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Enhancing Breeding Potential and Genetic Conservation: A Comprehensive Approach to Plus-Tree Selection for *Tilia amurensis* Improvement

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Abstract: The timber degradation and overexploitation of *Tilia amurensis* necessitate strategic genetic resource management. This study presents a comprehensive approach to plus-tree selection, focusing on growth evaluation. Drawing from the procedures developed for evergreen oak, it encompasses base population selection, criteria establishment, forest stand investigation, standardized measurements, and tree selection. This study advances the baseline selection methods by emphasizing growth differentiation through age and environmental adjustments. A total of 62 superior individuals were selected from 176 candidates across 20 populations, effectively expanding the geographical boundaries. This growth-centric improved approach offers practical insights for selective breeding and genetic conservation, and addresses the ecological characteristics of the species. This study underscores the need for further exploration of genetic differentiation and biological traits to provide a foundation for refining *T. amurensis* tree improvement programs. In a broader context, these findings contribute to the understanding and sustainable management of diverse broadleaf forests.

Keywords: plus-tree selection; tree improvement; growth standardization; *Tilia amurensis*

Citation: Lee, K.; Kim, I.-S.; Choi, W.-Y. Enhancing Breeding Potential and Genetic Conservation: A Comprehensive Approach to Plus-Tree Selection for *Tilia amurensis* Improvement. *Forests* **2023**, *14*, 1972. https://doi.org/10.3390/f14101972

Academic Editor: Keith Woeste

Received: 22 August 2023
Revised: 19 September 2023
Accepted: 27 September 2023
Published: 28 September 2023

1. Introduction

Forest environmental changes due to climate change and frequent accidental forest disasters, such as forest fires, insects, and diseases, have increased the importance of forest management [1,2]. The recent rise in forest health and adaptability issues highlights the necessity for hardwood utilization and research [3]. Hardwood forests are important resources for the forest industry; in addition, they have ecological functions [4]. *Tilia amurensis* Rupr., an important hardwood tree species found in temperate forests, is distributed throughout Korea, Japan, Northeastern China, and Far East Russia [5]. This species has high economic value for its timber and for nectar production [5,6]. The bark of this species is used as fiber material; this gives it the name 'bark tree' in Korea. In addition to its medicinal uses [7], the linden tree, of the genus *Tilia* in Europe, is one of the major ornamental shade and timber trees [4,8]. They play important roles in forest ecosystems [5]. In Korea, many imposing trees have grown in the past; however, these valuable resources have diminished owing to overexploitation for several decades. The destructive logging and utilization of this species has resulted in remnant resources with degenerated phenotypes. *T. amurensis* is still under threat of decreased habitat area and fragmentation due to climate change, similar to that observed for many hardwood species under pressure [5,9].

Tree improvement programs entail the conservation of genetic diversity and, in turn, the sustainable use of superior resources. Plus-tree selection following exploration of the natural population of the target species is an essential first step in forming the genetic basis of the subsequent tree improvement program [10]. In Korea, the selection of superior hardwood species, including *Tilia* species, for plus-tree selection began in the late 1980s to establish seed orchards, with the aim of providing reproductive materials for forest plantations. [11]. Recently, renewed selection criteria were established for plus-tree selection in evergreen oaks considering the intrinsic characteristics of the species [12]. This improved the existing selection criteria for broadleaved trees, which were rooted in the comparison tree method [11]. The modified selection criteria consist of three categories (growth, adaptability, and seed production) with different weights. *T. amurensis* shares a common challenge with evergreen oaks, namely, sparse distribution showing a mosaic structure in a mixed forest, in the application of the comparison tree method [13].

This study aimed to develop a systematic evaluation and selection method for superior *T. amurensis* resources, followed by natural conservation, for tree improvement. The objectives of this study were to (1) investigate the natural populations of *T. amurensis* in Korea, (2) establish selection criteria for plus trees, and (3) select superior resources for *T. amurensis* for use in tree improvement programs.

2. Materials and Methods

2.1. Site Characteristics of Base Populations of T. amurensis

Twenty base populations were selected from 15 regions based on the natural distribution of *T. amurensis* in Korea [14] (Figure 1; Table 1). Some populations were divided into two or three subpopulations when their environments differed, even though they were located in the same region. Topographic characteristics were classified into three types—A, B, and C—to reflect the environmental characteristics that affected the tree phenotype. The population was classified as type A when it was located in a piedmont or a gentle hilly area near the valley. Type B is a mountainside or ridge surrounded by neighboring mountains, in which the environment is relatively favorable for tree growth. Type C is a ridge with an unfavorable environment for tree growth owing to factors such as wind disposal.

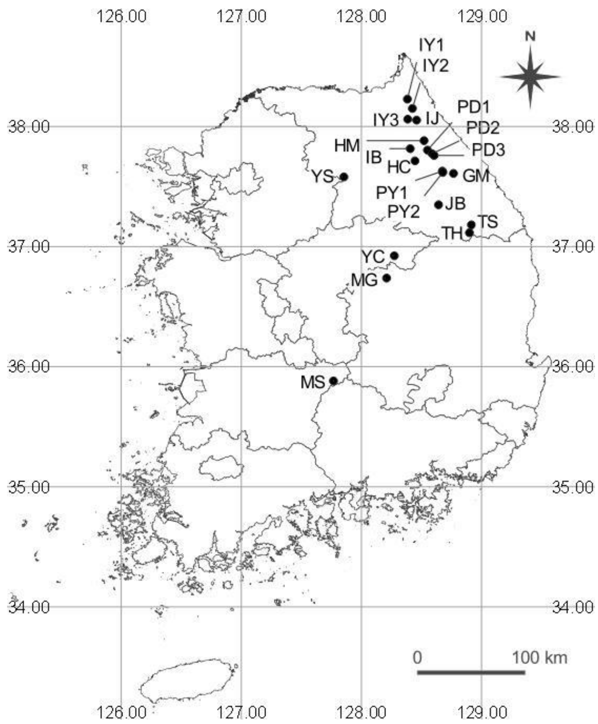

Figure 1. Locations of the twenty base populations of *T. amurensis* in Korea (mapping was performed using QGIS 3.10.6 (open-source project) [15] with a Korean administrative area map provided by GIS Developer (Seoul, Korea) [16]).

Table 1. Locations and altitudes of the twenty base populations of *T. amurensis*.

Base Population	Latitude (°)	Longitude (°)	Altitude (m)	Mean Annual Temp (°C)	Mean Annual Prep (mm)
IY I	38.22825	128.3829	514–605		
IY II	38.15035	128.42455	683–861		
IY III	38.06105	128.38555	491–527	10.4	1204.6
IJ	38.05325	128.45945	738–838		
IB	37.8156	128.4065	568–682		
HM	37.8822	128.52135	903–1264	10.8	1338.9
HC	37.714	128.4458	1023–1175		
PD I	37.8032	128.54995	1029–1347		
PD II	37.77375	128.58865	999–1305		
PD III	37.7593	128.60555	989–1408	10.2	1182.9
PY I	37.62865	128.67385	873–1056		
PY II	37.618	128.6778	1170–1393		
GM	37.60935	128.76655	703–846	13.5	1444.9
JB	37.3481	128.64235	641–735	10.7	1126.6
TS	37.18105	128.91605	1249–1400	9.0	1308.0
TH	37.11565	128.89945	1005–1169		
YS	37.5599	127.4624	573–648	11.7	1383.6
YC	36.9237	128.27395	726–977	11.7	1334.0
MG	36.73785	128.2097	592–629	11.9	1294.9
MS	35.8816	127.7673	714–1403	11.6	1105.3

Candidate trees were selected within a minimum distance of 30 m to avoid genetic correlations. The site conditions of the base populations were assessed to understand the characteristics of the forest stands with *T. amurensis*. A circular plot with a radius of 7 m, centered on each candidate tree, was investigated, to determine the ambiguity in delineating the spatial boundary of the population. This ambiguity was due to the sporadic distribution of *T. amurensis*.

The relative frequency of *T. amurensis* in the base population (P_a) was calculated by dividing the sum of the grades for each candidate tree by those of all surveyed species within the population.

$$P_a = \sum G_{a_i} / \sum G_s$$

$\sum G_{a_i}$: the sum of the grades assigned based on the occurrence frequency of *T. amurensis* within the i-th plot, $\sum G_s$: the sum of the grades assigned to each species in the population.

In the candidate tree evaluation stage, the investigation team consisted of two or three researchers with rich experience in tree improvement research to minimize the variations caused by technical errors in evaluating qualitative adaptive characteristics. The assessment process was standardized in advance through simulative selection.

2.2. Selection Criteria Establishment through Modification

The selection criteria for *T. amurensis* were fundamentally based on the Enforcement Decree of the Creation and Management of Forest Resources Act in Korea, similar to that for evergreen oak [11]. The selection criteria for *T. amurensis* followed the scheme developed for evergreen oaks to differentiate superior individuals in terms of their growth and adaptive characteristics. The growth characteristics were subdivided into two traits: superior growth (SG) and superiority of tree form (STF) (Table 2). The significant difference in this study from those on evergreen oaks is that the SG evaluation used a quantitative index based on growth measurement data instead of ocular evaluation. This evaluation method, with improved objectivity, reflected the species characteristics; it allowed us to measure the tree age and site index. The volume index was calculated from the height and diameter at breast height (DBH) and corrected for age and site conditions.

Table 2. Selection criteria and weights of characteristics of plus-tree selection in *T. amurensis* modified from the evergreen oak selection criteria [10].

Category (Weight)	Characteristic (Weight)	Remark
Growth (0.7)	Superiority of growth (SG) (0.3)	Determined by growth measurements of height and diameter
	Superiority of tree form (STF) (0.4)	Determined by considering the number of stems, stem straightness, and timber height
Adaptability (0.3)	Adaptability to disturbance (AD) (0.15)	Determined by considering the adaptability to disturbance or damage by abiotic and biotic stresses
	Adaptability to environment (AE) (0.15)	Determined by considering the vitality and adaptability to the environments of the site

Regarding adaptation characteristics, the state of disturbance and the health of the candidate tree and the companion species were selected as indicators to evaluate the ecological stability of the base population. The adaptability characteristics were subdivided into adaptability to disturbance and environment, which were evaluated based on the same principle as that used for the evergreen oak evaluation [11]. Adaptability to disturbance (AD) was used to evaluate the resilience to biotic and abiotic stresses. This can be inferred from the symptoms or damage caused by pests, diseases, or artificial disturbances detectable in stems, branches, or leaves [11]. Adaptability to the environment (AE) was used to evaluate the vitality of trees in a given environment as inferred from the overall leaf growth conditions [11]. The weight of each trait was determined based on its relative importance among the target traits.

2.3. Evaluation of Candidate Trees Using Selection Criteria and Standardization

The SG was evaluated from growth measurements corrected for the environment and age of each tree, for comparing candidate trees after standardization. To consider the environmental factors affecting individual tree growth, each local site index was assigned using a simplified site index presumption method [17]. It was recorded as four (when the estimated local site index was 16), three (when the site index was 14), two (when the site index was 12), and one (when the site index was 10). The volume index of each candidate tree was corrected using the estimated site index and the age measured using core analysis. The correction coefficient was set based on the forest yield table of *Quercus mongolica* [18], which occupies a similar status in the forests to *T. amurensis*, because a forest yield table for *T. amurensis* has not been developed. The correction coefficient at each age was set, with tree volume at site index 3 at age 35 as the standard at the inflection point of annual mean growth (Table 3). Specifically, the revised HD^2_{35y} was calculated by multiplying the measured height (H) and squared DBH (D^2) for each tree and applying the correction coefficients for the five-year growth intervals (Table 3). As an example, for the candidate tree IY I-01, whose age was 46, the simple average annual HD^2/y was 0.0197 (= 0.9061 ÷ 46) and HD^2_{35y} was 0.0207 (=0.0197×1.049). The correction coefficient for these candidates (1.049) was estimated from the correction coefficient of 1.060 at site index 2.5 (0.875 + (1.252 − 0.875)÷ 2), reflecting the rate of change from age 45 to 46.

STF, AD, and AE were evaluated by assigning values of 5 for very good, 4 for good, 3 for average, 2 for poor, and 1 for very bad, according to each criterion. The STF was evaluated using multiple factors that comprehensively influenced the tree-form Thcharacteristics. These factors included stem straightness, branching height, natural branch drop, branch angle, thickness, and internode length. The AD was evaluated based on stand history, including traces of forest fires or artificial damage. The AE was determined by comprehensively evaluating the adaptation and vitality of candidate trees to pests or environmental factors, including climate and soil. The characteristics related to adaptability can be identified through the observation and sensory evaluation of each tree and its surrounding site, based on the status of leaves, stems, branches, fallen objects, and soil conditions, as well as by a professional investigation team [10].

Table 3. Correction coefficients based on the tree age and rate of volume change using forest stand yield table.

Site Index	20	25	30	35	40	45	50	55	60	65	70
4	1.129	0.946	0.814	0.728	0.673	0.625	0.593	0.573	0.547	0.538	0.522
3	1.590	1.297	1.287	1.000	0.921	0.875	0.814	0.778	0.761	0.723	0.714
2	2.049	1.842	1.669	1.460	1.348	1.252	1.168	1.130	1.095	1.062	1.030
1	3.185	2.915	2.500	2.188	2.058	1.946	1.846	1.751	1.667	1.592	1.592

The generalization value was calculated for each candidate tree to standardize the trees residing in disparate site conditions. The generalization process followed the method described in a previous study [11]. GV_p generalizes the measurement of a candidate tree at the population level. GV_t is the generalized value of the tree at the level of the entire population, for the subsequent adjustment of the number of selected trees in each population; it prevents possible bias toward trees in more favorable sites.

$$GV_{p,i} = \frac{X_i - X_p}{SD_p}$$

$GV_{p,i}$ is the generalized value at each population level for the i-th individual, X_i is the measurement for the i-th individual, X_p is the average of the measurements in each population, and SD_p is the standard deviation of the measurements in the population.

$$GV_{t,i} = \frac{X_i - X_t}{SD_t}$$

$GV_{t,i}$ is the standardized value at the total population level of the i-th individual, X_i is the measurement of the i-th individual, X_t is the average of the measurements in the total population, and SD_t is the standard deviation of the measurements of the whole population.

The summation index of the generalized value (*GVI*) for each individual was calculated according to the weights (Table 2) of each characteristic in the growth and adaptation categories.

$$GVI_{p,\ i} = \sum \left(GV_{p,\ i,\ j} \times c_j\right)$$

$$GVI_{t,\ i} = \sum \left(GV_{t,\ i,\ j} \times c_j\right)$$

$GVI_{p,\ i,\ j}$ and $GVI_{t,\ i,\ j}$ are the generalized values of the j-th characteristic of the i-th individual in each population and in the entire population, respectively, and c_j is the weight of the j-th characteristic.

The weighted generalized value of each individual (GVI_w) was computed to apply the baseline selection. The weights were assigned as 0.7 for $GVI_{p,\ i}$ and 0.3 for $GVI_{t,\ i}$.

$$GVI_w = \left(GVI_{p,\ i} \times 0.7\right) + \left(GVI_{t,\ i} \times 0.3\right)$$

3. Results

3.1. Site Characteristics of Base Populations

Fifteen regions comprising 20 sites were selected as base populations for *T. amurensis* (Table 4). The forest stands were investigated to understand the characteristics of the target species. The investigation included the abiotic and biotic characteristics of the population, such as the topographic characteristics, proportion of *T. amurensis*, and accompanying species composition.

Table 4. Site characteristics of the twenty base population of *T. amurensis*.

Population	Topographic Type	Soil Depth	Rock Exposure	Slope	Wind Exposure	P_a (%)	
						U	L
IY I	A	***	****	**	***	13.5	3.2
IY II	B	****	***	***	**	12.8	2.6
IY III	A	****	***	****	****	20.2	0
IJ	A	***	**	**	*	11.1	2.8
IB	A	***	***	**	**	3.5	1.6
HM	C	***	***	*****	***	19.7	6.4
HC	B	****	**	**	***	18.6	6.2
PD I	C	****	*	***	****	28.0	5.4
PD II	B	****	**	***	***	25.1	1.1
PD III	B	***	*	**	****	23.2	1.6
PY I	B	***	**	****	**	18.7	9.9
PY II	C	***	**	**	***	31.2	3.7
GM	C	***	**	***	***	5.2	0
JB	B	***	***	***	***	4.6	0
TS	C	****	*	*	****	11.5	0
TH	B	****	*	**	**	17.2	11.6
YS	B	****	***	***	**	2.9	0
YC	B	****	***	***	**	16.9	1.0
MG	C	***	***	**	***	8.6	0
MS	C	***	***	***	***	4.8	0
						14.9	2.9

* Almost none, ** A little, *** average, **** high, ***** very high.

Regarding the topographic characteristics, Type A was lowland in the valley. The selected populations corresponding to Type A included IY I, IY III, IJ, and IB, which were relatively common in the northern part of the sites. Type B refers to mountain mid-sections or enclosed mountain plateau sites. It is located in the middle of mountains or mountain plateaus that ascend from lower valleys to mountaintops. These areas either have gentle slopes or are surrounded by mountains, providing relatively favorable conditions for tree growth. Populations corresponding to Type B included IY II, HC, PD II, PD III, PY I, JB, TH, YS, and YC. The C-type population consists of ridge lines. These populations are located on mountain ridges, where the terrain is rugged and characterized by excessive wind exposure, rocky conditions, and unfavorable conditions for tree growth. Under natural conditions, these areas have a limited number of individuals with well-developed morphologies. Most of the populations around the Baekdudaegan hike trail, which is a major mountain range in Korea, belong to this type. HM, PD I, PY II, GM, TS, MG, and MS were classified as Type C.

The dominant species in the population varied depending on the growth region, site, and population history. *Q. mongolica*, *T. amurensis*, *Fraxinus rhynchophylla*, *Acer pictum* var. *mono*, and *Cornus controversa* were found in the upper canopy. The *T. amurensis* occupancy rate was 14.9%.

3.2. Candidate Tree Evaluation and Standardization

SG, a characteristic of growth, was evaluated from the revised HD^2, based on tree height and DBH (Table S1). Tree height exhibited significant differences between the base populations and among the individuals within the populations (Table 5). Overall, populations located in deep valleys surrounded by mountains and protected from wind exposure showed favorable growth. Notably, TH (average tree height 24.1 m, type B) and IJ (19.6 m, type A) were representative sites with good tree height. In contrast, TS (12.4 m, type C), MS (14.0 m, type C), and JB (14.0 m, type B) showed poor height. DBH exhibited good growth in YS (41.7 cm, type B), PD II (37.2 cm, type B), and IJ (35.9 cm, type A).

Individuals selected from type C populations that encountered poor site conditions, such as high wind exposure and shallow soil depth, generally showed stunted growth with dense age rings.

Table 5. Evaluation of the *T. amurensis* base population based on the selection criteria.

Population	Number of Candidate Trees	Height (m)	DBH (cm)	Average Tree Age	STF	AD	AE
IY I	11	16.5 ^{bcdef}	27.54 ^{abcd}	42.8 ^{d}	4.59 ^{a}	4.56 ^{a}	4.61 ^{a}
IY II	8	18.3 ^{abc}	27.43 ^{abcd}	55.0 ^{abcd}	4.50 ^{a}	4.54 ^{a}	4.53 ^{a}
IY III	7	18.4 ^{ab}	29.67 ^{abc}	50.6 ^{bcd}	4.64 ^{a}	4.81 ^{a}	4.47 ^{a}
IJ	10	19.6 ^{ab}	35.92 ^{a}	63.0 ^{abc}	4.60 ^{a}	4.60 ^{a}	4.17 ^{a}
IB	7	17.3 ^{abcde}	28.14 ^{abcd}	46.6 ^{cd}	4.71 ^{a}	4.57 ^{a}	4.53 ^{a}
HM	10	14.6 ^{def}	26.89 ^{abcd}	78.8 ^{ab}	4.40 ^{a}	4.40 ^{a}	4.80 ^{a}
HC	15	16.7 ^{bcdef}	26.99 ^{abcd}	48.9 ^{cd}	4.63 ^{a}	4.40 ^{a}	4.58 ^{a}
PD I	10	16.8 ^{bcdef}	37.18 ^{a}	99.5 ^{ab}	4.00 ^{a}	4.80 ^{a}	4.50 ^{a}
PD II	10	17.8 ^{abcd}	28.40 ^{abc}	60.3 ^{abc}	4.75 ^{a}	4.55 ^{a}	4.70 ^{a}
PD III	7	12.4 ^{f}	21.84 ^{bcd}	40.3 ^{d}	4.64 ^{a}	4.43 ^{a}	4.43 ^{a}
PY I	12	16.4 ^{bcdef}	30.57 ^{ab}	67.8 ^{abc}	4.66 ^{a}	4.43 ^{a}	4.73 ^{a}
PY II	13	14.7 ^{cdef}	30.24 ^{ab}	81.9 ^{a}	4.58 ^{a}	4.65 ^{a}	4.73 ^{a}
GM	5	16.4 ^{bcdef}	20.56 ^{cd}	45.8 ^{cd}	4.80 ^{a}	4.60 ^{a}	4.40 ^{a}
JB	5	14.0 ^{def}	24.68 ^{abcd}	39.4 ^{d}	4.44 ^{a}	4.60 ^{a}	4.60 ^{a}
TS	11	12.4 ^{f}	18.75 ^{d}	41.2 ^{d}	3.97 ^{a}	4.91 ^{a}	4.43 ^{a}
TH	12	24.1 ^{a}	28.51 ^{abc}	44.7 ^{d}	4.50 ^{a}	4.58 ^{a}	4.81 ^{a}
YS	5	18.4 ^{abcdef}	41.70 ^{a}	57.0 ^{abcd}	4.40 ^{a}	4.74 ^{a}	4.34 ^{a}
YC	7	16.9 ^{abcdef}	31.43 ^{a}	52.9 ^{abcd}	4.36 ^{a}	4.36 ^{a}	4.64 ^{a}
MG	6	14.0 ^{ef}	24.73 ^{abcd}	38.5 ^{d}	4.17 ^{a}	4.42 ^{a}	4.67 ^{a}
MS	5	13.4 ^{ef}	25.38 ^{abcd}	53.6 ^{abcd}	4.00 ^{a}	4.60 ^{a}	4.60 ^{a}
Summary	176	16.6	28.45	57.2	4.47	4.58	4.56

The use of distinct superscript letters indicates significant differences among the populations as examined via the Kruskal–Wallis test ($p < 0.01$).

The overall average of HD^2_{35y}, based on a site index of 3 (16 in the forest yield table) after 35 years of age, was 0.0204. There were significant differences between and within the populations. YS (0.0472, type B), TH (0.0356, type B), IJ (0.0355, type A), and IY I (0.0274, type A) showed higher values, whereas populations such as TS (0.0106, type C), PD II (0.0073, type B), HM (0.0097, type C), and PY II (0.0109, type C) exhibited values that were approximately half those of the former group. In addition, there were significant differences within populations, with a difference of more than tenfold in HD^2_{35y} values between TH-09 (0.0089) and TH-05 (0.1245) in TH and between YS-03 (0.0074) and YS-05 (0.1230) in YS. The large difference within the population supported the larger weight of the generalized value within the population (0.7). STF was evaluated through ocular assessment, considering traits such as stem straightness, clear bole height, branch angle, and branch thickness. Based on this assessment, individual trees were assigned rank values ranging from 1 to 5. The mean STF was 4.47. PD II (4.80), GM (4.80), and IB (4.71) exhibited high values, whereas TS (3.97) and MK (4.17) showed low values. Overall, the values were substantial with relatively small differences from the average population values.

For AD and AE adaptation-related traits, indices were established by considering factors such as forest stand history and repeated negative disturbances. For AD (average 4.58), TS (4.91), IY III (4.81), and PD I (4.80) exhibited higher values, whereas YC (4.36), PDIII (4.40), and HM (4.40) showed lower values. In the latter case, the influence of natural habitats was considered more significant than artificial interference, whereas in the former case, the influence of cultivation or artificial damage, such as logging, was more pronounced because of the relatively close proximity and easy access to these populations. AE was measured based on the extent of damage caused by pests or various hazards, as well as adaptability to the habitat, resulting in an average value of 4.56. HM (4.80); PY I

(4.73), PY II (4.73), and PD II (4.70) showed the highest values. These groups belong to the mountainous and rocky type (Type B) of the Odaesan region, considered the main distribution area of *T. amurensis* in Korea; they show a relatively higher health status. In contrast, IJ (4.17), YS (4.34), and GM (4.40) exhibited slightly lower values. There were no significant differences between the populations in the adaptability indices according to the Kruskal–Wallis test.

During the calculation of the generalized value (GV) of the candidate tree characteristics, a distinction was made between the average values at the individual population level and those at the whole level. GV_p was calculated by applying the average of individual measurements by population, assuming differences in biotic and abiotic factors between populations. GV_t was calculated using the average of the measured values in the entire population, assuming that the influence of the environmental factors between populations was negligible. GVI_w is a compromise value that considers the influence of environmental factors between populations, with a weight of 0.7 for GVI_p and 0.3 for GVI_t. The weight used to compromise GVI_p and GVI_t was based on the values for evergreen oak species, which had similar sparse distribution characteristics [11]. GVI_p, GVI_t, and GVI_w, the summation indices of the generalized values, were obtained through weighted averages (Table 2) and standardization processes, according to the importance of each characteristic (Table S1).

3.3. Plus-Tree Selection through Baseline and Adjustment

To select approximately 60 plus trees from among the 176 candidate trees, the baseline of GVI_w was set to 0.3374 to apply a 35% selection intensity (Figure 2). The final adjustment and selection of plus trees were performed considering factors such as the number of trees selected by region and population, the uniqueness of populations, and selection bias caused by local site differences within populations (Table 6).

The baselines for GVI_p and GVI_t were set for comparing selection patterns among the three indices, including GVI_w (Figure 2). In the six populations, namely, IY II, IY III, IJ, PD I, JB, and YS, the number of individuals that showed higher values than the baseline for GVI_p, GVI_t, and GVI_w were the same (Table S1). IY I, HC, PD II, PY I, and YC showed similar sizes for the selected trees based on the three indices. In the case of HM, GM, and MS, no candidate tree surpassed the baseline set by GVI_t. In the case of TS and MG, each had only one individual with a value higher than the baseline set by GVI_t among the candidate trees. In HM, four individuals (nos. 4, 5, 7, and 10) showed higher GVI_p values than the baseline, whereas no individual met the criteria based on GVI_t. In GM, one individual (no. 1) showed higher GVI_p values, but none met the criteria based on GVI_t. For TS, three individuals (nos. 4, 6, and 9) were included based on GVI_p, but only no. 6 was included based on GVI_t; three individuals (nos. 4, 6, and 9) were included based on GVI_w. In MG, two individuals (nos. 3 and 4) belonged to the top 35% based on GVI_p, but only no. 4 met the criteria based on GVI_t. However, there were populations with a larger number of trees above the baseline of GVI_t than those above GVI_p. In the cases of IB and TH, more individuals were selected based on GVI_t than on GVI_p. In IB, one individual (no. 5) was selected based on GVI_p, whereas three individuals (nos. 4, 5, and 7) were selected based on GVI_t. In TH, four individuals (nos. 3, 5, 11, and 12) were selected based on GVI_p, whereas six individuals (nos. 1, 3, 4, 5, 11, and 12) were selected based on GVI_t.

Sixty-two individuals had GVI_w values greater than the baseline value. Among them, the population with the largest number of selected individuals (6 out of 15 candidates) was the HC. IY I and PY I had 5 individuals each, while IY II (8 candidates), IY III (7 candidates), IJ (10 candidates), TH (12 candidates), and YC (7 candidates) had 4 individuals each.

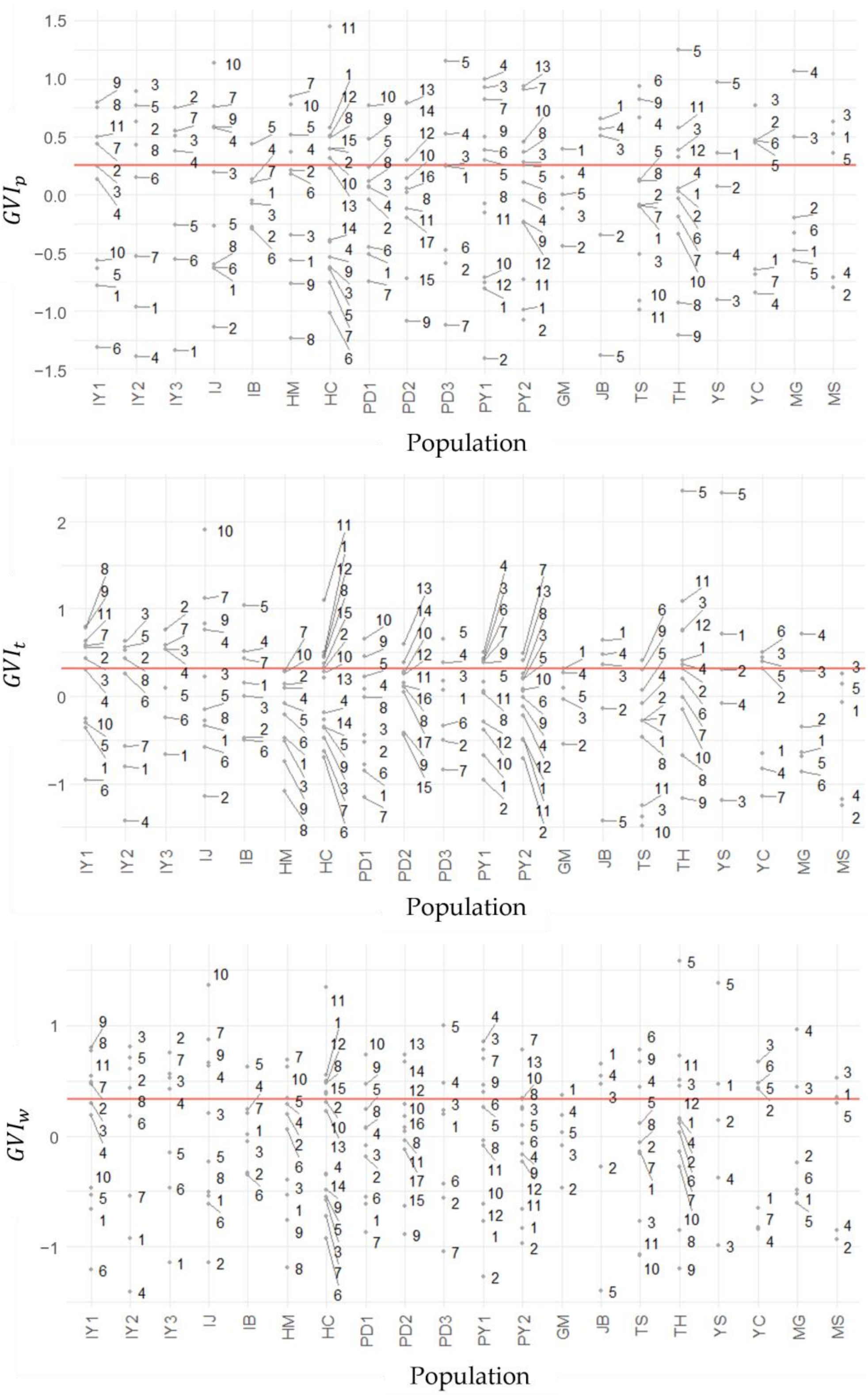

Figure 2. Evaluation of candidate trees within base populations using GVI_p, GVI_t and GVI_w. The numbers within each population represent the IDs of candidate trees. The red line indicates the baseline for 35% selection intensity in each index.

Table 6. Trees selected via baseline selection of GVI_p, GVI_t, GVI_w, and adjustment.

Population	Number of Candidate Trees	Selected Tree IDs in the Upper 35%			Final Plus-Tree ID	Percentage of Selected Trees	
		Selection Based on GVI_p	Selection Based on GVI_t	Selection Based on GVI_w		GVI_w Baseline	Final
IY I	11	2, 3, 7, 8, 9, 11	2, 3, 7, 8, 9, 11	2, 7, 8, 9, 11	2, 7, 8, 9	45.5	36.4
IY II	8	2, 3, 5, 8	2, 3, 5, 8	2, 3, 5, 8	2, 3, 5	50.0	37.5
IY III	7	2, 3, 4, 7	2, 3, 4, 7	2, 3, 4, 7	2, 3, 7	57.1	42.9
IJ	10	4, 7, 9, 10	4, 7, 9, 10	4, 7, 9, 10	4, 7, 9, 10	40.0	40.0
IB	7	5	4, 5, 7	5	4, 5, 7	14.3	42.9
HM	10	4, 5, 7, 10	-	5, 7, 10	2, 7, 10	30.0	30.0
HC	15	1, 2, 8, 10, 11, 12, 15	1, 2, 8, 11, 12, 15	1, 2, 8, 11, 12, 15	1, 8, 11, 12, 15	40.0	33.3
PD I	10	9, 10	9, 10	9, 10	8, 9, 10	20.0	30.0
PD II	10	12, 13, 14	13, 14	13, 14	12, 13, 14	20.0	30.0
PD III	7	1, 3, 4, 5	4, 5	4, 5	3, 4, 5	28.6	42.9
PY I	12	3, 4, 5, 6, 7, 9	3, 4, 6, 7, 9	3, 4, 6, 7, 9	3, 4, 6, 7	41.7	33.3
PY II	13	3, 5, 7, 8, 10, 13	7, 13	7, 10, 13	7, 8, 10, 13	23.1	30.8
GM	5	1	-	1	1, 4	20.0	40.0
JB	5	1, 3, 4	1, 3, 4	1, 3, 4	1, 4	60.0	40.0
TS	11	4, 6, 9	6	4, 6, 9	4, 5, 9	27.3	27.3
TH	12	3, 5, 11, 12	1, 3, 4, 5, 11, 12	3, 5, 11, 12	3, 5, 11, 12	33.3	33.3
YS	5	1, 5	1, 5	1, 5	1, 5	40.0	40.0
YC	7	2, 3, 5, 6	3, 5, 6	2, 3, 5, 6	3, 5, 6	57.1	42.9
MG	6	3, 4	4	3, 4	3, 4	33.3	33.3
MS	5	1, 3, 5	-	1, 3	1, 3	40.0	40.0
Number of trees	176	73	56	62	62	35.2	35.2

In contrast, IB and GM had only one individual with GVI_w values higher than the baseline, and populations such as PD I, PD II, and PD III had fewer individuals that surpassed the value. Therefore, considering unfavorable habitat conditions, such as poor soil or excessive exposure to wind in the vicinity of mountain ridges, we decided to include some individuals with substantial GVI_w values, although they did not reach the baseline. For IB, individuals that ranked second (no. 4 with GVI_w = 0.2462) and third (no. 7 with GVI_w = 0.2060) were included. For GM, individuals that ranked first (no. 1 with GVI_w 0.3725) and second (no. 4 with GVI_w 0.1877) were included. For PDI, the individual that ranked fourth among the excluded individuals with the highest GVI_w was included as an additional candidate (no. 8 with GVI_w of 0.0784) to compensate for the aged trees. For PD II, the individual with the highest GVI_w among the excluded individuals was included as the third selected candidate (PD II-12 with GVI_w 0.2840). For PD III the individual with the highest GVI_w among the excluded individuals was included as the third selected candidate (No. 3, GVI_w = 0.2303).

Meanwhile, 80 years was the highest age in the yield table applied to calculate the revised annual increment index for 35-year-old trees $\left(HD^2_{35y}\right)$. However, in this study, there were also eight candidate trees with ages of 100 or more, including PD I-07 (no. 7 of PD I) with an age of 184. The adjustment coefficient set based on age 70 was applied to 20 individuals aged 80 years or older; therefore, it was considered that HD^2_{35y} could have been underestimated for those individuals. The average HD^2_{35y} of individuals aged 80 years or older was 0.0120, which was significantly lower than the average of 176 individuals (0.0204). The average value of GVI_w in the older group was very low at -0.3221 (expected value 0) (Table S1). Among the 20 individuals, only two, HM-07 (age 85, GVI_w 0.6844) and PY I-06 (age 89, GVI_w 0.3967), had GVI_w values higher than the baseline. Among the eight individuals aged 100 years, only two individuals, PD I-08 (age 156, GVI_w 0.0784) and HM-02 (Age 122, GVI_w 0.1927; Figure 2), had a GVI_w greater than the average (0). Therefore, to compensate for the underestimation of GVI_w due to old age, individuals aged 80 years that showed GVI_w above the average were included as the final plus trees. These individuals were HM-02 and PD I-04 (age 84, GVI_w 0.0703).

In summary, among the 62 individuals that exceeded the baseline, nine were excluded because they were selected from populations with similar environments within the same growth zone. Nine individuals were selected considering their potential for underestimation owing to their advanced age or poor site conditions, such as rocky slopes or marginal distribution. After applying the baseline established based on GVI_w, factors such as site characteristics and local environmental differences by population and tree age were comprehensively considered; this resulted in the selection of a final set of 62 superior individuals. After the final selection with adjustment, the percentage of selected trees within each population ranged between 27.3% (TS) and 42.9% (IB, PD III, and YC), resulting in an average percentage of 36.3% and a standard deviation of 5.2%. The deviation decreased from 13.5% when the selection was solely determined based on the GVI_w baseline. This decrease in deviation showed that the adjustment procedure was appropriately performed to balance the selection percentage between populations.

The proportion of trees selected by topographic type was maintained in each selection step according to the insignificant difference observed in the X^2 test. The largest portion of selected trees belonged to type B, followed by types C and A, in all selection steps (Figure 3). The number of populations corresponding to types A, B, and C were four, nine, and seven, respectively.

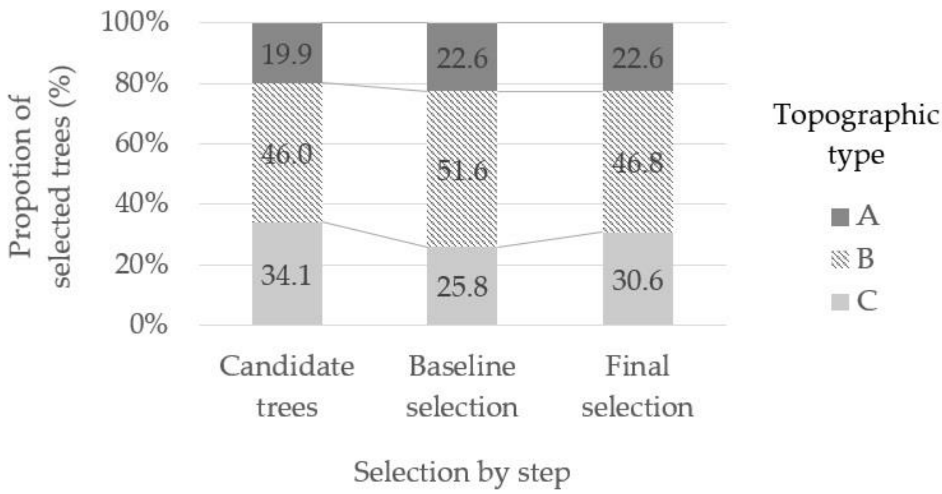

Figure 3. Proportion of selected trees by topographic type in each stepwise selection ((1) candidate tree selection, (2) baseline selection, (3) final selection).

The improvement in the characteristics by the final selection was verified by comparing the accepted and rejected trees (Table 7). All characteristics differed significantly between the mean values of the accepted and rejected trees ($p < 0.0001$). The selection effect on the targeted characteristics ranged from 104.4% to 196.1%.

Table 7. Comparison of average values of the targeted characteristics between accepted and rejected trees after final selection.

	Number of Trees	Height (m)	DBH (cm)	HD^2_{35y}	STF	AD	AE
Accepted (A)	62	18.0 ^{a}	31.90 ^{a}	0.02990 ^{a}	4.9 ^{a}	4.7 ^{a}	4.8 ^{a}
Rejected (R)	114	15.9 ^{b}	26.57 ^{b}	0.01525 ^{b}	4.2 ^{b}	4.5 ^{b}	4.5 ^{b}
A/R × 100 (%)	-	113.2	120.1	196.1	116.7	104.4	106.7

The use of distinct superscript letters indicates significant differences among the populations as examined via the ANOVA ($p < 0.001$).

4. Discussion

Our research scheme, including base population selection, the establishment of selection criteria, forest stand investigation, standardizing measurements of each candidate tree, and plus-tree selection, was fundamentally based on a recent selection procedure developed for evergreen oaks using ocular and baseline selection [11]. In this study, quantitative data for volume growth and qualitative data for tree form and adaptability were considered. Different selection methods were applied depending on the characteristics. In particular, the evaluation of growth characteristics was modified from a previous study on evergreen oaks, reflecting the species' characteristics, for improving the objectivity of the selection. Growth characteristics were assessed based on direct measurements, followed by revision. Tree form and adaptability were evaluated through ocular selection using a subject grading system. Next, the measured values of each characteristic were standardized, and a weighted average value for each candidate tree was calculated using the weight-by-characteristic method. A baseline was set based on the weighted average of the candidate trees by determining the selection intensity. The final plus trees were selected considering factors such as region, the number of trees selected by population, tree age, and possible bias due to local site variation within populations.

4.1. Site Characteristics of Base Populations

In tree improvement programs, it is desirable that breeding materials be selected under various environmental conditions to ensure the diversity and resilience of breeding material [19]. In this study, the base population was selected, and its topographic type was classified to retain superior individuals across various distributional ranges (Table 4). The characteristics of the *T. amurensis* base population were investigated, including biotic and abiotic factors such as topographic type and coexisting species in the stands. Basic information on the growth characteristics of the target species for tree improvement is required to determine the selection methodology or criteria [20,21]. Understanding the ecological aspects of this species is essential when using the selection materials in forest plantation management [22].

Among the 20 base populations explored, there were four populations of type A, nine of type B, and seven of type C. During the first attempt at plus-tree selection for *T. amurensis* in Korea in the early 2000s, most of the selected trees were located in type A from the limited northern part of the Baekdudaegan Mountain Ridge. In this study, the selection was made from a wider and more varied topography. Selection from divergent environments is desirable for generating genetic diversity at loci that affect target traits [23].

The *T. amurensis* occupancy rate (14.9%) was similar to that observed in previous studies on *Q. salicina* (12.3%) and *Q. glauca* (19.3%). The similar appearance patterns in the forest stand of the two taxa supported the idea that the framework of selection criteria can be cross-referenced because the selection criteria should be arranged according to the characteristics of the species [20]. In the vegetation structure of the temperate forest stand, *Q. mongolica* consistently showed the highest importance or a positive correlation with

T. amurensis [24,25]. In the upper layer, *T. amurensis* showed a high frequency following *Q. mongolica*, which is consistent with the observations in a previous study [24]. Both *T. amurensis* and *Q. mongolica* were rarely discovered in the middle or lower layers in this study. In a previous study conducted approximately 30 years ago in Korea [24], the importance of *Q. mongolica* was high in the middle layer, although it was investigated in a limited region near the northern population IY. *T. amurensis* appeared at a low frequency in the middle layer, with a large percentage of super-aged trees [24]. The vegetation status of *T. amurensis* in this study showed that both *T. amurensis* and *Q. mongolica* had a lower appearance rate in the lower layer than that observed in the past. However, sequential monitoring is required to determine whether these two species are more competitive than the others as succession proceeds.

4.2. Candidate Tree Evaluation and Standardization

The primary focus of this study was to establish techniques and criteria for selecting *T. amurensis* plus trees. The selection criteria for superior *T. amurensis* were based on the Enforcement Rule of the Forest Resource Development and Management Act and were further refined to investigate growth characteristics (e.g., height growth and morphological traits) and adaptation characteristics (e.g., ecological disturbance and tree health status).

Initially, 176 candidate trees were selected from 20 base populations, including 154 individuals from 16 major distribution populations in Baekdudaegan and 23 individuals from four additional peripheral populations (YS, YC, MG, and MS), for expanding genetic diversity. The growth and adaptation indices for each individual were standardized at the individual population (GVI_p) and overall population (GVI_t) levels and combined into an integrated index (GVI_w). Growth characteristics were assessed using the volumetric index as a quantitative indicator, which was standardized (HD^2_{35y}) based on the site index and tree age to consider the spatial and temporal environmental factors for candidate tree comparison.

The basic objective of the evaluation is to assess not only growth but also adaptability in plus-tree selection, considering the ecological characteristics of hardwood usually forming uneven and mixed forest [11,20]. Commonly used selection indicators for trees, such as tree height and diameter, are influenced by temporal and spatial environmental factors. Height and DBH per se, as phenotypic traits, are not suitable indicators for the selection of superior individuals because they are influenced by age or local site conditions, which can alter the genetic potential of tree growth [26], especially for species in uneven or mixed forests. Therefore, in this study, the 35-year-old average annual revised HD^2 (HD^2_{35y}), considering the tree's location and age, was selected as the indicator for the selection of plus trees, with the aim of minimizing the impact of environmental factors on growth characteristics. Forest yield tables for *T. amurensis* have not yet been established; therefore, the forest yield table for *Q. mongolica* was applied because the two species consist of a community with association [27,28], showing similar ecological requirements and conditions [29]. Correction coefficients were set based on the forest yield table, in terms of site index and age (Table 3). The revision of growth characteristics, considering age and site index, can be categorized as a regression selection system. The regression selection method is intricate but can be useful, especially for hardwood species in unevenly aged or mixed forests [20]. In this study, tree age was investigated using a visible annual ring, and the site index was determined from the forest yield table of *Q. mongolica*. This process of determining the growth index considering the tree's age and site conditions can be useful in selection practices in both natural populations and plantations for other species. Generalization, followed by evaluation, enabled an objective comparison of the growth of candidate trees from a heterogeneous environment. The harmonic mean of GVI_p and GVI_t, called GVI_w, was adopted as the final selection criterion to consider genetic and environmental factors and their interactions within each population. The weights assigned to GVI_p and GVI_t can be modified based on a sufficient understanding of the genetic and ecological characteristics of the species. In addition, the weights varied when a specific plantation area was planned, referring to seed zonation.

Qualitative traits were assessed through ocular evaluation. Ocular selection can be useful for improving multiple qualitative, morphological, and other characteristics [30]. Subject grading following ocular observation can be utilized by proficient graders with proper grading criteria [18]. The adaptability indices AD and AE, which were evaluated using this subject grading method, showed substantial average and insignificant differences in the overall populations. The distribution pattern of *T. amurensis* within the population formed subgroups with few individuals; therefore, most of the selected candidate trees had a rating of 'average' (3 points) or higher. If adjacent individuals below the standard had been chosen to enlarge the numerical pool, the variance between individuals and populations would have been larger, with a lower average. In addition, the differences between populations were more likely to offset each other rather than show directional differences. Tree improvement programs usually focus on improving growth traits, but improving forest health is also a major objective [31]. The habitat of *T. amurensis* is predicted to shrink and become fragmented by climate change [1]; therefore, securing breeding materials with stronger adaptability is emphasized. The selected candidates could be useful for genetic conservation, for improving the adaptability of *T. amurensis*.

4.3. Plus-Tree Selection through Baseline Selection and Adjustment

When setting the selection intensity, we aimed to secure 30 effective individuals and assumed a contribution rate of 50% [32]. Among the 11 populations with similar or equal numbers of individuals selected using GVI_p, GVI_t, and GVI_w, seven corresponded to topographic type B. These populations were located at relatively favorable sites for tree growth, such as flat ridges surrounded by nearby mountains, although they could also be on hillsides or mountain ranges. The lower number of trees selected based on GVI_t than GVI_w, as in TS, was because the average value of the candidates was relatively lower than that of the overall population. The exclusion of candidates based on GVI_t was generally found in populations corresponding to type C, where the terrain is steep and rugged, resulting in poor tree growth conditions owing to excessive wind exposure and rocky terrain. The population categorized as Type C naturally had a small number of individuals with good growth, and straight and well-formed trees. In other words, the exclusion based on GVI_t reflects the general inferiority of candidates within the scope of the entire population. The populations in which the number of individuals selected based on GVI_t was larger than those selected based on GVI_p were classified as topographic type A or B. These areas have vertical terrain with valleys and low-lying areas, exhibiting the typical characteristics of a gentle hilly area. Most previously selected trees were located on this type of terrain. Populations with more individuals with greater GVI_w than the average were mostly located in gentle hilly areas in the valley (Type A) or relatively favorable site conditions (Type B) surrounded by high mountains.

The final adjustment was to remedy the sole baseline selection, resulting in the majority of candidates being from the central region of *T. amurensis* distribution. We considered factors such as age and environmental differences that were not included in the typical selection criteria. Considering the candidates from the less favorable environment, the percentage of trees selected from Type A decreased and that from Type C increased, although the change was not statistically significant. Standardization and final adjustment helped us consider the candidate tree age or developmental stage, which should be considered in the selection of unevenly aged stands [33].

The rejected trees were also candidate trees within each population; therefore, the selection effect between the accepted trees and the natural population would be larger than that shown in this study (Table 7). In addition, it is worth noting that the rejected candidates also deserve to be used as breeding materials when greater diversity is required. Enabling the sufficient securing of breeding materials is one of the advantages of baseline selection after standardization in this selection scheme.

5. Conclusions

T. amurensis has high potential for utilization because of its special-purpose timber, pulpwood, landscape, and honey production. However, owing to its excessive exploitation and overutilization, existing resources have severely deteriorated, calling for urgent measures to secure and conserve superior resources, for their continuous improvement and utilization. Therefore, the aim of this study was to explore and select breeding populations through forest investigations to collect superior resources and establish a breeding base for the continuous improvement of *T. amurensis*.

The process of selecting plus trees requires consideration of the ecological and genetic characteristics of the target species, including appropriate selection criteria, measurement standardization, and variation within and between populations. In this study, we obtained fundamental data on the status of *T. amurensis* in temperate deciduous broadleaf forests in Korea. We developed a stepwise selection approach using various selection criteria and characteristics, by combining ocular and baseline selection methods for the selection of *T. amurensis* plus trees. The established approach enabled us to consider the growth patterns, vigor, dominance, and other aspects of *T. amurensis* under various site conditions. The selection for *T. amurensis* spatially expanded and diversified from previous selection in the valley (Type A) or hillside (Type B) to rocky slope dominance (Type C). This resulted in a base population composed of four Type A, nine Type B, and seven Type C populations. Based on the standardized values obtained through the selection criteria and weights by characteristics, 62 superior individuals were selected from 176 candidate trees in 20 *T. amurensis* base populations.

However, there are still limitations to this research owing to insufficient information on the biological characteristics of *T. amurensis*. Research on genetic differentiation between populations, experimental plantations, and G×E interactions in *T. amurensis* in the future will aid tree improvement in this species. Future studies will help refine the selection criteria or weights of characteristics.

This study on the selection and investigation of the base population of *T. amurensis* will be useful for future genetic and ecological studies on temperate deciduous broadleaf forests with *T. amurensis*. The results of our plus-tree selection can serve as basic information for the further development of selection guidelines or the preparation of test guidelines for plus trees. In addition, they can be directly used for genetic resource conservation, the establishment of breeding populations, and seed supply sources, including seed orchards and seed production areas, and are ultimately related to the plantation and forest management of broadleaf forests.

Supplementary Materials: The following supporting information can be downloaded from: https://www.mdpi.com/article/10.3390/f14101972/s1, Table S1: The evaluation and generalized values of candidate trees for the selection of *T. amurensis* plus trees.

Author Contributions: Conceptualization, I.-S.K. and W.-Y.C.; methodology K.L., I.-S.K. and W.-Y.C.; validation, K.L. and I.-S.K.; formal analysis, K.L. and W.-Y.C.; investigation, K.L. and W.-Y.C.; writing—original draft preparation, K.L.; writing—review and editing, K.L. and I.-S.K.; supervision, W.-Y.C.; project administration, I.-S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Institute of Forest Science (grant number FG0400-2019-01).

Data Availability Statement: The data are contained within the article or Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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