

DIFFERENT SEEDLING RAISING METHODS AFFECT CHARACTERISTICS OF MACHINE-TRANSPLANTED RICE SEEDLINGS

CHENG, S. R.^{1,2†} – ASHRAF, U.^{1,3†} – ZHANG, T. T.^{1,2†} – MO, Z. W.^{1,2} – KONG, L. L.¹ – MAI, Y. X.¹ – HUANG, H. L.¹ – TANG, X. R.^{1,2*}

¹*Department of Crop Science and Technology, College of Agriculture, South China Agricultural University, Guangzhou, Guangdong 510642, China*

²*Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture of the P. R. China, Guangzhou, Guangdong 510642, China*

³*Department of Agronomy, University of Agriculture Faisalabad 38040, Pakistan*

[†]*These authors have contributed equally to this work.*

**Corresponding author*

e-mail: tangxr@scau.edu.cn; phone/fax: +86-20-8528-0204-618

(Received 4th Oct 2017; accepted 12th Feb 2018)

Abstract. Present study was aimed to select the suitable seedling raising method with high seedling quality for the machine-transplanted rice. The seeds of two rice cultivars i.e., *Xiangyaxiangzhan* and *Wufengyou 615* were grown under four different seedling-raising methods i.e., the traditional seedling-raising method (TWSM), the dry seedbed nursery raising method (DSBM), the wet seedbed raising method (WSBM), and the substrate seedbed nursery raising method (SSBM). Results showed that the highest seed germination and seedling establishment rates were recorded in SSBM followed by DSBM, and TWSM. Rice seedlings grown in SSBM have maximum seedling length, longer leaves and leaf sheath as well as dry biomass than other establishment methods. Further, number of roots, root length, root surface area and root diameter were also found significantly ($P \leq 0.05$) higher than those seedlings which were established by normal farmer practice (TWSM). Additionally, higher proline, protein and soluble sugars contents and SPAD values (an indicator of chlorophyll contents) were also recorded in SSBM. Hence, SSBM would result in improved rice seedlings, compared to those under traditional nursery, dry bed nurse and wet bed nursery.

Keywords: *physiological character, root growth, rice nursery, seed germination, seedling-raising methods*

Introduction

Rice (*Oryza sativa*) is the most important cereal crop that feeds more than half of the world's population (Ruiz-Sánchez et al., 2010; Abid et al., 2015; Kargbo et al., 2016). Rice ranks first among the cereal crops in China and it has been cultivated for more than 7000 years ago, feeding more than 65% of the population of China as a staple food (Ivanic and Martin, 2008). In China, rice covers an area of 30.14 million hectare with an annual production of 204.24 million tons. Hence, rice production in China contributes to food security not only for China but also for the rest of the world (Yao et al., 2007; Ashraf et al., 2015).

Seedling transplanting has many advantages over direct seeding; however, manual transplantation of rice nursery is time consuming and slow process (Ehsanullah et al., 2014) as compared with mechanical transplantation. The

development of modern, large-scale, professional and commercial production of rice through mechanized planting plays a critical role to ensure efficient transplanting and maintaining rice productivity (Zhu et al., 2007). Presently, mechanized rice transplanting technology has been successfully adopted in Europe, America, Japan and many other developed countries, whilst in China, the mechanized transplanting is still not widely adopted. Recently, a report stated that mechanized rice transplantation was about 34% at the end of 2014 in China (Yang et al., 2003; Li, 2015).

Healthy and well-nourished seedlings with uniform growth (grown on PVC trays to load to the machine for transplanting) are the prerequisites for uniform transplantation in the field and these seedlings must meet certain technical standards in the system of mechanical transplanting of rice seedlings (Biswas et al., 2000). To develop standard seedlings, as required by the mechanized transplantation, the compatibility among seedlings cultivation/growing method, machine operational system, quality of the rice seedlings must be considered (Zhang and Gong, 2014). Conventional nursery raising is often practiced in paddy soils which is often silty in nature (Mishra and Salokhe, 2008; Li et al., 2014) which makes the manual transplantation difficult. Thus, rather growing rice nursery in the fields, nursery raising in PVC trays and/or in nursery mat is conducive for machine transplanting. Hence, for mechanized transplanting rice technology, indoor seedling/nursery raising on artificial media can also be practiced other than outdoor nursery (that may be threatened by environmental extremities). For indoor nursery raising, seedling growing medium may largely affect the growth and development of rice seedlings. Present study thus focused to test the effect of different nursery management methods on germination, morphological growth and physiological characters of rice seedlings with the aim to determine the best possible nursery raising method for machine-transplanted rice seedlings.

Materials and methods

Experimental site

The seedlings were raised in green house located at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou, China (23°09' N, 113°22' E and 11 m above the sea level) during 2015. The region has subtropical monsoonal type of climate normally with yearly average temperature ranges ranged from 21 to 29 °C and 70-80% relative humidity and high rainfall during May-July (Li et al., 2016; Mo et al., 2016). Moreover, the average temperature, rainfall, and relative humidity for experimental period was remained 28 °C, 19.17 mm and 79.60%, respectively.

Plant material and treatment description

Seeds of two local and widely cultivated rice cultivars i.e., *Xiangyaxiangzhan* and *Wufengyou 615*, were collected from the College of Agriculture, South China Agricultural University, Guangzhou, China. Prior to sowing, seeds of both rice cultivars were soaked in water for 48 h in dark chamber at room temperature. Seeds were sown in four different methods i.e., (i) traditional seedling-raising method in

which the seeds were directly sowed on the wet seedbed (TWSM) (taken as control), (ii) the dry seedbed raising method in which the seeds were sown in PVC trays covered with air dried soil collected from paddy fields, and applied with distilled water (DSBM), (iii) the wet seedbed raising method in which the seeds were sown in PVC trays covered with well cultivated puddled soil, collected from paddy fields (WSBM), and (iv) the substrate seedbed raising method in which seeds were sown in PVC trays covered with a substrate (agricultural organic waste product including rice husk, obtained from Lianyungang Heroda Fertilizer Technology Co., Ltd, China) and sprayed with distilled water (SSBM). The dimensions of PVC trays used for nursery rising was 58.5 cm × 28.5 cm. The seeding density of both *Xiangyaxiangzhan* and *Wufengyou 615* were 10000-12000 per m².

Determination of seed germination rate and seedling establishment percentage

To determine seed germination rate and seedling establishment percentage, seedlings from an area of 15 cm × 15 cm area from each establishment method were carefully uprooted (about 60 seedlings) at 15 days after sowing (DAS), washed thoroughly with tap water and then with distilled water and the seedlings were graded in to: a) full mature seedlings, and b) young seedlings (one-half of the height of the full mature seedlings). The number of full mature seedlings, young seedlings and non-germinated seeds were counted. The seed germination rate and seedling establishment percentage was recorded as:

Rice germination rate (%) = (number of full mature seedlings + number of young seedlings) / (total germinated and un-germinated seeds) × 100

Percentage of seedling establishment (%) = (Number of full mature seedlings / total number of germinated and un-germinated seeds) × 100

Morpho-physiological characteristics of rice seedlings

The seedlings of both rice cultivars at 10, 15 and 20 DAS were sampled for morpho-physiological parameters while for the estimation of physio-biochemical traits, the seedlings were separated into roots and shoots and stored at -80 °C.

Seedling length, first leaf sheath length, length of first and second leaves was measured with a measuring ruler. Seedling fresh and dry biomass (seedlings were placed in oven at 80 °C till constant weight) per 100 seedlings were recorded by an electric balance (Sartorius, Japan). The number of primary and secondary roots was counted manually whereas root length, root surface area and root diameter of 4 well mature seedlings were recorded by a root scanner (WinRHIZO, ProLA2400, Canada) according to Oršanić et al. (2011).

SPAD values of seeding

SPAD values characterized as chlorophyll contents, were recorded from 10 seedlings of each treatment with a SPAD meter 'SPAD-502' (Konica Minolta, Japan) according Asai et al. (2009) which provided a precise, rapid and non-destructive estimation of leaf chlorophyll contents.

Estimation of proline, protein and soluble sugar

The proline content was determined according to Celik and Atak (2012). Fresh leaf samples (0.3 g) were homogenized with 4 mL of 3% sulfosalicylic acid and heated at 100 °C for 10 min. After cooling, 2 mL of filtrate, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid was taken in a test tube and boiled in water bath at 100 °C for 30 min. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The solution was vortex mixed and 3 mL of the upper toluene extract was centrifuged at 4000 *g* for 5 min. The absorbance was read at 520 nm. Protein content was assayed using the coomassie brilliant blue G-250 (CBB-G) method as described by Aminian et al. (2013) and the absorbance was read at 595 nm. The soluble sugar contents of the rice leaves were estimated by the method of Karim et al. (2012) with minor modifications. Leaves (0.2 g) were ground with liquid nitrogen to a fine powder and then added 15 mL of distilled water to heat at 100 °C for 20 min. After cooling, 0.1 mL of filtrate and 5 mL of enthroned sulfuric acid solution were mixed and then heated at 100 °C for 10 min and the absorbance was recorded at 620 nm.

Experimental design and data analysis

Treatments were arranged according to completely randomized design (CRD) with three replications. The data were analyzed by an analysis of variance (ANOVA) technique with statistical software Statistix 8.0 (Analytical, Tallahassee, Florida, USA) while the differences among means were separated by least significant difference (LSD) test at the 5% significance level. The Sigma Plot 12.5 (Systat Software Inc., San Jose, CA, USA) was used to make the figures.

Results

Germination and seedling establishment

The highest germination rate and the percentage of seedling establishment were observed in SSBM treatment for both cultivars (*Fig. 1*). DSBM and SSBM significantly increased germination rate for *Xiangyaxiangzhan* by 9.69% and 10.53%, respectively, and for *Wufengyou 615* by 3.66% and 3.85%, respectively. However, WSBM treatment obviously reduced germination rate by 4.40% and 3.77% for *Xiangyaxiangzhan* and *Wufengyou 615*, respectively (*Fig. 1 A, B*). Seedling establishment was substantially increased in SSBM by 23.66% and 13.52% for *Xiangyaxiangzhan* and *Wufengyou615*, respectively. For *Xiangyaxiangzhan*, DSBM treatment significantly increased seedling establishment percentage but WSBM treatment show opposite trend. For *Wufengyou 615*, DSBM and WSBM did not affect the seedling establishment percentage significantly (*Fig. 1 C, D*).

Morphological growth

Different seedling establishment methods substantially affected morphological characters of both rice cultivars. For *Xiangyaxiangzhan*, seedling length, length of first leaf sheet, length of first and second leaf as well as dry biomass were recorded maximum in SSBM and the values were 0.40, 22.01, 66.81, 27.93, 45.37% and 14.49, 13.65, 77.37, 28.41, 26.00%, and 5.64, 0.72, 65.96, 19.77, 12.39%, higher than TWSM at 10 15 and 20 DAS, respectively (*Tables 1 and 2*).

Table 1. The seedling length and shoot dry weight of rice seedlings in response to different seedling raising methods

Cultivar	Treatment	Seedling length (cm)			Shoot dry weight (mg)		
		10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS
Xiangyaxiangzhan	TWSM	17.37 a	19.67 ab	21.82 a	10.80b	15.00b	23.40bc
	DSBM	16.52 a	19.76 ab	22.67 a	11.90b	21.00a	21.80c
	WSBM	17.39 a	18.03 b	19.36 b	10.90b	13.40b	25.20ab
	SSBM	17.44 a	22.52 a	23.05 a	15.70a	18.90a	26.30a
Wufengyou 615	TWSM	23.23 a	24.07 a	29.42 a	22.00a	26.90a	47.30a
	DSBM	18.96 b	19.99 b	23.30 c	13.70b	18.00b	33.10b
	WSBM	18.58 b	19.94 b	22.70 c	12.40b	17.00b	32.10b
	SSBM	19.06 b	23.24 a	25.03 b	20.00a	27.00a	47.40a

Different lowercase letters followed by the mean of different treatments in the same column within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

Table 2. Effects of different seedling raising methods on leaf and leaf sheath length

Cultivar	Treatment	First leaf sheath length (cm)			First leaf length (cm)			Second leaf length (cm)		
		10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS
Xiangyaxiangzhan	TWSM	3.18 b	3.59 b	4.19 a	2.35b	2.43b	2.82b	9.20b	9.47c	10.42c
	DSBM	3.62 a	4.08 a	4.13 a	3.23ab	3.33ab	3.68ab	9.47b	11.23ab	11.64ab
	WSBM	3.22 b	3.36 b	3.58 a	3.06ab	3.22b	3.51ab	10.53ab	10.53bc	11.17bc
	SSBM	3.88 a	3.90 ab	4.16 a	3.92a	4.31a	4.68a	11.77a	12.16a	12.48a
Wufengyou 615	TWSM	3.43 a	3.72 a	4.34 a	2.70a	2.88ab	3.57b	7.43b	9.83ab	10.28b
	DSBM	3.33 ab	3.72 a	4.16 ab	2.66a	2.91ab	3.40b	9.00a	9.18b	10.34b
	WSBM	2.94 b	3.34 a	3.58 b	2.31b	2.43b	2.76b	6.06c	9.00b	9.44c
	SSBM	3.09 ab	3.66 a	4.35 a	2.72a	3.01a	4.43a	8.39ab	10.81a	13.61a

Different lowercase letters follow by the mean of different treatments in the same column within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

For *Wufengyou 615*, seedling length was considerably reduced under all seedling establishment methods at all sampling intervals, compared with TWSM. Moreover, SSBM was found statistically at par ($P > 0.05$) with TWSM and/or higher than other seedling establishment methods regarding length of first leaf sheet (at all sampling intervals), first leaf length (at 10 and 15 DAS). The second leaf length were recorded maximum in DSBM at 10 DAS while at 15 and 20 DAS, the second leaf length was highest in SSBM. The dry biomass were also remained maximum in SSBM (higher than other two seedling establishment methods) but statistically similar ($P > 0.05$) with TWSM at 10, 15 and 20 DAS. Overall, the morphological parameters were increased with an increase in seedling age (from 10 to 20 DAS) in both *Xiangyaxiangzhan* and *Wufengyou 615*.

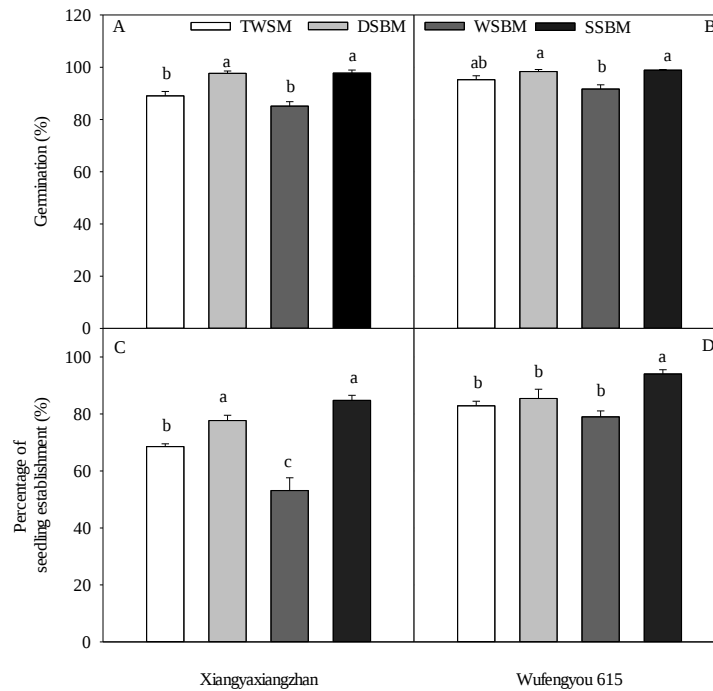


Figure 1. The seed germination rate and percentage of seedling establishment in response to the different seedling raising methods for Xiangyaxiangzhan (A, C) and Wufengyou 615 (B, D). Different lowercase letters followed by the mean of different treatments within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

Furthermore, differential responses of both rice cultivars regarding root morphological characters were noted under different seedling establishment methods. For example, root numbers, root length, root surface area and root diameter were higher in modified seedling establishment methods than TWSM whilst SSBM proved better than other methods (Table 3). For Xiangyaxiangzhan 44.41, 36.42, 19.81, 18.75 and 3.37, 13.52, 19.01, 33.33%, and 19.15, 23.24, 13.94, 85.71% higher root numbers, root length, root surface area and root diameter were recorded in SSBM at 10, 15 and 20 DAS, respectively. Similarly, for Wufengyou 615, 42.51, and 18.63, 19.81 % higher root numbers 31.95, 32.26, and 23.25% higher root length, 44.33, 45.99 and 40.83% higher root surface area, 9.68, 112.00 and 65.43% higher root diameter were recorded at 10, 15 and 20 DAS, respectively.

Table 3. Effects of different seedling raising methods on the morphological indexes of root

Cultivar	Treatment	Number of root			Number of the white root			Root length(cm)			Diameter (mm)		
		10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS	10 DAS	15 DAS	20 DAS
Xiangyaxiangzhan	TWSM	9.12c	14.83a	14.83b	1.86b	2.84c	2.77c	64.55b	84.00b	157.21b	0.32a	0.33a	0.42b
	DSBM	11.81ab	14.19ab	14.50b	10.37a	11.28b	11.90b	73.79b	93.06ab	178.42ab	0.32a	0.38a	0.47b
	WSBM	11.07bc	12.50b	13.00b	1.93b	2.89c	2.45c	69.59b	92.13ab	178.8ab	0.31a	0.35a	0.62ab
	SSBM	13.17a	15.33a	17.67a	11.89a	13.73a	15.38a	88.06a	95.36a	193.75a	0.38a	0.44a	0.78a

Wufengyou 615	TWSM	12.28b	15.67bc	16.00bc	2.67c	3.31c	3.82b	61.29b	74.05c	184.53c	0.31a	0.25b	0.81c
	DSBM	13.06b	16.33b	17.00b	11.84b	12.44b	15.25a	82.45a	83.95b	207.48ab	0.33a	0.46a	1.16b
	WSBM	13.33b	13.77c	15.00c	2.84c	3.29c	4.33b	71.05ab	91.14ab	203.09bc	0.30a	0.51a	1.16b
	SSBM	17.50a	18.59a	19.17a	13.48a	16.23a	16.27a	80.87a	97.94a	227.43a	0.34a	0.53a	1.34a

Different lowercase letters follow by the mean of different treatments in the same column within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

Physiological characters and SPAD values

Seedling raising methods variably affected physiological characters i.e., leaf proline, protein and soluble sugar contents as well as leaf chlorophyll contents (in terms of SPAD) values of both rice cultivars. Leaf proline contents were improved significantly ($P < 0.05$) in rice seedlings raised by SSBM method (except at 15 DAS in *Xiangyaxiangzhan*) than other raising method including TWSM at all sampling stages in both rice cultivars (Fig. 2).

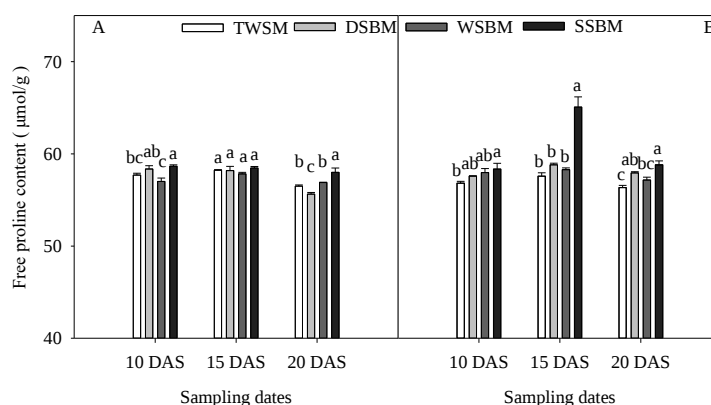


Figure 2. Effects of different seedling raising methods on proline content in leaves of seedlings for two cultivars (A: *Xiangyaxiangzhan*; B: *Wufengyou 615*). Different lowercase letters followed by the mean of different treatments within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

The leaf protein contents were remained highest in the seedlings raised by SSBM method which was remained statistically similar ($P > 0.05$) with WSBM and TWSM and minimum were recorded in DSBM at 10 DAS. At 15 DAS, maximum protein contents were recorded in the leaves of *Wufengyou 615* established by DSBM followed by TWSM, SSBM while minimum in WSBM (Fig. 3). Further, at 20 DAS, the leaf proline contents were remained statistically similar ($P > 0.05$) for all seedling establishment methods. The SSBM resulted in higher soluble sugar contents for both rice cultivars at 10 and 15 DAS, however, at 20 DAS; the maximum soluble sugars were recorded in TWSM whilst minimum in WSBM and DSBM in *Xiangyaxiangzhan*

and Wufengyou 615, respectively (Fig. 4). Overall, the soluble sugar contents were increased with increase in seedling age from 10 to 20 DAS for *Xiangyaxiangzhan*, and increased from 10 to 15 DAS and then reduced to 20 DAS in *Wufengyou 615*. Furthermore, all seedling raising methods remained statistically similar ($P > 0.05$) for leaf protein contents for *Xiangyaxiangzhan* at all sampling stages, while affected differently at 10 and 15 DAS for *Wufengyou 615* (Fig. 3). Regarding SPAD values, maximum values were recorded at SSBM at all sampling stages and the values of percentage increase were 15.21, 17.56, and 24.12% in *Xiangyaxiangzhan* at 10, 15 and 20 DAS (Fig. 5). For *Wufengyou 615*, highest SPAD values were recorded in the seedlings raised by SSBM method at all sampling stages and the values were significantly higher than other seedling raising methods and TWSM.

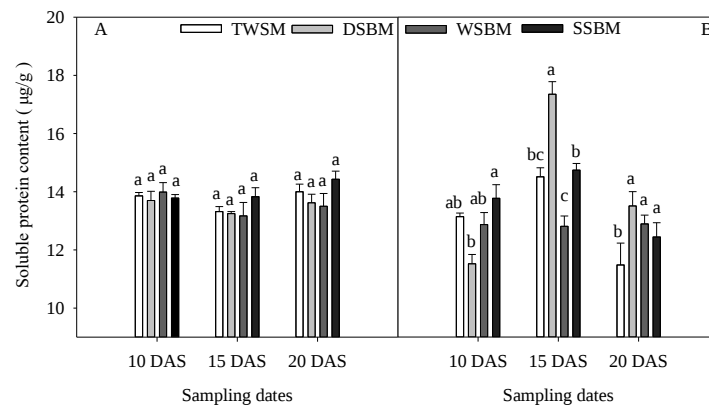


Figure 3. Effects of different seedling raising methods on soluble protein content in leaves of seedlings for two cultivars (A: *Xiangyaxiangzhan*; B: *Wufengyou 615*). Different lowercase letters followed by the mean of different treatments in the same column within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

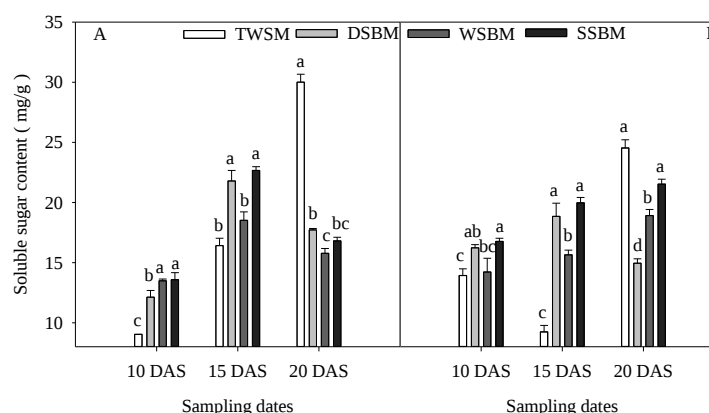


Figure 4. Effects of different seedling raising methods on soluble sugar content of seedling for two cultivars (A: *Xiangyaxiangzhan*; B: *Wufengyou 615*). Different lowercase letters followed by the mean of different treatments within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

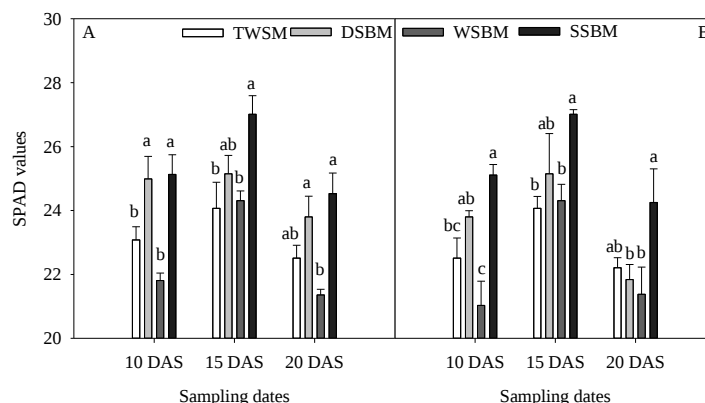


Figure 5. Effects of different seedling raising methods on SPAD values of seedling for two cultivars (A: Xiangyaxiangzhan; B: Wufengyou 615). Different lowercase letters followed by the mean of different treatments within one variety indicate significant difference at $P < 0.05$ level. TWSM: traditional seedling-raising method, DSBM: dry seedbed nursery raising method, WSBM: wet seedbed raising method, and SSBM: substrate seedbed nursery raising method

Correlation analysis

There was no significant correlation between shoot dry weight at 10 DAS and seedling establishment for *Wufengyou 615* (Fig. 6 A). Only *Xiangyaxiangzhan* presented a high positive correlation between shoot dry weight at 10 DAS and seedling establishment ($r = 0.6686$, $P < 0.05$), indicating same effects of different seedling raising methods on the shoot dry weight and seedling establishment for this variety. There was however a high ($P < 0.05$) positive correlation between shoot dry weight at 15 DAS and seedling establishment for both cultivars (Fig. 6 B) where the correlation coefficients (r) are 0.5792 ($P < 0.05$) for *Wufengyou 615* and 0.7849 ($P < 0.01$) for *Xiangyaxiangzhan*. This result suggests that the increased percentage of seedling establishment is closely linked to shoot dry weight. Further, shoot dry weight at 15 DAS was positively associated with leaf sheath length at all sampling dates, but the significance was only recorded for *Wufengyou 615* and 20 DAS in *Xiangyaxiangzhan* (Fig. 6 C, D, E). In addition, the positive associations were also recorded for the percentage of seedling establishment with germination rate, SPAD values at 15 DAS, number of roots and white roots at all dates for the two cultivars (Fig. 7).

Discussion

Rice seed germination and seedling growth is affected by the seed viability and vigour, nature of the growing medium, water application and the various other external factors. These external factors might be promoting or inhibitory and the effects of these factors on the rice nursery and/or seedling growth vary overtime. In this study, highest germination rate and seedling establishment in SSBM may possibly due to fertile growing medium of amorphous nature (non-compacted) with significant amount of organic matter. Our results are in concomitant with the findings of Mishra and Salokhe (2008), who found in reduced soil environments (under aerobic conditions), the coleoptile of the rice seeds were poorly developed

than flooded soil. Further, type of growing media and growing environments determine the quality of the seedlings growing in it for nursery raising (Wilson, 2001; Agbo and Omaliko, 2006), which have significant impacts on its later growth and performance in the field after transplanting (Baiyeri, 2006). Hence, higher seed germination and seedling establishment in SSBM indicates their relations with the type of growing medium.

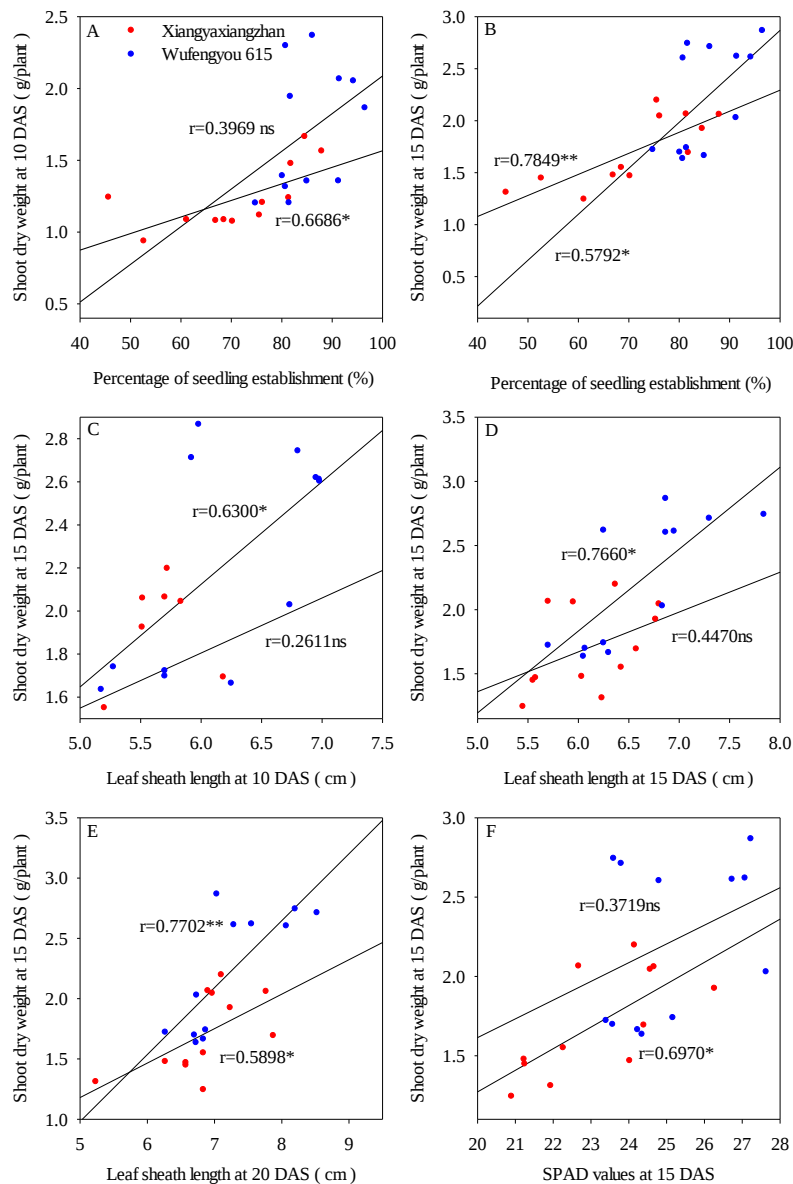


Figure 6. Correlation analyses between (A) shoot dry weight at 10 DAS and percentage of seedling establishment, (B) percentage of seedling establishment and shoot dry weight at 15 DAS, (C) shoot dry weight at 15 DAS and leaf sheath at 10 DAS, (D) shoot dry weight at 15 DAS and leaf sheath length at 15 DAS, (E) shoot dry weight at 15 DAS and leaf sheath length at 20 DAS, (F) shoot dry weight at 15 DAS and SPAD values at 15 DAS for Xiangyaxiangzhan and Wufengyou 615, respectively. ns: non-significant at $P < 0.05$ level; *: significant at $P < 0.05$ levels; **: significant at $P < 0.01$ levels

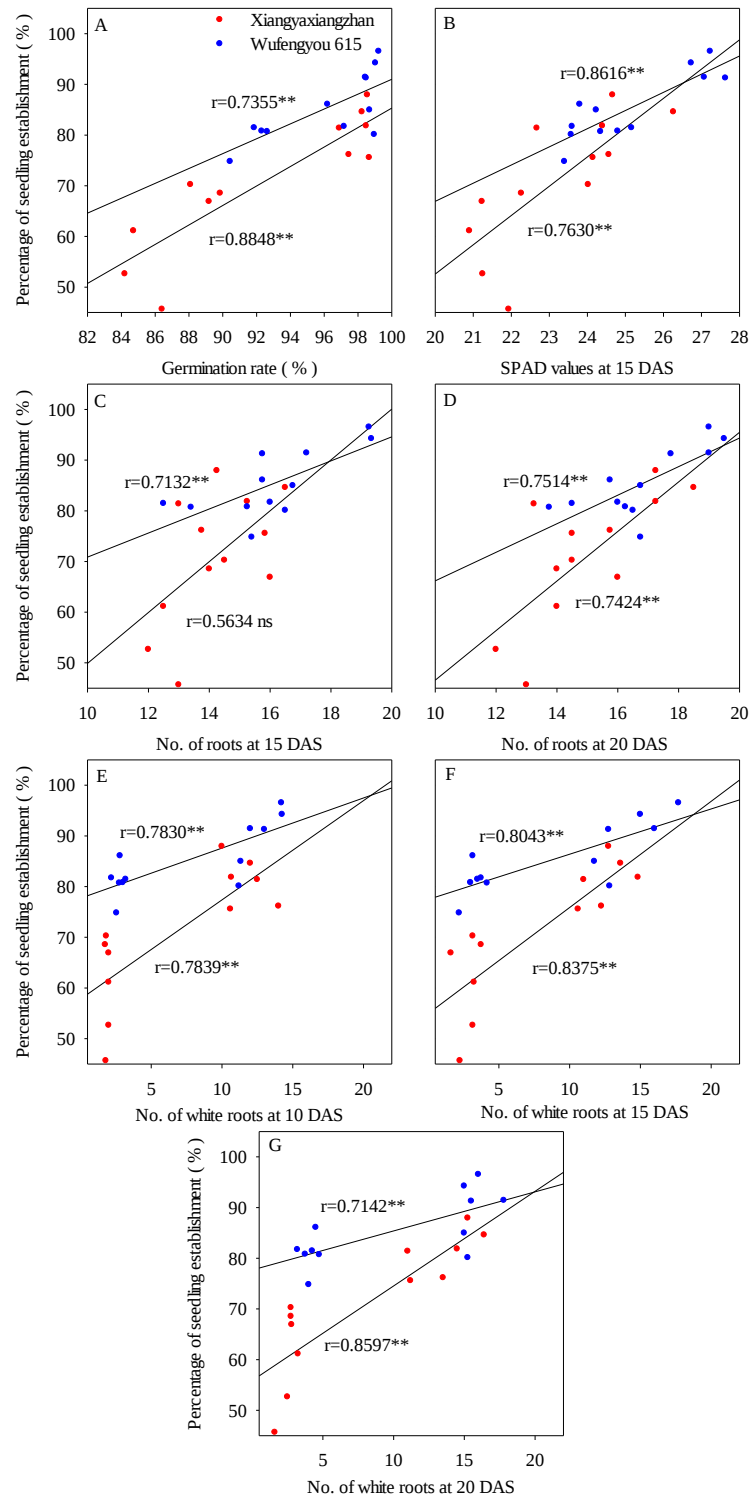


Figure 7. Correlation analyses between (A) percentage of seedling establishment and germination rate, (B) percentage of seedling establishment and SPAD values at 15 DAS, (C) percentage of seedling establishment and the No. of roots at 15 DAS, (D) percentage of seedling establishment and No. of roots at 20 DAS, (E) percentage of seedling establishment and the No. of white roots at 10 DAS, (F) percentage of seedling establishment and the No. of white roots at 15 DAS, and (G) percentage of seedling establishment and the No. of white roots at 20 DAS for Xiangyaxiangzhan and Wufengyou 615, respectively. ns: non-significant at $P < 0.05$ level; **: significant at $P < 0.01$ levels

Different seedling establishment methods substantially affected morphological characters of both rice cultivars. Development of shoot and leaves along with the growth of roots is prerequisite or transitory phase for rice seeds from heterotrophic to autotrophic level (Sasaki, 2004). Our results revealed that nursery management practices and type of nursery growing medium influenced growth and development of rice seedlings. Better root growth with higher root surface area in SSBM mimics in shoot growth with seedling length, leaf length and dry biomass. Ros et al. (2003) argued that rice seedlings establishment and their consequent growth not only related to above-ground morphological characters but also on the root growth and development. Raising healthy and well-established seedlings with well-flourished and healthy root system or simply, the morphogenesis of rice seedlings might have strong associations with nature and type of seedbed and/or growing media, number of seeds per area and soil conditions (Yamauchi and Biswas, 1997). Hence, root elongation under modified nursery raising methods, especially SSBM, showed that use of substrate (organic in nature) of rice seedlings favors root formation and seedling development. Furthermore, Subedi et al. (2013) also stated that nursery management practices considerably influence seedling characteristics of low land rice, whereas Panda et al. (1991) reported that seedlings raised in nutritious seed-bed would result in healthy and vigorous seedlings and performs better in the field conditions.

Moreover, seedling raising methods variably affected physiological characters i.e., leaf proline, protein and soluble sugar contents and leaf chlorophyll contents (in terms of SPAD) values of both rice cultivars. Overall, seedlings grown by substrate method also performed physiologically better than other nursery establishment methods, while all other modified seedling raising methods also proved better than TWSM (normally practiced by the farmers). Previously, Thakur et al. (2010) also reported rice establishment method induced regulations in physiological characters of rice seedlings. Higher chlorophyll contents in the leaves would lead to increased photosynthesis, hence improved seedling growth. Dark green leaves with higher SPAD values were observed in modified seedling establishment methods than TWSM. These findings are in accordance with Harper et al. (2004) and Thakur et al. (2010). Overall, the results of this study depicted that improved seedling establishment methods with better growing medium may induce multiple, positive and significant changes in morphogenesis, phenotype and growth of rice seedlings, however, evoking these changes and responses of rice nursery to the specific seed establishment methods yet need to be studied and their performance in the field conditions as well. Hence, keeping in view this point, field based evaluation of rice nursery managed by different rice nursery establishment methods would thus be focused in our subsequent experiments to draw a most comprehensive inference.

Conclusion

In sum, different seedling raising methods significantly affect rice seedling growth and seedling establishment. Among modified rice seedling raising methods, SSBM proved better for both *Xiangyaxiangzhan* and *Wufengyou 615* rice cultivars. Along with morphological growth, significant improvements were also observed in physiological characteristics of the seedlings of both rice cultivars. Hence, addition of organic manure contained material in the growing media of rice nursery may improve morpho-physiological features of rice seedlings for the purpose of better stand establishment in the paddy fields.

Acknowledgements. Founding provides by Agricultural research project of Guangdong Province (2011AO2020001), and Agricultural standardization project in Guangdong Province (4100F10003) is highly acknowledged.

REFERENCES

- [1] Abid, M., Khan, I., Mahmood, F., Aahraf, U., Imranm, M., Anjum, S. A. (2015): Response of hybrid rice to various transplanting dates and nitrogen application rates. – *Philippine Agric Scientist* 98(1): 98-104.
- [2] Agbo, C. U., Omaliko, C. M. (2006): Initiation and growth of shoots of *Gongronema latifolia* benth. Stem cuttings in different rooting media. – *African J Biotechnol* 5(5): 425-428.
- [3] Aminian, M., Nabatchian, F., Vaisi-raygan, A., Torabi, M. (2013): Mechanism of Coomassie Brilliant Blue G-250 binding to cetyltrimethylammonium bromide: an interference with the Bradford assay. – *Anal Biochem* 434(2): 287-291.
- [4] Asai, H., Samson, B. K., Stephan, H. M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shairaiwa, T., Horie, T. (2009): Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. – *Field Crops Research* 111(1): 81-84.
- [5] Ashraf, U., Kanu, A. S., Mo, Z. W., Hussain, S., Anjum, S. A., Khan, I., Abbas, R. N., Tang, X. R. (2015): Lead toxicity in rice: effects, mechanisms, and mitigation strategies—a mini review. – *Environ Sci Pollut Res* 22(23): 18318-18332.
- [6] Baiyeri, K. P. (2006): Seedling emergence and growth of pawpaw (*Carica papaya*) grown under different coloured shade polyethylene. – *International Agrophysics* 20(2): 77-84.
- [7] Biswas, J. C., Ladha, J. K., Dazzo, F. B., Yanni, Y. G., Rolfe, B. G. (2000): Rhizobial inoculation influences seedling vigor and yield of rice. – *Agronomy Journal* 92(5): 880-886.
- [8] Celik, O., Atak, C. (2012): The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. – *Turkish Journal of Biology* 36: 339-356.
- [9] Ehsanullah, Anjum, S. A., Ashraf, U., Rafiq, H., Tanveer, M., Khan, I. (2014): Effect of sowing dates and weed control methods on weed infestation, growth and yield of direct-seeded rice. – *Philippine Agric Scientist* 97(3): 307-312.
- [10] Harper, A. L., Von Gesjen, S. E., Linford, A. S., Peterson, M. P., Faircloth, R. S., Thissen, M. M., Brusslan, J. A. (2004): Chlorophyllide a oxygenase mRNA and protein levels correlate with the chlorophyll a/b ratio in *Arabidopsis thaliana*. – *Photosynthesis Research* 79(2): 149-159.
- [11] Ivanic, M., Martin, W. (2008): Implications of higher global food prices for poverty in low-income countries. – *Agricultural economics* 39: 405-416.
- [12] Kargbo, M. B., Pan, S. G., Mo, Z. W., Wang, Z. M., Luo, X. W., Tian, H., Hossain, M. F., Ashraf, U., Tang, X. R. (2016): Physiological basis of improved performance of super rice (*Oryza sativa*) to deep placed fertilizer with precision hill-drilling machine. – *Int J Agric Biol* 18: 797-804.
- [13] Karim, M. A., Komdo, T., Ueda, K., Higuchi, H., Nawata, E. (2012): Effect of NaCl treatment on growth and some physiological characteristics of a salt-tolerant soybean genotype AGS 313 bred in Bangladesh. – *Tropical Agri Develop* 56(4): 139-142.
- [14] Li, M. J., Aahraf, U., Tian, H., Mo, Z. W., Pan, S. G., Anjum, S. A., Tang, X. R. (2016): Manganese-induced regulations in growth, yield formation, quality characters, rice aroma and enzyme involved in 2-acetyl-1-pyrroline biosynthesis in fragrant rice. – *Plant Physiol Biochem* 103: 167-175.
- [15] Li, Q. D. (2015): Level of rice planting mechanization over 38%. – *Agriculture Machinery Technology Extension* 1: 12 (in Chinese).

- [16] Li, Y. X., Xing, X. M., Li, G. H. (2014): Study of the Quality and Growth Characteristics in Field About water Volume of Machine Transplanting Seedling. – 2014 National Youth Symposium on the Cultivation and Physiology of Crops: Yangzhou, Jiangsu, China (in Chinese).
- [17] Mishra, A., Salokhe, V. M. (2008): Seedling characteristics and the early growth of transplanted rice under different water regimes. – *Exp Agri* 44(03): 365-383.
- [18] Mo, Z. W., Li, W., Pan, S. G., Fitzgerald, T. L., Xiao, F., Tang, Y. J., Tang, X. R. (2015): Shading during the grain filling period increases 2-acetyl-1-pyrroline content in fragrant rice. – *Rice* 8(9): 1-10.
- [19] Oršanić, M., Drvodelić, D., Ugarković, D. (2011): Ecological and Biological Properties of Holm Oak (*Quercus ilex* L.) on the Island of Rab. – *Croatian J Forest Eng* 32(1): 41-42.
- [20] Panda, M. M., Reddy, M. D., Sharma, As. R. (1991): Yield performance of rainfed lowland rice as affected by nursery fertilization under conditions of intermediate deep water (15–50 cm) and flash floods. – *Plant and Soil* 132(1): 65-71.
- [21] Ros, C., Bell, R. W., White, P. F. (2003): Seedling vigour and the early growth of transplanted rice (*Oryza sativa*). – *Plant and Soil* 252(2): 325-337.
- [22] Ruiz-Sánchez, M., Aroca, R., Muñoz, Y., Polon, R., Ruiz-lozano, J. M. (2010): The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the ant oxidative response of rice plants subjected to drought stress. – *Journal of Plant Physiology* 167(11): 862-869.
- [23] Sasaki, R. (2004): Characteristics and seedling establishment of rice nursling seedlings. – *Japan Agricultural Research Quarterly* 38(1): 7-13.
- [24] Subedi, R. (2013): Nursery management influences yield and yield attributes of rainfed lowland rice. – *Journal of Sustainable Society* 2(4): 86-91.
- [25] Thakur, A. K., Uphoff, N., Antony, E. (2010): An assessment of physiological effects of system of rice intensification (SRI) practices compared with recommended rice cultivation practices in India. – *Experimental Agriculture* 46(01): 77-98.
- [26] Wilson, S. B., Stoffella, P. J., Graetz, D. A. (2001): Use of compost as a media amendment for containerized production of two subtropical perennials. – *Journal of Environmental Horticulture* 19(1): 37-42.
- [27] Yamauchi, M., Biswas, J. K. (1997): Rice cultivar difference in seedling establishment in flooded soil. – *Plant and Soil* 189(1): 145-153.
- [28] Yang, M. J., Yang, L., Li, Q. D. (2003): Agricultural mechanization system of rice production of Japan and proposal for China. – *Transactions of the CSAE* 19(5): 77-82.
- [29] Yao, F., Xu, Y., Lin, E., Yokozawa, M., Zhang, J. (2007): Assessing the impacts of climate change on rice yields in the main rice areas of China. – *Climatic Change* 80(3-4): 395-409.
- [30] Zhang, H. C., Gong, J. L. (2014): Research status and development discussion on high-yielding agronomy of mechanized planting rice in China. – *Scientia Agricultura Sinica* 47(7): 1273-1289 (in Chinese).
- [31] Zhu, D. F., Chen, H. Z., Xu, Y. C. (2007): Countermeasure and perspective of mechanization of rice planting in China. – *Northern Rice* 05: 13-18 (in Chinese).