

HIGH TRANSPLANT DENSITY CAUSE LOSS YIELD AND QUALITY DECREMENT BY AFFECTING PHOTOSYNTHESIS, DRY MATTER ACCUMULATION AND TRANSPORTATION IN SUPER RICE

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Abstract. Transplant density is an important factor which has impacts on rice growth and development in transplanted rice production system. Present study used three transplant densities in paddy field experiment and set as D1 (transplant spacing of 20 cm × 24 cm, about 2.085 hundred thousand hills for each hectare), D2 (transplant spacing of 20 cm × 20 cm, about 2.603 hundred thousand hills for each hectare) and D3 (transplant spacing of 20 cm × 16 cm, about 3.120 hundred thousand hills for each hectare). The results showed that high density (D3) not only reduced the net photosynthetic rate and *LAI* values at heading stage, but also decreased dry matter accumulation and transportation compared to D1 density. The highest yield was recorded in D1 density and the lowest yield was recorded in D3 density. Study also revealed that high density caused yield loss by decreasing the grain number and seed-setting rate. Furthermore, high density also increased chalk rice rate and chalkiness while reducing the amylose content. The activity of sucrose synthase (SS) in D3 was significantly lower than D1 and it might relate to the reduction of grain quality.

Keyword: rice, density, yield, quality, net photosynthetic rate, dry matter accumulation and transportation

Introduction

Rice (*Oryza sativa* L.) is a main crop which has been cultivated in China, its production plays an important role in Chinese food security. In recent years, because the area of arable lands is in rapid decline, how to maintain the rice productivity and promote the yield potential had been the main research direction for many experts in last decade (Tao et al., 2019; Tang et al., 2019). There are a lot of factors that could affect grain yield and quality of rice. For example, the transplant density is a key part which affects grain yield and quality significantly in rice production.

Rational close planting is one of the important strategies for high yield and good quality cultivation of rice. A suitable transplanted density not only could give full play to the strong tillering and self-regulation ability of rice population, reduce the competition of inter-row and inter-plant growth in rice population, and avoid the overgrowth of early growth, but also ensure that the rice field can make full use of light energy, carry out photosynthesis, accumulate more organic matter, thereby increasing

rice yield (Zhou et al., 2018; Huang et al., 2018; Tareq et al., 2018). In 2001, Yuan demonstrated that low plant density enlarged the growth space of above-ground and below-ground parts of rice, enlarges individual strength and panicle size, thus gaining high yield. The study of Hayashi et al. (2006) revealed that over-dense planting strengthens the competition of photosynthetic nutrients among rice individuals and promotes early flowering of rice. Furthermore, Mobasser et al. (2009) indicated that low density treatment could increase the conversion rate and output rate of stem and sheath substances and finally increased rice yield.

High yield and good quality of rice are the major goals in rice production and in transplanted rice production system, suitable transplant density is an important part to gain those two goals. Thus, we explored the process of photosynthesis, dry matter accumulation and transportation in order to study the response of super rice yield and quality under high transplant density in present study.

Materials and Methods

Plant materials and growing condition

A super hybrid rice variety, *Huahang31*, a well-known and widely grown in South China, were in planted during the late season (July-November) in 2010 and the early season (March-July) in 2011 at the College of Agriculture's Experimental Farm, South China Agricultural University (SCAU), Guangzhou, Guangdong Province, China (23°16' N, 113°23' E, elevation 11 m). The air temperature was shown in *Figure 1*.

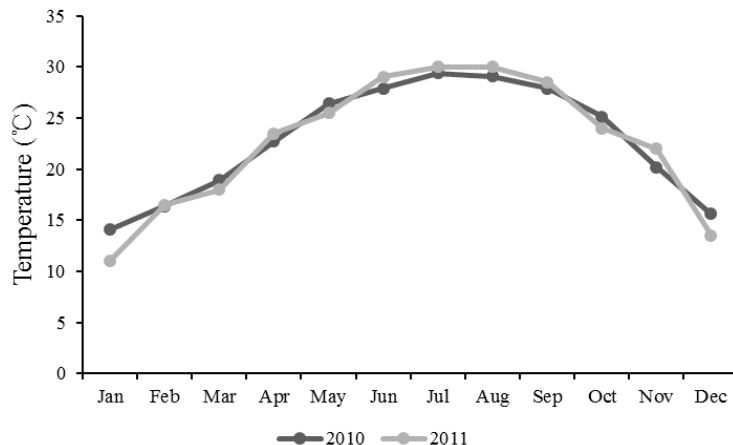


Figure 1. Mean monthly air temperature in Guangzhou during 2010 and 2011 (Annual average temperature in 2010 was 22.81°C and in 2011 was 22.63°C)

Before sowing, the seeds were soaked in water for 24 h, germinated in manual climatic box for the next 24 h, shade dried and the germinated seeds were sown in PVC trays for nursery raising. 15-day-old seedlings and 25-day-old seedlings were transplanted to the field in 2010 and 2011, respectively. The experimental soil has been planted rice for many years and it was sandy loam with of 16.80 g kg⁻¹ organic matter content, 1.032 g kg⁻¹ total N, 0.998 g kg⁻¹ total P, 13.598 g kg⁻¹ total K, 84.60 mg kg⁻¹ available N, 43.58 mg kg⁻¹ available P, and 106.10 mg kg⁻¹ available K.

Treatments descriptions

Three transplanted densities were applied at the experiment and set as blow.

- D1: Seedlings were transplanted at the rate of 2 seedlings per hill at a spacing of 20cm × 24cm, about 0.2085 million hills per hectare.
- D2: Seedlings were transplanted at the rate of 2 seedlings per hill at a spacing of 20cm × 20cm, about 0.2505 million hills per hectare.
- D3: Seedlings were transplanted at the rate of 2 seedlings per hill at a spacing of 20cm × 16cm, about 0.3120 million hills per hectare.

Commercial compound fertilizer (Total nitrogen contents TN = 15%, K₂O contents = 12%, P₂O₅ contents = 7.2%) was applied at the same amount of 1500 kg ha⁻¹. 80% of fertilizer was applied as basal and 20% was top-dressed at tillering stage.

Plant sampling and determination of biomass accumulation

At tillering stage, heading stage and maturity, the rice plants were harvested from fifteen hills in each plot. The leave, stems and grain were separated from the plants and dried Under the condition of 80°C respectively in order to get the estimation of dry matter. The output rate of stem matter (OPRS) was calculated as:

$$\text{OPRS} = \frac{(\text{stem weight at heading stage} - \text{stem weight at maturity})}{\text{stem weight at heading stage}} \quad (\text{Eq.1})$$

The conversion rate of stem matter (CRS) was calculated as:

$$\text{CRS} = \frac{(\text{stem weight at heading stage} - \text{stem weight at maturity})}{\text{grain weight at maturity}} \quad (\text{Eq.2})$$

The fresh grains were separated and collected from the rice plants 7th day after heading stage, washed with double distilled water and stored Under the condition of -80°C for the estimation of sucrose synthase (SS), soluble starch synthase (SSS) and Bound starch synthase (GBSS).

Determination of photosynthesis, SPAD and Leaf area index (LAI)

Portable photosynthesis system (LI-6400, LI-COR, USA) was used to determine net photosynthetic rate at 09:00–10:30 a.m. according to the standard method (Pan et al., 2016). SPAD meter ‘SPAD-502’ (Konica Minolta, Japan) was used for precise, rapid and non-destructive estimation of leaf chlorophyll contents. At tillering stage, heading stage and maturity, the rice plants were harvested from ten hills in each plot. The leaves were separated from main plants and the green leaves area was measured by a leaf area meter (CID-202, Lincoln, NE, USA) to determine the leaf area index (LAI).

Determination of activities of sucrose synthase (SS)

The activity of SS was measured according to the method described by Li et al. (2008). The activity of SS was determinate in both saturating and selective (limiting) conditions. The limiting assay consisted of the same reaction mixture except that 10 mmol L⁻¹ Pi was added and the concentrations of uridine diphosphate glucose

(UDPG), fructose, and glucose-6-P were reduced to 2, 2 and 10 mmol L⁻¹. The reaction was terminated by using 1 mol L⁻¹ NaOH and the mixture was boiled to degrade any unreacted fructose-6-P. After cooling, 0.20 mL of 0.1% (w/v) resorcinol in 95% ethanol and 0.80 mL of 9 mmol L⁻¹ HCl were added and the tubes were incubated at 80°C for 10 min. Three separate extractions of grain powder were assayed for the activities of enzymes. The activities of SS were shown as specific activities (g⁻¹ FW h⁻¹).

Determination of activities of soluble starch synthase (SSS) and granule-bound starch synthase (GBSS)

The activity of SSS and GBSS was determined by Nakamura and Yuki (1992). Twenty shelled grains of the same part were accurately weighed and placed in a pre-cooled mortar. The supernatant was extracted with 4 ml extract (containing 100 mmol L⁻¹ Ph7.5 Hepes-NaOH buffer, 8 mmol L⁻¹ MgCl₂ 6H₂O, 2 mmol L⁻¹ EDTA-Na₂, 12.5% (v/v) glycerol, 1% (w/v) PVP-40, 50 mmol L⁻¹ mercaptoethanol) by grinding in an ice bath. The supernatant was extracted by centrifugation at 12000 G for 30 min.

SSS activity determination: 1.5 ml centrifuge tube was taken and 20 ml crude enzyme solution was added in turn into 20 ml crude enzyme liquid, 36 µl reaction liquid A (containing 50 mmol L⁻¹ pH7.4 Hepes-NaOH, Hepes-NaOH, 1.6 mmol L⁻¹ ADPG, 0.7 mg amylose and 15 mmol L⁻¹ DTT) was reacted at 30°C for 20 minutes and then boiling water for 30 s to stopped reaction and ice bath cooled; 20 µl reaction liquid B (containing 50 mmol L⁻¹ pH7.4 Hepes-NaOH, 4 mmol L⁻¹ mmol L⁻¹ MgCl₂6H₂O and 1.2 unit pyruvate kinase reacted at 30°C for 20 minutes, then boiling water terminated the reaction for 30 seconds and centrifuged for 10000 g for 10 minutes. The supernatant was extracted at 60 µl and added to 43 µl reaction liquid C (containing 50 mmol L⁻¹ pH7.4 Hepes-NaOH, 10 mmol L⁻¹ glucose, 20 mmol L⁻¹ MgCl₂6H₂O, 2 mmol L⁻¹ NADP, 1.4 unit hexokinase and 0.35 unit glucose-6-phosphate dehydrogenase) for 10 minutes. The absorbance at 340 nm was determined after reaction at 30°C.

GBSS activity determination: The subsoil of SSS crude extract was washed twice with 1 ml extract and centrifuged for 20 minutes at 4°C for 3000 g. Then the crude enzyme solution of GBSS was obtained by centrifuging the 3 ml extract at 4°C for 24 hours and 12000 g at 2°C for 30 minutes. A 1.5 ml centrifugal tube was used, 20 µl GBSS crude enzyme was added in turn, 36 µl reaction liquid A (containing 50 mmol L⁻¹ pH7.4 Hepes-NaOH, 1.6 mmol L⁻¹ ADPG, 15 mmol L⁻¹ DTT), the other two reaction liquids and reaction process were the same as SSS determination.

Estimation of rice yield and related attributes

At maturity stage, the rice grains were harvested from ten unit sampling area (10.00 m²) in each plot and then threshed by machine. The harvested grains were sundried and weighed in order to determinate the grain yield. Twenty hills of rice from different locations in each plot were sampled for estimating the average effective panicles number per hill. Then, three hills representative plants were taken for estimation of the yield related traits.

Estimation of grain quality

Dry grains were stored at room temperature for two months to determine grain quality attributes. About 1.00 kg rice grains from each treatment was taken from storage and brown rice rate was determined by rice huller (Jiangsu, China) while brown rice and

head rice rates were calculated by using a Jingmi testing rice grader (Zhejiang, China). Grains with chalkiness and chalkiness degree were determined using an SDE-A light box (Guangzhou, China) while an Infratec-1241 grain analyzer (FOSS-TECATOR) was used to measure the grain amylose and protein contents.

Statistical analysis

Data were analyzed by using statistical software ‘Statistix 8.1’ (Analytical Software, Tallahassee, FL, USA) while differences amongst means were separated by using least significant difference (LSD) test at 5% probability level. Graphical representation was conducted via Sigma Plot 14.0 (Systat Software Inc., California, USA).

Result

Yield and yield components

As shown in Table 1, transplant densities affected rice yield and its components differently. High density could significantly increase panicle number and the highest panicle number was recorded in D3 in both years. However, we observed that the lowest yield was recorded in D3 for 2010 and 2011 and the yield in D3 was significantly lower than D1. The decrement of yield was due to the reduction in both grains number and seed setting rate. Compared to D1, D3 treatment remarkably reduced seed setting rate and grain number. Moreover, there was no significant difference among D1, D2 and D3 in 1000-grain weight.

Table 1. Effect of transplant densities on rice yield and its components

Year	Transplant density	Panicle number 10 ⁴ hm ⁻²	Grain number per panicle	Seed setting rate (%)	1000-grain weight (g)	Yield (t ha ⁻²)
2010	D1	270.83b	132.33a	88.56a	22.99a	7.30a
	D2	271.67b	133.67a	88.18a	23.37a	6.92b
	D3	295.14a	120.00b	87.90b	23.26a	7.02b
2011	D1	230.67c	153.33a	91.25a	23.43a	7.70a
	D2	273.33b	138.00b	88.18b	23.49a	7.55a
	D3	298.00a	117.67c	88.57b	23.29a	7.12b
Analysis of variance						
Density (D)		**	**	ns	ns	ns
Year (Y)		ns	ns	*	**	*
D × Y		ns	ns	ns	*	ns

Data are expressed as the mean of three replications. Values followed by different letters within the same column are significant different at $P < 0.05$ probability level. NS means non-significant differences; * and ** indicated significant differences at 0.05 and 0.01 probability levels, respectively. The same as belowed

Grain quality and its attributes

As shown in Table 2, there was no significant difference among D1, D2 and D3 in both brown rice rate and head rice rate. However, compared to D1 treatment, D2 and D3 treatments significant increased chalky rice rate in 2010 and 2011. D3 treatment also

increased chalkiness remarkably for both years. Furthermore, the lower amylose content was recorded in D3 than D1 for 2010 and 2011.

Table 2. Effect of transplant densities on grain quality and its attributes

Year	Transplant density	Brown rice rate (%)	Head rice rate (%)	Chalky rice rate (%)	Chalkiness (%)	Amylose content (%)
2010	D1	81.72a	44.64a	4.00b	2.13b	18.07a
	D2	81.55a	43.64a	5.33a	3.35a	18.10a
	D3	81.82a	45.80a	5.67a	3.6a	17.77b
2011	D1	80.67a	41.99a	8.00b	5.10b	18.00a
	D2	80.57a	40.65a	10.67a	5.33ab	18.00a
	D3	80.80a	42.67a	11.33a	5.70a	17.80b
Analysis of variance						
	Density (D)	**	ns	*	*	*
	Year (Y)	**	**	**	*	ns
	D × Y	ns	ns	*	**	ns

Net photosynthetic rate, LAI and SPAD values

The difference in transplant density could significantly affect rice photosynthesis (Figure 2). At tillering stage, heading stage and maturity in 2010, the net photosynthetic rate in D3 was significantly lower than D1 whilst in 2011, only at heading stage the net photosynthetic rate in D3 was remarkably lower than D1. Compared to D1, D3 treatment significantly decreased LAI values at heading stage for both years. Furthermore, we observed that the SPAD values in D2 and D3 were remarkably lower than D1 at heading stage and maturity in 2010. However, in 2011, there was no significant difference among D1, D2 and D3 in all growth stages.

Dry matter accumulation and transportation

As shown in Table 3, different densities affected dry matter weight differently. At tillering stage, D3 density significantly decreased leaf weight and stem weight compared to D1 in 2010. The leaf weight, stem weight and grain weight at both heading stage and maturity in D3 were remarkably lower than D1 for 2010 and 2011. Furthermore, we observed that high density (D3) had negative impact on transportation of stem matter while the lowest OPRS and CRS were also recorded in D3 treatments for both years while there was no significant difference between D1 and D2.

Enzymes involved sucrose biosynthesis

As shown in Figure 3, different densities affected sucrose biosynthesis in grain in terms of SS, SSS and GBSS activities. For SS activity, D3 and D2 had lower acidity than D1 in 2010 while the trend in 2011 was recorded as: D1 > D2 > D3. Compared to D1, D3 treatment also significant decreased the GBSS and SSS activity in 2010 whilst in 2011, there was no significant difference among D1, D2 and D3 in both GBSS and SSS activity.

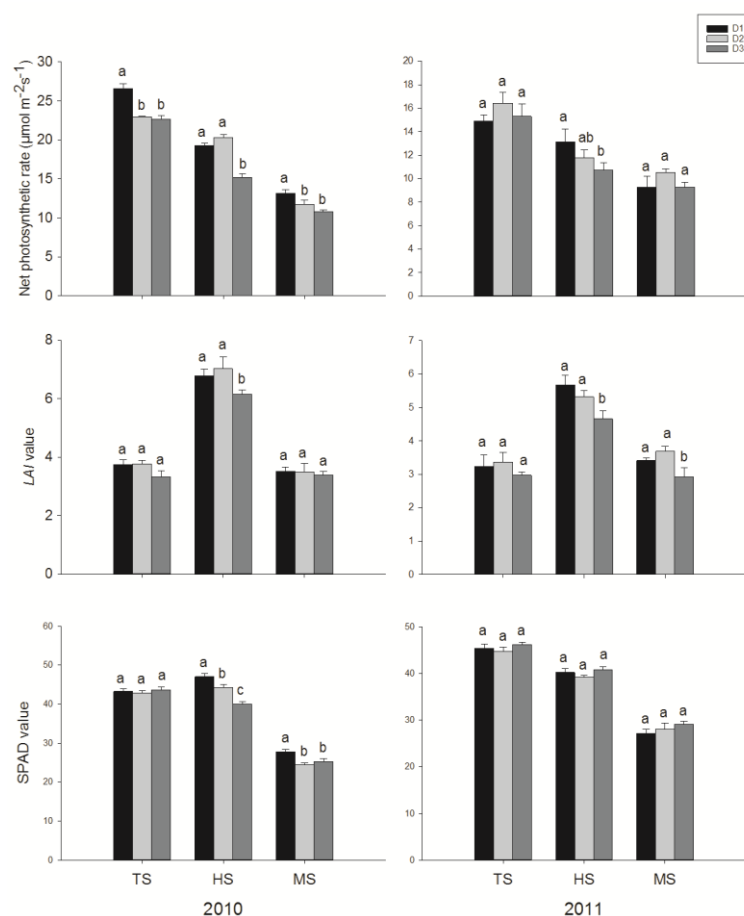


Figure 2. Effect of transplant densities on net photosynthetic rate, LAI and SPAD values. Capped bars represent S.E. of three replicates. Means sharing a common letter don't differ significantly at ($P \leq 0.05$) according to least significant difference (LSD) test for both the years. The same as below

Table 3. Effect of transplant densities on dry matter accumulation and transportation

Year	Transplant density	TS		HS			MS			OPRS (%)	CRS (%)
		Leaf weight (g hill ⁻¹)	Stem weight (g hill ⁻¹)	Leaf weight (g hill ⁻¹)	Stem weight (g hill ⁻¹)	Grain weight (g hill ⁻¹)	Leaf weight (g hill ⁻¹)	Stem weight (g hill ⁻¹)	Grain weight (g hill ⁻¹)		
2010	D1	7.74a	6.16a	14.07a	29.69a	9.02a	10.69a	24.36a	36.10a	17.95a	19.68a
	D2	6.38b	5.73a	13.11a	25.25b	6.62b	9.07b	19.27b	33.08a	23.68a	22.60a
	D3	4.97c	4.77b	8.50b	15.95c	4.71c	7.43c	15.62b	29.31b	2.07b	1.34b
2011	D1	7.22a	6.16a	11.85a	22.02ab	18.49a	11.85a	18.74a	47.66a	14.90a	11.24a
	D2	6.90a	5.42a	12.21a	23.15a	17.88ab	12.21a	19.94a	53.52a	13.87a	10.01a
	D3	6.59a	6.34a	9.46b	18.64b	12.45b	9.46b	16.51b	38.25b	11.43b	8.26b
Analysis of variance											
Density (D)		**	ns	**	**	**	**	**	*	**	**
Year (Y)		ns	ns	ns	ns	**	ns	ns	**	**	**
D × Y		ns	ns	ns	*	ns	ns	ns	**	**	**

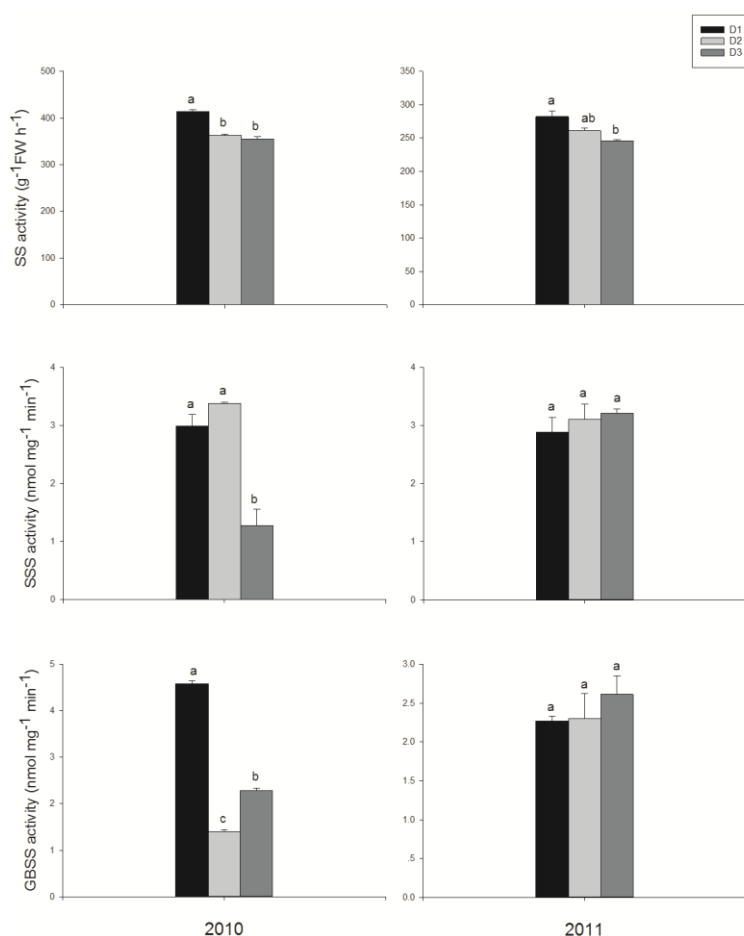


Figure 3. Effect of transplant densities on SS, SSS and GBSS activity

Discussion

A suitable plant density is one of the decisive factors for harvesting higher grain yield in rice production (Peng et al., 2018; Lee et al., 2018). Normally, the transplant density determines the competition of nutrients such as photosynthesis and fertilizer uptake among rice individuals in the middle and late growth stages among different rice individuals. In our study, we observed that compared to D1, D2 density significantly decreased rice yield in 2010 but there was no difference between D1 and D2. The D3 density remarkably reduced yield by decreasing the grain number and seed setting rate in both years. Present study showed that high density was able to significantly increase panicle number in paddy field, but it would also have negative impacts on other yield components and then still cause the yield loss without yield increment. Similar result was also reported by Zhao (2011) who demonstrated that the yield of super rice, *Y-liangyou 302*, was mainly affected by panicles and spikelets per panicle, while the spike length and 1000-grain weight did not show any significant influence on yield and high nitrogen and low planting density is conducive to increase the yield of rice variety *Y-liangyou 302* in north region of Guangxi. The further reason in reduction of yield might be the increment in competition for photosynthesis among rice individuals under high density. Present study showed that D3 treatment not only decreased the net

photosynthetic rate, but also reduced the *LAI* values. Leaf area index (*LAI*) is an important parameter in crop growth status monitoring and yield forecasting (Xue and Wang, 2004). Normally, the values of *LAI* and net photosynthetic rate represent the intensity of population photosynthesis. In our study, the result indicated that the lower population photosynthesis under high density induced the decrement in dry matter accumulation and inadequate supply of dry matter eventually led to a decline in production. Our finding was also consistent with the research of Sultana et al. (2001) who indicated that dry matter accumulation was an important factor in determining rice yield.

Furthermore, worse grain quality was recorded in D3 treatment than D1. Present study revealed that not only D2 and D3 densities would increase both chalky rice rate and chalkiness, but also D3 density would significantly decreased amylose content. The increment in chalkiness and chalky rice rate might be due to the decrement of net photosynthetic rate at heading stage and the matter transportation from stem to grain. The study of Mo et al. (2015) showed that photosynthesis during the filling stage might had a great impact on grain chalkiness of rice. In 2015, the study of Zhou et al. (2015) found that there was a positive correlation among low amylose content, high degree of endosperm chalkiness and percentage of grains with chalkiness in grain and similar conditions were also observed in our study. The lower amylose content in D3 than D1 could be explained by the reduction in SS activity. Moreover, lower OPRS, CRS and net photosynthetic rate in heading stage also could be responsible for amylose content decrement.

Conclusion

Present study showed that high transplanted reduced the rice photosynthesisism decreased the dry matter accumulation and transportation and finally caused the loss of grain yield. For revealing the mechanism of rice yield and quality formation by transplant density treatments, much work should be done at molecular and physiological level at the field trials.

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APPENDIX



Figure A1. *Photo of the experiment*