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Effects of Seed Size and Sand Burial on Germination and Early Growth of Seedlings for Coastal *Pinus thunbergii* Parl. in the Northern Shandong Peninsula, China

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Abstract: This paper examines the effects of seed size and the depth of sand burial on seed germination and seedling development for *Pinus thunbergii*. Parl. Seeds from 20- to 30-year old trees grown in the coastal area of Yantai were divided into three size categories (large, medium, and small). The seeds were sown in pots with different depth of sand, and their germination and seedling growth during the first month were investigated. Results showed that large seeds possessed the highest 1000-seed weight and soluble sugar concentration. Large and medium seeds had a higher germination rate, germination index, vigor index, and seedling biomass than small seeds. With the increase in seed size, root mass ratio, root/shoot ratio, specific root length, and specific root area decreased, whereas leaf mass ratio increased. Sand burial depth significantly influenced seed germination and seedling growth, and the highest germination rate and seedling biomass were achieved with 2–3 cm sand burial. We also found that seedling biomass was positively related to germination rate, germination index, and vigor index, but was negatively related to mean germination time. Moreover, seedling biomass was negatively correlated with root mass ratio and root/shoot ratio, but positively correlated with leaf mass ratio, specific root length, and specific root area. The results suggest that seed size and sand burial depth are key factors in the regeneration of the coastal *P. thunbergii* forest.

Keywords: seed germination; seedling growth; biomass allocation; root index; forest regeneration

1. Introduction

At a global scale, the area of planted forests increased rapidly between 1990 and 2015 from 4.06 to 6.95% of the total forest area [1]. As an important component of global forest resources, planted forests are playing an increasingly important role in timber production, environment improvement, landscape rehabilitation, and climate change mitigation [2]. China has the largest area of planted forests in the world. Although the quality of planted forests in China in general is perceived low, planted forests in the coastal area play a significant role in reducing natural disasters, stabilizing shore lines, and sustaining biodiversity [3–5]. How to renew coastal protection forests without destroying their ecological functions is an important research topic [5,6].

Pinus thunbergii Parl. forests are the most important coastal protection forests in the Shandong Peninsula, China. Most of these forests were planted in the early 1970s. Much research has been conducted on natural regeneration of the *P. thunbergii* protection forests. In particular, the effects of seed tree age and salt stress [7], water stress [8], and sand burial [9] on seed germination have been systematically studied. One of the important findings from these studies is that seed tree age played an important role in the seed germination of *P. thunbergii*. Moreover, previous studies showed that undergrowth in the forest [10] and distance from the shoreline [11] had important effects on the natural regeneration of *P. thunbergii*.

P. thunbergii is a light demanding tree species, and natural regeneration of *P. thunbergii* forest requires large canopy openness [5,12]. However, in order to maintain their protection functions, thinning of *P. thunbergii* forest is forbidden in Chinese coastal zones, and this had led to high stand density and low understory light intensity. Therefore, the natural regeneration of *P. thunbergii* is very poor. Some artificial regeneration measures are necessary in order to achieve successful regeneration of the coastal *P. thunbergii* protection forests.

The purpose of this paper is to examine the effects of seed size and sand burial depth on seed germination and the early growth of seedlings of coastal *P. thunbergii*. Results from this study can help forest managers to select the best seeds and sowing method for producing sturdy seedlings, thereby contributing to the successful regeneration of coastal *P. thunbergii* protection forests and the sustainment of their protection functions.

Seed size is a key trait in many aspects of plant fitness. How plants determine their seed size has been under debate in recent years [13]. Seed size has an impact on germination and survival under stressful circumstances [14]. Plants from larger seeds, compared with those from smaller seeds, show a competitive advantage on germination and seedling stress tolerance [14,15]. Baraloto et al. [16] showed that seed size influenced performance within and among eight neotropical tree species. A short germination time can cause *P. thunbergii* seedlings to have a longer growth time and larger individuals, which is vital for seedling establishment in the natural environment [9]. Larger seeds have more nutrients, which can improve the germination and seedling tolerance of *Caragana microphylla* Lam. and *Hedysarum leave* Maxim under unfavorable conditions [17]. However, Chen and Maun [18] found that seed size had no effects on germination for *Cirsium pitcher* Torr.& A. Gray. Seedlings from smaller seeds of *Eperua grandiflora* (Aubl.) Benth. and *Vouacapoua americana* Aubl. grow faster than seedlings from larger seeds [16]. Venable and Brown [19] claimed that the successful establishment of plants derived from larger seeds had advantages over that derived from smaller seeds only under adverse circumstances. However, Chacón and Bustamante [20] indicated that any attribute that increased germination (e.g., large seeds) would be advantageous for *Cryptocarya alba* (Molina) Looser, independent of the prevailing abiotic conditions.

Sand burial depth has an obvious effect on seed germination rate and germination time [9,17]. Burial at an appropriate depth stimulates seed germination more so than if seeds were placed on the soil surface [18,21–23] due to the moist environment [24]. Excessive burial may prevent seed germination and subsequent seedling survival due to the lack of oxygen, light, and lower temperature in the soil [25]. In addition, an optimal seed germination time is vital for seedling establishment in the natural environment [26]. Therefore, clarifying sand burial, the seed size of sand dune species, and their interaction is essential for the natural regeneration in coastal sand dune forest ecosystems.

Regarding *P. thunbergii*, the importance of seed size and the effect of sand burial on seed germination and seedling growth are not well understood. In this study, *P. thunbergii* seeds of different sizes were buried at varying sand depths in pots to assess the effects of seed size and sand burial depth on seed germination and the early growth of seedlings.

2. Materials and Methods

2.1. Study Area

This study was carried out at the Forestry Experimental Station of Shandong Agricultural University, China (36°10′09′′ N, 117°09′24′′ E). The site has a warm temperate continental monsoon climate. The mean annual temperature is 12.8 °C, and the minimal and maximal temperature is −6.9 °C and 37.5 °C, respectively. The average annual precipitation varies from 600 to 700 mm, with precipitation mainly occurring in the summer. The frost-free period is 186.6 days with an effective accumulated temperature of about 4283 °C. The mean annual relative humidity is 65%.

2.2. Seed Collection

Seeds were collected from 40 *P. thunbergii* trees of 20–30 years old in Kunyu Mountain, Yantai, Shandong Province at 200 m above sea level in late September of 2016. These seed trees originated from seeds collected from the coastal forests planted in the early 1970s. No significant difference in average seed size was found among different trees. Therefore, the seeds collected from different trees were mixed and treated as one sample. Seeds were sorted into three seed size categories, large (SI), medium (SII), and small (SIII), using soil sieves with mesh widths of 1.63 and 2.26 mm.

The 1000-seed weight of each seed size group was measured by 100-seed method. A subsample of 30 seeds in each seed size group was selected, and their maximum length and width were measured using a vernier caliper. The sample seeds from each size group were dried in an oven at 120 °C for 30 min, and then dried at 75 °C for 48 h. After these seeds were pulverized, the concentration of soluble sugar and starch was determined by anthrone–H_2SO_4 colorimetry [27].

2.3. Seed Germination Test

A completely randomized factorial design was used to test the effect of seed size and sand burial depth on seed germination and seedling growth. Randomly selected seeds from different size groups were sowed at depths of 1.0, 2.0, 3.0, and 4.0 cm in plastic pots filled with sand taken from the coastal *P. thunbergii* forest around Muping Coastal Environment Research Station, Chinese Academy of Sciences (36°27′15″ N, 121°41′57″ E). The plastic pots were 20 cm high and 22 cm in diameter; the sand was sieved to remove debris and seeds before use. Twenty seeds were sowed in each pot, and each treatment was replicated five times.

The experiment started on 10 May 2017 and ended on 18 June 2017. All pots were well watered during this period. After the first seed germinated, germination was assessed every day. The germination rate (Gr, %), the mean germination time (MGT, in days), the germination index (GI), and the vigor index (V_i) were calculated. These indexes were calculated as follows:

$$G_r = G_1/N \times 100 \tag{1}$$

$$MGT = \sum(D \times n_i)/\sum n_i \tag{2}$$

$$GI = \sum(n_i/D) \tag{3}$$

$$Vi = S \times Gi \tag{4}$$

G_1 is the number of germinated seeds by the end of the germination test, and N is the total number of seeds sown. D is the number of days counted from the beginning of the test, and n_i is the number of seeds that germinated on the day (D). S is seedling dry mass.

2.4. Seedling Growth Test

After the seed germination experiment, the seedlings continued to grow in the pots until 15 July 2017. During this period, the average daily maximum radiation dose was 256.5 MJ·m^{-2}. On 15 July, one seedling was randomly selected from each pot, and its height and diameter at the surface were measured. The selected seedlings were then removed from the pots and washed free of sand. For each of these seedlings, the roots were arranged and floated on shallow water in a glass tray (24 cm × 32 cm), scanned (on an HP Scanjet 8200), and analyzed with an image analyzer (Delta-T Area Meter Type AMB2; Delta-T Devices, Cambridge, UK). Total root length (TRL, mm) and total root surface area (TRSA, mm^2) were determined after the above analysis. The leaf, stem, and root components of each seedling were dried at 75 °C for 48 h. The dry biomass of leaf, stem, and root was weighed using an electronic balance (0.1 mg accuracy). Individual seedling biomass (SB, leaf +

stem + root mass, g), leaf mass ratio (LMR, leaf mass/seedling biomass), stem mass ratio (SMR, stem mass/seedling biomass), root mass ratio (RMR, root mass/seedling biomass), root/shoot ratio (RSR, root mass/(leaf + stem biomass)), specific root length (SRL, total root length/root mass), and specific root area (SRA, total root surface area/root mass) were calculated to measure the treatment effects.

2.5. Data Analysis

Effects of seed size on 1000-seed weight, seed maximum length, seed maximum width, soluble sugar concentration, and starch concentration were tested by a one-way ANOVA. The indexes of seed germination and seedling growth were tested by a two-way ANOVA, with seed size and sand burial depth as the source of variations. Least-significant difference (LSD) multiple comparisons were conducted when there were significant differences. Bivariate correlation analyses were used to investigate relationships among different indexes of seed germination and seedling growth. All the indexes of seed germination and seedling growth were analyzed by principal component analysis (PCA). All statistical analyses were conducted using SPSS for Windows 13.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Characteristics of Seeds in Different Size Groups

There were significant differences among different size categories with respect to 1000-seed weight (F = 1572.26, $p < 0.01$), seed maximum length (F = 80.69, $p < 0.01$), seed maximum width (F = 194.46, $p < 0.01$), and soluble sugar concentration (F = 62.90, $p < 0.01$), whereas no significant difference was detected in starch concentration among size groups (F = 3.36, $p > 0.05$). The 1000-seed weight, seed maximum length, and seed maximum width increased as seed size increased (SI > SII > SIII, $p < 0.01$, Figure 1). Regarding soluble sugar concentration, SI showed a higher value than SII and SIII ($p < 0.01$), while SII was similar to SIII ($p > 0.05$).

3.2. Effects of Seed Size and Sand Burial Depth on Seed Germination

There were significant differences in Gr, MGT, GI, and Vi among different seed size groups (Table 1). SI was similar to SII in Gr, GI, and V_i ($p > 0.05$), but these values were higher than they were in SIII ($p < 0.05$, Figure 2). Regarding MGT, SI was similar to SIII ($p > 0.05$), and both showed a higher MGT than did SII ($p < 0.05$, Figure 2). Significant effects of sand depth on Gr and MGT as opposed to GI and V_i were found (Table 1). With the increase in burial depth, Gr increased at first and then decreased, whereas MGT showed the opposite trend (Figure 2). Gr at a 1 cm depth was similar to that 4 cm ($p > 0.05$), and both were lower than that at 2 cm ($p < 0.01$). MGT at 1 cm was higher than that at 2 and 3 cm ($p < 0.05$) but was similar to that at 4 cm. However, there were no significant interactions between seed size and burial depth in these above indexes (Table 1).

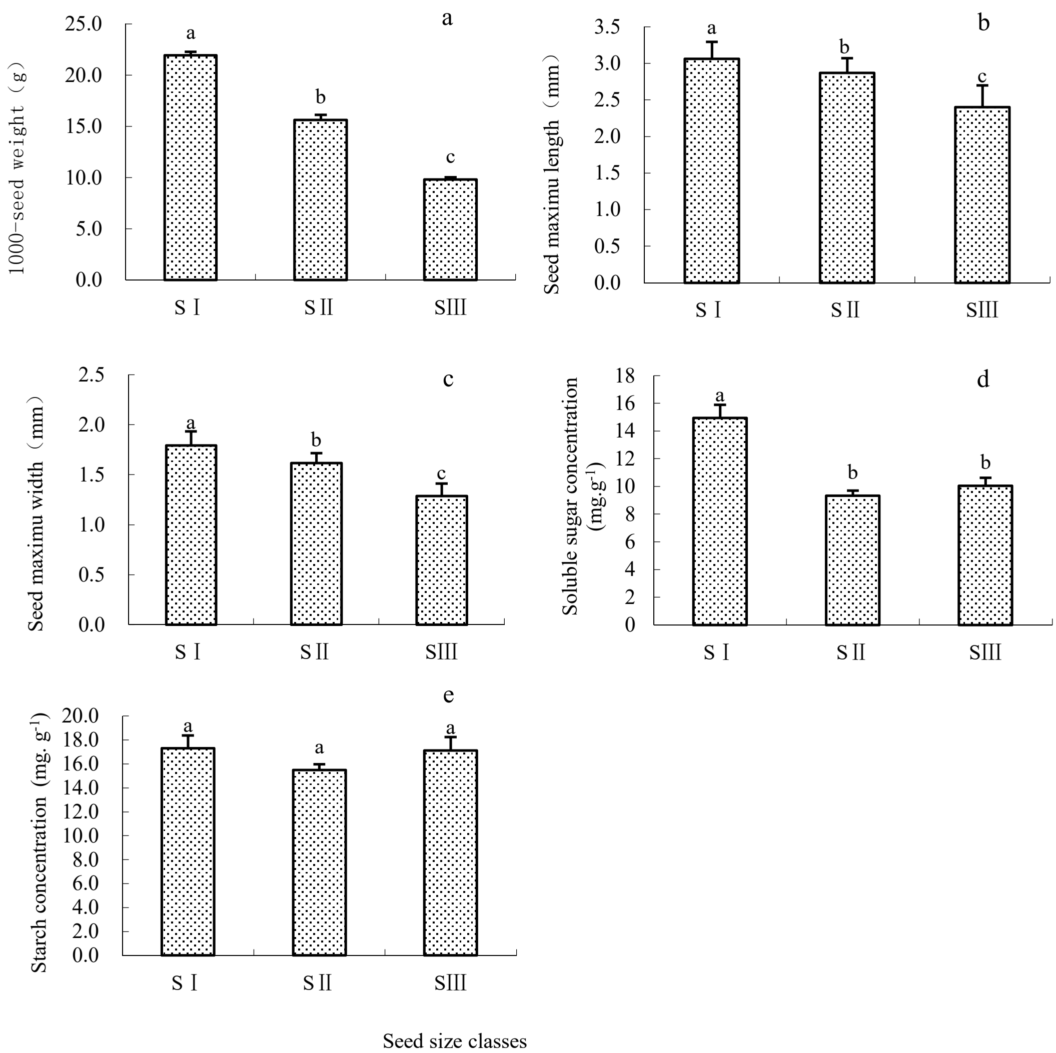

Figure 1. Characteristics of seeds of *Pinus thumbergii* Parl. in different size groups. (**a**): 1000-seed weight; (**b**): seed maximum length; (**c**): seed maximum width; (**d**): soluble sugar concentration; (**e**): starch concentration. SI: large seeds; SII: medium seeds; SIII: small seeds. Values are means ± standard deviation (SD). Means with different letters are significantly different from each other ($p < 0.05$).

Table 1. Two-way ANOVA analyses of the effects of seed size, sand burial depth, and their interaction on seed germination and seedling characteristics.

Indexes	Seed Size		Sand Burial Depth		Seed Size × Sand Burial Depth	
	F Value	*p* Value	*F* Value	*p* Value	*F* Value	*p* Value
Germination rate	8.43	<0.01	5.70	<0.01	1.21	0.35
Mean germination time	11.92	<0.01	3.41	<0.05	1.75	0.17
Germination index	5.08	<0.05	1.14	0.36	1.44	0.26
Vigor index	22.16	<0.01	3.06	0.07	1.57	0.22
Seedling height	58.74	<0.01	19.56	<0.01	5.62	<0.01
Ground diameter	27.70	<0.01	3.25	<0.05	3.05	<0.05
Seedling biomass	38.85	<0.01	4.52	<0.05	1.32	0.30
Root mass ratio	20.50	<0.01	0.34	0.80	2.25	0.09
Stem mass ratio	4.64	<0.05	0.83	0.50	0.46	0.83
Leaf mass ratio	18.47	<0.01	0.28	0.84	2.39	0.07
Root/shoot ratio	17.28	<0.01	0.26	0.85	1.92	0.14
Specific root length	75.12	<0.01	8.21	<0.01	6.16	<0.01
Specific root area	210.40	<0.01	5.53	<0.01	2.65	0.05

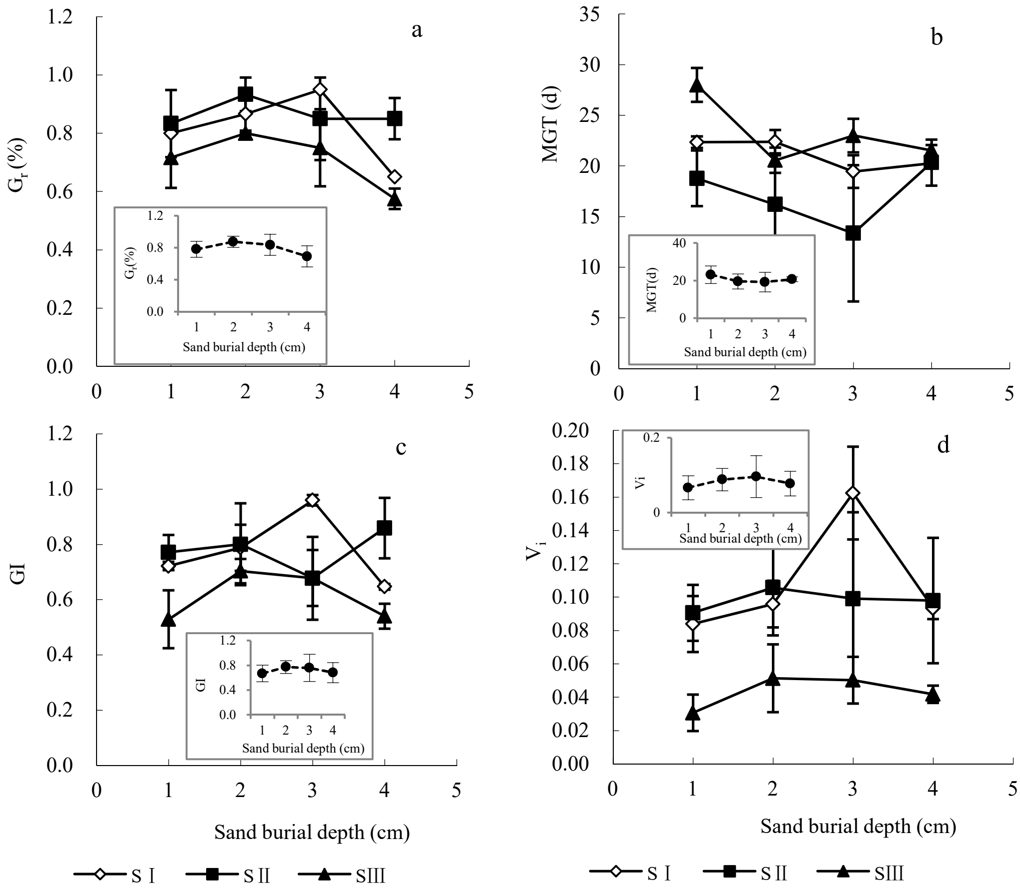

Figure 2. Effects of seed size and sand burial depth on seed germination of *Pinus thumbergii.* G_r (**a**): germination rate; MGT(**b**): mean germination time; GI (**c**): germination index; V_i (**d**): vigor index. SI: large seeds; SII: medium seeds; SIII: small seeds. Values are means ± standard deviation (SD). Inset panel: variation of mean values of three seed size groups with sand burial depth for G_r, MGT, GI, and V_i.

3.3. Effects of Seed Size and Sand Burial Depth on Seedling Growth

Both seed size and burial depth showed significant differences in seedling height, ground diameter, and biomass (Table 1). Among different size groups, SI was similar to SII in the indexes mentioned above, whereas they were higher than SIII ($p < 0.01$, Figure 3). With increased burial depth, seedling height and biomass increased and reached the maximum at a 3 cm depth and then decreased. However, seedling ground diameter increased with increased burial depth for SI and SII, while the values for SIII decreased gradually (Figure 3). Significant interaction effects between seed size and burial depth were found on seedling height and ground diameter but not on biomass (Table 1). For each sand burial depth, large and medium seeds, compared with small seeds, led to larger seedlings (both in height and in ground diameter) (Figure 3).

3.4. Effects of Seed Size and Sand Burial Depth on Seedling Biomass Allocation

Seed size showed significant effects on RMR, SMR, LMR, and root/shoot ratio (Table 1). Among different size groups, SI was lower than SII and SIII in terms of RMR and root/shoot ratio ($p < 0.05$), and SII was similar to SIII ($p > 0.05$, Figure 4). The order of LMR was SI > SII > SIII ($p < 0.05$). In terms of SMR, SII was lower than SI and SIII ($p < 0.05$), but no significant differences between the other two were found. However, there were no significant differences in RMR, SMR, LMR, or root/shoot ratio between different sand burial depths. Moreover, no significant interaction effects were found between seed size and burial depth on RMR, SMR, LMR, and root/shoot ratio (Table 1).

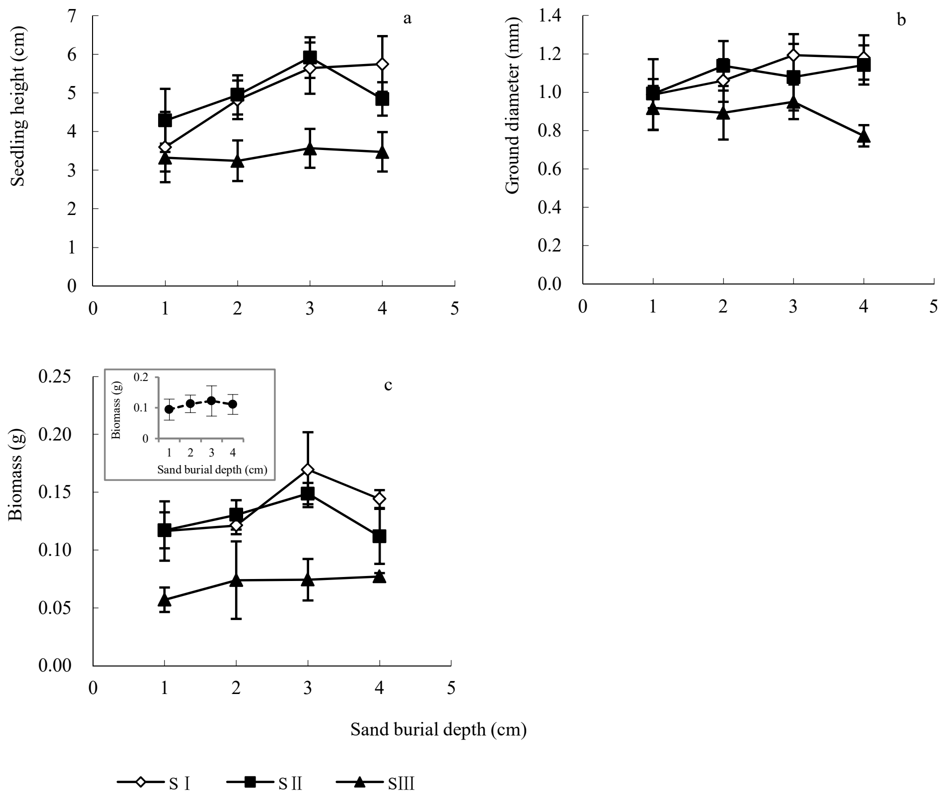

Figure 3. Effects of seed size and sand burial depth on seedling growth. (**a**): seedling height; (**b**): ground diameter; (**c**): biomass. SI: large seeds; SII: medium seeds; SIII: small seeds. Values are means ± standard deviation (SD). Inset panel: variation of mean values of three seed size groups with sand burial depth for biomass.

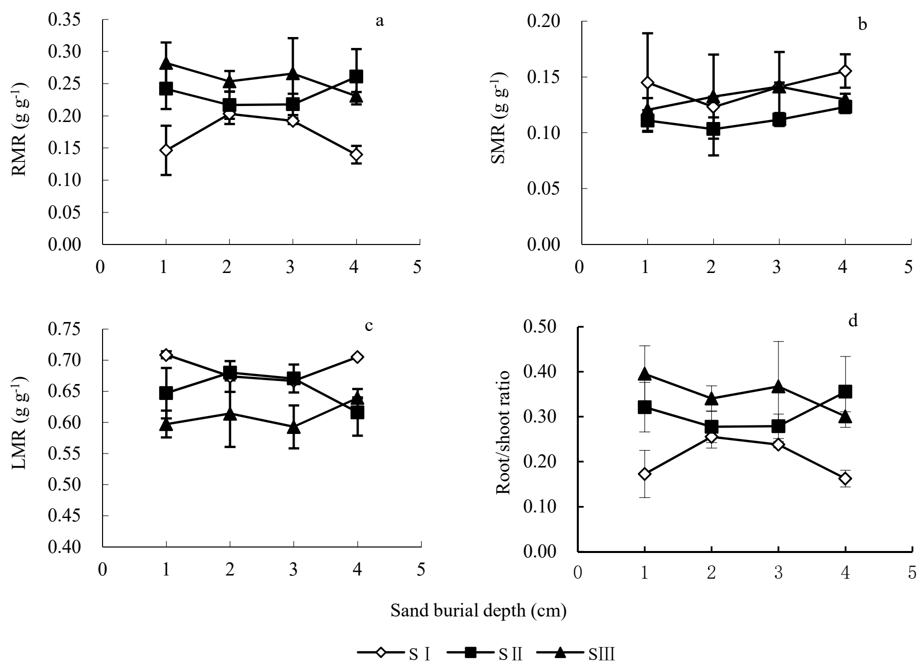

Figure 4. Effects of seed size and sand burial depth on seedling biomass allocation of *Pinus thumbergii.* RMR (**a**): root mass ratio; SMR (**b**): stem mass ratio; LMR (**c**): leaf mass ratio; root/shoot ratio (**d**). SI: large seeds; SII: medium seeds; SIII: small seeds. Values are means ± standard deviation (SD).

3.5. Effects of Seed Size and Sand Burial Depth on Seedling Root Characteristics

Seed size and burial depth both had significant impacts on SRL and SRA (Table 1). Larger seeds resulted in higher values of SRL and SRA ($p < 0.01$, Figure 5). With the increase in burial depth, SRL and SRA increased at first and then decreased (Figure 5). The interaction between seed size and burial depth had a significant effect on SRL but not on SRA (Table 1). As the depth of burial increased, the bigger the seed size was, the bigger the SRL was (Figure 5).

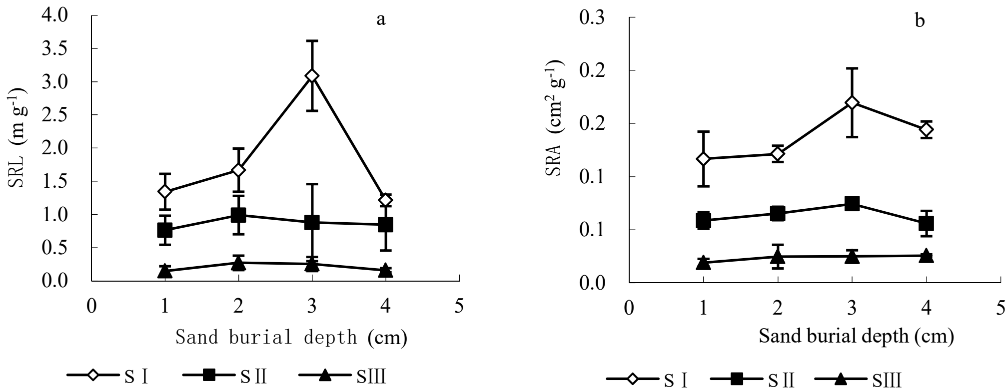

Figure 5. Effects of seed size and sand burial on seedling root characteristics of *Pinus thumbergii.* SRL (**a**): specific root length; SRA (**b**): specific root area. SI: large seeds; SII: medium seeds; SIII: small seedsp. Values are means ± standard deviation (SD).

3.6. Correlation Analysis of Seed Germination and Seedling Growth Indices

Among seed germination indexes, SB was significantly positively correlated with G_r, GI, and V_i and negatively correlated with MGT (Table 2). No significant correlation was shown between G_r, GI, and MGT or between SMR and LMR except that MGT showed a negative correlation with LMR. However, V_i negatively correlated with RMR and positively correlated with LMR. G_r, GI and V_i were significantly positively correlated SRL and SRA except G_r and SRA. Among growth indexes, seedling biomass was negatively correlated with RMR and root/shoot ratio and was positively correlated with LMR, SRL, and SRA but not with SMR. Significant negative correlations were found between SMR and LMR and between SRL and SRA.

The indices of seed germination and seedling growth were analyzed by PCA (see Table 3). The first and second principal components together represented 65.58% of the total variation. The first principal components (40.57%) were mainly related to seedling growth, including SB, SH, RMR, LMR, RSA, SRL, and SRA. The second principal components (25.01%) were associated with seed germination, including G_r, GI, and V_i.

Table 2. Correlations among seed germination and seedling characteristics of *Pinus thumbergii* Parl. (Pearson correlation coefficient). SB: seedling biomass; SH; seedling; height; GD: ground diameter; G_r: germination rate; MGT: mean germination time; G_i: germination index; V_i: vigor index; RMR: root mass ratio; SMR: stem mass ratio; LMR: leaf mass ratio; RSR: root/shoot ratio; SRL: specific root length; SRA: specific root area.

Index	SB	SH	GD	G_r	MGT	GI	V_i	RMR	SMR	LMR	RSR	SRL	SRA
SB	1.00	**0.83** **	−0.23	**0.48** **	**−0.56** **	**0.51** **	**0.82** **	**−0.56** **	−0.12	**0.68** **	**−0.58** **	**0.79** **	**0.83** **
SH		1.00	−0.04	0.41 *	**−0.52** **	**0.47** *	**0.69** **	**−0.49** **	0.01	**0.54** **	**−0.49** **	**0.67** **	**0.73** **
GD			1.00	0.05	0.05	0.07	−0.11	−0.11	**0.44** *	−0.10	−0.12	−0.11	−0.15
G_r				1.00	−0.32	**0.81** **	**0.80** **	−0.15	−0.22	0.27	−0.17	**0.57** **	0.32
MGT					1.00	−0.15	−0.35	0.24	0.27	**−0.39** *	−0.27	−0.18	−0.25
GI						1.00	**0.86** **	−0.31	0.06	0.31	−0.33	**0.69** **	**0.44** *
V_i							1.00	**−0.40** *	−0.12	**0.50** **	**−0.42** *	**0.86** **	**0.68** **
RMR								1.00	**−0.42** *	**−0.89** **	**1.00** **	**−0.57** **	**−0.72** **
SMR									1.00	−0.04	**−039** *	0.11	0.19
LMR										1.00	**−0.90** **	**0.57** **	**0.70** **
RSR											1.00	**−0.58** **	**−0.71** **
SRL												1.00	**0.90** **
SRA													1.00

Notes: The values statistically significant were marked in bold. Levels of significance are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

Table 3. Coefficient, eigenvalue, variance contribution rate, and accumulated contribution rate of principal components. SB: seedling biomass; SH; seedling; height; GD: ground diameter; G_r: germination rate; MGT: mean germination time; G_i: germination index; V_i: vigor index; RMR: root mass ratio; SMR: stem mass ratio; LMR: leaf mass ratio; RSR: root/shoot ratio; SRL: specific root length; SRA: specific root area.

Index	The First Principal Component	The Second Principal Component	The Third Principal Component	The Fourth Principal Component
SB	0.74	0.46	−0.27	−0.27
SH	0.65	0.43	−0.08	−0.30
GD	−0.08	0.05	0.93	−0.06
G_r	0.12	0.89	0.04	−0.20
MGT	−0.33	−0.14	0.01	0.87
GI	0.27	0.89	0.13	0.08
V_i	0.49	0.85	−0.13	−0.08
RMR	−0.94	0.03	−0.24	−0.06
SMR	0.28	−0.15	0.65	0.53
LMR	0.90	0.05	−0.06	−0.20
RSR	−0.94	0.01	−0.25	−0.02
SRL	0.68	0.63	−0.14	0.19
SRA	0.86	0.32	−0.16	0.15
Eigenvalue	5.27	3.25	1.56	1.36
Variance contribution rate (%)	40.57	25.01	11.99	10.44
Accumulated contribution rate (%)	40.57	65.58	77.56	88.00

4. Discussion

Seed size is an important morphological trait that may affect the population regeneration process. In this paper, different size of seeds showed significant differences on 1000-seed weight, maximum length, and maximum width, and the order of these indexes was SI > SII > SIII. During seed germination and seedling growth, SI and SII had higher G_r, GI, V_i, seedling height, ground diameter, and biomass than SIII, which indicated that large seeds of *P. thumbergii* showed obvious advantages on seed germination and seedling growth than small seeds. Our results were similar to those of Wang et al. [15] and Larios et al. [14]. Moreover, they found that larger seeds led to a higher survival rate of seedlings [14,15]. Soluble sugar is an important nutritional component of plant seeds and plays a significant role during the process of seed germination. Therefore, higher soluble sugar concentration of SI may be a main reason for the superiority of large seeds over small ones in seed germination and seedling growth. However, Chen and Maun [18] found that seed size does not show significant effects on seed germination and seedling emergence. The probable reasons for the above results may be related to physiological requirements, the structural limitations of seeds, and the microenvironment surrounding the seeds. Recent studies have shown that the environment experienced by plants during seed formation had important effects on seed characteristics [13], such as temperature [28], stand density, age of the mother tree [12], and hormone levels of the mother tree [29]. Therefore, seed size needs to be integrated with the external environmental factors of seed formation in explaining the natural regeneration of forests.

With the increase in burial depth, G_r increased at first and then decreased, whereas MGT showed the opposite trend. Intermediate burial depth was favorable for seed germination of *P. thumbergii*, which was consistent with the results of Wang et al. [15], Liu et al. [22], and Zhu et al. [23]. A high germination rate at an appropriate depth were related to greater humidity around these seeds [22,23]. The decrease in germination rate at the 4 cm burial depth may be related to insufficient oxygen in the sand [25]. In addition, the increase in germination time would lead to excessive consumption of energy contained within seeds, which affected the survival rate of subsequently seedlings [18,22]. In this paper, seedling biomass was significantly negatively correlated with seed mean germination time. Therefore, the duration of seed germination is an important mechanism determining seedling establishment.

With the increase in sand burial depth, seedling height and the ground diameter of seedlings from large seeds increased gradually, while seedlings from small seeds did not change significantly or even decreased, which was perhaps related to the high concentration of storage substances in large seeds [30]. In addition, the longer time of larger seeds between actual germination and seedling emergence from the sand was also perhaps an important reason for the larger seedling height and diameter. Seed size and sand burial depth together affected the growth of *P. thunbergii* seedlings.

Biomass allocation is important for plants to adapt to different habitats. In this paper, the seedlings from large seeds had higher LMR. Moreover, seedling biomass and height were positively correlated with LMR. The increase in leaf investment was beneficial for plants to improve photosynthetic capacity, growth rate [31], and light interception ability under the forest [32]. Thus, high investment in leaf biomass was an important mechanism for the rapid growth of seedlings from large seeds. Gross [33] suggested that large seedlings could maintain a size advantage in the early growth stage in competitive or vegetated cover. Saeed and Shaukat [34] found that the seedlings of *Senna occidantalis* L. from large seeds had a higher survival rate. High SMR could increase stem elongation of seedlings, which made them less vulnerable to sand burial [35,36]. RMR decreased with the increase in seed size, but the changes in SRL and SRA showed the opposite trend. Seedling biomass was positively correlated with SRL and SRA. Thus, increased SRL and SRA may partially compensate for the loss in root biomass under stress conditions [37]. Chen and Maun [18] argued the ability to grow longer roots for seedlings from large seeds would be of adaptive significance on sand dune systems because it would enhance absorption of water and nutrients. However, sand burial showed no significant effects on biomass allocation of *P. thunbergii* seedlings. Frosini et al. [38] found no significant differences on root/shoot ratio of *Sporobolus virginicus* (L.) Kunth among sand burial depths. With the increase in burial depths, Sykes and Wilson [39] reported four New Zealand sand dune species did not change significantly; six species decreased root/shoot ratios, whereas 19 species increased these ratios. These results indicate that the response of biomass allocation to sand burial is closely related to plant species and growing environments. High leaf investments (higher LMR) and good root morphology (higher SRA and SRL) in large seeds are important ways for *P. thunbergii* seedlings to adapt to coastal sand burial and low light.

The strong correlations between different indicators of seed germination performance and seedling growth imply that some indictors can be used to select the best material for the regeneration of *P. thunbergii* forests. For the selection of seeds, the most important indicator is 1000-seed weight, because larger seeds were apparently superior to smaller ones with respect to germination performance and seedling growth. According to the results of principal component analysis, the vigor index is the most important indicator of germination performance because it had the highest correlation coefficient with seedling biomass. Among the indicators of seedling growth, seedling biomass, SRL, and SRA appeared to be the most relevant. RMR was negatively correlated with SRA and SRL, indicating a trade-off between root biomass allocation and root morphology. Under low root biomass investment, the ability of seedlings to absorb water and nutrients was increased by improving root morphology. Therefore, 1000-seed weight, the vigor index, seedling biomass, and root morphology can help us to evaluate the quality of the seedlings.

5. Conclusions

For many plant species, large seeds can produce large and sturdy seedlings, which are more resistant to environmental stresses [40]. In this study, we found that larger seeds of *P. thunbergii* were more advantageous with respect to seed germination and the early growth of seedlings. Correlation analysis showed that the characteristics of seed germination had significant impacts on the initial development of seedlings. The results also revealed that the proper choice of sand burial depth could significantly improve seed germination and seedling growth. A conclusion we can draw from this study is that seed size and sand burial depth are the two most important factors to be considered in the production of seedlings of coastal *P thunbergii* in Shandong Peninsula. Regarding the assessment of the quality of seedlings, close attention should be paid to seedling biomass and root morphology.

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