
Genetic Differentiation among Seed Samples from Provenances of Pinus sylvestris L.

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Summary

Differentialiation is studied among seed samples from nine Scots pine forest stands or seed orchards. The results are based on the allele frequencies obtained at ten enzyme gene loci. Pairwise statistical comparisons between the samples indicated significant deviations for nearly all pairs. The genetic distances reflected similar tendencies but were shown to allow more reliable interpretations of the genetic differences between samples and the efficiency of single loci in revealing such differences. A new method of subpopulation differentiation was applied to measure and illustrate the genetic differentiation among the samples and their complements instead of in pairs. The presented graphs clearly pointed out loci reflecting maximum differentiation and thus can serve as a criterion for the selection of the best-suited single locus or multilocus combination. The proposed methods can be utilized equally well for genotype frequencies, which, however, requires larger sample sizes. As a result of the measure of subpopulation differentiation, the seed orchard crops were proven to show greater genetic differentiation than the samples representing the seed of forest stands.

Key words: Differentiation, allele frequency, provenance, forest, seed, Pinus sylvestris L.

Zusammenfassung


Introduction

In this country, the marketed reproductive material of coniferous species still originates exclusively from generative propagation. By law, this category is termed "selected reproductive material". In effect, such seed originates from collections in phenotypically selected forest stands or from particular breeding populations, i.e. seed orchards which are laid out to reduce pollination from the outside and are managed to produce abundant crops of seed. At present, in Scots pine (Pinus sylvestris L.), clonal seed orchards cover nearly 300 ha or 0.4% of the total area of selected Scots pine seed stands. The area devoted to clonal seed orchards, is, however, increasing continuously, as is the productivity. Seed stands are located in designated regions of provenance which are supposed to be ecologically homogeneous. The clones being used for seed orchard establishment descend vegetatively from phenotypically selected trees in forest stands within regions of provenance. In the present paper,
the term provenance designates the origin of seed samples from both forest stands and seed orchards.

Characterization of reproductive material in the strict form of unique identification of its origin is not practicable, because it would require that each existing provenance be genetically inventoried and that it have distinct identifiable characters which are obtained in the same way in the offspring populations. Even if seed are collected in different parts of one and the same forest stand, the resulting material can be expected to show genetic heterogeneity. The same is true between flowering periods as a result of varying fertilities, mating system effects and also early stage viability selection. Such heterogeneities over time were studied in Scots pine seed orchards and found to occur in most cases (e.g. Möller-Starck et al. 1983, Möller-Starck and Ziehe 1984, Möller-Starck 1983).

Therefore, a characterization of forest provenances is practicable only by genetic methods of differentiation among the studied material. This can occur in the form of an assignment of sexually reproduced material to parental populations or to any other samples in question in order to monitor genetic differences among the respective samples (for survey of limitations and recent results of the "identification" of forest reproductive material see Gregorius 1983, Gregorius, Hattemer and Bergmann 1984, Möller-Starck 1983).

The aim of the present paper is to investigate differentiation among seed samples from forest stands and clonal seed orchards of Scots pine. It is self-evident that any method of genetic differentiation requires the existence of environmentally independent genetic markers.

Material and Methods

Seed samples

Nine seed probes from five Bavarian regions of provenance were genotyped, each probe being represented by 120 seeds drawn randomly from the bulked crop of forest stands (STAND) or single clonal seed orchards (S.O.):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ref. No. and design. of region of provenance/location of seed orchards</th>
<th>Seed category</th>
<th>Flowering period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85118 Steigerwald</td>
<td>STAND</td>
<td>1979</td>
</tr>
<tr>
<td>2</td>
<td>85119 Selb</td>
<td>STAND</td>
<td>1979</td>
</tr>
<tr>
<td>3</td>
<td>85119 Selb/Ebrach I</td>
<td>S.O.</td>
<td>1979</td>
</tr>
<tr>
<td>4</td>
<td>85119 Selb/Ebrach II</td>
<td>S.O.</td>
<td>1979</td>
</tr>
<tr>
<td>5</td>
<td>85119 Selb/Weissenstadt</td>
<td>S.O.</td>
<td>1979</td>
</tr>
<tr>
<td>6</td>
<td>85119 Falkenberg</td>
<td>STAND</td>
<td>1977</td>
</tr>
<tr>
<td>7</td>
<td>85120 Mid. Bavaria/Rothenburg</td>
<td>S.O.</td>
<td>1979</td>
</tr>
<tr>
<td>8</td>
<td>85122 Alpes/Laufen</td>
<td>S.O.</td>
<td>1980</td>
</tr>
<tr>
<td>9</td>
<td>85123 Schrobenhausen</td>
<td>STAND</td>
<td>1979</td>
</tr>
</tbody>
</table>

The geographical origin of the nine seed probes is marked in Figure 1.

The two seed orchards Ebrach I and II (probe 3 and 4) were established by the same clones but differ with respect to the actual number of replicates per clone (for details see Möller-Starck 1985). These seed orchards and the orchards Rothenburg and Laufen (probes 7 and 8) are located outside of the provenance region.

Genotyping

Each seed was genotyped separately for its haploid endosperm (macro gametophyte) and the corresponding diploid embryo, which allows the determination of the female and male contributions as an ordered pair (Möller-Starck 1976). The data were originally used to test ways of identifying components of mixtures (Möller-Starck 1984). The genetic control of the enzyme systems leucine aminopeptidase, glutamate oxaloacetate transaminase, malate dehydrogenase, and shikimate dehydrogenase (LAP, EC 3.4.11.1, GOT, EC 2.6.1.1, MDH EC 1.1.1.37, SKDH, EC 1.1.1.25) was verified by analysing seeds from controlled crosses (Möller-Starck 1982a, b and unpublished; see also Rudin and Ekberg 1978, Chung 1981 for GOT, and Szmidt and Yazdani 1984 for SKDH in Scots pine). The multilocus-genotypes were scored simultaneously by horizontal starch gel elec-
puter program GSEED (GALLER et al., in preparation). The concept of GREGORUS and ROBERUS (1988) was applied to measure the genetic differentiation among the seed probes.

Results and Discussion

Allele frequencies

In Figure 2, the allele frequencies are given exemplarily at a gene locus which reflects pronounced deviations among the studied seed samples. The illustrated distributions refer to the combined frequencies among the female and the male genetic contributions to the seeds. Two alleles appear to be comparatively frequent in nearly all samples (MDH- D₁ and D₂), and one is either rare or not represented in all probes (MDH-D₃). The latter phenomenon also occurs in most of the obtained frequency distributions of alleles and/or genotypes at the gene loci. It includes the appearance of unique alleles/genotypes. Due to low class frequencies, a reliable qualitative distinction on the basis of unique single- or multilocus types was not practicable, so that only quantitative criteria remained for discrimination among the seed probes. In the given example, outstandingly high or low frequencies are indicated for the samples 5 and 8 with respect to the allele MDH-D₅, for the samples 2, 5, 7 in the case of D₆, and particularly for sample 5 with respect to D₄.

Other available criteria for discrimination are (1) the allele frequencies in either the female or the male gametic contributions to the seeds and (2) the genotype frequencies at each of the ten gene loci. The latter can be expected to reveal larger deviations between samples due to the much greater number of different types per locus as compared to the alleles. However, in many cases this would imply overly small numbers of genotypes in single frequency classes and thus restrict the applicability of statistical test procedures. Moreover, genotype frequencies among seeds are less suitable for differentiation studies, since they may vary heavily with the mating conditions, which, in turn, may depend strongly on environmental fluctuations. To achieve a maximum number of samples within each frequency class, it was appropriate to pool the combined female and male allele frequencies.

Deviations between pairs of seed samples

Statistical tests

Viewing the ten gene loci, in 35 of the 36 pairs which can be formed from nine samples, the two seed lots differed significantly from each other in their frequency distributions of alleles at one or more loci according to the G-test. Only the pair of samples 6 and 9 could not be discriminated statistically. Exemplarily for other loci, in Table 1 results are surveyed for two of them, MDH-B and MDH-D. In the case of MDH-B outstandingly low frequencies of two out of three alleles were obtained, even after pooling: Since the G-test is not sufficiently accurate in such cases, the exact a-values were calculated with the rare alleles pooled according to the optimum exact test for $2 \times 2$ tables (KENDALL and STUART 1961, Vol. 2, sec. 33.24).

Significant deviations are evident for 15 pairs for MDH-B and for 24 pairs for MDH-D. Both gene loci together already enable the statistical discrimination of 28 of the 36 pairs. Comparatively low a-values, i.e. high probabilities that the two samples do not originate from populations with identical allele frequency distributions, are obtained in the case of MDH-D for the pairs 3–5, 3–6, 3–7, 4–6, 5–8, 5–9.

Table 1. - Results of heterogeneity test calculated for all pairs of seed samples for two selected gene loci, MDH-D and MDH-B (upper and lower diagonal half resp.): For MDH-D, the G-values are given. For MDH-B the exact a-values were calculated because of too small expected allele frequencies. In the case of MDH-D, the alleles 1 and 3 were pooled and for MDH-B the alleles 2 and 3. Data refer to the combined female and male allele frequencies.

<table>
<thead>
<tr>
<th>Seed sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>12.74 **</td>
<td>0.36 ns</td>
<td>2.54 ns</td>
<td>62.21 **</td>
<td>0.42 ns</td>
<td>16.90 ***</td>
<td>7.91 *</td>
<td>0.78 ns</td>
</tr>
<tr>
<td>2</td>
<td>0.751 ns</td>
<td>-</td>
<td>12.40 **</td>
<td>4.09 ns</td>
<td>24.64 **</td>
<td>8.80 *</td>
<td>1.78 ns</td>
<td>18.95 ***</td>
<td>7.81 *</td>
</tr>
<tr>
<td>3</td>
<td>0.108 ns</td>
<td>0.028 *</td>
<td>-</td>
<td>2.27 ns</td>
<td>65.05 ***</td>
<td>0.90 ns</td>
<td>14.98 ***</td>
<td>11.48 **</td>
<td>0.55 ns</td>
</tr>
<tr>
<td>4</td>
<td>0.324 ns</td>
<td>0.113 ns</td>
<td>0.682 ns</td>
<td>-</td>
<td>44.69 **</td>
<td>1.15 ns</td>
<td>6.51 *</td>
<td>11.25 **</td>
<td>0.60 ns</td>
</tr>
<tr>
<td>5</td>
<td>0.122 ns</td>
<td>0.372 ns</td>
<td>0.001 ***</td>
<td>0.006 **</td>
<td>-</td>
<td>52.77 ***</td>
<td>32.32 ***</td>
<td>48.07 ***</td>
<td>54.26 ***</td>
</tr>
<tr>
<td>6</td>
<td>0.030 *</td>
<td>0.123 ns</td>
<td>0.001 ***</td>
<td>0.001 ***</td>
<td>1.000 ns</td>
<td>-</td>
<td>13.08 **</td>
<td>6.91 *</td>
<td>0.36 ns</td>
</tr>
<tr>
<td>7</td>
<td>0.030 *</td>
<td>0.123 ns</td>
<td>0.001 ***</td>
<td>0.001 ***</td>
<td>1.000 ns</td>
<td>1.000 ns</td>
<td>-</td>
<td>29.83 **</td>
<td>10.51 **</td>
</tr>
<tr>
<td>8</td>
<td>0.447 ns</td>
<td>0.173 ns</td>
<td>0.531 ns</td>
<td>1.000 ns</td>
<td>0.011 *</td>
<td>0.002 **</td>
<td>0.000 **</td>
<td>-</td>
<td>10.33 **</td>
</tr>
<tr>
<td>9</td>
<td>0.285 ns</td>
<td>0.686 ns</td>
<td>0.004 **</td>
<td>0.021 *</td>
<td>1.000 ns</td>
<td>0.500 ns</td>
<td>0.500 ns</td>
<td>0.036 *</td>
<td>-</td>
</tr>
</tbody>
</table>

* 0.05 > P ≥ 0.01; ** 0.01 > P ≥ 0.001; *** P < 0.001; ns = not significant
4—7. Analogously, in the case of MDH-D, outstanding high G-values are apparent for all combinations which include sample 5 and to a lesser degree also sample 8. The opposite holds particularly for the pairs 4—8, 5—6, 5—7, 6—7 for the first locus and for 1—3, 1—6, 4—9, and 6—9 for the second. This indicates trends in favor of single samples (e.g. sample 3 or 9), but there is no general tendency observable within seed samples from seed orchards, within forest stands, or within certain provenance regions.

Summarizing results of all studied gene loci, among the pairs of samples which can be statistically discriminated simultaneously on the basis of six or even seven loci, the samples 3, 5, 7, and 8 appear more often than the others. These samples all originate from seed orchards. The samples 2, 6, and 9 rarely appear in this category but are included more frequently among those pairs which can be discriminated on the basis of two or fewer loci. They all originate from forest stands. However, these findings merely indicate a tendency and do not yet permit general interpretation in terms of a systematic discrimination between samples from forest stands and those from seed orchards.

The results of statistical testing would provide more information if the ranking of the G-values could be used in order to quantify deviations between the respective frequency distributions and thus to indicate gene loci which are particularly effective or ineffective in revealing such deviations. Unfortunately, appropriate multivariate analysis to solve this problem does not seem to exist.

**Genetic distances**

The genetic distance was used to quantify the heterogeneity between two frequency distributions of alleles on a continuous scale: Values of zero indicate two identical samples, while values equaling one signify the absence of common alleles in the respective samples. In Table 2, the genetic distances are given for the same gene loci and the same combined allele frequencies as in Table 1.

On the average, the genetic distances obtained for the gene locus MDH-D are much higher than those for MDH-B. As in the case of the G-values in Table 1, comparatively high values are obtained for the locus MDH-B in the case of the pairs 3—6, 3—7, and for MDH-D again for all combinations which include sample 5. Minimum values are evident for MDH-B for the pairs 5—6, 5—7, 5—9, 6—7, and for MDH-D in the case of the pairs 1—3, 2—7, 5—9.

There is once again no clear indication of trends visible only in seed samples from forest stands as compared to those from seed orchards. The results obtained for MDH-D suggest the more general statement that sample 5 is genetically different from the remainder.

The given genetic distances quantitatively confirm the tendencies demonstrated by the set of G- or a-values in Table 1. It is obvious that the distance values are much greater for MDH-B than for MDH-D. Here and also in the case of other loci, this trend is less apparent in the results of the statistical tests. The reason for such a phenomenon could be the dependency of test statistics on the manner in which the single samples are distributed over the frequency classes, while the applied distance measure is entirely independent of this. Both loci, MDH-B and MDH-D, reflect a considerable number of statistically significant deviations (15 and 24 resp.) even though the genetic distances indicate a much higher level of genetic heterogeneity for MDH-D than for MDH-B.

**Genetic differentiation among seed samples**

The measure of Gregorius and Robers (1988) for subpopulation differentiation was utilized to describe genetic differentiation among the studied samples as a whole instead of in pairs. Like the calculated genetic distances, this measure is based on the frequency distribution of alleles or genotypes, but here the frequencies observed in one sample are contrasted with the weighted averages of the frequencies among the remaining samples. Hence each seed sample is considered to be a subpopulation, and differentiation is measured by the genetic distance between it and the other eight samples, which are combined to form the respective complement population.

In Figure 3, the genetic differentiation among all seed samples is based on the combined female and male allele frequencies and plotted for all ten gene loci as well as for the gene pool, i.e. the weighted average of these loci. In each graph, the radius of the dotted circle is equal to the average level of differentiation at that particular locus. The given scale measures the average proportion of alleles in which any sample differs from the remainder. For instance, a radius of the value 0.2 indicates that each seed sample differs from the lumped remainder on the average by 20% of its alleles. Consequently, the larger the radius of the dotted circle, the more effective the respective locus is in reflecting genetic differentiation.

As can be seen in Figure 3, the gene locus GOT-A, and to some extent the loci MDH-A, MDH-B, and SKDH-B, are not suitable for revealing differentiation among the
samples. The opposite holds particularly for MDH-D and also for GOT-B, GOT-C, and SKDH-A. It is self-evident that the latter category of gene loci contributes to a much greater extent to the sample differentiation in the gene pool graph than the others.

Analogously, for each graph the radii of the sample-specific solid sectors are equal to the proportion of alleles in which a sample differs from the lumped remainder at the respective gene locus. The sample with the largest radius and thus the greatest amount of differentiation between it and the remainder is always placed at the top of the respective graph, followed by the other samples in the sequence of decreasing radii. Some of the loci deviate markedly in the differentiation between single samples and the respective remainder, as is the case, for instance, with samples 5 and 2 at GOT-C.

The relative sizes of the samples are represented by the angles of the sectors; they do not differ here due to the constancy of the sample size.

The variation between the radii of the nine sectors within each graph, which appears as steepness of the snail-like figures, is proportional to the differentiation among the respective samples at that particular locus. For instance, LAP-B indicates a much greater differentiation of sample 8 as compared to samples 5 and 3 than does GOT-B or MDH-A. Graphs which reveal an outstandingly great differentiation, as evident especially in the case of MDH-D with respect to sample 5, facilitate determination of those loci which can be used for optimal differentiation of single samples in contrast to the remainder. Generally, the closer the sector radii approach the center, the more representative of the remainder is the genetic information of the re-

Figure 3. — Genetic differentiation among nine seed samples at the level of the combined female and male allele frequencies for ten gene loci and their entire gene pool. Probes 2, 3, 4, 5, 7, 8 originate from seed orchards, probes 1, 2, 6, 9 from forest stands.
spective samples and the smaller is the chance for a reliable discrimination of those samples from the remaining.

Viewing the differentiation among samples within the single locus graphs, an above-average differentiation is indicated especially for sample 5 at MDH-D and sample 8 at LAP-B, but also for instance for sample 8 at GOT-B, sample 3 and /or 5 at GOT-B, C, MDH-B, C, SKDH-A, B, sample 9 at MDH-C.

In the ten-locus gene pool graph, samples 3, 5, and 8 show an above-average differentiation due to their predo-
minance in differentiation at several loci. These results make it clear that, due to the pronounced differentiation with respect to these samples, a reliable discrimination be-
tween them and the remainder can be expected. Contrary to this, particularly the sectors of samples 1 and 6 are locat-
ed relatively close to the center of the gene pool figure, so that these samples share the largest part of the genetic in-
formation with the remainder and thus are difficult to dis-
criminate from the others. This concerns the 10-locus level and not necessarily the differentiation at the single locus level. For instance, sample 1 shows average or slightly above-average differentiation at MDH-A, D and sample 6 at MDH-B and SKDH-A.

The gene pool graph reveals a remarkable phenomenon: All samples from seed orchards (No. 3, 4, 5, 7, 8) are more differentiated than the samples from stands, which means that they contain a smaller part of the common genetic in-
formation of the respective complement of Scots pine seed samples.

Conclusions

In this study, the difficulty lay in the fact that genetic differentiation was considered among samples from sexual-
ly reproducing populations rather than from small, well-
developed mixtures of vegetatively propagated cultivars. The chances of finding qualitative differences due to the exist-
ence of sample-specific unique alleles were low, since the majority of known alleles in Scots pine is present in each sample: At the studied ten enzyme loci, at total of 43 genes was found in this and other studies by the author in Scots pine populations in this country. The total number of al-
leles in the nine seed samples varied between 53 and 70% of this value. In the rare case that a unique allele had been present, an ad hoc discrimination could not have been made, because the extremely small frequency of such unique single or multilocus types would have required multi-
plies of the actual sample sizes.

The investigation of quantitative differences was focused on the frequency distributions of alleles instead of ge-
notypes in order to increase the frequencies of the types in the samples to be compared. Also, allele frequencies de-
pend to a much lesser extent on characteristics of the repro-
ductive system than the genotypic frequencies and thus allow a better representation of the parental population. The alleles contributed by the seed parents depend strongly on the mode of cone collection, i.e. on the method of selection and the number of harvested trees, while the alleles con-
tributed by the pollen parents are better suited for a cha-
racterization of the parental populations. Moreover, pollen contributions may also provide biased information, since pollen may have immigrated from neighbouring populations. In the present study both frequencies were combined; the more sample-specific information as reflected by the fe-
male frequencies is utilized to a lesser degree in favour of a better representation of frequencies of allelic types.

In spite of these limitations, considerable genetic differ-
ences were revealed between the seed samples. The relative-
ly small number of ten enzyme gene loci enables the dis-
crimination of nearly all pairs of samples by statistical test procedures. This also includes the pair of samples 3—4 (e.g. MDH-C), which, even though they originate from two different seed orchards, are nevertheless genetically very closely related, since the majority of the parental clones are identical.

The calculated genetic distances reflect similar tenden-
cies, but in addition they clearly indicate that one of the two gene loci under consideration reveals much greater ge-
netic differences between pairs of samples than the other. It appears that statistical testing alone may not suffice for a reliable interpretation of data, because of its depend-
ency on the manner of distribution of the types over the frequency classes. Contrary to the applied test statistics, the continuous measure of genetic distance allows a listing of pairs of samples in a hierarchical sequence on a scale between one and zero.

The applied measurement of subpopulation differentia-
tion permits a contrasting of any samples at any single locus with the complement population, i.e. the respective remaining seed probes. The graphs both illustrate and quantify the obtained differentiation. The graphs also al-
low the systematic selection of those loci which reflect the maximum level of differentiation with respect to certain seed samples of interest. The efficiency of differentiation can be increased, if the graphs are utilized to select com-
binations of loci in order to optimally characterize sam-

da by multilocus types with respect to alleles or gene-
types. This requires larger sample sizes than those which were available for the present study. It is self-evident that the graphs also clearly indicate those loci which reflect the largest variation in the levels of differentiation among the samples.

It was the intention of the present paper to propose and to apply suitable methods for studying differentiation among seed samples. Due to the limited sample size, the full information potential of the presented methods could not be used. Nevertheless, this still resulted in a quite ef-

effective genetic characterization of the seed samples. Con-

sidering the samples originating from the two population types, namely forest stands and breeding populations, i.e. seed orchards, the following trend is indicated: All samples from forest stands show a lesser level of differentiation in the gene pool graph than any sample from seed orchards in accordance with the expectation that those samples contain more of the actual common genetic information. This can tentatively be explained by differences in the effective population sizes, which amount to many hundreds or more trees in the first category and to not more than one hun-
dred different clonal genotypes in the latter, and possibly also by the mode of selection of the required clones.

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sche Forschungsgemeinschaft, Bad Godesberg.
Narrow-Crowned Variants of Mulanje Cedar (Widdringtonia nodiflora Powrie) in Malawi

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Summary

Narrow-crowned, thin-branched, and thin-barked variants of this indigenous fine-timber tree are reported. The probable genetic basis of these variants is discussed and their potential practical value in indigenous silviculture and arboriculture is indicated.

Key words: Widdringtonia, Mulanje Cedar, Crown form.

Zusammenfassung

Es wird über schmalkrone, fein-astige und dünnborkige Varianten der in Malawi einheimischen Werholzbaumart Widdringtonia nodiflora Powrie berichtet. Die wahrscheinliche genetische Basis dieser Varianten wird diskutiert und auf ihren potentiellen praktischen Wert für den heimischen Waldbau und die Züchtung hingewiesen.

Introduction

Mulanje Cedar (Widdringtonia nodiflora (L.) Powrie syn. W. wygelti Rendle), the National Tree of Malawi, is one of five species of native African Cypresses taxonomically close to the Australian Callitris and the monotypic N. African genus Tetracanthis (Chapman, 1961). This species was first reported by Alexander Wygottt in 1891 from Mt. Mulanje in erstwhile Nyasaland (now Malawi) whence its common first name. It yields a durable fragrant timber much like that of the true Cedars (Cedrus spp.) and hence the second name. Departmental exploitation of natural forests of this Cedar on Mt. Mulanje by the colonial government for timber began around 1900 and continued for about 50 years. Simultaneously plantations of it were also raised first on Zomba Mt. 70 km north of Mulanje Mt. and subsequently elsewhere in the country.

Material

During my very first tour of the Zomba Mt. Widdringtonia plantations on 21st August, 1985, I noticed a few narrow-crowned, heavy branched typical Cedar trees in a 1907 plantation in Compt. 34F. In their crown shape, stem form and bark colour, these offtype trees were so strikingly different from the even-aged typical neighbouring trees that not till twigs bearing seed cones could be collected by tree climbers from their crowns could I be certain that they too were Mulanje Cedar but of a different kind.

No records exist as to the seed source used for raising this 79-year old plantation but it is very likely that the seed would have come from the Thuchila plateau on Mt. Mulanje where departmental extraction in Cedar forests was in progress at that time. As far as can be ascertained from the compartment register one thinning was done in this stand in 1962. At present there are approximately 300 Cedar trees standing in this 0.12 ha plot out of which about a dozen are atypical narrow-crowned variants.