WITCHES’ BROOM OF ALEPO PINE (*PINUS HALEPENSIS* MILL.) AND ITS USE FOR NEW ORNAMENTALS

Petar Vrgoc
Sv. Anton
51511 Malinska
Croatia
petar.vrgoc@ri.hinet.hr

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**Abstract**

The aim of this research was to select new cultivars among *Pinus halepensis* Mill. Witches’ Broom progenies. The most compact Witches’ Broom-3 had the smallest shoots, cones and seeds. Witches’ Broom-1 was slightly less compact with slightly larger shoots, cones and seeds. Witches’ Broom-2 was the least compact with larger shoots, cones and seeds. Significant differences (p ≤ 0.01) were found for the number of shoots per node, shoot length, needle length, length and width of cones and length and width of seeds between the three different Witches’ Brooms and between the Witches’ Brooms and normal crowns. The denser the Witches’ Broom crown, the denser and shorter were the shoot lengths and lengths and widths of its cones and seeds, while the number of shoots per node was larger. As a result of Cluster analyses and graphical analyses of cluster means of 3 clustering variables, it was determined that height, internode length and number of branches per 1-cm were quantitative characters of Witches’ Brooms and normal progenies. As the Witches’ Brooms only have female flowers, pollination occurred by the normal crown of the same tree or neighbouring trees. The inheritance from Witches’ Broom has gone into segregation resulting in smaller heights and smaller internode lengths than the same characteristics obtained by segregation of normal progeny originating from the normal crown of the same tree. Cluster analyses appeared to be very suitable for cluster recognition among Witches’ Broom or normal progeny for the purpose of quantitative inheritance confirmation. Among Witches’ Brooms progenies 12 plants were selected of different shapes: *globosus*, *procumbens*, *pyramidalis*, *ovalis* and *divaricatus*.

**1. Introduction**

Witches’ Brooms were noticed on Aleppo pine (*Pinus halepensis* Mill.), Austrian pine (*Pinus nigra* Arnol) and Maritime pine (*Pinus pinaster* Ait.) in 1994. We investigated the Witches’ Brooms on Aleppo pines on three trees with one Witches’ Broom in each of their crowns. The trees were found along the Adriatic Coast in Croatia. One of the trees was found on the island of Hvar (Witches’ Broom-1) and the second close to the city of Crikvenica (Witches’ Broom-2). Witches’ Broom-3 was found on the island of Rab.

When looked at from a distance the Witches’ Broom resembles a small part of the crown of a tree which appears denser, is kidney-shaped or round in form, and looks greener than the normal part of the crown. When we come sufficiently close we can see that it is in fact only one branch which branches out, forming its own crown. Only the female flowers regularly flower in the Witches’ Brooms, while the normal parts of the same trees have both male and female flowers.

Momose (1967) reported on Witches’ Brooms on Japanese Red pine (*Pinus densiflora*) and announced that the progeny cultivated from the collected seed of grafted...
Witches’ Brooms showed a variation of phenotypes, by segregation of characteristics in 19 normal plants, 10 Witches’ Brooms and 1 abnormal small plant. The author also explained that these Witches’ Brooms are of a genetic (hereditary) and not parasitic nature.

Johnson et al. (1968) reported on the generative progenies of Witches’ Brooms on Pinus banksiana, P. strobus, P. resinosa and P. clausa and reported statistically significant differences in height between the progenies of Witches’ Brooms and normal trees and also differences between the progenies of different Witches’ Brooms. This prompted the idea that generative progenies of Witches’ Brooms, from Aleppo pines, might have variable phenotypes, among which it would be possible to select desirable ones.


Grasso (1969) was the first to give a report on Witches’ Broom on Aleppo pine. He observed this phenomena near Bari (Italy) and studied the generative progeny from seeds collected in its crown and from the normal part of the tree. He noticed variability in the progenies of Witches’ Broom and reported on the genetic nature of this phenomenon.

The aim of this study was to analyse the possibility of using Witches’ Brooms for breeding Aleppo pine. As Witches’ Brooms have no male flowers, the genetic variability of Witches’ Brooms progenies was expected to originate from a cross between normal Aleppo pines and the Witches’ Brooms.

2. Materials and methods

2.1 Plant material

Shoots and ripe cones were collected from the normal parts of the crowns of three Aleppo pine trees and from the Witches’ Brooms growing on them. Twenty branch tips and ten cones, randomly selected from each crown, were used as the standard.

All together 3383 seeds (from the normal parts of the crowns and from the Witches’ Brooms) were X-rayed. An identification seed number was recorded for each seed and was the same as the identification number for the sapling and older seedling. In this way monitoring of plant origin was enabled, with regard to the cone and seed from which the plants originated. X-ray photographs provided great help for separation of germinable seeds, measuring variables on the seeds and monitoring of plant origin.

The seeds from the Witches’ Broom-3 could not be evaluated as germinable and this sample was excluded from further research. Further research of seed samples encompassed 2551 seeds from tree-1 and from tree-2.

At the end of the first vegetation season 753 saplings were successfully cultivated in multi-pot containers. The aim of the cultivation was for the plants to develop as quickly as possible by transplanting them into larger pots before the start of the second year of cultivation.

In order to form samples of the appropriate size the following were retained in the experiment: every second plant from the normal crown from tree-1, thus forming a sample of 59 seedlings, every fifth seedling was retained from the normal crown from tree-2, and thus a sample of 68 seedlings was formed. All the plants grown from the seeds of the Witches’ Broom-1, 81 plants, and from Witches’ Broom-2, 160 plants, were retained in the experiment until the end of the second year of cultivation.
2.2. Variables

Number of shoots at node, length of shoots and length of needles, were measured together with, length and width of cones. The length and width of the seeds were measured on the seed X-ray photographs.

Upon germination the number and length of cotyledons were measured. At the end of the second year: height of plant, internode length, number of branches per 1-cm and length of needles was measured. Besides this a visual evaluation of the habitus of each plant was made.

2.3. Statistics

Variables were measured separately for each tree and separately for each part originating from Witches’ Broom and from the normal part of the crown. It was, therefore, possible to apply different statistical methods for the compartment of Witches’ Brooms and normal crowns as well as for the compartment of the progeny of the different groups.

To check the variables normality, skewness and kurtosis of the distributions were analysed according to Spiegel and Stephens (1999).

The MANOVA and Tukey-Test were performed for the compartment of the groups.

Cluster analyses of the variables and Factor analyses were performed in order to determine the most distinctive variables (the strongest factors) among two-year-old progenies.

Cluster analyses of progenies in the three most distinctive variables were also performed. The method of Cluster analysis used was graphic association into a “branching tree”. The model “Manhattan-distance” was found the most suitable with the method of pooling of “Weighted-pair-group-average”. The method of Cluster analyses explains Manly (1986).

The Mann-Whitney-U-Test and the Wald-Wolfowitz-Runs-Test were used for compartment of clusters among themselves, Spiegel and Stephens (1999).

3. Results

By a simple visual evaluation differences can be observed between Witches’ Brooms and normal crowns, and between Witches’ Brooms from different trees. The most compact Witches’ Broom-3 has the smallest shoots, cones and seeds. The Witches’ Broom-1 is slightly less compact and has slightly larger shoots, cones and seeds. The Witches’ Broom-2 is the least compact and has larger shoots, cones and seeds (Table 1).

Significant differences (p ≤ 0.01) were found for number of shoots per node, shoot length, needle length, length and width of cones and length and width of the seeds between three different Witches’ Brooms and between Witches’ Brooms and normal crowns. As the Witches’ Broom crown was denser, shoot length, length and width of its cones and seeds were shorter. Unexpectedly shoots of the normal crown-1 were shorter than Witches’ Broom-1 shoots. In addition, needle length of normal crown-1 was shorter than the needle length of Witches’ Broom-1. More seeds were found in larger cones.

Analysis of the seed X-ray photographs showed that none of the seeds from the Witches’ Broom-3 could be evaluated as germinable (except a few seeds that germinated and died in the test prior to this study). Witches’ Broom progenies were derived from the seeds of Witches’ Broom-1 and Witches’ Broom-2.

The saplings of Witches’ Brooms were smaller with fewer and smaller cotyledons. Among the progeny of the normal part of crown-1 and crown-2 were 6 to 9 cotyledons, while for the progeny of Witches’ Broom-1 it ranged from 5 to 7 and for Witches’ Broom-2 from 4 to 8.

Two-year-old progeny from the seeds of normal crowns grew into normal plants.

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Witches’ Brooms progeny could be divided into two visually recognisable groups, one with plants looking normal and one with dwarf plants. The term “plants looking normal” in this article describes plants which look normal but can not be accepted as normal without prior statistical analyse. Significant differences (p ≤ 0.01) were found in the height of normal plants, plants looking normal and dwarf plants from tree-1 and for analogous progeny groups from tree-2 (Table 2).

Variables used for the Cluster analysis were plant height (as the strongest factor), number of branches per 1-cm of crown height and internode length. Plants originating from each Witches’ Broom and from each of the normal crowns were analysed separately. Figure 1 and Figure 2 show arithmetic means of three clustering variables of Cluster analyses of the tree-1 progeny. Clusters from 1 to 4 are dwarf plants and clusters from 5 to 7 are plants from Witches’ Broom-1 seeds, whose growth looks normal. Normal progeny from the normal crown of tree-1 is arranged in clusters from 8 to 11. From the first to the last cluster, there is a gradual increase in height and internode length and gradual decline in crown density, as shown in the graphic presentation (Figures 1 and 2).

From Witches’ Broom-1 progeny four clusters of plants were separated with lower average height and internode length from the smallest average for the clusters of normal plants. At the same time, these four clusters of plants had a higher average number of branches per 1-cm than the highest average for this variable in clusters of normal plants. Thus, among the progeny of Witches’ Broom-1, four clusters of dwarf plants were selected with compact crowns and different density. Three such clusters of dwarf plants were selected by the same method for Witches’ Broom-2. The denser Witches’ Broom-1 gave lower and denser progeny.

Significant differences in plant height (p ≤ 0.05 or intensifier) were determined in all cases of comparison of neighbouring clusters originating from tree-1 and from tree-2, in the results of the Mann-Whitney-U-Test and the Wald-Wolfowitz-Runs-Test.

The gradual increase, from smaller to greater, from the first to the last cluster, indicates the quantitative heredity of height, crown density and uniformity of crown density in the progeny of Witches’ Brooms and the progeny of the normal parts of the crowns.

The frequencies of the height of normal progeny are normally distributed. The frequencies of the height of progeny of Witches’ Brooms are polymodally distributed, with the highest mode on the part of the progeny of dwarf growth, and several lower modes on the part of progeny which look normal (Figure 3). Consequently, the main factors of heredity are clearly visible, influencing the height and crown density of Witches’ Broom progenies.

Because of the absence of stamens the Witches’ Brooms are wind pollinated by the pollen from normal trees. Therefore the inherited dwarf growth comes from Witches’ Broom and expresses its strongest influence in the form of the highest mode. Another influence of normal growth comes from the normal crown and expresses its strongest influence in the form of the lowest mode. As plant height (dwarf or normal) comes in quantities, several modes appeared within the height distributions of Witches’ Broom progenies (Figure 3).

Twelve candidates for new cultivars were selected from the progeny of the two Witches’ Brooms in the third year of cultivation. Among these selected plants different forms were present: recumbent (procumbens), pyramidal (pyramidalis), oval (ovalis), global (globosus) and irregularly branched form (divaricatus).

4. Discussion

Duffield and Wheat (1963), Fordham (1967), Grasso (1969), Johnson et al. (1965 and 1968), Panetsos (1981) and Waxman (1975) investigated Witches’ Brooms on conifers and their findings and results were the starting point for this study. During the first visual analyses of Witches’ Brooms on Aleppo pines, indications were found that these Witches’ Brooms were the products of somatic bud mutation. The above authors
described Witches’ Brooms on conifers, also caused by somatic bud mutation. They confirmed the genetic character of the Witches’ Brooms by the following findings: the occurrence of only one Witches’ Broom on each tree, the presence of female and absence of male flowers, and cones and seeds which are smaller than in the normal part of the tree. Among their progeny there were markedly dwarf plants. These authors visually evaluated one part of the plants in the progeny of Witches’ Brooms as normal and the other part as abnormal.

One part of the plants from the seeds of Witches’ Broom-1 and 2 was referred, in this article, as plants looking normal. By statistical comparison of the characteristics of normal plants and plants which looked normal it was determined that the plants which looked normal in height were intermediary to normal plants and dwarf plants. The average lower height of plants that looked normal, with a similar number of branches as the normal plants, resulted in a denser crown in plants evaluated as looking normal. Thus, it was demonstrated that regarding plants which looked normal as normal plants, without statistical comparison, is not justifiable. However, a more detailed insight into the mechanism of heredity of characteristics from Witches’ Broom was still not achieved.

By Cluster analysis of progenies of the two Witches’ Brooms, in three variables which express height and crown density, clusters of plants were differentiated. For these clusters arithmetic means were analysed, and according to the formed clusters photographs of the plants were classified in analogous groups. This method provided a completely new insight into the quantitative heredity of the plant height, length of internodes and crown density among the progenies of two Witches’ Brooms. Candidates for the new cultivars were selected from the clusters separated by Cluster analysis.

The hypothesis that the Witches’ Brooms are of genetic character and that their morphological characteristics are transferred by generative means to progeny was confirmed. The Witches’ Brooms are a suitable source for the cultivation of progeny among which candidates for new cultivars can be selected.

Witches’ Brooms (and normal parts of crowns) and their progenies represent excellent material for research of inheritance from the male or female parent. Further research with deployment of gene markers and other molecular tools would be another challenge that may offer opportunity for broader understanding of the occurrence of Witches’ Brooms. Search for the cause or mechanism of spontaneous Witches’ Broom formation and the discovery of an artificial way to produce them could provide a new and original approach to breeding.

References
Table 1. Means of characteristics of shoots, cones and seeds from Witches’ Brooms and normal parts of Alepo pine trees

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tree - 1</th>
<th>Tree - 2</th>
<th>Tree - 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal part of tree N = 20</td>
<td>Witches’ Broom N = 20</td>
<td>Normal part of tree N = 20</td>
</tr>
<tr>
<td>Number of shoots at node</td>
<td>mean 1.0</td>
<td>3.0</td>
<td>3.0</td>
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<td></td>
<td>St. dev. 0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Length of shoot (mm)</td>
<td>mean 16</td>
<td>50</td>
<td>82</td>
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<td></td>
<td>St. dev. 3.05</td>
<td>14.44</td>
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<td>Length of needle (mm)</td>
<td>mean 63</td>
<td>78</td>
<td>85</td>
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<td></td>
<td>St. dev. 6.58</td>
<td>12.83</td>
<td>10.06</td>
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<td>Length of cone (mm)</td>
<td>mean 55</td>
<td>34</td>
<td>70</td>
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<td></td>
<td>St. dev. 7.62</td>
<td>2.98</td>
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<td>Width of cone (mm)</td>
<td>mean 32</td>
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<td>32</td>
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<td></td>
<td>St. dev. 2.83</td>
<td>0.67</td>
<td>2.00</td>
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<td>Length of seed (mm)</td>
<td>mean 5.6</td>
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<td>6.4</td>
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<td></td>
<td>St. dev. 0.42</td>
<td>0.48</td>
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<td>Width of seed (mm)</td>
<td>mean 3.0</td>
<td>2.2</td>
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<td></td>
<td>St. dev. 0.25</td>
<td>0.26</td>
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Table 2. Means of characteristics of plant height and crown density of 2-year-old Witches’ Broom and normal progeny of Alepo pine

<table>
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<tr>
<th>Variables</th>
<th>Tree - 1</th>
<th>Tree - 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal progeny N = 59</td>
<td>Witches’ Broom progeny Plants looking normal N = 68</td>
</tr>
<tr>
<td></td>
<td>Plants looking normal N = 36</td>
<td>Dwarf plants N = 45</td>
</tr>
<tr>
<td>Height (mm)</td>
<td>mean 497</td>
<td>424</td>
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<tr>
<td></td>
<td>St. dev. 88.19</td>
<td>94.83</td>
</tr>
<tr>
<td>Internode length (mm)</td>
<td>mean 115</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>St. dev. 76.37</td>
<td>79.82</td>
</tr>
<tr>
<td>Number of branches per 1-cm</td>
<td>mean 0.8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>St. dev. 0.27</td>
<td>0.29</td>
</tr>
<tr>
<td>Length of needles (mm)</td>
<td>mean 49</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>St. dev. 25.67</td>
<td>26.24</td>
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</table>
Figure 1. Plant height and internode length among clusters of two-year-old progeny of Witches’ Broom-1 (black) and normal part of the same tree (grey). Standard deviations are added above each bar.

Figure 2. Number of branches per 1-cm of plant height among clusters of two-year-old progeny of Witches’ Broom-1 and normal part of the same tree. Standard deviations are added above each bar.

Figure 3. Unimodal height distributions (from the seeds of normal crowns) and polymodal height distributions (from the seeds of Witches’ Brooms) of two-year-old progenies.