

Article

Plus Tree Selection of *Quercus salicina* Blume and *Q. glauca* Thunb. and Its Implications in Evergreen Oaks Breeding in Korea

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Abstract: This study was conducted to select plus trees of two evergreen oaks, *Quercus salicina* and *Q. glauca*, in Korea. Evergreen oaks are distributed in subtropical region in Korea and have recently emerged as one of the alternative tree species against climate change. Accordingly, a tree breeding program is underway to foster evergreen oaks as a reforestation species for the future. Through intensive survey on the distribution range, 15 stands (8 for *Q. salicina*, 3 for *Q. glauca*, and 4 for both species) were selected as base populations. To select candidate trees, we developed a subjective grading system with six characteristics in three categories and introduced a weighted generalized value (GVI_w) to compare superiority of candidate trees. The candidate trees were screened using baseline value '0', i.e., if $GVI_w > 0$, then accepted and if $GVI_w < 0$, then rejected. After then, adjustment was conducted to avoid biasing the selection of plus trees for a particular location. Through this process, 44 candidate trees in *Q. salicina* and 41 candidate trees in *Q. glauca* were selected as plus trees. Finally, the results and implications were discussed in relation to evergreen oak breeding in Korea.

Keywords: tree improvement; evergreen oak; phenotypic selection; selection criteria; seed orchard; generalized value; conservation

1. Introduction

The genus *Quercus* is one of the most important angiosperms in the northern hemisphere in terms of species diversity, ecological dominance, and economic value. Oaks are dominant members of a wide variety of habitats including temperate deciduous forest, temperate and subtropical evergreen forest, subtropical and tropical savannah, and subtropical woodlands [1,2].

In South Korea, six deciduous oaks (*Q. acutissima* Carruth., *Q. variabilis* Blume, *Q. mongolica* Fisch. Ex Ledeb., *Q. serrata* Thunb. Ex Murray, *Q. dentata* Thunb. and *Q. aliena* Blume) and five evergreen oaks (*Q. myrsinifolia* Blume, *Q. acuta* Thunb., *Q. glauca* Thunb., *Q. salicina* Blume, and *Q. gilva* Blume) are naturally distributed. While the deciduous oaks are growing all over the country, from temperate to subtropical regions, evergreen oaks are restricted to subtropical regions, the southernmost part of Korea [3]. Traditionally, deciduous oaks have been used as a wood resource (timber, media for mushroom cultivation, charcoal, etc.) and their nuts are also used for starch production, i.e., acorn jelly. Evergreen oaks are also used as a timber production in subtropical region, but the portion is very small. Accordingly, deciduous oaks have more attention in tree breeding program than evergreen oaks [4].

As global warming progresses, the distribution and composition of forest tree species are expected to change. According to climate change scenario in Korea, it is predicted that the distribution range of conifers will decrease while the range of subtropical tree species will expand northward [5,6].

The assisted migration and development of alternative tree species were suggested as countermeasures against global warming [7,8]. In Korea, evergreen oaks have recently emerged as one of the alternative tree species against climate change. Moreover, there have been reports that the leaf, branch, bark, and acorn of evergreen oaks contain useful substances that can be used for food or medicine, which have attracted more attention [9,10]. Thus, as a preliminary study, plus tree selection and establishment of seedling seed orchard of *Q. glauca* had been conducted with small scale mainly in Jeju island [11]. After that, Korea Forest Service established a plan for additional plus tree selection and expansion of seed orchard of evergreen oaks, which was major motivation for conducting this study.

Although the utilization potential of evergreen oaks is highly evaluated, there is a problem that the amount of available resources is relatively limited compared to other common species. For example, they are restrictedly distributed in a narrow area of subtropical region with small population size and low appearance frequency. There is also a need to prepare measures to conserve genetic resource. Thus, in the process of reviewing the breeding plan, it is highly recommended to consider the utilization and genetic resources conservation together. Accordingly, unlike a previous study conducted by considering only growth characteristics [11], this study aimed to consider growth, adaptability, and seed production characteristics simultaneously.

Tree breeding is an integral part of modern silviculture used to increase the economic profit through enhanced wood production. In first-generation tree improvement programs, mass selection or phenotypic selection would be the first step in breeding cycle [12]. The philosophy underlying plus tree selection is that the favorable deviation of plus tree from the population mean is due at least partly to genetic rather than environmental or random effects [13]. There are several methods for plus tree selection, i.e., ocular or subjective method, comparison tree method, baseline method, and regression method [14,15]. The comparison tree method is generally preferred and widely applied in even-aged, pure-species stands. In uneven-aged and mixed-species stands, most common for hardwoods, a grading system is required than standard comparison tree method. The regression or baseline method aims to adjust tree scores for differences in ages, competition and/or environmental gradients. The ocular or subjective method is used when environmental noise is too large to be overcome by regression methods or visual inspection will be effective at locating superior trees. A determination of the best selection techniques depends on several factors, including species characteristics, past history, the present condition of the forest, variability and inheritance pattern of important characteristics, and objectives of the particular tree improvement program [12,16].

As deciduous oaks are dominant or co-dominant tree species with high density in natural stands, comparison tree method is usually used for plus tree selection. However, evergreen oaks have a characteristic of being scattered in a mixed forest with a low density, making it difficult to apply the comparison tree method. Regression method or baseline method would be considered as alternatives. However, the data needed to obtain a baseline or regression equation for the target traits was not accumulated at present. Thus, we have to consider ocular selection method for plus tree selection in evergreen oaks. Ocular method rely upon a subjective assessment where trees that appear to be better than average are chosen as plus trees without measuring the candidate or neighboring trees [14,15]. Actually, simple direct (ocular) selection method was used for plus tree selection in previous study on *Q. glauca* [11]. They were selected 35 plus trees only based on growth traits such as height, diameter, stem straightness, and timber height in lowlands and valleys of Mt. Halla in Jeju island. It seemed to achieve the selection objectives relatively easily using ocular selection method. However, they were not present the detailed selection criteria and methods. In addition, it was regrettable to overlook the characteristics such as flowering, fruiting, resistance to pests or diseases, and adaptability to environment for production of improved seed and reforestation. In other case, Stringer et al. [17] compared the growth and stem quality of 19-year-old progeny from superior and comparison trees in *Q. rubra*. They suggested that selecting several phenotypically above-average candidate trees may be more effective than rigorously selecting a smaller number of phenotypically superior trees. Combining these two cases, it seemed that development of some modified selection method was required for this

study, because the selection work has to be operated at several stands with different environments in the entire distribution and the selection results have to be evaluated objectively by some standard.

Accordingly, we firstly tried to make selection criteria and method for evergreen oaks. Then, we applied the method to plus tree selection of *Q. salicina* and *Q. glauca* for case study. Finally, the results and implications were discussed in relation to evergreen oak breeding in Korea.

2. Materials and Methods

2.1. Selection of Base Populations

To screen candidate stands and select base populations of *Q. salicina* and *Q. glauca*, some relevant literatures had been searched [3,18–22]. However, there were limitations in understanding of population size, density and growth performance of target species, which were needed for the selection of base populations. To supplement this, we had interviewed with related researchers and experts to get more information. In addition, consideration was also given not to overlap with the stands selected in previous study [11].

Through this process, 25 stands (15 for *Q. salicina* and 10 for *Q. glauca*) were screened. After then, preliminary survey was conducted to determine if they were appropriate or not as a base population. In the fields, we examined stand size, frequency and density of target species, growth state, existence of candidate trees, and environmental condition. These findings were used to select base populations as well as set up the selection criteria and method.

For selection of base population, the priority was given to whether there were enough individuals to perform selection and whether there were candidate trees that met the selection criteria. For example, a stand with small size and no available candidate trees was excluded. Finally, 15 stands were selected as a base population, i.e., 8 for *Q. salicina*, 3 for *Q. glauca*, and 4 for both species (Figure 1, Table 1).

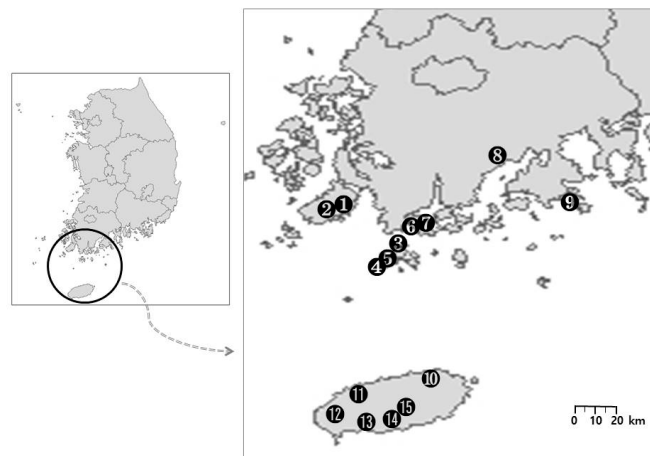


Figure 1. Location map of base populations for candidate tree selection in *Quercus salicina* and *Q. glauca*. The numbers in the black circle indicate the No. of stands shown in the Table 1.

2.2. Selection Criteria and Methods

In Korea, selection criteria and method for broad-leaved tree species are already established and have been used to plus tree selection of several tree species [23]. However, the method is based on comparison tree method, which is not suitable to evergreen oaks as mentioned above. Through literature review and in-depth discussion, a subjective grading system was developed and applied for plus tree selection of evergreen oaks.

Firstly, the dominant trees that occupy the upper crown canopy of the stand were selected as a candidate trees. The interval among adjacent candidate trees was kept at 30~50 m to avoid selection of

relatives. In addition, selection of candidate trees was made to include as diverse location as possible to avoid biased selection from a particular site.

Table 1. List of 15 candidate stands of *Q. salicina* and *Q. glauca* stands used in this study.

Region	Stand	Latitude (°N)	Longitude (°E)	Altitude (m)	Target Species	Mean Annual Temperature (MAP, °C)	Mean Annual Precipitation (MAP, mm)	Relative Humidity (RH, %)
Jindo	1. Sacheon1	34.4701	126.3087	108	<i>Q. salicina</i>	139	1568.5	78.2
	2. Sacheon2	34.4618	126.3211	154	<i>Q. salicina</i>			
Wando	3. Yesong	34.1555	126.5744	58	<i>Q. salicina</i>	14.1	1532.7	72.5
	4. Jeongja	34.1612	126.5095	37	<i>Q. salicina</i>			
	5. Buhwang	34.1507	126.5348	72	<i>Q. salicina</i>			
	6. Jangjoa	34.3555	126.7177	86	<i>Q. glauca</i>			
	7. Daeya	34.3655	126.7160	80	<i>Q. salicina</i>			
Boseong	8. Yulpo	34.6791	127.0846	62	<i>Q. salicina</i>	13.6	1779.9	70.7
Goheung	9. Sayang	34.4627	127.4493	33	<i>Q. glauca</i>	13.6	1453.4	69.7
Jeju-si	10. Seonhul	33.5191	126.7129	113	<i>Q. salicina</i>	15.5	1456.9	73.3
	11. Napup	33.4339	126.3304	90	<i>Q. glauca</i>			
	12. Cheongsu	33.2969	126.2534	105	<i>Q. salicina</i> <i>Q. glauca</i>			
Seguipo-si	13. Gamsan	33.2573	126.3565	125	<i>Q. salicina</i>	16.2	1850.8	70.7
	14. Seoho	33.3018	126.5250	602	<i>Q. glauca</i>			
	15. Sanghyo	33.3294	126.5857	465	<i>Q. salicina</i>			

To select candidate trees, we developed subjective grading system with six characteristics in three categories (Table 2). Each characteristic was rated and scored as 5 grades, i.e., 5—very good, 4—good, 3—moderate, 2—poor, and 1—very poor. The grade was given by relative superiority or degree compared to the average of the stand.

Table 2. Selection criteria and weights of characteristics used for candidate tree selection in *Quercus salicina* and *Q. glauca*.

Category (Weight)	Characteristic (Weight)	Remark
Growth (0.5)	Superiority of Growth (SG) (0.30)	Determined by considering growth status of shoot, branch, crown, and stem.
	Superiority of Tree form (STF) (0.2)	Determined by considering number of stem, stem straightness, and timber height.
Adaptability (0.2)	Adaptability to Disturbance (AD) (0.08)	Determined by considering adaptability to disturbance or damage by abiotic and biotic stresses
	Adaptability to Environment (AE) (0.12)	Determined by considering vitality and adaptability to the environments of the site.
Seed production (0.3)	Superiority of seed production (SSP) (0.18)	Determined by considering current status and recent history of seed production including buried or stand floor seed.
	Potential of seed production (PSP) (0.12)	Determined by considering seed production capacity reflecting the advantages and disadvantages of current micro-environmental conditions affecting seed production.

Growth category was aimed to evaluate the superiority of growth (SG) and superiority of tree form (STF). The SG was determined by considering growth status of shoot, branches, crown, and stem. This is an indicator for selecting large and well-growing individuals. The STF was determined by considering number of stem, stem straightness, and timber height. This is an indicator for selecting individuals with straight stem and high timber height for long-log production.

Adaptability category was aimed to evaluate the adaptability to disturbance (AD) and adaptability to environment (AE). The AD was determined by considering adaptability to disturbance such as biotic (pests or diseases) and abiotic (artificial disturbance) stresses. The symptoms or damages by pests or

diseases were immediately identified by ocular observation. Artificial disturbance usually appeared in the form of collecting leaves or small branches, even stem for medicine. It was also possible to intuitively determine whether there was an impact on tree growth by ocular observation. The grade was scored by considering these two factors together. Thus, this is an indicator for selecting individuals with superior adaptability or resilience to disturbance. The AE was determined by considering vitality and adaptability to a given environment. When a tree does not adapt well to a given environment, its vitality was decreased. The adaptability can be indirectly identified through observations of growth state of leaves, i.e., small and fewer leaves in crown and/or abnormal growth responses of leaves such as curling, bending, marginal browning, chlorosis, and shedding. The grade was scored considering all aspects mentioned above. Thus, this is an indicator for selecting individuals with superior adaptability to environment.

Seed production category was aimed to evaluate the superiority of seed production (SSP) and potential of seed production (PSP). The SSP was determined by considering current status and recent history of seed production. First, the average level of flowering or fruiting of the stand was determined and the grade was scored according to the relative level of flowering or fruiting of an individual. However, the amount of flowering or fruiting usually varied from year to year. To compensate for this, the grade was adjusted considering the amount of buried or floor seed under the crown of individual tree. Thus, this is an indicator for selecting individuals with superior seed production. The PSP was determined by considering seed production capacity reflecting the advantages and disadvantages of micro-environment affecting flowering or fruiting such as spacing, light intensity, soil moisture, and humidity. The exact values of these factors cannot be accurately known without measuring equipment, but experienced foresters can intuitively determine whether the place is favorable or unfavorable for flowering or fruiting. The grade of PSP was scored considering these judgement results and SSP grade together. Thus, PSP is an indicator for selecting individuals with high potential of seed production.

In a grading system, weights of the characteristics were generally determined by its heritability and economic worth [16]. However, since there is no information on the heritability of evergreen oaks, breeding goal and expected economic worth were only considered for determining the weights of each category and characteristic in this study. The primary objective of this breeding program is to improve growth rate and stem straightness for timber production. Thus, the highest weight was given to growth category. Seed production was considered the second most important in terms of supply of materials for reforestation as well as food and medicine. So, the second highest weight was given to seed production category. Usually, adaptation is not well placed in a grading system, because it is automatically taken into account through common garden test of selected trees [16]. However, since evergreen oaks have the urgency of genetic resources conservation as mentioned before, adaptability category was included in this study considering adaptability to various environments for assisted migration or reforestation outside of current habitats range. However, it was judged that its importance in current state was relatively lower than those of others, so the lowest weight was given to adaptability category. The detailed weights of each category and characteristic were inevitably determined through discussion of relevant experts and researchers (Table 2).

In principle, individuals above the moderate grade in all characteristics were selected as candidates. Exceptionally, however, if the number of candidates to be selected was small and/or the growth-related characteristics were excellent, an individual with lower than moderate grade in some characteristic included in the candidates.

This subjective grading system is frequently used for hardwoods but is successful only if the grader is experienced and dedicated to finding the best tree possible [16,24]. In order to minimize the problem of deviation caused by technical errors or prejudices when selecting candidates, the survey team composed of three experienced researchers and standardized the research method through simulation selection according to the selection criteria before starting work. Two investigation teams were operated for candidate tree selection. Each team consisted of 3 people, i.e., selector, evaluator/recorder,

and GPS manager. Selector was primarily responsible for selection and marking of candidate trees. Evaluator/recorder was responsible for evaluation and recording the grade of each characteristic in each candidate tree. GPS manger had recorded and managed the positional information. In all cases, if there were some problem in selection process, i.e., whether to select or not, how to rank, and etc., three persons worked together to draw a conclusion. To reduce the deviation of candidate tree selection, all members of each team had a meeting to evaluate and adjust daily results after finishing work every day.

2.3. Data Analysis

Since the primary goal of this study was to secure as many candidate trees as possible from various candidate stands, candidate selection was made on each stand basis according to the selection criteria and methods. However, it was suggested that it would be appropriate to combine geographically adjacent stand with similar environment into a group. If each stand was treated separately in the process of data analysis for plus tree selection, candidate trees selected under similar environmental conditions were more likely to be included into breeding population. The reason could be understood by looking at the relevant analysis method presented later in this section. For *Q. salicina*, Sacheon1 and Scaheon2 were tied together because they were adjacent to each other with a ridge in between them. Gamsan and Cheongsu, Seoho and Sanghyo, and Buhwang and Jeongja were also tied together respectively, because they are geographically adjacent under similar environment. For *Q. glauca*, Cheongsu and Gamsan were also tied for the same reason. The linear distance between stands tied together was ranged 1.4~10.4 km. All subsequent analysis was performed based on this group (Table 3).

Table 3. The adjusted groups and the number of candidate trees selected in each group in *Quercus salicina* and *Q. glauca*.

<i>Q. salicina</i>				<i>Q. glauca</i>			
Stand	No. of Candidates	Group	Remark	Stand	No. of Candidates	Group	Remark
Seonhul	11	A		Seonhul	15	A	
Cheongsu	9	B	+Gamsan	Cheongsu	33	B	+Gamsan
Seoho	14	C	+Sanghyo	Napup	10	C	
Sacheon	26	D	Sacheon1 + Sacheon2	Jeongja	9	D	
Yesong	10	E		Jangjoa	12	E	
Buhwang	8	F	+Jeongja	Sayang	3	F	
Daeya	6	G					
Yulpo	3	H					
Total	87	8			82	6	

Since a grading system was applied to select candidate trees in this study, the scoring data of measured characteristics were highly skewed to 4 or 5 grades. Thus, prior to performing the analysis, the data was transformed using logarithm function to improve normality [25–27]. Even after data transformation, the normality was not improved significantly while the homoscedasticity was satisfied. In this case, the robustness of analysis of variance (ANOVA) would be weakened. So, a non-parametric method such as the Kruskal-Wallis (KW) test was generally recommended as an alternative. However, in case of small sample size like this study, the ANOVA test will be a better option than KW test, even for non-normal data [28]. Moreover, when using rank data, the result of KW test was very similar to that of ANOVA F test. Since the grade score measured in this study was a kind of rank in each characteristic, it was considered possible to use ANOVA and multiple comparison methods. Thus, one-way ANOVA with fixed effect model and post hoc multiple comparison analysis were performed to examine difference across the group means and specific difference between pairs of groups, respectively. For multiple comparison analysis, Fisher's least significant difference (LSD) with Bonferroni adjustment was applied. To understand the relationship among six characteristics as well

as height and diameter at breast height of candidate trees, correlation analysis was performed with Spearman rank correlation. All statistical analyses were performed using R software version 4.0.0.

Although we obtained scores applying grade system in each characteristic, the mean and distribution of the measurements were different among characteristics as well as base populations. If we used original grade scores measured for candidate selection, it was possible that individuals from a specific stand or group with overall high grade are more likely to be selected as candidates. In other words, if such an erroneous biased selection was made, it does not meet the purpose of this study to select candidate trees evenly from various stands as possible. Therefore, normalization (rescaling) of the data was carried out before applying the weights for each characteristic according to the selection criteria.

There were several methods typically used for normalization, i.e., scaling to a range, clipping, log scaling, z-score, etc. Depending on the nature of the data and the purpose of the study, researchers usually select and use the appropriate method [25–27]. In this study, we normalized the data by dividing the deviation by mean. When applying this method, the mean of the new data was set to '0' and the each value was reversely adjusted according to the relative value of the original mean. This feature was thought to be more suitable to our purpose, because we could select candidates more evenly from various stands or locations than other methods.

Then, we could consider two kinds of mean value for normalization, i.e., each population level (GV_p) and total population level (GV_t) like the below formula.

$$GV_{p,i} = (X_i - \bar{X}_p) / \bar{X}_p \quad (1)$$

where, $GV_{p,i}$ is a generalized value of i_{th} individual, X_i is a grade score i_{th} individual and \bar{X}_p is a mean value of a corresponding characteristic in each population.

$$GV_{t,i} = (X_i - \bar{X}_t) / \bar{X}_t \quad (2)$$

where, $GV_{t,i}$ is a generalized value of i_{th} individual, X_i is a grade score i_{th} individual and \bar{X}_t is overall mean value of a corresponding characteristic in total population.

The next question was which mean value was more effective for candidate selection. GV_t was assumed that the environmental differences among stands was negligibly small. If we only considered GV_t , it would be easy to compare and rank overall selected candidates, but it may lead to biased selection from a specific stand with superior properties without consideration of environmental differences. However, environmental impact on fitness and growth of provenances or families is already well known in several cases [16,29]. Thus, it is necessary to combine GV_p in candidate tree selection, which was assumed environmental differences among stands. Moreover, since the genetic test has not been conducted, the G×E interaction effect cannot be estimated. To compensate for this problem, we decided to temporarily apply the weighted generalized value, i.e., $GV_w = 0.7 \times GV_p + 0.3 \times GV_t$. These weights are not from a scientific basis but from a conceptual one suggested by researchers in the sense that consideration of each population level was more important in achieving the purpose of this study.

In actual data analysis, we firstly calculated GV_p and GV_t of each characteristic in each individual, respectively. To obtain the summation index of generalized value of each characteristic for each individual, GVI_p and GVI_t were calculated with GV_p and GV_t multiplying by the weights of each characteristic, respectively (Table 2).

$$GVI_{p,i} = \sum (GV_{p,i} \times C_j) \quad (3)$$

where $GVI_{p,i}$ is a generalized value of individual based on GV_p value and GV_j is a weight of corresponding characteristics.

$$GVI_{t,i} = \sum (GV_{t,i} \times C_j) \quad (4)$$

where $GVI_{t,i}$ is a generalized value of individual based on GV_i value and C_j is a weight of corresponding characteristics.

Then, the weighted generalized value of each individual (GVI_w) was calculated as like $GVI_w = 0.7 \times GVI_p + 0.3 \times GVI_t$. The candidate trees were truncated using GVI_w of each individual with baseline value '0', i.e., if $GVI_w > 0$, then accepted and if $GVI_w < 0$, then rejected. Finally, plus trees were selected through the adjustment process to avoid biasing the selection of candidate trees for a particular stand or group.

3. Results and Discussion

3.1. Characteristics of Base Populations

The base populations were horizontally distributed in the inlands and islands region of southern coast of Jeonnam-do, and southern parts of Jeju-do. The latitudinal range was from 33.33° (Sanghyo) to 34.47° N (Sacheon) and the most of altitudinal range was below 200 m above sea level, except for Seoguipo (465 m) and Seoho (602 m). The mean annual temperature (MAT), mean annual precipitation (MAP), and relative humidity (RH) of base populations were ranged 13.6~16.2 °C, 1453.4~1850.8 mm and 69.7~78.2%, respectively. These areas belonged to warm temperate and subtropical region, which were characterized by high temperature, high precipitation, and high humidity in Korea (Figure 1, Table 1).

In overall range of both species, most stands were discontinuously distributed with relatively small size and low appearance frequency, which would lead to restriction of genetic exchange and promote of genetic differentiation. The flowering and fruiting are well done in most stands, however, seedlings for next generation were not well observed within a stand, except for some forest gaps. This phenomenon was commonly found in mature and old-growth forests with closed canopy [30,31]. In addition, both species were being pushed out of competition with other companion species, such as *Distylium racemosum*, *Acer palmatum*, *Ficus erecta*, *Machilus therbergii*, *Litsea japonica*, *Celtis sinensis*, etc.

In most stands, except for Seonhul and Cheongsu, it was estimated that inbreeding was likely to occur due to limitation of pollen-mediated gene flow by low dominance and frequency of target species within a stand. Moreover, the morphological variation of leaves was observed. For example, *Q. salicina* typically has leathery, narrow-lanceolate, taper-pointed leaves having mostly entire margins with a few marginal teeth near the apex. The front side of leaves is glossy and the reverse side is grayish green, which is easily distinguishable from other evergreen oaks. However, in Yesong, Daeya, and Sacheon, there were variations on leaf size, shape, and reverse side color different from the typical characteristics. In relation to this, some varieties like *Q. salicina* var. *latifolia* and *Q. glauca* var. *nudata* were described as an ecotype [4]. Thus, it was difficult to assert that there were some degree of genetic differentiation among stands in target species.

While well managed stands generally showed good growth performances, the stands experienced with illegal or destructive logging had many multi- or crooked stems due to reproduction by sprouts [19]. Thus, it was also necessary to consider the history of disturbance of stand for candidate tree selection. For example, even if an individual had multi-stem, it is possible to select as a candidate if the growth characteristics of each stem is good.

Considering the investigation results, it was highly suggested that more active measures are needed for sustainable use of evergreen oaks. Although the distribution of evergreen oaks is expected to expand due to climate change [5,6], it is possible that the distribution will not expand as expected due to competition with other broad-leaved tree species. Even if it is possible to create new habitat by natural migration, the genetic diversity may decrease due to the founder effects, i.e., limited number of individuals will contribute in the formation of next generation and genetic drift may result in the loss or fixation of some allele within stand [16]. Thus, it was suggested that assisted migration by supplying seeds from seed orchard with reasonable genetic diversity [7,8]. To this end, this study

attempted to select plus trees from as many stands and/or individuals as possible to secure genetic diversity, if the selection criteria were met.

3.2. Selection of Candidate Trees

According to selection criteria and methods, intensive survey and selection were conducted to each base population. Since we tried to assess all trees appearing within a stand as possible, there were large differences in the number of assessed trees per site depending on the stand size as well as density and frequency of the target species. For example, in small stands such as Yulpo, Goheung, and Gamsan, it was ranged approximately 50~100. In large stands such as Seonhul, Seoho, and Cheongsu, it was ranged approximately 250~300. A total of 169 candidate trees were primarily selected in two target species (Table 3). While the selection intensity of *Pinus* species with large pure stands was generally ranged 0.5~1.0% [23], the range in this study was approximately 5~8%. As a result of sampling and analyzing of candidate trees using increment borer, most of them were belonged to age class IV (30~40 years old). It indicated that they were mature enough to be selected. Although optimum selection age may vary depending on heritability and genetic correlation of target trait, more than 50% of rotation age is thought as an appropriate selection age to reduce the risk of early selection [12,23].

In *Q. salicina*, 87 candidate trees were selected from 8 groups (12 stands). The highest number of candidate trees was selected in Sacheon (26 individuals) and the smallest one was Yulpo (3 individuals). In *Q. glauca*, 82 candidate trees were selected from 6 groups (7 stands). While the highest numbers of candidate trees were obtained in Cheongsu (33 individuals), Sayang had the smallest candidate trees (3 individuals).

Although there were some limitations to discuss the differences among groups because the values were just only measured from selected candidate trees, it was observable significant differences among groups in some characteristics (Table 4). In *Q. salicina*, the mean values of superiority of growth (SG), adaptability to disturbance (AD), superiority of seed production (SSP) and potential of seed production (PSP) were significantly different among groups, but superiority of tree form (STF) and adaptability to environment (AE) were not. In *Q. glauca*, all characteristics except for adaptability to environment (AE) were significantly different among groups. These differences might reflect the differences in local environment and growth situation among groups.

Table 4. Multiple comparisons of group mean among candidate trees in *Quercus salicina* and *Q. glauca* by Fisher's least significant difference (LSD) with Bonferroni adjustment.

Species	Group	Characteristics Used for Candidate Tree Selection						Growth Arranged after Finishing of Selection	
		Superiority of Growth (SG)	Superiority of Tree Form (STF)	Adaptability to Disturbance (AD)	Adaptability to Environment (AE)	Superiority of Seed Production (SSP)	Potential of Seed Production (PSP)	Height (HT)	Diameter at Breast Height (DBH)
<i>Q. salicina</i>	A	4.45 ^{ab}	4.09 ^a	4.18 ^{ab}	4.45 ^a	3.36 ^c	3.73 ^{ab}	14.8 ^{bc}	30.1 ^{bc}
	B	4.67 ^{ab}	4.11 ^a	3.89 ^b	4.33 ^a	3.78 ^{abc}	4.00 ^{ab}	13.9 ^c	28.8 ^{cd}
	C	4.88 ^a	4.14 ^a	4.57 ^a	4.42 ^a	3.43 ^{bc}	3.36 ^b	16.9 ^c	37.4 ^{ab}
	D	4.58 ^{ab}	4.50 ^a	4.19 ^{ab}	4.42 ^a	3.92 ^{ab}	4.27 ^{ab}	13.9 ^c	29.7 ^c
	E	4.50 ^{ab}	4.40 ^a	4.10 ^{ab}	4.60 ^a	3.80 ^{abc}	3.70 ^{ab}	13.7 ^c	23.1 ^{cd}
	F	4.00 ^b	4.25 ^a	3.88 ^b	4.25 ^a	3.63 ^{abc}	4.00 ^{ab}	10.5 ^d	20.3 ^d
	G	4.33 ^{ab}	4.33 ^a	3.83 ^b	4.33 ^a	4.17 ^{ab}	4.67 ^a	11.3 ^d	24.7 ^{cd}
	H	4.33 ^{ab}	4.33 ^a	3.67 ^b	4.33 ^a	4.37 ^a	4.33 ^{ab}	16.7 ^{ab}	50.0 ^a
	Pr.(>F)	*	ns	**	ns	**	**	**	**
Mean	4.53	4.30	4.14	4.41	3.76	3.97	14.1	29.6	
<i>Q. glauca</i>	A	4.33 ^a	4.00 ^{ab}	3.80 ^{ab}	4.00 ^a	3.53 ^b	3.60 ^b	14.7 ^a	26.8 ^{bc}
	B	4.33 ^{ab}	4.24 ^a	4.03 ^{ab}	4.15 ^a	3.91 ^{ab}	3.91 ^{ab}	12.4 ^b	23.6 ^c
	C	4.20 ^{ab}	3.60 ^{ab}	4.30 ^a	4.20 ^a	4.30 ^a	4.50 ^a	15.2 ^a	40.7 ^a
	D	3.67 ^b	4.11 ^{ab}	3.56 ^b	3.89 ^a	3.89 ^{ab}	3.67 ^{ab}	8.8 ^c	17.3 ^d
	E	4.33 ^a	4.17 ^{ab}	3.92 ^{ab}	4.33 ^a	4.17 ^{ab}	4.58 ^a	10.8 ^{bc}	22.9 ^{cd}
	F	4.33 ^{ab}	4.33 ^a	3.67 ^{ab}	4.33 ^a	3.67 ^{ab}	3.67 ^{ab}	11.0 ^{bc}	33.7 ^{ab}
	Pr.(>F)	*	*	**	ns	*	**	**	**
Mean	4.24	4.10	3.94	4.13	3.92	3.99	12.5	25.9	

Significant differences ($p < 0.05$) are indicated by different letters among groups. ns indicate not significant. *, and ** represent a significant at 95% and 99% probability level.

Although mean values of height (HT) and diameter at breast height (DBH) of candidate trees in each group were presented together (Table 4), candidate tree selection was made by only six characteristics. In other words, HT and DBH had no effect on candidate tree selection because they were arranged after finishing of selection. To understand the relationships among these variables, correlation analysis was conducted in each species (Table 5). Although the explanatory power was low due to weak correlation, a rough tendency for significantly correlated variables was examined.

Table 5. Spearman rank correlations among variables measured from candidate trees of *Quercus salicina* (upper diagonal) and *Q. glauca* (lower diagonal).

Variable	Superiority of Growth (SG)	Superiority of Tree Form (STF)	Adaptability to Disturbance (AD)	Adaptability to Environment (AE)	Superiority of Seed Production (SSP)	Potential of Seed Production (PSP)	Height (HT)	Diameter at Breast Height (DBH)
SG	-	-0.087 ^{ns}	0.320 ^{**}	0.114 ^{ns}	-0.052 ^{ns}	-0.171 ^{ns}	0.330 ^{**}	0.350 ^{**}
STF	-0.050 ^{ns}	-	0.010 ^{ns}	0.332 ^{**}	-0.049 ^{ns}	0.094 ^{ns}	0.021 ^{ns}	0.043 ^{ns}
AD	0.257 [*]	0.083 ^{ns}	-	-0.099 ^{ns}	-0.354 ^{**}	-0.279 ^{**}	0.321 ^{**}	0.178 ^{ns}
AE	0.235 [*]	0.086 ^{ns}	0.140 ^{ns}	-	-0.035 ^{ns}	-0.076 ^{ns}	0.071 ^{ns}	0.088 ^{ns}
SSP	-0.057 ^{ns}	-0.132 ^{ns}	0.149 ^{ns}	0.250 [*]	-	0.434 ^{**}	0.170 ^{ns}	0.083 ^{ns}
PSP	-0.051 ^{ns}	-0.300 ^{**}	0.152 ^{ns}	0.300 ^{**}	0.441 ^{**}	-	0.273 ^{**}	0.040 ^{ns}
HT	0.353 ^{**}	-0.215 ^{ns}	0.227 [*]	0.069 ^{ns}	-0.122 ^{ns}	-0.080 ^{ns}	-	0.713 ^{**}
DBH	0.256 ^{ns}	-0.298 ^{**}	0.228 [*]	0.197 ^{ns}	0.225 ^{ns}	0.267 ^{**}	0.597 ^{**}	-

^{ns} indicate not significant. *, and ** represent a significant at 95% and 99% probability level.

In both species, there were no correlations between SG and STF in growth category and between AD and AE in adaptability category. However, there was a significant correlation between SSP and PSP in seed production category. It meant that candidate trees with higher fruiting at present were also graded having higher potential of reproduction, although the evaluation criteria were different.

In *Q. salicina*, AD had significant positive correlation with SG and significant negative correlations with SSP and PSP. It indicated that the candidate trees with high adaptability to disturbance also showed good growth and development, but the seed production was not. AE showed positive correlation with STF, which indicated that the candidate trees with high adaptability to environment had good tree form. HT of candidate trees had significant positive correlation with SG, AD and PSP. DBH of candidate trees also had significant positive correlation with SG. It meant that the candidate trees with superior growth, high adaptability to disturbance and high potential of seed production had higher HT and/or DBH.

In *Q. glauca*, AD had significant positive correlation with SG like *Q. salicina*. AE had significant positive correlation with SG, SSP, and PSP. It indicated that the candidate trees with higher adaptability showed higher growth and seed production. Meanwhile, STF had negative correlation with PSP, which indicated that candidate trees with superior tree form showed low potential of seed production. HT had significant positive correlation with SG and AD. DBH had significant positive correlation with AD and PSP but significant negative correlation with STF. It meant that the candidate trees with superior growth and high adaptability to disturbance had higher HT and/or DBH. However, candidate trees with higher STF or DBH showed lower PSP unlike *Q. salicina*.

By analogy with the results of this survey and the relevant research [32], it seemed to be related to the characteristics of the species. Initial adaptation of *Q. glauca* is favorable as a pioneer species, but if the stand is stabilized, it is disadvantageous in competition with other species. *Q. salicina* is estimated to be advantageous in competition once it is adapted because it has higher adaptability in relatively broad environments as a generalist species.

3.3. Data Analysis and Plus Tree Selection

The weighted generalized value of each individual (GVI_w) was calculated as $GVI_w = 0.7 \times GVI_p + 0.3 \times GVI_t$. The candidate trees were truncated with baseline value '0', i.e., if $GVI_w > 0$, then accepted and if $GVI_w < 0$, then rejected (Figure 2).

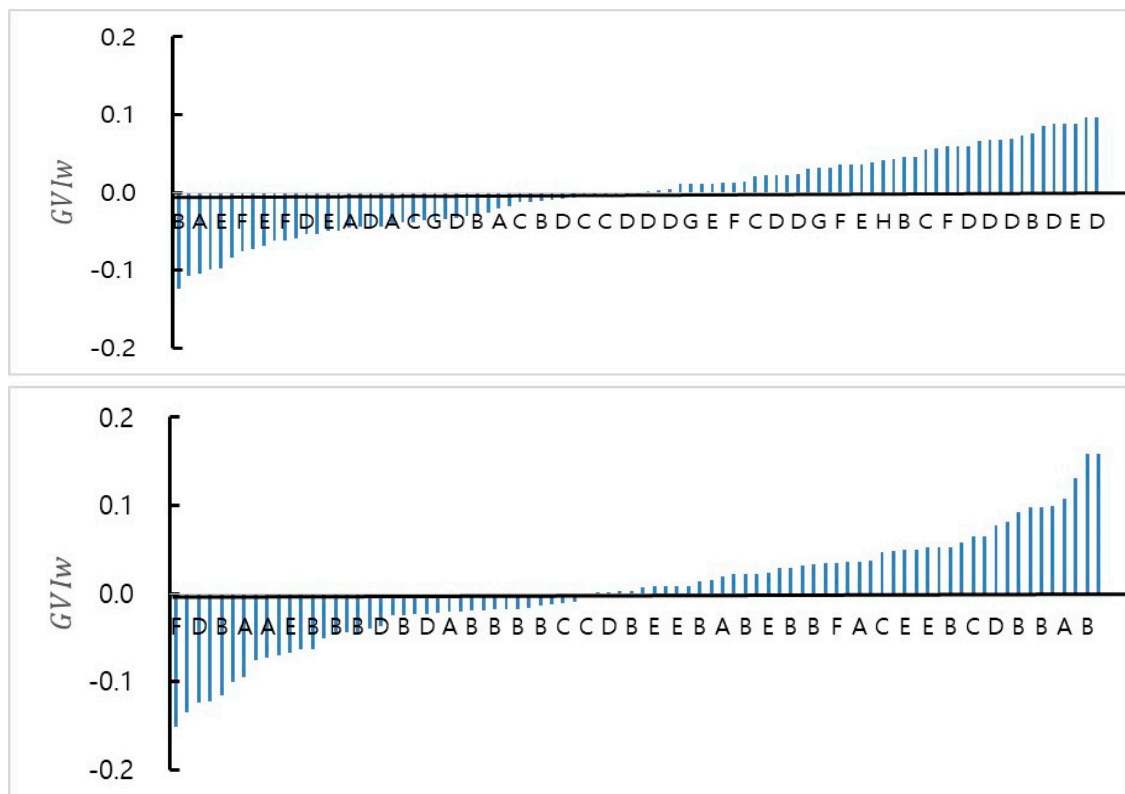


Figure 2. Truncation selection of candidate trees based on GVI_w values in *Quercus salicina* (top) and *Q. glauca* (bottom). If $GVI_w > 0$, then accepted and if $GVI_w < 0$, then rejected. Capital letters indicate the group to which each individual belongs.

In *Q. salicina*, 43 candidate trees were accepted and 44 candidate trees were rejected. In *Q. glauca*, 42 candidate trees were accepted and 40 candidate trees were rejected (Table 6). In group A of *Q. salicina*, for example, GVI_p and GVI_t of No. 4 candidate tree were 0.0242 and -0.0200 , respectively. If we only considered GVI_p the candidate tree would be accepted (>0), but if GVI_t was only considered, the candidate tree would be rejected (<0). However, if we applied $GVI_w (=0.0109)$ suggested in this study, it was classified as an accepted candidate (>0).

Table 6. Final list of plus trees selected by truncation ($GVI_w > 0$) and adjustment in *Quercus salicina* and *Q. glauca*.

<i>Q. salicina</i>			<i>Q. glauca</i>		
Group	Accepted by Truncation ($GVI_w > 0$)	Final Selection by Adjustment	Group	Accepted by Truncation ($GVI_w > 0$)	Final Selection by Adjustment
A	1, 4, 11	1, 4, 6, 8, 11	A	1, 5, 7, 10, 11, 12, 15 2, 5, 8, 9, 10, 12, 13, 14,	1, 3, 5, 7, 10, 12, 15 2, 4, 5, 8, 9, 12, 13, 15,
B	1, 2, 3, 8	1, 2, 3, 8	B	15, 17, 20, 21, 22, 23, 24, 27, 29	17, 20, 21, 22, 23, 24, 27, 29
C	2, 3, 4, 9, 12	2, 3, 4, 8, 9, 12, 14	C	1, 4, 6, 7, 8	1, 4, 6, 7, 8
D	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 19, 20, 22, 25	2, 3, 4, 6, 8, 9, 10, 11, 13, 19, 20, 22, 25	D	2, 6, 7, 9	2, 4, 6, 7, 9
E	1, 5, 6, 7, 8, 10	1, 5, 7, 8, 10	E	2, 3, 4, 7, 8, 9	2, 4, 7, 8, 9, 12
F	1, 3, 4, 8	1, 3, 4, 8	F	1, 3	1, 3
G	2, 3, 4, 5	2, 3, 4, 5	Total	42	41
H	1, 2	1, 2			
Total	43	44			

The final selection was decided through some adjustment process considering the imbalance of number of individuals selected, the specificity of stand identified at investigation, and biased selection according to the differences in micro-environment within the group.

The major adjustments performed in *Q. salicina* were as follows (Table 6). In group A, only 3 out of 11 candidate trees were included in accepted group. To balance the number of candidate trees among groups, No. 6 (−0.0054) and No. 8 (−0.0179) with higher GVI_w among rejected group were included in accepted group. In group C, Seoho (602 m) and Sanghyo (465 m) stand were located in highland area comparing other stands (less than 200 m). To consider specificity of stand and balance the number of candidate trees among groups, No. 8 (−0.0050) and No. 14 (−0.0050) with higher GVI_w among rejected group were included in accepted group. In group D, Sacheon1 and Sacheon2 stand were adjacent with a ridge in between them. However, the micro-environment was a little different, i.e., soil depth, direction of slope, and topology of Sacheon1 is better than Sacheon2. So, they were initially treated as a separate stand. Later, however, they were grouped into a group considering geographic proximity. Accordingly, 85.7% of candidate trees in Sacheon1 (6 out of 7) were accepted whereas only 47.4% of candidate trees of Sacheon2 (9 out of 19) were accepted. To balance the number of candidate trees within group, adjustment was made to include No. 5 (0.0033) and No. 7 (0.0047) with small GVI_w in the rejected group.

The following adjustments had been made in *Q. glauca* (Table 6). In Cheonsu stand in group B, No. 8~13, 15, and 20~24 candidate trees were selected from relatively good sites with deep soil depth and low gravel ratio. All except for No. 11 candidate tree were included in accepted group. To avoid biased selection to a specific site condition, two candidate trees with lower GVI_w , No. 10 (0.0172) and No. 14 (0.0188), were adjusted into rejected group. An opposite case was found in group D. No. 3~5 were selected in relatively poor site of Jeongja stand. Accordingly, all of them were rejected by GVI_w truncation. To broaden genetic variability, adjustment was made to include No. 4 candidate tree in accepted group. In the same sense, No. 3 candidate tree (0.0208) in Jangjoa stand of group E were adjusted into rejected group and No. 3 (−0.0228) and 11 (0.0067) in Seonhul of group A were adjusted into accepted group and rejected group, respectively.

Although the primary goal of this study was to select plus trees, the conservation aspect was also considered. Thus, we tried to include as many individuals as possible from stands of various environments. To this end, the selected stands were divided into groups as well as the adjustment was performed as above. Nevertheless, we cannot be sure that enough genetic diversity will be included in a breeding population. Thus, additional studies are required to evaluate genetic diversity, i.e., comparison of genetic diversity between natural stands and candidate trees including rejected trees. If genetic diversity is lower than expected, adding some rejected trees to breeding population may be an option.

Through adjustment process, 44 candidate trees in *Q. salicina* and 41 candidate trees in *Q. glauca* were finally selected as plus trees. In order to compare the selection effect of plus tree, the mean values of six characteristics, height and diameter growth between accepted group (plus trees) and rejected group (Table 7). On average, the accepted group was excellent in all characteristics in both species, i.e., 103.2~112.9% in *Q. salicina* and 105.0~112.8% in *Q. glauca*. This tendency was the same in HT and DBH, even though they were not considered directly at the time of selection. It was interpreted as the result of indirect selection of HT and DBH in relation to selection of SG, AD, or STF as shown in the correlation analysis (Table 5). It was meaningful that similar selection effects were obtained without direct measuring or selecting growth-related traits like other general selection methods.

Table 7. Comparison of means values of six characteristics, height and diameter growth between accepted trees (plus trees) and rejected trees in *Quercus salicina* and *Q. glauca*.

Species	Class	Mean Values of Each Characteristic						Height (HT)	Diameter at Breast Height (DBH)
		Superiority of Growth (SG)	Superiority of Tree Form (STF)	Adaptability To Disturbance (AD)	Adaptability to Environment (AE)	Superiority of Seed Production (SSP)	Potential of Seed Production (PSP)		
<i>Q. salicina</i>	Accepted (A)	4.72	4.48	4.20	4.57	3.89	4.20	14.1	30.9
	Rejected (R)	4.33	4.12	4.07	4.26	3.63	3.72	14.0	28.3
	A/R (%)	109.0	108.7	103.2	107.3	107.2	112.9	100.7	109.2
<i>Q. glauca</i>	Accepted (A)	4.49	4.20	4.05	4.34	4.15	4.10	12.9	27.2
	Rejected (R)	4.00	4.00	3.83	3.93	3.68	3.88	12.1	24.5
	A/R (%)	112.3	105.0	105.7	110.4	112.8	105.7	106.6	111.0

3.4. Implications in Evergreen Oak Breeding

Evergreen oaks are only distributed in the southernmost part of Korean peninsula and the natural stands are discontinuously scattered with small size [3,4]. The genetic conservation for evergreen oaks is also highly recommended [11,33]. Accordingly, it is difficult to secure large stand for seed production area or seed stand. Thus, establishment of multi-purpose seedling seed orchard (MPSSO) for seed production and genetic conservation by mass selection was suggested as an alternative.

There are several selection methods, but the most important thing is to find the best method for the target species [16]. Comparison tree selection had been widely used in most conifer species as well as deciduous oak species in Korea [23].

In this study, some modified selection criteria and method were applied for plus tree selection. It was primarily due to the characteristics of the target species as mentioned above. Another reason was the results of Stringer et al. [17]. They suggested that selecting several phenotypically above-average candidate trees may be more effective than rigorously selecting a smaller number of phenotypically superior trees in *Quercus rubra*. Reflecting these aspects, the truncation of candidate trees for plus tree selection was performed with baseline value of GVI_w in this study.

Through this process, 44 candidate trees in *Q. salicina* and 41 candidate trees in *Q. glauca* were finally selected as plus trees. Since it was found that this method could provide a selection effect similar to direct selection on growth-related traits, it was expected that application of this methodology could be expanded to other tree species if the conditions were met.

Most tree improvement programs are designed to provide continued gain through each of many cycles of improvement. A selection procedure that involves many cycles of selection and breeding is known as recurrent selection (RS). A number of recurrent selection schemes have been devised to utilize general combining ability (GCA) and, in some cases, specific combining ability (SCA) [12,16].

In case of *Q. salicina* and *Q. glauca*, simple recurrent selection (SRS), was suggested as a strategy for advanced generation. Although SRS is less efficient at achieving genetic gains than other forms of recurrent selection, i.e., RS for GCA and RS for SCA, it is considered to be a more reasonable approach matched the breeding goal and current situation of *Q. salicina* and *Q. glauca*. It could serve the purpose of increasing the average of genetic gain in each generation and maintaining the genetic variation in the selected population as much as possible.

Another reason for making this suggestion is the aspect of forest policy in Korea. The reforestation tree species are classified into two categories, i.e., major vs minor tree species. The major tree species is a species with high demand and large planting area, i.e., deciduous oaks, Pines, Larches, and so on. The minor tree species is the opposite. Evergreen oaks and most of other broad-leaved trees are belonged to this category. Accordingly, RS-GCA and RS-SCA strategy are mainly applied in major tree species. In practical aspects, it is difficult to invest a lot of efforts and expenses into minor tree species [29,34].

Nevertheless, to compensate the disadvantage of SRS and use of GCA information for advanced generation breeding, operation of several MPSSO was suggested as an alternative. At present,

a discussion is underway to determine the location and number of sites for establishment of MPSSO in Korea.

4. Conclusions

Although evergreen oaks, including *Q. salicina* and *Q. glauca*, are restricted to subtropical regions on the southernmost part of the Korean peninsula, it is considered as an alternative species for adaptation to climate change in the future. So, Korea Forest Service is being pursued to expand the selection of plus trees and establishment of seed orchard to simultaneously consider conservation and utilization of evergreen oaks. However, a selection methodology for evergreen oaks has not been established to support this. Through this study, we tried to find a suitable methodology for the plus tree selection of evergreen oaks. The usefulness of our method was confirmed through application of plus tree selection in *Q. salicina* and *Q. glauca*. Thus, it is expected that all processes and results thus far will be used as a guideline for plus tree selection as well as conservation of evergreen oaks.

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