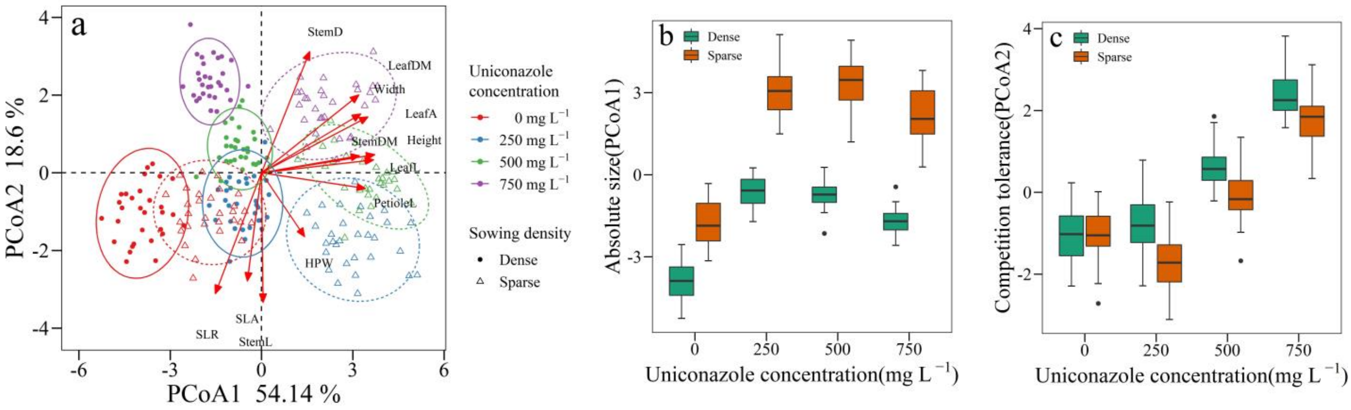

Figure 5. The result of principal coordinate analysis in two sowing densities using treatment with uniconazole. (**a**) the result of principal coordinate analysis. Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Arrows represent principal component loadings for each attribute. "Height" is seedling height, "Width" is seedling width, "StemL" is stem length, "StemD" is stem diameter, "LeafDM" is leaf dry matter, "StemDM" is stem dry matter, "LeafA" is leaf area, "LeafL" is leaf length, "PetioleL" is petiole length, "SLR" is stem/leaf ratio, "SLA" is special leaf area, "HPW" is height per width of seedling. (**b**) Boxplot showing seedling trait variation in absolute size in response to uniconazole treatment in two sowing densities. (**c**) Boxplot showing seedling trait variation in pressure tolerance in response to uniconazole treatment in two sowing densities.

3.5. Integrated Evaluation of Seedling Performance during Transplant Shock and Mature Stages

Transplant shock is a period of growth stagnation unique to transplanted crops. The sturdiness of transplanted seedlings affects the speed of plant recovery. The shoot of seedling growth and leaf expansion showed an apparent period of growth arrest of about 15 days (Figure 6a,c,d,f). Then, the shoot growth showed differences with more dry matter accumulation with higher doses of uniconazole. Root growth did not show significant growth arrest. In brief, uniconazole treatment can significantly enhance dry matter accumulation and leaf area expansion of plants after transplant. However, the relative growth rate decreased in 250 mg L^{-1} uniconazole and then rebounded with higher doses of uniconazole treatment (Figure 7a,b). The NAR of seedlings was increased at 750 mg L^{-1} uniconazole; other concentrations did not show a significant increase (Figure 7c).

In the mature stage, except for 1000-grain weight and main effective pots, all the yield-related traits showed notable differences between the control and uniconazole treatments in the two sowing densities. With the uniconazole treatments, the seeds per silique and number of effective branches were increased compared with no uniconazole treatment (Table 4). The yield loss caused by the sowing density can be reduced by uniconazole treatment, from a gap of 30% without uniconazole to 13% with a 750 mg L^{-1} uniconazole treatment compared with the control or the treatment between the two densities. The branch effective pots offered the most contributions to the increased yield, which showed obvious changes among different treatments (Table 4). In order to find the relationship between seedling characteristics affected by uniconazole and yield components, we performed partial correlation analysis, in which the sowing density influence was excluded. The results of partial correlation analysis show that the competition tolerance showed a significant correlation with yield, especially with the branch effective pots and seed per silique. The "absolute size" (PCoA1) of seedlings seemed to have nothing to do with yield. In the transplant shock and mature stages, the "competition tolerance" (PCoA2) showed impressive correlation with growth and yield (Table 5). This result suggests that seedling tolerance to high sowing density can be improved by uniconazole treatment to increase yield.

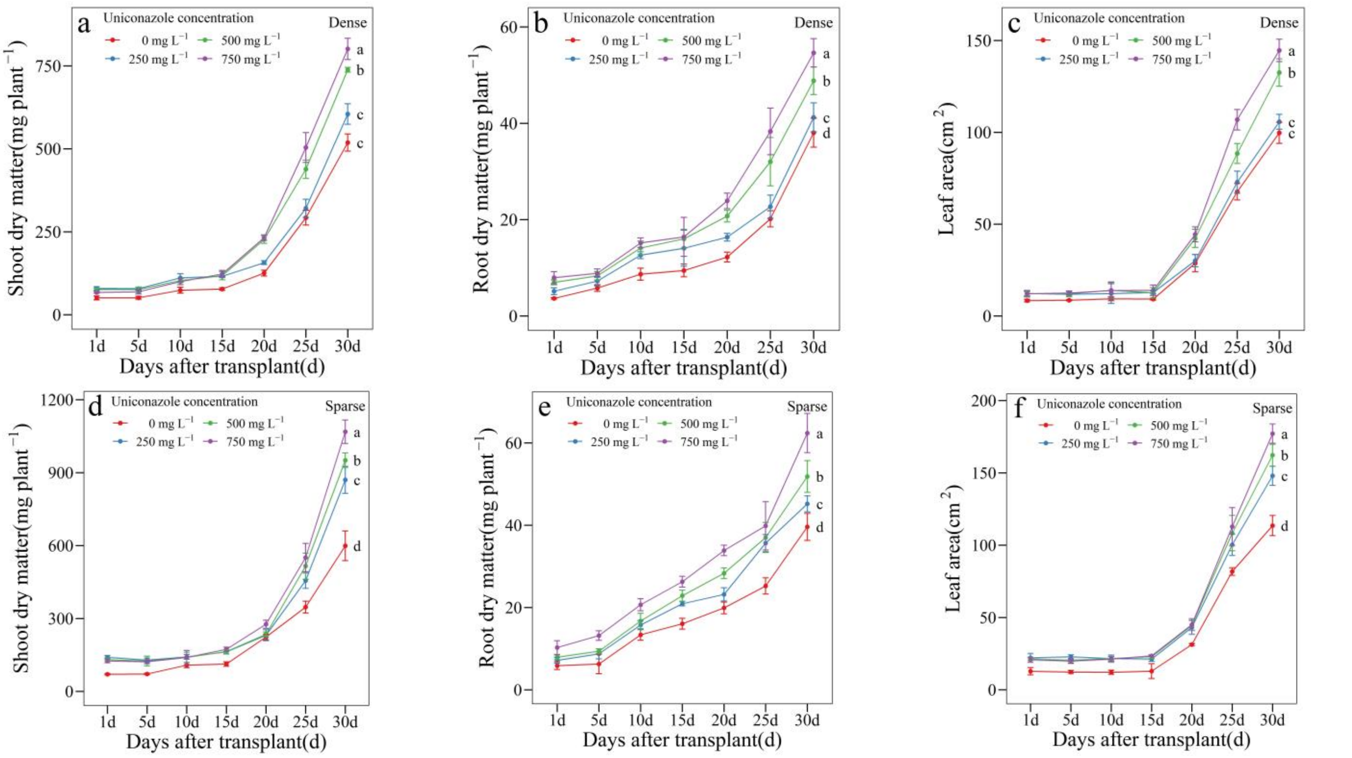

Figure 6. The growth of seedlings after transplant to pots for 30 days. Values are the seedlings sown at dense or spare density of shoot dry matter (**a**,**d**), root dry matter (**b**,**e**), and leaf area (**c**,**f**) during the 30 days after transplanting. Spare and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters in the same sowing density at 30 d.

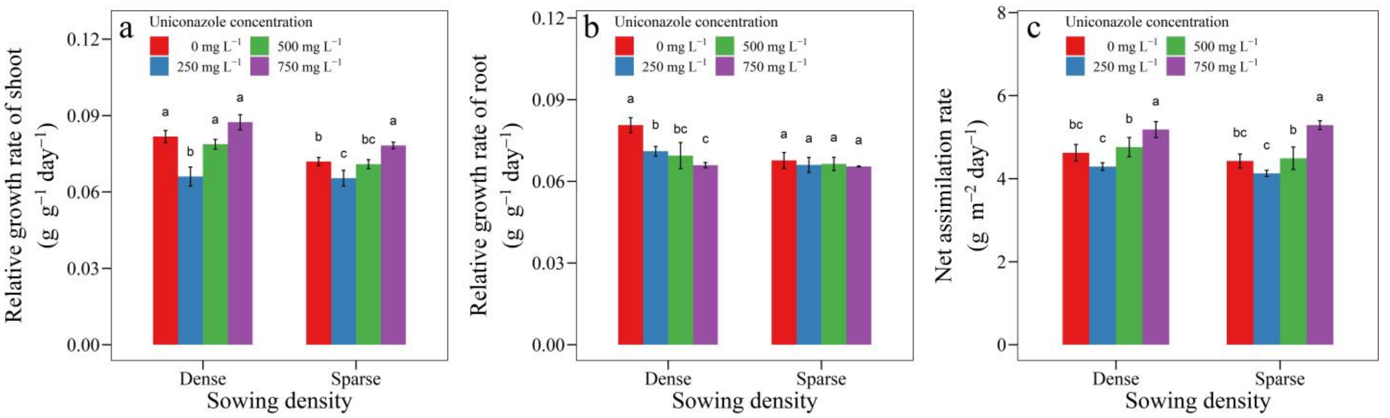

Figure 7. Growth indicators of seedlings at the 30th day after transplant from two densities with different uniconazole treatments. Values represent the relative growth rate of shoot (**a**), the relative growth rate of root (**b**), and the net assimilation rate (**c**). Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters in the same sowing density at 30 d.

Table 4. Effects of uniconazole on yield and yield components of rapeseed in two sowing densities.

Density	Concentration	Effective Branches	Main Effective Pods	Branch Effective Pods	Seeds per Silique	1000-Grain Weight	Grain Yield
Dense	0 mg L^{-1}	6.11 c	74.25 b	199.13 f	20.64 d	4.13 c	18.08 f
	250 mg L^{-1}	7.33 b	83.00 a	249.67 e	21.28 cd	4.14 bc	21.83 e
	500 mg L^{-1}	7.43 b	83.33 a	320.17 cd	23.53 ab	4.24 abc	24.98 d
	750 mg L^{-1}	7.38 b	85.29 a	366.83 b	23.61 ab	4.37 a	28.08 c
Spare	0 mg L^{-1}	6.67 bc	85.80 a	309.20 d	22.44 bc	4.27 abc	26.62 c
	250 mg L^{-1}	7.00 bc	85.14 a	342.00 c	23.35 ab	4.34 a	28.00 c
	500 mg L^{-1}	7.00 b	85.83 a	372.00 b	23.83 a	4.38 a	30.64 b
	750 mg L^{-1}	8.50 a	86.17 a	413.86 a	24.22 a	4.32 ab	32.52 a
F-value							
density		1.863 ns	16.820 **	245.631 **	16.395 **	8.8561 **	254.999 **
concentration		7.548 **	6.761 **	116.771 **	13.297 **	3.326 *	79.321 **
density × concentration		2.482 ns	4.606 **	7.621 **	1.961 ns	1.651 ns	4.895 **

Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown by different letters in the same column. Probability levels are ns, *, and ** for not significant, 0.05, and 0.01, respectively.

Table 5. Partial correlation analysis of seedling growth characteristics between pre and post transplant.

Stage	Indicator	Absolute Size (PCoA1)		Competition Tolerance (PCoa2)	
		Correlation	*p*-Value	Correlation	*p*-Value
Transplant shock stage	RGR of shoot	−0.3148	0.4916	0.6777	0.0943
	RGR of root	−0.3685	0.4160	−0.5784	0.1737
	NAR	0.1698	0.7159	**0.9171**	**0.0036**
	Growth of dry matter	0.6835	0.0905	**0.8398**	**0.0181**
	Extension of leaf area	0.5933	0.1602	**0.8662**	**0.0117**
Yield components	Effective branches	0.5427	0.2082	0.7470	0.0537
	main effective pots	0.4543	0.3058	0.5761	0.1758
	branches effective pots	0.5359	0.2150	**0.9095**	**0.0045**
	seed per silique	0.1804	1.5561	**0.8115**	**0.0267**
	1000-grain-weight	0.4275	0.3387	0.7026	0.0783
	yield	0.5486	0.2022	**0.9108**	**0.0044**

Sparse and dense represent sowing densities of 400 and 800 seeds per tray, respectively. Significant effects ($p \leq 0.05$) are shown in bold font.

4. Discussion

Seedlings can plastically respond to competition using well-documented strategies, including "confrontational" vertical growth, shade tolerance, or lateral avoidance [36,37]. However, how these responses influence transplant has seldom been studied in rapeseed. In general, many studies, such as those in rice [38], tomato [39], and corn [40], have found increasing density will cause seedling dry matter to decrease. Our results confirm this conclusion, showing a clear drop in rapeseed seedling dry matter with increasing seeding density (Table 2). The height and stem length of the seedlings showed clear overgrowth in their vertical growth (Table 1). The leaves of the seedlings displayed clear signs of growth restriction, such as reductions in leaf area and leaf length (Table 1). Thinner stems are more vulnerable to damage during transplantation, and it is difficult to grow straight after transplanting. As a result, we urge that seedlings be as strong as possible. Yet, the sowing density clearly has a negative effect on this. However, increasing sowing density is an inevitable problem in actual crop production because of the economic issues or transplant efficiency problems [41]. With a seeding density below 400 seeds per tray, it is challenging for seedlings to knot like a blanket, which cannot meet the needs of mechanical transplanting in production practice. Additionally, our biomass results showed that increasing the sowing density appropriately can increase the biomass per unit area, which can reduce the expensive cost of nursery seedlings as well. In order to maintain biomass, 400–800 seeds per tray kept it at a similar level; a too high or too low sowing density will cause a decline in biomass (Table 2). Therefore, we believe that when resources are scarce, the population can only support a specific amount of biomass, and too high or too low sowing densities are detrimental to the biomass. However, concentrating on the individual, higher planting density resulted in a considerable reduction in seedling dry matter. In addition, seedling architecture and biomass allocation were greatly altered. Seedlings were shorter, more compact, and had slender stems. At the same time, the stem/leaf ratio increased, the leaf dry weight ratio decreased, and more resources were devoted to competitive development (Tables 1 and 2). Competition for resources does not lead to higher growth, but to differentiation within individual plant growth. Therefore, alleviating the internal competitive pressure, enhancing the accumulation of individual dry matter, and optimizing the dry matter distribution is a key component of improving population quality.

Uniconazole as a plant growth regulator is widely used to regulate growth and improve resistance [42]. In our work, we used the method of seed coating to apply uniconazole, which has been used in many other crop productions [43,44]. The results show that uniconazole can significantly increase seedling dry matter (Figure 3a). Because of the improvement of individual seedling growth, the biomass in the tray also increased (Figure 5d,e). Simultaneously, uniconazole can optimize seedling architecture, raise stem diameter and leaf area, and reduce excessive elongation of the stem and petiole (Figures 1, 2 and 4). According to this optimization of structure, the rapeseed seedlings showed a greater competition tolerance to the high sowing density, which caused a remarkable gain in yield and reduced the yield loss in higher sowing densities (Figure 5, Table 4). During the transplant shock stage, the RGR showed the relative speed of the seedlings compared with the initial state, but the uniconazole did not always show a positive influence on the seedling recovery. The 250 mg L^{-1} uniconazole treatment led to a decrease in RGR (Figure 7a,b). These results suggest that lower doses of uniconazole could exacerbate the competitive pressure induced by the increase in dry matter, leading to uncontrolled growth in the later nursery stage and delayed growth after transplanting. However, all uniconazole treatments enhanced the rapeseed yield production compared with the control, meaning that the dry matter of rapeseed seedlings before transplant has an important influence on the yield contribution (Figure 3, Table 4). In the higher dose treatments, the RGR and NAR were recovering and increasing. The late stage of seedling growth is more strongly influenced by greater dosages of uniconazole, which results in a more suitable high-density population structure for transplantation. The result of principal coordinate analysis and partial correlation analysis demonstrated that the competition tolerance of seedlings in nursery trays is substantially correlated with the rapid growth of seedlings after transplanting and the components of yield at maturity (Figure 5, Table 5).

5. Conclusions

In our study, we found the uniconazole treatment could effectively alleviate the decrease in seedling quality caused by high sowing density and enhance yield. We showed that more than 500 mg L^{-1} of uniconazole can effectively control the overgrowth of seedlings at a high sowing density. Moreover, 750 mg L^{-1} uniconazole treatment can further lessen the yield gap between the sowing densities at 400 and 800 seeds per tray. However, as a well-known gibberellin inhibitor, excessive doses of uniconazole could have a negative impact on seed germination and even growth [45,46]. Hence, on safety grounds, we conservatively recommend 5 mL of 500–750 mg L^{-1} uniconazole polyvinyl alcohol solution per 100 g of seeds in production practice. This is just the beginning of this technology and we accept that further development of field management techniques in conjunction with this technology will lessen the negative effects of high-density seedling rearing.

Author Contributions: X.Z. and S.L. designed the research. X.Z., Y.Z., Z.W. and C.G. performed the experiments and data collection. X.Z., S.L., Q.Z. and G.Y. contributed to data analysis and wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program of China (2018YFD1000900), the Major Project of Basic Science (Natural Science) Research in Colleges and Universities in Jiangsu Province, China (21KJA210003), and Demonstration and Promotion Project of Key Technologies for Improving Quality and Efficiency of Rapeseed, Yangzhou, China (YN202229).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors gratefully acknowledge the laboratory staff for their contributions, continued diligence, and dedication to this research. The valuable comments of the editor and all anonymous reviewers are greatly appreciated.

Conflicts of Interest: The authors declare no conflict of interest.

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