Use of Peroxidase Taxonomical Values in Determination of Hazel Genotypes

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Abstract: Hazel's shrubs are distributed in the north of Iran. No genetically, study has been done on coylus in order to determine genetically segregation in natural stands. The aim of this study was to determine the relationship between morphological characters of leaves and involucres with peroxidase enzymatic bands. Two natural habitats of hazels were chosen in Makash and Fandangos in the north of Iran and the shape and size of leaves and involucres were determined in 23 hazels. In addition, the qualitative alteration of peroxidase was determined in shoots in summer. Results indicated that hazels are grouped in 13 different genetically classes. Use of the taxonomic similarity coefficient (Spi) and taxonomic distances (D) drew the cladogram. Then, morphological alteration was conformed to their location on a cladogram. Results indicated that diversity of leaves shape and involucres had conformity with their location on the cladogram. In conclusion, observed morphological alterations have genetically origin.

Key words: Hazel • Involucres • Leaf • Genetically segregation • Proxidase

INTRODUCTION

Corylus is native to Europe, Minor Asia and Caucasian [1]. Although from many years ago, it grows more in the northern hemisphere in Japan, China, Iran and Turkey [2, 3]. In the Ice Age (17000-18000 BC), Corylus was one of the first species that moved to the north of Europe. Hazel had very vast distribution in Europe in the period 7500 to 8000 BC. Then its distribution decreased in 5500 BC because of human activities [4]. There are three species of Corylus in Iran that are C. avellana and C. maxima which are distributed in the north of Iran and C. pontica which is distributed in the centre and the west of Iran [5].

Hazel stands show high variability in northern habitats. The objective of this study was determination of genetically segregation in Corylus species. Genetically segregation is one the factors which is critical in establishment of plants in nature [6-9]. Enzymatic analysis is one the methods for genetically segregation. Enzymatic studies have been used in forest trees since 1971 [4]. Different enzyme has been used in genetically segregation such as glutamate oxalate transferase, isocitrate dehydrogenase, menadione reductase, phospho glucomutase, phospho glucosomerase, shikimate dehydrogenase (10) and peroxidase (Korori, 2002). Peroxidase has a special importance. It is very suitable for genetically segregation studies because it has many isoenzymatic bands. Many times peroxidase has been used for isolation and identification of inter species and intra species alteration in ferns [9] and broad leaves [8, 10, 11]. Mrzakowa [9] improved application of enzymes in taxonomy of plants and relations among different species by use of peroxidase. In a cladogram time is not important. By use of cladograms there will be a network of dandrogams, which as a priority and coming next, ancestor and interspecies unities are not concerned [3].

MATERIALS AND METHODS

Study Area: Sampling was done from two natural habitats of Corylus in Fandogloog and Makesh habitats.

- Fandogloog with height of 1400 m above sea level was located on Aria hills next to Fandiglo village in Ardabile province on the route from Aradabil to Astara. Its climate according to Dumbarton system is very humid and ultra cold. This area has three dry months in year. Surface soil texture is loam-silt with 6.8 acidity.
Makesh habitat with height of 1400-1500 m above sea level was located in Talesh in 30 Km in south of city between Makesh and Agolar villages. Its climate according to Dumbarton system is very humid. There is no dry season in this area. Soil has loam texture with 6.9 acidity. 23 trees including 8 samples on Fandogloo (F1-F8) and 15 samples on Makesh (M1-M15, MB indicates individuals that were on downside of main road) habitats were selected.

Methods: The shape and size of leaves and involucres (± 001 m): It was determined if the size of involucres is more or less that of the ripened fruit size. Peroxidase enzymatic assay was done on shoot samples. Enzymes were extracted with Tris-Ascorbic and then analyzed by use of acryl amide (pH=8.6) and Tis-Glycine electrolyte in a vertical electrophoresis [11-13]. Gels were stained with 2% benzidine (7). After staining, each individual was identified according to its isoenzymatic bands.

Analysis: Distance of each band was measured from the loading point and coded. Measured codes were used in determination of the taxonomic similarity coefficient (sphi) according to this formula:

$$sphi = \frac{(a \times d) - (b - c)}{\sqrt{(a + b)(c + d)(a + c)(b + d)}}$$

In this formula, a shows number of isoenzymatic bands in two samples. B and C indicate the number of bands, which are present in one sample and absent in another. D shows the number of bands which are absent in both of samples [9]. Taxonomic distances (D) is measured based on taxonomic similarity coefficient (sphi) according to this formula: $D = \sqrt{1 - sphi}$. Cladogram was drawn based on taxonomic distances values [9].

RESULTS

Involucres dimension assayed in relation to ripened fruits. Measurements showed that involucres dimension shows a great diversity. Their size varied from half to two times of fruit dimensions. There was a great diversity in leaf morphology. Some of them had same length and width and there was spherical while others had pointed morphology and their length was two time of width. Figure 1 shows the isoenzymatic bands of peroxidase in hazel. It seems there were thirteen isoenzymatic patterns in assayed trees. According to these patterns, hazels were grouped in thirteen genetically classes (Table 1).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Individuals</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>M0-M2-F2</td>
</tr>
<tr>
<td>2</td>
<td>F3</td>
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<tr>
<td>3</td>
<td>M1</td>
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<td>4</td>
<td>MB1-MB5-M10-M1</td>
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<td>5</td>
<td>F4, F5</td>
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<tr>
<td>6</td>
<td>MB2-MB2-F4</td>
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<tr>
<td>7</td>
<td>MB3</td>
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<tr>
<td>8</td>
<td>M6</td>
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<tr>
<td>9</td>
<td>MB0</td>
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<td>10</td>
<td>M8</td>
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<td>MB-</td>
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<td>12</td>
<td>MB-</td>
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<tr>
<td>13</td>
<td>F5</td>
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</table>

(F= Fandogloo individuals, M= Makesh individuals, MB= individuals in the downside of main road in Makesh)

The first band was the fastest band and moved 85.75 mm. This band is specific of 13th and was observed in individuals number 5 in Fandogloo habitat. The second band moved 80.85 mm and observed in genotypes number 2,3,4,5,6,7,8,9,10,11,12 and 13. The third band moved 78.9 mm and observed in genotypes number 4, 8 and 13. The fourth band which moved 75.95 mm was observed in genotypes number 1,2,3,6,8,9,10, 11, 12, and 13. The fifth band moved 73.6 mm and observed in genotypes number 1-6, 11 and 12. The sixth band, which was the lightest band, moved 71 mm and was observed in all genotypes except to genotype number 7. The absence of this band in genotype number 7 was used as a critical factor in determination of this genotype. The band number 12 moved 44.1 mm and was observed in genotypes number of 1,2,4,5,6,8-13. The band number 13 with movement of
Fig. 2: The main axis drawn based on the Taxonomic distances (D) amount

Fig. 3: Drawing of the first minor axis according to Taxonomic distances (D) amount

\[
\begin{align*}
D_{12,10} &= 0/74 & D_{12,11} &= 0/75 \\
D_{6,12} &= 0/57 & D_{7,12} &= 0/58 \\
D_{7,10} &= 0/64 & D_{7,11} &= 0/61 \\
D_{6,10} &= 0/71 & D_{6,11} &= 0/56
\end{align*}
\]

Fig. 4: Drawing of the second minor axis according to Taxonomic distances (D) amount

39.2 was considered as a general band and was observed in all genotypes. In addition, band number 14 with movement of 37.75 was a general band. The band number 15, 16 and bloke of number 17 was regarded as heavy bands because of a little movement. The band number 15 with movement of 24.5 mm was considered as a general band and was observed in all individuals. The band number 16 with movement of 19.6 mm was observed only in genotypes number of 1 and 2. The band-bloke number of 17 was considered as a general band-bloke and moved 7.35 mm.

Fig. 5: Leaf and involucres dimension, the morphology of genotype number 1 which is seen in M9, M9 and F2 individuals

Fig. 6: Leaf and involucres dimension, the morphology of genotype number 2 which is seen F13 individuals

Taxonomic similarity coefficient (sphi) and taxonomic distances (D) were measured for all of individuals. At first two individuals that had the most taxonomic distances were put in two extremities of horizontal axis. Genotype number of 1 and 13 with D=1 had the most taxonomic distances. The least taxonomic distance was 0.47 that belonged to genotype number of 2. Then this genotype settled between genotype number of 1 and 13 in near to genotype number of 1. Results indicated no individuals in near to genotype number of 13. The least D for genotype of number 13 was 0.63, which belonged to genotype of number 6. The distance between genotype of number 1 and 13 from genotype of number 6 was the same. Then this genotype settled between them. Due to extreme distance of genotype number of 13 with other genotypes, it was removed from main axis and put on a alternative axis. The branching position is on genotype of number 1 position is or very closed to its position. According to D, the nearest genotype to number 6 is number 5. Comparison of the amount of D1-5=0.65 and D3-5=0.75 indicated that genotype of number 5 settles between number 6 and 1 and near to number 1. The position of other genotypes was determined on the axis. What must be pointed out is that the amount of D is not absolute and can be used for a specific genotype. The amount of D is estimated by use of different peroxidase bands. This is shown in Figure 2. The second branching axis locates between position of genotypes number 6 and 7. Kinds of branching and related information are given in Figure 3. Final cladogram is shown in Figure 4.

DISCUSSION AND CONCLUSION

There are many methods to classify trees Recently Enzymes are used in tree classification. Salehi et al. [14] used peroxidase for beech stem form classification. In this study the hazels individuals are classified by use of peroxidase isoenzymatic bands.
Fig. 7: Leaf and involucres dimension, the morphology of genotype number 3 which is seen in M3 and M4 individuals.

Fig. 8: Leaf and involucres dimension, the morphology of genotype number 4 which is seen MB, MB, MB, and M individual.

Fig. 9: Leaf and involucres dimension, the morphology of genotype number 5 which is seen in F1 and F3 individuals.

Fig. 10: Leaf and involucres dimension, the morphology of genotype number 6 which is seen BM1, BM12, and F1 individuals.

Fig. 11: Leaf and involucres dimension, the morphology of genotype number 7 which is seen in BM2 individuals.

Fig. 12: Leaf and involucres dimension, the morphology of genotype number 8, which is seen M1 individual.

Fig. 13: Leaf and involucres dimension, the morphology of genotype number 9 which is seen in MB5, MB, and MB9 individuals.

Fig. 14: Leaf and involucres dimension, the morphology of genotype number 10 which is seen F6 individual.
The whole trend of genotype alteration is seen on the morphology of hazel fruit. In genotype of number 1 (including individuals of M9-M6-F2) and genotype of number 2 (including individual of F9) leaves are tall and pointed (Figures 5, 6). The morphology of leaves in genotype of number 3 is spherical. (Figure 7) but in genotype of number 4 (including individuals of MB6-M3), genotype of number 5 (including individuals of F1-F3), genotype of number 6 (including individuals of MB1-F4), genotype of number 7 (including individuals of MB2), genotype of number 8 (including individuals of M1) leaves are spherical and pointed (Figures 8, 9, 10, 11, 12). As shown in figures the length of involucres gradually decreases from genotype number 1 toward genotype number 8. Then in genotype number 1 & 2 involucres are taller than nut but in genotype number 7 and 8 involucres is shorter than nut (Figures 5-6, 11-12). This kind of morphological changes in shape of involucres is intensified in alternative axis in the genotype number 9 including MB5- MB7- MB9 (Figures 13- 15). In this genotype, even in immature nut the involucres are cleft. Maximum of this kind of alteration was seen in the genotype of number 13 in which complete involucres did not exist (Figure 16). Results of this study was a confirmation for Mrzakwawa studies [9] who used peroxidase isoenzymatic bands and related cladogram for showing relation among ferns individuals. In conclusion, drawing of cladogram by use of isoenzymatic bands facilitates the understanding of relation among Corylus stands.

REFERENCES

4. www.ars-grin.gov/ ars/ Pacwest/ Corvallis/ negr/ Corylus/CoryinFo.html.