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Analysis of Cone and Seed Characteristics from Different Mating Design Strategies of *Pinus densiflora* for. *multicaulis*

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Abstract

This study aimed to enhance seed productivity and secure genetic resources for *Pinus densiflora* for. *multicaulis*. We analyzed the characteristics of cone and seed generated by control pollination between *Pinus densiflora* (PD) and *Pinus densiflora* for. *multicaulis* (PDM). The highest number of cone scales (63.0) was obtained from the self-pollinated (sp) PDM clone B (PDM-sp-B), whereas the lowest number of cone scales (44.7) was obtained from two combinations designated as PDM-A×PD-075 and PDM-A×PD-0111. Both female parents of the hybrids were PDM-A. The highest seed production capacity (80.8) was obtained from the open-pollinated (op) PDM clone B (PDM-op-B). The seed potentials of PDM-B×PD-0111, PDM-op-A, and PDM-sp-B were 67.4, 66.5, and 63.1, respectively. The highest number of fertile scales (41.5) was obtained from PDM-op-B, and the lowest number of fertile scales (28.8) was obtained from PDM-A×PD-075. The total number of aborted ovules and 1st aborted ovules was not statistically significant in the mating design. The cross combination of PDM-B×PD-0111 had the highest number (34.8) of filled seeds and the lowest number of 2nd aborted ovules (5.2) and empty seeds (9). PDM-op-B had the highest number of developed seeds (47.6), although the number of empty seeds was the highest (41.2). Therefore, we conclude that the mating design of PDM-B×PD-0111 is useful for future breeding programs to improve seed yield of PDM. Our results showed that there was a strong correlation between the following two parameter pairs: number of scales and number of fertile scales, and the number of fertility scales and seeds potential (r=0.89 and r=0.84, respectively; both $p<0.01$).

Key Words: *Pinus densiflora*, *Pinus densiflora* for. *multicaulis*, cone production, mating design, seed characteristics

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Introduction

Pinus densiflora Siebold & Zucc is geographically distributed throughout Korea, Japan, and Northeastern China. This species is distributed across the entire Korean peninsula except on the northern highlands (Mirov 1967; Woo 2003), and is classified into six ecotypes based on the morphological characteristics within the distribution region (Han et al. 2007). *P. densiflora* is a major forest species in Korea with economic and cultural importance. Therefore, extensive research has focused on pine improvement, and genetically excellent seeds have been produced through superior tree selection, seed orchard development, and progeny tests (Zobel and Talbert 1984; Sang et al.1991).

P. densiflora for. *multicaulis* Uyeki is a variety of pine and is called Cheonjisong, Manjisong, and Joseon Dahaengsong. A native population has not been reported, and it very rarely grows as an individual tree (Woo 2003). The stem is divided into various branches from the root zone as a sprout shape, and the crown has a characteristic scallop or hemisphere shape. By contrast, pine trees generally have a very high crown height. For these reasons, *P. densiflora* for. *multicaulis* has a very high value as an ornamental species. Although the morphological characteristics of *P. densiflora* for. *multicaulis* are shown in native conditions due to various reason, the unique shape does not follow the mother tree shape, and progeny appear similar to *P. densiflora* in the next generation. Several parameters have been reported to distinguish between *P. densiflora* and *P. densiflora* for. *multicaulis*, including isoenzymes (Hwang and Lee 1996), chromosome morphology (Kim et al. 1990), and morphological characteristics of *P. densiflora* for. *multicaulis* (Woo 2003).

Previous studies used randomly selected individuals to investigate the morphological characteristics of seeds and cones in *P. densiflora* and *P. densiflora* for. *multicaulis*. However, studies on the morphological characteristics, cones, and seed characteristics or traits in clones obtained by artificial control pollination between *P. densiflora* and *P. densiflora* for. *multicaulis* have not been reported.

Timber quality in pine species can be improved through control pollination (Kim et al. 2003). It is necessary to study seed characteristics with different mating designs between *P. densiflora* and *P. densiflora* for. *multicaulis*. Under these conditions, growth trait variations can be confirmed.

P. densiflora for. *multicaulis* has a lower growth rate, seed production capacity, seed maturity rate, germination rate, seedling height, and root diameter than other pine trees (Kim et al. 1989). This study aimed to identify best lines of pine trees to improve seed productivity and secure genetic resources by examining the characteristics of conifers and seeds produced by different mating designs between *P. densiflora* for. *multicaulis* (PDM) and *P. densiflora* (PD).

Materials and Methods

Mating design

We selected eight combinations of pine trees for mating with two *P. densiflora* (PD) clones (0111 and 075) and two

Table 1. Mating design of *Pinus densiflora* and *P. densiflora* for. *multicaulis*

Mating design	Artificial crossing		
	Yes/No	Female parent	Male parent
PDM-op-A	No	*P. densiflora* for. *multicaulis*	
PDM-op-B	No	*P. densiflora* for. *multicaulis*	
PDM-sp-A	Yes	Clone A, *P. densiflora* for. *multicaulis*	Clone A, *P. densiflora* for. *multicaulis*
PDM-sp-B	Yes	Clone B, *P. densiflora* for. *multicaulis*	Clone B, *P. densiflora* for. *multicaulis*
PDM-A×PDM-B	Yes	Clone A, *P. densiflora* for. *multicaulis*	Clone B, *P. densiflora* for. *multicaulis*
PDM-B×PDM-A	Yes	Clone B, *P. densiflora* for. *multicaulis*	Clone A, *P. densiflora* for. *multicaulis*
PDM-A×PD-075	Yes	Clone A, *P. densiflora* for. *multicaulis*	Clone 075, *P. densiflora*
PDM-A×PD-0111	Yes	Clone A, *P. densiflora* for. *multicaulis*	Clone 0111, *P. densiflora*
PDM-B×PD-075	Yes	Clone B, *P. densiflora* for. *multicaulis*	Clone 075, *P. densiflora*
PDM-B×PD-0111	Yes	Clone B, *P. densiflora* for. *multicaulis*	Clone 0111, *P. densiflora*

PD, *Pinus densiflora*; PDM, *Pinus densiflora* for. *Multicaulis*; sp, self-pollination; op, open pollination.

P. densiflora for. *multicaulis* (PDM) clones (A and B) (Table 1). The control groups of cones were sampled from the open pollinated *P. densiflora* for. *multicaulis*.

Cone analysis

Morphological analyses were conducted by separating the scales from the 12 cones (4 cones×repetition) using the methods of Bramlett et al. (1977) and Lee et al. (1984). Segregated scales were classified as a fertile scale or a sterile scale. The productive potential of the cone was determined by counting aborted ovule and developed seed. Mature seeds were classified as filled seeds or empty seeds after immersing in water for 24 hours. In the first and second years, embryos were grouped according to their size and morphological characteristics.

Statistical analysis

A SAS enterprise guide (SAS Institute, ver. 9.2) program was used to test the significance of the traits. Duncan's multiple test was performed when the mean value was significant ($p < 0.05$). Pearson correlation coefficients were tested at significance levels of 1% and 5% using the same program.

Results and Discussion

Cone analysis

Investigating the characteristics of pine cones and seeds enables the analysis of actual seed production and potential seed production capacity, and identifies and quantifies the extent and type of loss in the seed development stage (Bramlett et al. 1977). These analyses also provide information that is needed to evaluate control pollination programs and resulting seed production (Choi et al. 2007). The average number of scales among mating designs (50.8) was confirmed in self-pollination of clone B (PDM-sp-B) (Table 2). In the combinations of PDM-A×PD-075 and PDM-A×PD-0111, they had the lowest number of scales (44.7). The average number of fertile scales was 33.9. The highest number of fertile scales (41.5) was investigated in open pollination (PDM-op-B) and the lowest number of scales (28.8) was identified in PDM-A×PD-075, which was a combination of a *P. densiflora* for. *multicaulis* mother tree (PDM-A). Compared with previous studies such as that of Choi et al. (2007), our results indicated that the number of fertile scales (39.4) tended to be specify the number of fertile scales reported pine trees; however, it was a similar trend as that reported by Kwon et al. (1990), which was 34.0.

Control pollination research in pine trees uses open pollination and multiple artificial pollination. There was no dif-

Table 2. Seed potential factors of cones in different mating designs

Mating design	Number of scales			Seed potential	Number of aborted ovules			Number of developed seeds		
	Total	Fertile	Sterile		Total	1st year	2nd year	Total	Empty seeds	Filled seeds
PDM-op-A	53.4^{abc}	34.2^{abc}	19.3^{b}	66.5^{ab}	31.7ns	16.7ns	15.0^{ab}	34.8^{ab}	29.7^{ab}	5.1^{d}
PDM-op-B	59.0^{ab}	41.5^{a}	17.5^{bc}	80.8^{a}	33.2	17.9	15.4^{a}	47.6^{a}	41.2^{a}	6.4^{d}
PDM-sp-A	48.2^{c}	32.3^{abc}	15.9^{bc}	52.0^{bc}	28.4	15.1	13.4^{ab}	23.6^{b}	20.6^{bc}	2.9^{d}
PDM-sp-B	62.8^{a}	38.7^{ab}	24.1^{a}	63.1^{ab}	23.7	9.7	14.0^{ab}	39.4^{ab}	33.3^{ab}	6.1^{d}
PDM-A×PDM-B	45.4^{c}	29.9^{bc}	15.6^{bc}	42.0^{c}	22.4	12.0	10.4^{ab}	19.6^{b}	17.9^{bc}	1.7^{d}
PDM-B×PDM-A	49.2^{bc}	34.4^{abc}	14.8^{bc}	53.8^{bc}	25.8	16.2	9.6^{ab}	28.0^{ab}	25.9^{ab}	2.1^{d}
PDM-A×PD-075	44.7^{c}	28.8^{c}	15.9^{bc}	43.9^{c}	22.8	11.7	11.1^{ab}	21.1^{b}	5.2^{c}	15.9^{bc}
PDM-A×PD-0111	44.7^{c}	31.6^{bc}	13.1^{c}	49.6^{bc}	26.6	15.1	11.4^{ab}	23.0^{b}	9.1^{c}	13.9^{c}
PDM-B×PD-075	46.2^{c}	30.7^{bc}	15.6^{bc}	55.4^{bc}	26.8	11.2	15.6^{a}	28.7^{ab}	8.3^{c}	20.3^{b}
PDM-B×PD-0111	54.6^{abc}	37.0^{abc}	17.6^{bc}	67.4^{ab}	23.7	18.4	5.2^{b}	43.8^{a}	9.0^{c}	34.8^{a}
Mean	50.82	33.91	16.94	57.45	26.51	14.40	12.11	30.96	20.02	10.92

PD, *P. densiflora*; PDM, *P. densiflora* for. *multicaulis*; sp, self-pollination; op, open pollination.

ference in the number of cone scales in open pollination and multiple artificial pollination; however, the seed-to-ovule ratio was higher in artificial pollination than open pollination (Iwaizumi et al. 2008). Self-pollination (sp) is conducted using controlled quality and quantity of pollen, and results in lower numbers of seeds due to ecological or evolutionary factors such as cone growth conditions. The average number of sterile scales was 16.9 in 8 breeding combinations. The highest number of sterile scales (24.1) were produced by self-pollination of B (PDM-sp-B), whereas the lowest number (13.1) was produced by crossing PDM-A× PD-0111.

The mean of seed potential per cone was 57.5. The highest value was recorded in PDM-op-B (80.8), whereas the lowest value was recorded in PDM-A×PDM-B (42.0). The seed potential is the biological limit of the number of available productive seeds per cone, and seed productivity can be reduced as the quantity of fruiting rate increases (Kim and Hur 2013). The average number of aborted ovules was 26.5, and it was 14.4 in the first year. The aborted ovule number was higher in the first year than the second year for essentially all crossing combinations. The first year of aborted ovule is due to a lack of pollen and stink bug–induced damage during the cone development stage (Lee et al. 1984). The second year of aborted ovule is caused by insect-induced damage, such as stink bug–induced damage before seed coat development (Kim et al. 2013). The seed potential can be used to select the best crossing combination by selecting for the greatest number of seeds produced and the lowest number of secondary aborted ovules, because the number of aborted ovules did not significantly differ between the total and the first year. The highest number of secondary aborted ovules was recorded in open pollination of PDM-op-B (15.4), whereas the lowest number was recorded in PDM-B×PD-0111 (5.2), which resulted from the crossing combination where PDM-B was selected as the mother tree. The number of filled seeds was the highest (34.8) and the number of empty seeds was the lowest (9.0) in the PDM-B×PD-0111 combination.

The highest number of mature seeds (47.6) was recorded in the open pollination combination B (PDM-op-B) and PDM-B×PD-0111; however, the number of empty seeds also was the highest (41.2) in this combination. Sufficient quantities of high-quality pollen are required to maximize the production of mature, fully grown seeds. Tissue damage caused by cone insects needs to be minimized to elevate mature seed productivity to potential seed productivity (Bramlett et al. 1977). The difference in seed characteristics between the upper and lower crown in open pollination and control pollination was due to differences in pollen quality and quantity (Iwaizumi and Takahashi 2012). Differences in seed maturity in an individual clone of a mating design may be related to the number of empty seeds. Lethal genes in the seed embryo, pathogenic cone insects, and pathogenic fungi have been suggested to cause the production of empty seeds (Bramlett et al. 1977).

Open pollination produces many seeds in *P. densiflora* for. *multicaulis*, but it is considered disadvantageous to obtain numerous filled seeds because of the correspondingly large number of empty seeds. The number of mature seeds collected from open-pollinated *P. densiflora* (clone PD-075, PD-0111) was similar to that of open-pollinated PDM, but the number of filled seeds was four to five times greater (data not shown). Therefore, it will be possible to increase the seed fertility ratio of *P. densiflora* for. *multicaulis* by cross pollination with *P. densiflora*. Our study indicates that the best combination to increase seed potential and mature seed ratio is the PDM-B×PD-0111 combination. Seed yield can be affected by fertilization failure due to pollen quantity and quality or cone degeneration (Kim et al. 2002). Therefore, it is necessary to consider the number of filled seeds along with seed production capacity when designing the mating strategy.

Correlation

We examined the correlations between cone and seed factors (Table 3). There was a strong positive correlation between the number of scales and the number of fertile scales ($r=0.89$, $p<0.01$). The number of fertile scales was strongly positively correlated with seed potential ($r=0.83$, $p<0.01$) and the number of mature seeds ($r=0.79$, $p<0.01$). Seed potential was strongly positively correlated with the number of filled seeds and empty seeds ($r=0.83$ and $r=0.80$, respectively, $p<0.01$). Similar trends were observed for other mating designs with high seed potential and seed yield, such as PDM-op-B, but were not observed in the PDM-B×PD-0111 combination. We observed a negative correlation between the number of secondary

Table 3. Correlation coefficients of cone and seed characteristics

	NS	NFS	NSS	SP	NAO	NAO1	NAO2	NDS	NES	NFSE
NS	1.00									
NFS	0.89**	1.00								
NSS	0.53**	0.08	1.00							
SP	0.83**	0.84**	0.24	1.00						
NAO	0.34**	0.23	0.31*	0.45**	1.00					
NAO1	0.34**	0.33**	0.13	0.42**	0.77**	1.00				
NAO2	0.19	0.04	0.35**	0.29*	0.80**	0.24	1.00			
NDS	0.71**	0.79**	0.08	0.83**	-0.12	-0.02	-0.18	1.00		
NES	0.69**	0.71**	0.17	0.80**	0.06	0.03	0.06	0.84**	1.00	
NFSE	-0.01	0.09	-0.17	0.01	-0.33**	-0.09	-0.42**	0.22	-0.34*	1.00

NS, number of scales; NFS, number of fertile scales; NSS, number of sterile scales; SP, seed potential; NAO, number of aborted ovules; NAO1, number of aborted ovules in the 1st year; NAO2, number of aborted ovules in the 2nd year; NDS, number of developed seeds; NES, number of empty seeds; NFSE, number of filled seeds.
*$p < 0.05$, **$p < 0.01$.

Table 4. Seed germination rate and seedling growth characteristics from the mating design strategy

Mating design	Germination rate (%)	Seeding height (cm)		
		Mean	Max.	Min.
PDM-op-A	25	8.9	3.5	14.2
PDM-op-B	34	7.5	4.0	12.6
PDM-sp-A	40	5.8	3.0	7.8
PDM-sp-B	32	8.1	5.1	12.4
PDM-A×PDM-B	50	5.7	3.5	7.7
PDM-B×PDM-A	40	6.6	3.0	9.4
PDM-A×PD-075	14	8.9	4.0	13.2
PDM-A×PD-0111	18	8.4	6.3	10.8
PDM-B×PD-075	44	9.1	4.0	11.4
PDM-B×PD-0111	51	8.2	5.0	11.8

PD, *P. densiflora*; PDM, *P. densiflora* for. *Multicaulis*; sp, self-pollination; op, open pollination.

aborted ovules and the number of filled seeds. Previous research into developing pine plus trees reported that the negative correlation between developed seed ratio and aborted ovules was caused by insufficient pollen supply, and the number of fertile scales was strongly positively correlated with seed efficiency (correlation coefficient 0.80) (Choi et al. 2007).

Germination and seedling growth

We analyzed the germination rate after 30 days from sowing and growth characteristics of eight-month-old seedlings produced by different mating designs (Table 4). The germination rates of seeds produced by open pollination combinations PDM-op-A and PDM-op-B were 25% and 34%, respectively, which were below the average germination rate for all mating designs. Mating designs that had germination rates above 50% were PDM-B×PD-0111 and PDM-A×PDM-B. The germination rate of *P. densiflora* ranges from 78.7-97.3% depending on the stand (Choi et al. 2007). The germination rate of *P. densiflora* (PD) seeds collected from the Acha mountains was 80.8-88.6% depending on the slope (Chung et al. 2010). We observed that the germination rate of *P. densiflora* for. *multicaulis* control pollination was lower than that reported in previous pine research. In *P. thunbergii*, different storage periods caused approximately two-fold differences in initial germination rates, and differed by approximately 14% in final germination rates (Hong et al. 2006).

The PDM-A×PDM-B combination had the highest germination rate, but the average seedling height was lower that all other mating designs. The PDM-A×PD-0111 combination had the highest germination rate and the seedling height was higher than the average value of other combinations. Our results indicate that control pollination of *P. densiflora* for. *multicaulis* with *P. densiflora* may improve seed productivity of PDM. We observed that clone B of *P. densiflora* for. *multicaulis* (PDM-B) mediated greater improvement in seed productivity than clone A, and it may be selected and utilized as a mother tree for F1 seed production. Therefore, we conclude that the best mating design is PDM-B×PD-0111, which showed the highest of seed production capacity and yield of developed seeds.

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