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Variation of Seed Viability among Cone Harvest Times at Two Clonal Seed Orchards of *Chamaecyparis obtusa*

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Abstract

The timing of seed harvesting is an important decision in the management of seed orchards because it affects seed quality and yield. To investigate the effect of cone harvest time on seed quality and determine the optimal harvesting time, cones were regularly collected in seven times and germination tests were performed at each harvest time in two clonal seed orchards of *Chamaecyparis obtusa*. As cones developed, the percentage of seed germination increased before cone moisture content began to decrease significantly. The moisture contents of cones were highest at the first collection as 68.3% and 67.3% in Jeju and Gochang seed orchards respectively. At this time, germination speed was slowest, indicating poor seed vigour. The highest germination was found at the second stage in Jeju (36.5%) and at the seventh stage in Gochang (28.6%) seed orchard. The germination speed increased as cone moisture content decreased. Additionally, changes of seed vigour differed among the developmental stages in both seed orchards. Consequently, the optimal cone harvest time of *C. obtusa* seed orchards in Jeju was early September when high germination percentage was obtained. In Gochang seed orchards, late October was optimal cone harvest time when the germination speed was fast and the cone moisture content decreased.

Key Words: cone moisture content, seed maturation, seed viability, seed vigour, hinoki

Introduction

Chamaecyparis obtusa (Siebold and Zucc.) Endl. is an economic tree species used in afforestation in South Korea and widely planted in the southern regions since its introduction from Japan in the early 20th century (Korea Forest Service 2021). It occupies 27.8% of the area afforested over the past five years in South Korea due to its high wood quality. Moreover, the demand for *C. obtusa* has increased as the effect of forest healing has attracted attention. The demand requires the production of large quantities of seeds and seedlings (Kang et al. 2014). But the seed production is very irregular, and the germination is normally very poor. Most of the *C. obtusa* seeds used in the national afforestation programme of South Korea are produced in clonal seed orchards.

Seed quality changes during seed development and maturation. As seeds mature various seed quality compo-

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nents are attained sequentially. In general, germination ability develops first, during the seed-filling phase. In the case of orthodox seeds, desiccation tolerance is obtained next. Thereafter, seed vigour, which is related to rapid germination and stress resistance, is obtained. Longevity during storage is attained at the end of the developmental period (Bewley et al. 2013).

There have been various opinions about when seed quality is at its highest. Firstly, there is the traditional hypothesis claiming that seed quality is highest when it reaches physiological maturity at the end of the seed-filling phase where the seed's dry weight reaches its maximum. Thereafter seed quality gradually decreases as the nutritional supply from the maternal plant is stopped (Shaw and Loomis 1950; Harrington 1972). On the other hand, other reports found that seed quality improved after seeds reached their maximum seed dry weight (Rao et al. 1991; Wilson and Trawatha 1991; Hay et al. 1997; Probert et al. 2007; Hay et al. 2010), and maturity was achieved after being separated from the mother tree (Leprince et al. 2017). Ellis (2019) reported that the time of maximum dry weight of seeds does not necessarily indicate the time of the highest quality and that this is rather affected by the environment or genotype-environment interaction during the development and maturation of seeds.

To assess the quality of seeds, both viability and vigour should be determined. Seed viability is the ability of seeds to germinate and produce seedlings under favourable conditions. It can be measured as the germination percentage obtained during a standard germination test as described by the International Seed Testing Association (ISTA). Seed vigour is a more complex concept which represents the vigour displayed by the seed in unstable and stressful conditions and the speed and uniformity of seed germination and seedling growth (Marcos-Filho 2015).

Seed vigour usually continues to increase after germination capacity reaches its maximum. It should thus be considered along with seed viability when determining seed quality because it affects the storability of seeds and the robustness of seedlings. ISTA defines seed vigour as the sum of the properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments (Finch-Savage and Bassel 2016). Therefore, seed vigour cannot be measured as a single property of germination ability. Methods to examine seed vigour include the accelerated aging test the electrical conductivity test and the cold test yet there is no standardized method. Germination speed, which is quantified by T_{50} (Coolbear et al. 1984) or mean germination time (Soltani et al. 2015) can also indicate seed vigour.

Cone harvest time is an important decision affecting the efficiency of seed production. The timing of cone harvesting is a major factor affecting the quality yield and storability of seeds. The optimal time for seed harvest is when most of the seeds have a high quality and yield, while the loss during harvest and storage is minimized (Black et al. 2006). Early harvesting may result in low quality and yield and seeds being vulnerable to fungal infections due to their high moisture content (Kelly and George 1998). On the other hand, late harvesting can lead to seed hardening or deterioration due to excessive drying, exposure to insect damage, or losses due to seed dispersal (Tak et al. 2006; Karrfalt 2008; Likoswe et al. 2008).

The optimal time for seed harvest is suggested from mid-September to early October (Bae et al. 2012) in natural stands of *C. obtusa*. There have been a few reports on the relationship between seed maturation and harvesting time. However, the cone harvest time at seed orchards is not determined clearly, and the effect of cone harvest time on the seed quality of *C. obtusa* is still unclear.

The main aim of this study is to determine the optimal cone harvest time to avoid loss of seed production in clonal seed orchards of *C. obtusa* by 1) measuring cone moisture content among different harvest times in two clonal seed orchards (young and old) of *C. obtusa*, 2) analysing the correlation between cone moisture and seed quality (*i.e.*, germination percentage and germination speed), and 3) finding an index for optimal cone harvest time at different locations (island and inland) of seed orchards in South Korea.

Materials and Methods

Seed harvest and cone moisture content

Cones were harvested at two clonal seed orchards of *C. obtusa* at Jeju and Gochang in South Korea. The Jeju seed orchard was located on Jeju Island in the southernmost region of the Korean Peninsula (33°18'25" N, 126°32'49" E, 590 m), and the Gochang seed orchard was located on an inland area in the southwestern part of the Korean Peninsula (35°19'09" N, 126°33'22"E, 33 m). The Jeju seed orchard (old) was established in 1973 and the Gochang seed orchard (young) was set up in 2015. The seed trees comprising the two clonal seed orchards were propagated from the same gene pool of selected plus trees.

The first cone harvest from the two seed orchards was carried out in the fourth week of July in 2021. The first harvest time was designated as H0 and cones were harvested seven times every three weeks until the first week of December named H21 H42 H63 H84 H105 and H126 (*i.e.* the number indicates the number of days since the first collection). Fifteen individuals were randomly selected in each seed orchard to consider the clone as a block factor to remove the effect of genotype and test the effect of harvest time.

For each tree, more than 30 cones were collected evenly from all four sides of the crown to reduce sampling errors due to differences in branch direction and height. The harvested cones were dried in a drying chamber at 15°C and 10% relative humidity until the cone moisture content was below 10%. The seeds were extracted and stored in polyethylene sealing bags at 4°C for about two months before the germination test. The seed of *C. obtusa* is known as orthodox and tolerant for dry storage, and as there was no difference in germination between freshly harvested and stored seeds. The reason why dry-stored seeds were used was to make the seeds similar condition to the practical seed process since the seeds should be stored for several months between the time of harvest and supply to the nursery.

In order to analyse the effect of harvest time on cone moisture, two or more cones per harvest time were randomly selected from each individual and then the cone moisture content was measured using a moisture analyser (AND MX-50, Seoul, Republic of Korea).

Seed germination test and germination parameters

Standard germination tests were performed to evaluate seed quality at each harvest time. For each harvest time, a total of 400 seeds per individual (four replicates of 100 seeds) were randomly sampled and subjected to the germination test. Seeds were placed on moist filter paper in Petri dishes and watered with distilled water. The Petri dishes were kept at 25°C temperature and 40% relative humidity for 28 days. Seeds with a radicle growing more than 2 mm were considered as germination. The number of germinated seeds was recorded every day.

Seed viability was estimated as the percentage of germinated seeds. Germination percentage (*GP*) was calculated as follows:

$$GP = \frac{N}{S} \times 100\,(\%)$$

where N is the final number of germinated seeds and S is the total number of seeds.

Seed vigour was estimated as the median number of days required to achieve 50% of the final germination percentage (T_{50}). A lower T_{50} value indicates greater seed vigour. The T_{50} was calculated using the following formula suggested by Coolbear et al. (1984) and modified by Farooq et al. (2005):

$$T_{50} = T_i + \frac{(\frac{N}{2} - N_i)(T_j - T_i)}{(N_j - N_i)}\,(\text{Days})$$

where N is the final number of germinated seeds, N_i and N_j are cumulative numbers of germinated seeds by adjacent counts at times T_i and T_j, respectively ($N_i < N/2 < N_j$).

Statistical analysis

A generalized linear mixed model with a binomial distribution and logit link function and pairwise *post-hoc* tests were used to test the effect of harvest time on germination percentage. An ANOVA and a *post-hoc* Tukey test were used to test the effect of harvest time on T_{50}. These models included harvest time as a fixed factor and individual as a random effect.

An analysis of correlation was conducted to test whether there was a relationship among cone moisture content, germination percentage and T_{50} at different harvest times. Since each combination of variables did not satisfy the assumption of a bivariate normal distribution, Spearman's rank correlation coefficient was used to test for significance. The T_{50} cannot be calculated when the number of germinated seeds is less than two, so observations below two were excluded from the rank correlation analysis. All statistical analyses were performed using R 4.0.2 (R Core Team, 2020).

Results

Relationship between cone moisture content and harvest time

The moisture content of cone consistently decreased from H0 (fourth week of July) to H126 (first week of December) in both seed orchards of *C. obtusa* (Fig. 1). In the mature seed orchard of Jeju Island, the moisture content of cones was highest at H0 (68.3%) and then gradually decreased by the developmental stage of H126 (45.0%). The first significant difference was detected at H42 (62.6 %). After that, the difference was not significant until H84 (fourth week of October). The cone moisture content decreased rapidly from H105 (54.9%) to H126 (45.0%), and some seeds started to disperse naturally at the stage.

In the young seed orchard of Gochang (inland), the cone moisture content was peak at H0 (67.3%) and gradually decreased until H105 (58.3%) (Fig. 1). The first significant decrease was detected at H63 (first week of October) and the second drop was remarkable at H126 (38.5%) (Fig. 1). Unlike Jeju, the cone did not open completely in Gochang.

Effect of cone harvest time on germination parameters

Germination percentage was classified as six and five groups based on Turkey's HSD test and there were significant differences among the groups in Jeju and Gochang seed orchards, respectively (Fig. 2). Germination percentage was almost zero at H0 (0.5%) and peak at H21

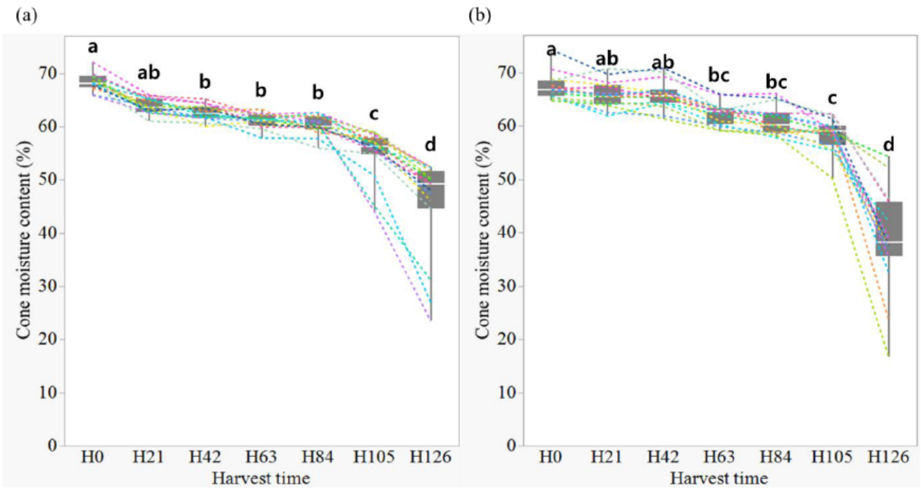

Fig. 1. Moisture content of cone according to harvest time in Jeju (a) and Gochang (b) seed orchards of *C. obtusa*. Dotted lines represent the mean values of the individual tree, and each boxplot represents a summary of harvest time. Different letters above the boxplots represent significant differences among harvest times according to Tukey's HSD tests at $\alpha=0.05$.

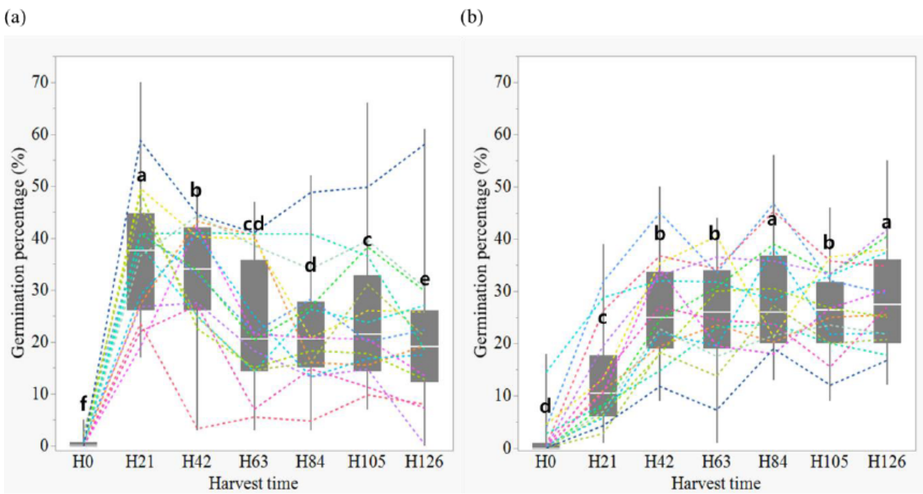

Fig. 2. Germination percentage according to cone harvest time in clonal seed orchards of *C. obtusa*; (a) Jeju and (b) Gochang. Different letters above the boxplots represent significant differences among harvest times according to pairwise *post-hoc* tests at $\alpha=0.05$.

(36.5%). But the germination speed at H21 was very slow as indicated by the high T_{50} value (14.6 days) (see also Fig. 3). After H21, the germination percentage was decreased by H63 (24.2%) and then steady until H128 (22.8-21.8%) in the old seed orchard of Jeju (Fig. 2). But the germination percentage in the young seed orchard of Gochang was increase continuously from H0 (1.8%) to H126 (28.6%).

The T_{50} were significantly different ($p < 0.001$) among cone harvest times and individuals in both seed orchards (Fig. 3). The variation of T_{50} among individuals was larger in Gochang (young) than Jeju (old) seed orchard (Fig. 2 and 3). The T_{50} was dropped down at the stage of H0 (21 days) and H42 (8.2 days) but steady after H42 stage in Jeju seed orchard (Fig. 3) indicating the speed of germination was getting faster and stable after H42 (6.9-6.0 days). The T_{50} in the young seed orchard of Gochang fell more slowly than Jeju which was from 20.8 days (H0) to 7.8 days (H84) and consistent until H126 stage (7.4 days). This result implies that the cone collection time should be different in both seed orchards, *i.e.*, faster in Jeju than Gochang.

Correlation between cone moisture content and seed germination

The cone moisture content and seed germination had a negative correlation each other (Table 1), which was non-significant in Jeju seed orchard but statistically significant at the 5% significance level (Spearman's r=-0.567) in Gochang seed orchard (Table 1). However, the correlation between cone moisture content and T_{50} was positive and highly significant in Jeju (Spearman's r=0.692, $p < 0.001$) and Gochang (Spearman's r=0.759, $p < 0.001$) seed orchards (Table 1). These results indicate that germination speed increased as cone moisture content decreased.

Discussion

To assess the quality of seeds produced in each harvest times, germination percentage was calculated to estimate seed viability, and T_{50} for seed vigour. The seed quality of seeds changed as cone moisture content decreased at differ-

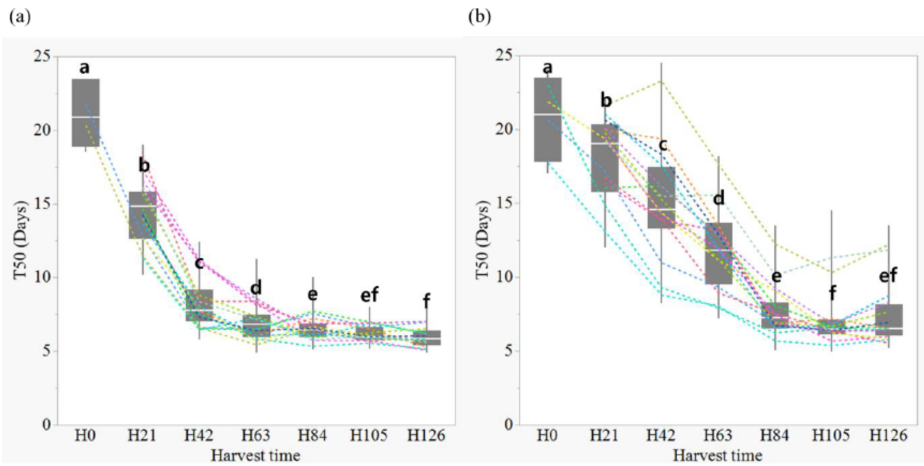

Fig. 3. Average of T_{50} among clones according to cone harvest time in clonal seed orchards of *C. obtusa*; (a) Jeju and (b) Gochang. Different letters above the boxplots represent significant differences among harvest times according to pairwise Tukey's HSD tests at $\alpha=0.05$.

Table 1. Spearman's rank correlation coefficient among cone moisture content, germination percentage and T_{50} in clonal seed orchards of *C. obtusa* at Jeju (above diagonal) and Goching (below diagonal)

	Cone moisture content	Germination percentage	T_{50}
Cone moisture content	-	0.176^{NS}	0.692 ***
Germination percentage	-0.567 ***	-	0.146^{NS}
T_{50}	0.759 ***	-0.539 ***	-

NS, not significant at $\alpha=0.05$; ***, significant at $\alpha=0.001$ probability level.

ent harvest times. In Jeju, seed viability increased before cone moisture content decreased significantly. Seeds collected before H21 (late August) had poor seed vigour, which can lead to poor performance when producing seedlings and be harmful during storage. Seeds can have germination ability before they reach physiological maturity but may require artificial (post-harvest) drying to germinate in conifer species (Bewley et al. 2013).

The seed quality was significantly different among cone harvest time in Jeju and Gochang seed orchards. Also, the variation among individuals in seed viability was larger in Jeju (old) than in Gochang (young), and *vice versa* in seed vigour. The seeds from Gochang seed orchard acquired viability and vigour later compared to those from Jeju. Thus, there is a need for a flexible decision of harvest timing depending on seed orchard locations.

The difference of seed quality between seed orchards may be due to various reasons. Firstly, this may be due to differences in climate between the two locations. Jeju and Gochang have differences in air temperature and summer rainfall. In 2019, the average of air temperature from January to February in Jeju was about 7°C that was higher than that in Gochang, and the rainfall from July to August was about 360 mm higher in Jeju. Ellis (2019) argued that the interaction among genotype, soil condition and climate affects seed quality. Follow-up studies should thus investigate the effect of climate on the maturation of seeds in *C. obtusa* seed orchards. Secondly the *C. obtusa* seed orchard in Jeju is about 40 years older than that of Gochang. Although several studies have reported that the age of mother tree affects seed quality (Espahbodi et al. 2007; Williams 2009; Viglas et al. 2013; Mao et al. 2014), there are no reports on the effect on seed maturation. Therefore, further research is needed on the effect of tree age on seed quality, and the timing of pollination and fertilization.

At both seed orchards the maximum germination was approximately 30 to 35%. It is known that *C. obtusa* produces significant amount of anatomically unsound seeds (Matsuda et al. 2015). We performed cutting tests of non-germinated seeds and most of them were empty seeds (data not shown). Therefore, the maximum germination was inevitably low even when the seeds were mature enough.

Since the maturation of seeds is largely affected by annual climate it is more important to develop an indicator to determine the right time to harvest than to specify a certain calendar date of the year. The fact that the relationship between germination and cone moisture content differs at two clonal seed orchards indicates that it would be necessary to monitor changes in climates during pollination and fertilization and cone moisture contents over time at each seed orchard. The cone moisture content is closely related to seed quality and is easy to measure (Tanaka 1984). Cone moisture content is strongly related to seed quality and decreases as the cone develops after desiccation tolerance is attained (Tanaka 1984; Bewley et al. 2013). The moisture content level at which the cone reaches its maximum may also differ between species and genotypes.

The moisture content of cones was high during the early developmental stages, and the seeds had low viability and vigour at this stage (Fig. 1 and 2). As seeds matured, germination ability increased and cone moisture content decreased, but not significantly. Furthermore, in case of Jeju, when the cone was dried, the quality of seeds started to decrease. Therefore, the proper time of cone harvest of *C. obtusa* in Jenu is early September when high germination ability is obtained. In Gochang, the optimal harvest time is late October when the germination speed (T_{50}) is fast and the cone moisture content decreases. In conclusion, the optimal cone harvest could depend on the quantity of seed harvest and/or the quality of seedlings produced. This means that early September is the best when many seeds are needed, on the other hand, late October is optimal when uniform seedlings should be produced.

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