Effects of seedling age and cultivation density on agronomic characteristics and grain yield of mechanically transplanted rice

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Delayed transplantation frequently occurs in mechanically transplanted rice in China, leading to a significant reduction in grain yield. Thus, determining how to compensate grain yield loss is crucial for improving rice cultivation technology. A field experiment was conducted to investigate the effects of cultivation density and seedling age on agronomic traits and grain yield of mechanically transplanted rice. With increasing seedling age, rice tiller number, pre-anthesis dry matter accumulation, remobilization efficiency and contribution to grain yield, as well as post-anthesis photosynthesis amount decreased, causing reductions in the number of effective panicles, the total number of grains per panicle, the sink capacity per tiller, and grain yield. In rice transplanted at 30- and 35-day seedling ages, increasing cultivation density significantly enhanced the number of effective panicles and grain yield. Additionally, there existed strong, positive correlations between sink capacity per tiller and pre-anthesis dry matter remobilization efficiency and pre-anthesis dry matter contribution to grain yield. We conclude that in addition to cultivation density, enhancing the amount of pre-anthesis dry matter and the remobilization efficiency could be feasible for mitigating grain yield loss caused by delayed transplantation.

With the increasing scarcity of the rural labour force, mechanical transplantation has become a prevalent and simplified cultivation method to replace hand transplantation in Chinese rice production. Many researchers have demonstrated that the achievement of high grain yield for mechanically transplanted rice requires an optimal seedling age1. Shen et al. reported that among all agronomic practices influencing grain yield of mechanically transplanted rice, seedling age is the most important factor followed by the use of fertilizer and sowing density2. Currently, the delay of transplantation events frequently occurs in wheat-rice rotation districts in China. These delays are attributed to the late harvest of the former crop, the deficiency in the number of transplanting machines, and unfavourable weather conditions3–5. The seedling age of mechanically transplanted rice even exceeds 30 days in some regions. Today, some regions are facing the challenges of grain yield fluctuations resulting from delayed mechanical transplantation3,5,6. Based on previous research and local rice production, the suitable seedling age for mechanically transplanted rice is commonly considered to be less than 25 days5.

When the seedling age is more than 25 days, rice seedlings are usually named “delayed transplanted rice” or “rice with old seedling age.” Delaying transplantation can detrimentally impact rice growth and grain yield formation, mainly due to poor tiller occurrence, shortened vegetative duration, and decreased dry matter accumulation6. Accordingly, how to effectively compensate for the grain yield loss caused by delaying transplantation has become an important issue for some researchers. Lampayan et al. found that lowering the seeding rate could improve the grain yield of rice with old seedling age7. Yu et al. suggested that for mechanically transplanted rice, adopting dry seedling-nursery technology was instrumental in enhancing seedling quality and prolonging seedling age suitable for transplantation8. Hu et al. reported that an optimum nitrogen application pattern (a split application ration of 4:3:3 before transplantation, at tillering and at panicle initiation stages) could significantly enhance the nitrogen utilization efficiency and grain yield of delayed mechanically transplanted rice9. These
It has been established that planting density plays an important role in improving population structure, promoting the efficient use of sunlight and regulating rice tiller occurrence and grain yield formation10–12. Wang et al. found that the maximum grain yield was produced at 25 cm × 17 cm spacing for mechanical hill direct sown rice13. Qian et al. reported that the achievement of high grain yield for mechanically transplanted rice cultivars with small, medium, and large panicles required 4 seedlings per hill, 3 seedlings per hill, and 2 seedlings per hill, respectively14. Xu et al. showed that an increase in the mechanical transplanting density could significantly enhance the grain yield of rice cultivars suitable for close-planting15. Furthermore, it has been verified that the number of effective panicles significantly reduced for rice undergoing delayed transplantation, due to the repression of tiller occurrence, thereby leading to decreased grain yield6. Accordingly, increasing the tiller number may be a feasible method for offsetting the grain yield loss of delayed transplanted rice. Whether enhancing cultivation density (i.e., seedling number per hill) can adequately promote the number of effective tillers in rice populations and eventually decrease yield loss under delayed transplantation conditions remains unexplored. However, in regards to optimal seedling age and old seedling age, there is little information available on how cultivation density impacts the utilization efficiency of preanthesis stored photoassimilates and post-anthesis newly created assimilates. Conducting related research could provide a reference for guiding the management of mechanically transplanted rice cultivation.

The objectives of this research were (1) to determine the effect of cultivation density on tiller occurrence, pre-anthesis dry matter remobilization and utilization efficiencies, post-anthesis photosynthetic products use efficiency, sink capacity per tiller, and the grain yield component of rice with optimal and old seedling ages; (2) to ascertain whether increasing the cultivation density could significantly compensate for the grain yield loss of mechanically transplanted rice incurred by delaying transplantation; and (3) to clarify the relationship between the utilization efficiency of photoassimilates and sink capacity.

### Results

**Performances of the main growth duration and tiller occurrence in rice with different seedling ages at normal and high densities.** With the increase of seedling age, the booting, heading, and maturity stages of rice were markedly postponed. However, these parameters remained unchanged at normal and high densities for rice with different seedling ages, indicating that cultivation density did not have an influence on the duration of rice growth (Table 1). In 2013, the booting, heading, and maturity stages of rice with 30- and 35-day seedling ages were delayed by 3–6 days, 2–3 days, and 1–3 days, respectively, when compared with those of rice with a 25-day seedling age. In 2014, these stages were delayed by 3–5 days, 2 days and 1–2 days, respectively. From the 6th day to the 50th day after transplanting, theiller numbers of rice at high cultivation density were much greater than those at normal cultivation density in both study years, regardless of rice seedling age (Fig. 1). For rice with a 25-day seedling age, the tiller numbers at high cultivation density were 25.94% and 29.04% higher than those at normal cultivation density in 2013 and 2014, respectively (Fig. 2). For rice with a 30-day seedling age, the tiller numbers at high cultivation density were 29.70% and 30.36% higher than those at normal cultivation density in 2013 and 2014, respectively (Fig. 2). For rice with a 35-day seedling age, the tiller numbers at high cultivation density were 40.55% higher than those at normal cultivation density in 2013 and 2014, respectively (Fig. 2).

**Dry matter productivity and utilization characteristics in rice with different seedling ages at normal and high densities.** At the heading stage, the total dry matter weight, as well as weights of the leaf, stem and sheath, and panicle, at normal density were greater than those at high density in both study years, irrespective of seedling age (Table 2). The parameters mentioned above showed similar tendencies (except for the leaf dry matter weight) at each rice maturity stage in both years. In 2013 and 2014, the dry matter weights of the

| Year | Treatment | Dates for different growth stage |
|------|-----------|---------------------------------|
|      | Seedling age | Cultivation density | Booting | Heading | Maturity |
| 2013 | 25 d       | ND                   | 21-Jul  | 16-Aug | 11-Oct   |
|      |            | HD                   | 21-Jul  | 16-Aug | 11-Oct   |
|      | 30 d       | ND                   | 24-Jul  | 17-Aug | 13-Oct   |
|      |            | HD                   | 24-Jul  | 17-Aug | 13-Oct   |
|      | 35 d       | ND                   | 27-Jul  | 19-Aug | 14-Oct   |
|      |            | HD                   | 27-Jul  | 19-Aug | 14-Oct   |
| 2014 | 25 d       | ND                   | 23-Jul  | 20-Aug | 13-Oct   |
|      |            | HD                   | 23-Jul  | 20-Aug | 13-Oct   |
|      | 30 d       | ND                   | 25-Jul  | 20-Aug | 14-Oct   |
|      |            | HD                   | 25-Jul  | 20-Aug | 14-Oct   |
|      | 35 d       | ND                   | 28-Jul  | 22-Aug | 15-Oct   |
|      |            | HD                   | 28-Jul  | 22-Aug | 15-Oct   |

Table 1. Dates for booting, heading and maturity stages of rice with different seedling ages at normal and high densities. ND = normal density, HD = high density.
leaf, stem and sheath, and panicle, and the total weight at heading stage for rice with 30- and 35-day seedling ages showed a decreasing trend compared to that for rice with a 25-day seedling age. Similar results (excluding the dry matter weight of the leaf) were also observed at the maturity stage.

Irrespective of seedling age, the leaf dry matter remobilization amount, remobilization efficiency, contribution to grain yield, and post-anthesis photosynthesis amount decreased while the stem and sheath dry matter remobilization amount, remobilization efficiency, contribution to grain yield, and post-anthesis photosynthesis contribution to grain yield were enhanced at high density, compared with those at normal density. In 2013, the corresponding values for leaf were 15.67%, 10.31%, 12.94%, and 1.47%, respectively. They were 11.29%, 7.87%,

Figure 1. Daily precipitation and mean air temperature from sowing to maturity.

Figure 2. Tiller number of rice with different seedling age at normal and high cultivation densities from the 6th d to the 50th d after transplanting. (a) ND = normal density. (b) HD = high density.
| Year | Treatment | Cultivation density | Leaf | Stem and sheath | Panicle | Total | Leaf | Stem and sheath | Panicle | Total |
|------|-----------|---------------------|------|-----------------|--------|-------|------|-----------------|--------|-------|
| 2013 | 25 d ND   | 0.808a 1.595a | 0.532a | 2.935a | 0.356a | 1.530a | 2.034a | 3.920a |
|      | HD       | 0.755b 1.450b | 0.493a | 2.698b | 0.392a | 1.340b | 1.950b | 3.682b |
|      | 30 d ND  | 0.783a 1.385a | 0.436a | 2.604a | 0.461a | 1.372a | 1.740a | 3.573a |
|      | HD       | 0.741a 1.258b | 0.419a | 2.418b | 0.465a | 1.216b | 1.702a | 3.383b |
|      | 35 d ND  | 0.731a 1.212a | 0.393a | 2.336a | 0.451a | 1.210a | 1.574a | 3.235a |
|      | HD       | 0.683b 1.130b | 0.384a | 2.197a | 0.435a | 1.125a | 1.498b | 3.058b |
|      | Seedling age 25d | 0.782a 1.523a | 0.513a | 2.817a | 0.374a | 1.435a | 1.992a | 3.801a |
|      | Seedling age 30d | 0.762a 1.322b | 0.428a | 2.511b | 0.463a | 1.294b | 1.721b | 3.478b |
|      | Seedling age 35d | 0.707b 1.171c | 0.389b | 2.267c | 0.443a | 1.168c | 1.536c | 3.147c |
|      | Normal density | 0.774a 1.397a | 0.454a | 2.625a | 0.423a | 1.371a | 1.783a | 3.576a |
|      | High density | 0.726a 1.279b | 0.432a | 2.438b | 0.431a | 1.227b | 1.717b | 3.374b |
| 2014 | 25 d ND   | 0.821a 1.612a | 0.578a | 3.011a | 0.368a | 1.545a | 2.205a | 4.118a |
|      | HD       | 0.788b 1.570a | 0.523a | 2.881b | 0.412a | 1.551a | 2.016b | 3.979b |
|      | 30 d ND  | 0.801a 1.525a | 0.501a | 2.827a | 0.428a | 1.491a | 1.981a | 3.900a |
|      | HD       | 0.774a 1.474b | 0.483a | 2.731a | 0.412a | 1.420a | 1.968a | 3.800b |
|      | 35 d ND  | 0.751a 1.424a | 0.435a | 2.610a | 0.461a | 1.400a | 1.701a | 3.562a |
|      | HD       | 0.725a 1.382a | 0.400a | 2.507b | 0.472a | 1.300b | 1.685a | 3.457b |
|      | Seedling age 25d | 0.805a 1.591a | 0.551a | 2.946a | 0.390a | 1.548a | 2.111a | 4.049a |
|      | Seedling age 30d | 0.788a 1.500b | 0.492a | 2.779b | 0.420a | 1.456a | 1.975a | 3.850b |
|      | Seedling age 35d | 0.738b 1.403c | 0.418b | 2.559c | 0.467a | 1.350b | 1.693b | 3.510c |
|      | Normal density | 0.791a 1.520a | 0.505a | 2.816a | 0.419a | 1.479a | 1.962a | 3.860a |
|      | High density | 0.762a 1.475a | 0.469a | 2.706b | 0.432a | 1.424a | 1.890b | 3.745b |

Table 2. Above-ground dry matter weight per tiller for rice with different seedling age at normal and high densities. Values followed by different letters represent significant difference at \( p < 0.05 \). ND = normal density, HD = high density.

8.90%, and 0.48%, respectively, in 2014. With increasing seedling age, the post-anthesis photosynthesis contribution to grain yield decreased while the other parameters increased across the two study years (Table 3).

Sink capacity per tiller and the grain yield component of rice with different seedling ages at normal and high densities. As shown in Fig. 3, compared to that at normal density, sink capacity per tiller decreased at high density. Sink capacity per tiller decreased with increasing seedling age. The change tendencies of the parameters of grain yield components were similar in both study years (Table 4). The numbers of primary and secondary branches per panicle and the total number of grains per panicle at high density were found to be 6.14%, 7.47%, and 6.21% lower, respectively, than those at normal density in 2013. In 2014, the corresponding values were 9.38%, 10.07%, and 7.03%, respectively. Conversely, the number of effective panicles per square metre decreased across both study years. For rice with a 25-day seedling age, there was no significant difference in grain yield between normal density and high density across both study years (Table 3). The numbers of primary and secondary branches per panicle and the total number of grains per panicle at high density were found to be 6.63% and 13.10% in 2013 and 7.67% and 6.82% in 2014, respectively.

Relationships between sink capacity and pre-anthesis dry matter remobilization and post-anthesis photosynthesis utilization characteristics. The relationships between sink capacity per tiller and the utilization efficiency of pre-anthesis dry matter and post-anthesis photosynthesis under high and normal densities are shown in Fig. 4. There were positive relationships between sink capacity per tiller and pre-anthesis dry matter remobilization amount, remobilization efficiency, contribution to grain yield and the amount of post-anthesis photosynthesis transport per tiller. Conversely, sink capacity per tiller was negatively related to the contribution of post-anthesis photosynthesis to grain yield.

Across all seedling ages, sink capacity per tiller was positively correlated with the amounts of pre-anthesis dry matter remobilization and post-anthesis photosynthesis transport per tiller (Fig. 5). For rice with a 25-day seedling age, sink capacity per tiller was negatively related to the preanthesis dry matter contribution to grain yield and positively related to the post-anthesis photosynthesis contribution to grain yield. In rice with 30- and 35-day seedling ages, sink capacity per tiller was positively related to the pre-anthesis dry matter contribution to grain yield and negatively related to the post-anthesis photosynthesis contribution to grain yield.
Discussion
Effects of cultivation densities on growth duration and tiller occurrence of mechanically transplanted rice with different seedling ages. Previous reports showed that rice jointing, heading, and maturity stages under mechanical transplantation were delayed compared with those under hand transplantation, with this difference increasing with the delay of mechanical transplantation. In the present study, rice booting, heading, and maturity stages were delayed by 1–6 days with increasing seedling age. The rice booting stage was delayed more than the other growth stages. Compared to that of rice with a 25-day seedling age, the booting stage was delayed only by 2–3 days for rice with a 30-day seedling age and 5–6 days for rice with a 35-day seedling age, suggesting that the rice vegetative stage is obviously shortened with the delay of mechanical transplantation. In contrast, cultivation density did not show any influence on rice growth stage regardless of seedling age. This phenomenon indicates that seedling age could be an important factor determining rice growth stage under mechanical transplantation patterns, which is in agreement with past research.7,16.

Table 3. Pre-anthesis dry matter remobilization traits and post-anthesis newly created photosynthesis in rice with different seedling age at normal and high densities Values followed by different letters represent significant difference at \( p < 0.05 \). ND = normal density, HD = high density.

| Year | Treatment | Leaf | Stem and sheath | Post-anthesis photosynthesis contribution to grain yield (%) |
|------|-----------|------|-----------------|----------------------------------------------------------|
| 2013 | 25 d ND   | 0.452a | 55.94a | 30.09a | 0.065a | 4.08a | 4.33a | 0.985a | 65.58a |
|      | HD       | 0.363b | 48.08b | 24.91b | 0.110a | 7.59b | 7.55b | 0.984a | 67.54b |
|      | 30 d ND   | 0.322a | 41.12a | 24.69a | 0.013a | 0.94a | 1.00a | 0.969a | 74.31a |
|      | HD       | 0.276b | 37.25b | 21.51b | 0.042a | 3.34b | 3.27a | 0.965b | 75.21a |
|      | 35 d ND   | 0.280a | 38.30a | 23.71a | 0.002a | 0.17a | 0.17a | 0.899a | 76.12a |
|      | HD       | 0.248b | 36.31a | 22.26a | 0.005a | 0.44b | 0.45b | 0.861b | 77.29a |
|      | Seedling age 25d | 0.408a | 52.14a | 27.54a | 0.087a | 5.75a | 5.91a | 0.958a | 66.54a |
|      | Seedling age 30d | 0.299b | 39.24b | 23.12b | 0.027b | 2.08b | 2.13b | 0.967a | 74.76b |
|      | Seedling age 35d | 0.264c | 37.34b | 23.01b | 0.003c | 0.30c | 0.31c | 0.880b | 76.69c |
|      | Normal density | 0.351a | 45.39a | 26.44a | 0.027a | 1.91a | 2.01a | 0.951a | 71.56a |
|      | High density | 0.296b | 40.71b | 23.02b | 0.052a | 4.09b | 4.07b | 0.937b | 72.91a |
| 2014 | 25 d ND   | 0.453a | 55.18a | 27.84a | 0.067a | 4.16a | 4.12a | 1.107a | 68.04a |
|      | HD       | 0.376b | 47.72b | 25.18b | 0.019b | 1.21b | 1.27b | 1.098b | 73.54b |
|      | 30 d ND   | 0.373a | 46.57a | 25.20a | 0.034a | 2.24a | 2.30a | 1.073a | 72.49a |
|      | HD       | 0.362a | 46.77a | 24.38a | 0.054a | 3.66a | 3.64a | 1.069a | 71.99a |
|      | 35 d ND   | 0.290a | 38.62a | 22.91a | 0.024a | 1.69a | 1.90a | 0.952a | 75.20a |
|      | HD       | 0.253a | 34.90b | 19.66b | 0.082b | 5.93b | 6.38b | 0.950a | 73.93a |
|      | Seedling age 25d | 0.415a | 51.52a | 26.57a | 0.043a | 2.70a | 2.76a | 1.103a | 70.67a |
|      | Seedling age 30d | 0.368b | 46.67b | 24.79b | 0.044a | 2.94a | 2.97a | 1.071b | 72.24b |
|      | Seedling age 35d | 0.272c | 36.79c | 21.29c | 0.053a | 3.78a | 4.16a | 0.951b | 74.56c |
|      | Normal density | 0.372a | 47.03a | 25.52a | 0.042a | 2.74a | 2.86a | 1.044a | 71.62a |
|      | High density | 0.330a | 43.33b | 23.25b | 0.052a | 3.50b | 3.64b | 1.039a | 73.12a |

Figure 3. Effects of seedling age and cultivation density on sink capacity per tiller of rice. (a) Values followed by different letters represent significant difference at \( p < 0.05 \). (b) ND = normal density. (c) HD = high density.
It is indisputable that rice tiller occurrence is depressed with increasing seedling age, thereby resulting in reduced tiller numbers\textsuperscript{16–19}. In general, the seedling nursery of mechanically transplanted rice is characterized by a greater sowing amount. With the prolongation of transplantation, the growth of rice seedlings is evidently suppressed due to intensive competition among individual plants under limited growing space in seedling nursery beds, which further hampers rice tiller occurrence after being transplanted to the paddy field\textsuperscript{16,20}. In the present study, rice tiller number markedly decreased with an increase in seedling age regardless of density, suggesting that rice seedling age could play a vital role in determining tiller occurrence. In addition, cultivation density is also an important factor influencing rice tiller number. Increasing the seedling number per hill or the distance among rows has been shown to be feasible in enhancing the tiller numbers of the rice population\textsuperscript{21,22}. Data in our study also showed an increase in tiller number through enhanced cultivation density regardless of seedling age. However, it is notable that, as for rice with a 25-day seedling age, the increased magnitude of the average tiller number induced by increasing cultivation density was smaller than that of rice with 30- and 35-day seedling ages. These results reveal that an enhancement in cultivation density exerts a positive role in minimizing the reduction of rice tiller number incurred by delaying transplantation.

| Year | Treatment | Cultivation density | No. of the primary branch per panicle | No. of the secondary branch per panicle | No. of effective panicles per square meters | No. of total grains per panicle | Seed-setting rate (%) | 1000-grain weight (g) | Grain yield (kg ha\textsuperscript{-1}) |
|------|-----------|----------------------|--------------------------------------|----------------------------------------|-----------------------------------------|-------------------------------|----------------------|------------------------|-------------------------------|
| 2013 | 25 d ND   | HD                   | 9.88a                                | 19.43a                                 | 415.50a                                 | 100.06a                     | 83.85a               | 24.69a                 | 7290.89a                     |
|      |           | HD                   | 9.83a                                | 18.01a                                 | 453.65b                                 | 94.27b                      | 82.19a               | 24.79a                 | 7482.67a                     |
|      | 30 d ND   | HD                   | 10.22a                               | 16.28a                                 | 384.34a                                 | 97.06a                      | 81.88a               | 24.67a                 | 6451.82a                     |
|      |           | HD                   | 9.22b                                | 15.56a                                 | 443.83b                                 | 91.06b                      | 81.76a               | 24.89a                 | 6879.83b                     |
|      | 35 d ND   | HD                   | 10.35a                               | 14.50a                                 | 249.99a                                 | 96.29a                      | 80.44a               | 24.77a                 | 4802.41a                     |
|      |           | HD                   | 9.53a                                | 12.91a                                 | 336.60b                                 | 89.85b                      | 80.70a               | 24.99a                 | 5431.43b                     |
|      | Seedling age 25d HD  | 9.86a                           | 18.72a                               | 434.58a                                 | 97.17a                      | 82.02a                      | 24.74a               | 7386.78a                     |
|      | Seedling age 30d HD  | 9.72a                           | 15.92b                               | 414.08b                                 | 94.06a                      | 81.82a                      | 24.78a               | 6665.83b                     |
|      | Seedling age 35d HD  | 9.94a                           | 13.71c                               | 293.29c                                 | 93.07a                      | 80.57b                      | 24.88a               | 5116.92c                     |
|      | Normal density HD  | 10.15a                          | 16.74a                               | 349.94a                                 | 97.80a                      | 82.06a                      | 24.71a               | 6181.71a                     |
|      | High density HD  | 9.53a                           | 15.49a                               | 411.36b                                 | 91.73b                      | 80.88a                      | 24.89a               | 5597.98b                     |
| 2014 | 25 d ND   | HD                   | 12.50a                               | 25.20a                                 | 448.74a                                 | 112.61a                     | 95.10a               | 24.79a                 | 9434.71a                     |
|      |           | HD                   | 12.00a                               | 24.60a                                 | 490.76b                                 | 106.36b                     | 96.02a               | 24.33a                 | 9694.85a                     |
|      | 30 d ND   | HD                   | 12.20a                               | 24.90a                                 | 427.66a                                 | 110.50a                     | 95.49a               | 24.83a                 | 8874.44a                     |
|      |           | HD                   | 11.30a                               | 20.90b                                 | 483.06b                                 | 103.00b                     | 94.44a               | 25.16a                 | 9554.78b                     |
|      | 35 d ND   | HD                   | 9.75a                                | 12.75a                                 | 304.70a                                 | 101.00a                     | 94.97a               | 24.40a                 | 5712.86a                     |
|      |           | HD                   | 7.92b                                | 11.02b                                 | 380.50b                                 | 92.04b                      | 95.25a               | 25.09a                 | 6399.66b                     |
|      | Seedling age 25d HD  | 12.25a                          | 24.90a                               | 469.75a                                 | 109.46a                     | 95.56a                      | 24.56a               | 9564.78a                     |
|      | Seedling age 30d HD  | 11.75a                          | 22.90b                               | 455.36b                                 | 106.75a                     | 94.97a                      | 25.00a               | 9214.61b                     |
|      | Seedling age 35d HD  | 8.84b                           | 11.89c                               | 342.60c                                 | 96.52b                      | 95.11a                      | 24.75a               | 6056.26c                     |
|      | Normal density HD  | 11.48a                          | 20.95a                               | 393.70a                                 | 108.04a                     | 95.19a                      | 24.67a               | 8004.00a                     |
|      | High density HD  | 10.41b                          | 18.84b                               | 451.44b                                 | 100.45b                     | 95.24a                      | 24.86a               | 8549.76b                     |

Table 4. Agronomic traits and grain yield of rice with different seedling age at normal and high cultivation density. Values followed by different letters represent significant difference at $p < 0.05$. ND = normal density, HD = high density.

Effects of cultivation densities on the utilization efficiency of pre-anthesis dry matter stored in vegetal organs and post-anthesis photoassimilates of mechanically transplanted rice with different seedling ages. For rice that experienced delayed transplantation, we observed the amount of above-ground dry matter accumulation after transplantation substantially decreased due to inhibited photosynthetic ability compared with rice transplanted on time\textsuperscript{6,19}. We also observed that the above-ground dry matter weight of mechanically transplanted rice from heading to maturity decreased with an increase in seedling age. Furthermore, our study further verified that the amount of pre-anthesis dry matter remobilization, efficiency and contribution to grain yield, as well as the amount of post-anthesis photosynthesis, were reduced significantly with increasing rice seedling age. One possible reason for this phenomenon is the shortened vegetative growth of delayed mechanically transplanted rice, which not only adversely influences pre-anthesis dry matter accumulation but also constrains post-anthesis photosynthesis\textsuperscript{1,6}. In addition, previous work showed that the remobilization efficiency and contribution to grain yield of dry matter stored in the leaf, stem and sheath before the rice heading stage reduced significantly with increasing seedling numbers per hill\textsuperscript{15}. Our results indicated that increasing cultivation density significantly reduced the amount of pre-anthesis dry matter remobilization, efficiency and contribution to grain yield, and the amount of post-anthesis photosynthesis. With increasing seedling age and cultivation density, both the amounts of pre-anthesis dry matter remobilization and post-anthesis photosynthesis decreased. Nonetheless, it is notable that while the pre-anthesis dry matter contribution to grain yield decreased, the post-anthesis photosynthesis contribution to grain yield increased. We infer that the amount
of pre-anthesis dry matter remobilization decreased more than that of post-anthesis photosynthesis, thereby resulting in a reduced pre-anthesis dry matter contribution to grain yield and an increased post-anthesis photosynthesis contribution to grain yield.

**Effects of cultivation densities on grain yield and yield components of mechanically transplanted rice with different seedling ages.** Previous studies demonstrated that delaying transplantation triggered a decrease in rice grain yield due to a marked reduction in the number of effective panicles and grain number per panicle.\(^1\) In the present study, we observed that with the enhancement of seedling age, rice grain yield and sink capacity per tiller pronouncedly declined because of the significant decreases in the number of effective panicles per square metre and the number of grains per panicle. This could be attributed to inhibited tiller occurrence and shortened vegetative growth for delayed transplanted rice.\(^1\) It is notable that increasing cultivation density significantly enhanced grain yield for rice with 30- and 35-day seedling ages, but it showed a nonsignificant influence on that of rice with a 25-day seedling age. Despite a reduced grain number per panicle, the increase of cultivation density could effectively compensate for rice grain yield loss resulting from delayed transplantation. Additionally, we found that with increasing seedling age or cultivation density, the significant reduction in grain number per panicle was primarily attributable to the marked decrease in the number of secondary branches per panicle (Table 4).

**Agronomic channels for compensating for the grain yield loss of mechanically transplanted rice resulting from delaying transplantation.** Considering the detrimental effect on rice grain yield due to delaying transplantation, researchers have made many attempts to improve the grain yield of mechanically transplanted rice.\(^1\) Hu et al. and Liu et al. confirmed that a moderate increase in nitrogen top-dressing at the rice panicle initiation stage could mitigate the adverse effect and offset partial grain yield losses caused by delaying
transplantation\textsuperscript{9,23}. Since the significant reduction in grain yield was primarily attributed to the decrease in the number of effective panicles for delayed transplanted rice, enhancing cultivation density might minimize the decrease in magnitude of the number of effective panicles and compensate for grain yield loss. However, this still lacks evidence because related reports are limited, especially for mechanically transplanted rice. Our study confirmed that for rice that experienced delayed transplantation, increasing the cultivation density (enhancing the seedling number per hill from 3 to 6) significantly enhanced the number of effective panicles despite a marginal decrease in the total number of grains per panicle, thereby compensating for grain yield loss. This result indicates that a rational enhancement of cultivation density could effectively expand a rice population and improve grain yield, particularly for rice plants that received inhibiting effects because of delayed transplantation.

Apart from the number of effective panicles, sink capacity per tiller (determined by the number of total grains per panicle and one thousand grain weight) is also an important factor determining rice grain yield\textsuperscript{24,25}. Our study indicated that the increase of cultivation density enhanced the number of effective panicles but decreased sink capacity per tiller. Therefore, increasing sink capacity per tiller is essential for further improvement of grain yield with delayed transplantation. As a rule, the carbohydrate supply for grain filling mainly relies on two resources: (1) dry matter stored in vegetative organs before heading stage, and (2) newly created photosynthesis from heading to maturity stage, during which the contribution of the former to final grain yield accounts for approximately 30\textpercent\textsuperscript{26–30}. Currently, increasing evidence has demonstrated that non-structural carbohydrates, the main source of the dry matter stored in vegetative organs, not only function as an assimilate supply for grain filling but also play a critical role in enhancing sink activity during rice grain filling\textsuperscript{31,32}. This fully embodies the importance of dry matter accumulated in vegetative organs before heading for determining sink capacity and final grain yield\textsuperscript{33,34}.

In the present study, we further found that in regard to mechanical-transplanted rice that experienced delayed transplantation, enhancing the amount of pre-anthesis dry matter mobilization and the contribution to grain

Figure 5. Relationships between sink capacity per tiller and utilization efficiency of pre-anthesis dry matter and post-anthesis photosynthesis in rice with different seedling ages.
yield was an effective channel to increase sink capacity per tiller regardless of cultivation density. As a result, agronomic measurements such as irrigation and fertilizer managements should be utilized to promote pre-anthesis dry matter accumulation and remobilization. Related research still needs further exploration and will be of great significance for guiding rice production.

Materials and Methods

Plant materials and experimental design. The field experiments were carried out at Yutai (35°00’N, 116°39’E), Shandong, China, during rice growth stages (from May to October) in 2013 and 2014. The site belongs to typical wheat-rice rotation cropping district in China. The daily average temperature and precipitation during rice growth season of both years were shown in Fig. 1. The means of daily average temperature in 2013 was 1.28 °C higher than that in 2014, particularly the means from the 83rd day to the 100th day after sowing. The precipitation in 2013 was 42.01 mm, which was 39.46 mm higher than that in 2014. A locally cultivated japonica rice cultivar Shengdao19, which had been bred by Shandong Rice Research Institute, was employed as experimental material. The experiment was arranged as a completely randomized split-plot design with three replications. Rice plants with three differed seedling ages (representing the days from sowing to transplantation) were assigned as the main plot, with two cultivation densities being randomly allotted to sub-plot. Three seedling ages were 25d, 30d and 35d, respectively. Planting 3 seedlings per hill and 6 seedlings per hill were regarded as two different cultivation densities (normal density and high density). To keep independent irrigation and fertilization managements for rice plants with three seedling ages, the main plots were separated by 50-cm-wide ridges with plastic film inserted into soil at a depth of 30 cm.

Cultivation management. For all treatments, rice seeds were uniformly sown on 23 May under mechanical transplantation pattern. Rice plants were transplanted on 17 June, 22 June and 27 June, respectively, by a transplanter (KURATO, SPU-60) made in Japan. The distances between rows and hills were 30 cm and 12 cm, respectively, with three seedlings per hill and 6 seedlings per hill as normal cultivation density and high cultivation density. The size of each sub-plot was 30 m². Fertilization and other agronomic managements were implemented according to local rice cultivation practices. The total N application amount was 270 kg ha⁻¹ with an N, P and K application rate of 2:1:1.

Sampling and determination. The tiller numbers of 20 fixed hills from each plot were counted from the 6th day to the 50th day after transplantation at an internal of 3 days. Rice growth stages of each plot were recorded timely. Representative plants of 6 hills from each plot were sampled at rice heading and maturity stages. The above-ground parts of these plants were oven-dried at 105 °C for 0.5 h and divided into three segments, including leaf blades, stems and sheaths and panicles. Subsequently, these samples were oven-dried at 80 °C until constant weight and weighed to acquire dry matter weights, respectively. At maturity, rice plants of 10 m² from each plot were harvested excluding border plants to determine grain yield with moisture content 14%. Thirty Representative hills of rice plants from each plot were sampled to determine the number of effective panicles, total and filled grains per panicle and 1000-grain weight.

Data analysis. Based on the parameters mentioned above, the amount of pre-anthesis dry matter remobilization and efficiency, the dry matter contribution to grain yield, the amount of post-anthesis photosynthesis, the post-anthesis photosynthesis contribution to grain yield, and sink capacity per tiller were calculated using the following equations:

\[
\text{Pre-anthesis dry matter remobilization amount (g)} = \text{Dry matter weight of the leaf or stem and sheath per tiller at heading} - \text{Dry matter weight of the leaf or stem and sheath per tiller at maturity}.
\]

\[
\text{Pre-anthesis dry matter remobilization efficiency (%) = 100 × dry matter remobilization amount/dry matter weight per tiller at heading.}
\]

\[
\text{Pre-anthesis dry matter contribution to grain yield (%) = 100 × dry matter remobilization amount/dry weight of the grains per tiller at maturity - dry weight of the grains per tiller at heading).}
\]

\[
\text{Post-anthesis photosynthesis amount (g) = Dry matter weight of the above-ground per tiller at maturity - Dry matter weight of the above-ground per tiller at heading.}
\]

\[
\text{Post-anthesis photosynthesis contribution to grain yield (%) = (Dry matter weight of the above-ground per tiller at maturity - Dry matter weight of the above-ground per tiller at heading)/(dry weight of the grains per tiller at maturity - dry weight of the grains per tiller at heading).}
\]

\[
\text{Sink capacity per tiller = (Number of total grains per panicle × one thousand grain weight)/1000.}
\]

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**Author Contributions**

Q.L. and X.Z. designed the research. Q.L. and C.X. performed experiments. Q.L. and J.L. analyzed the data. Q.L. prepared and wrote the paper.

**Additional Information**

**Competing Interests:** The authors declare that they have no competing interests.

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