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GENETICS AND PLANT BREEDING

Genetic Diversity in *Quercus leucotrichophora* Populations Through RAPD Markers

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ABSTRACT

Quercus leucotrichophora is an evergreen tree which is usually twisted and has wiry branches. Genetic diversity is an essential component for the effective implementation of a tree improvement program. Four populations collected from Jhungi Suket, Taradevi Shimla, Bhatwari-Taknor Range, Nohra-Shimla) of *Q. leucotrichophora* were studied using RAPD markers. Ten primers were screened out of which five showed polymorphism. Five primers were used to amplify the individuals from each population. In a multi population descriptive analysis, the total diversity (Ht) in the four populations of *Quercus leucotrichophora* ranged from 0.1620 to 0.3215 with the mean 0.2253. The within population gene diversity (Hs) ranged from 0.0928 to 0.2204 with the mean 0.1542. The among population differentiation (Gst) ranged from 0.0304 to 0.3177 with the mean 0.3155. The gene flow observed among the populations was 7.9677, which is greater than 1 indicating higher levels of gene flow in the studied populations. Highest similarity co-efficient of 0.1793 was observed between populations Jhungi Suket and Nohra Shimla. The highest percentage of polymorphic loci (89.06%) was found in the population from Jhungi Suket. RAPD markers indicated that the frequencies of the presence of these markers correlated with the result of UPGMA analysis. The genetic diversity of all the four populations from different regions were found to be low, so if the trees of one place is damaged by any means the trees of similar genetic diversity from other places could be planted. Genetic diversity also helps us to study the general characteristics of a species and also particular species can be free from diseases. In spite of the relatively short distances between populations three clearly distinct regional groups of populations could be identified. These findings have implications for decisions on *in-situ* and *ex-situ* genetic conservation and for forest management planning and practices.

Highlights

- Four populations collected from Jhungi Suket, Taradevi Shimla, Bhatwari-Taknor Range, Nohra-Shimla) of *Q. leucotrichophora* were studied using RAPD markers
- The genetic diversity of all the four populations from different regions were found to be low

Keywords: RAPD, Genetic diversity, *Quercus leucotricophora*

The geographic range and distribution of populations represent important factors in analyzing genetic diversity because they give an indication of probable movement of organisms between populations and subsequent gene flow. An estimate of the overall population size provides a measure of the potential genetic diversity within the population. Populations that are isolated with very low levels of interchange show high levels of genetic divergence (Hunter 2002). When these isolated populations are somehow lost then their specific genetic component is also lost along with them. Genetic diversity is essential for populations to adapt to the ever-changing environment. Variations can lead to certain individuals possessing certain unique characteristics that are suited to their environment. Genetic diversity is essential for the evolution of a species and for subsequently providing a broader base for reproduction.

Quercus leucotrichophora is an evergreen tree with

wiry and twisted branches. The focus of this study was on *Q. leucotrichophora* due to its numerous uses such as providing fodder for cattle, being used as firewood, etc.

RAPD markers are convenient in analyzing genetic diversity and have been extensively used in population diversity analysis (Saini *et al*. 2010).

MATERIALS AND METHODOLOGY

Plant Material

The samples for determining population diversity of *Quercus leucotrichophora* were collected from various regions of India. The leaf samples for Population 1 were collected from Jhungi Suket located in Himachal Pradesh. The samples for Population 2 were collected from Taradevi Shimla located in Himachal Pradesh. The samples for Population 3 were collected from Bhatwari-Taknor Range located in Uttarakhand. The samples for Population 4 were collected from Nohra-Shimla of Himachal Pradesh. The samples which were kept in polythene bags at -80°C were taken out from the deep freezer. For better results, the young and disease free leaves were cut and the veins were removed to facilitate easy crushing. The leaves were taken in a glass beaker and washed with tap water to remove the contamination and were dried by spreading them on top of a tissue paper. The samples were then used for DNA extraction.

DNA Isolation and PCR Amplification

Genomic DNA was extracted from the young leaves by using the protocol of Stange *et al.* (1998) with some modifications: Quantification of the extracted DNA samples was done with the help of bio-photometer.

Table 1: Final primers selected after PCR for *Quercus leucotrichophora*

Sl. No.	Primers Used	Base Sequences (5′-3′)
1	M-119	ATT GGG CGA T
2	M-156	GCC TGG TTG C
3	M-184	CAA ACG GCA C
4	M-198	GCA GGA CTG C
5	OPA-2	TGC CGA GCT G

At first, the bio-photometer was set for DNA measurement protocol. Based on the bio-photometer reading, the best replicate of each sample was selected and the required volume of ultrapure autoclaved water was calculated accordingly. Dilutions of the samples were prepared for RAPD analysis prior to amplification using PCR.

Data Analysis

The total genetic diversity and diversity within population was calculated using POPGENE Version 1.32 (Yeh, *et al.* 1999).

Here, $Ht = 1 - \Sigma\, p_i^{\,2}$

Where, p_i is the mean frequency of the i^{th} allele at a locus. The total genetic diversity (Ht) can be partitioned into the genetic diversity within populations (*Hs*) and the genetic diversity among populations (*Dst*).

Thus,

$$Ht = H_S + Dst$$

H_S is the weighted (by the population sample size) mean of the genetic diversity of each population.

Gst = the proportion of genetic diversity due to the among population component. Thus,

$$Gst = \frac{Dst}{Ht}$$

RESULTS

Multi-Population Descriptive Statistics

In a multi-population descriptive analysis, the number of alleles observed was 2.000 and the effective number of alleles ranged from 1.214 to 1.5907 with the mean of 1.348.

Table 2: Summary of Genetic Variation Statistics for all loci in *Quercus leucotrichophora*

Locus	Sample Size	na*	ne*	h*	I*
M-119	30	2.000	1.5907	0.3506	0.5266
M-156	30	2.000	1.2624	0.1678	0.3013
M-184	30	2.000	1.4350	0.2606	0.4114
M-198	30	2.000	1.2140	0.1661	0.2956
OPA-2	30	2.000	1.2372	0.1598	0.2743
Mean	30	2.000	1.3480	0.2209	0.3618
St Dev.		0.0000	0.1465	0.1861	

** na = Observed number of alleles; * h = Nei's (1973) gene diversity; * I = Shannon's Information index (Lewontin, 1972).*

The total diversity (Ht) in the four populations of *Quercus leucotrichophora* ranged from 0.1620 to 0.3215 with the mean 0.2253. The within population gene diversity (Hs) ranged from 0.0928 to 0.2204 with the mean 0.1542. The among-population differentiation (Gst) ranged from 0.0304 to 0.3177 with the mean 0.3155. The gene flow observed among the populations was 7.9677, which is greater than 1 indicating higher levels of gene flow in the populations studied.

Table 3: Nei's Analysis of Gene Diversity in Subdivided Populations

LOCUS	Ht	Hs	Gst	Nm
M-119	0.3215	0.2204	0.3177	2.3939
M-156	0.1658	0.1603	0.0304	16.7774
M-184	0.2345	0.1627	0.1954	7.5109
M-198	0.1945	0.1407	0.2158	4.1030
OPA-2	0.1620	0.0928	0.2062	9.0532
Mean	0.2253	0.1542	0.3155	7.9677
St. Dev	0.0220	0.0054		

Ht = Total Diversity; Hs = Within population Gene Diversity; Gst = Among Population Differentiation; Nm = Gene Flow.

SINGLE POPULATION DESCRIPTIVE ANALYSIS

Population 1

In a single descriptive analysis, the number of alleles observed in the population of Jhungi Suket located in Himachal Pradesh ranged from 1.6429 to 2.0000 with the mean 1.8906 and the effective number of alleles ranged from 1.0443 to 1.3574 with the mean 1.0443. The number of polymorphic loci was 57 and the percentage of polymorphic loci was 89.06%.

Table 4: Single Population Descriptive Analysis of Population 1

Locus	na*	ne*	h*
M-119	2.0000	1.3574	0.2422
M-156	1.9167	1.1687	0.1403
M-184	1.9167	1.2564	0.1930
M-198	2.0000	1.1619	0.3582
OPA-2	1.6429	1.0443	0.0414
MEAN	1.8906	1.0443	0.0414

** na = Observed number of alleles; * ne = Effective number of alleles (Kimura and Crow, 1964); * h = Nei's (1973) gene diversity.*

The number of polymorphic loci: 57

The percentage of polymorphic loci: 89.06%

Population 2

In a single descriptive analysis, the number of alleles observed in the population of Taradevi Shimla in Himachal Pradesh ranged from 1.0000 to 2.0000 with the mean 1.4375 and the effective number of alleles ranged from 1.0000 to 1.6482 with the mean 1.1129. The number of polymorphic loci was 28 and the percentage of polymorphic loci was 43.75%.

Table 5: Single Population Descriptive Analysis of Population 2

Locus	na*	ne*	h*
M-119	2.0000	1.6382	0.3827
M-156	1.4167	1.1751	0.1201
M-184	1.2500	1.1285	0.0838
M-198	1.0000	1.0000	0.0000
OPA-2	1.5000	1.1129	0.0874
MEAN	1.4375	1.1129	0.0874

** na = Observed number of alleles; * ne = Effective number of alleles (Kimura and Crow, 1964); * h = Nei's (1973) gene diversity.*

The number of polymorphic loci: 28

The percentage of polymorphic loci: 43.75%

Population 3

In a single descriptive analysis, the number of alleles observed in the population of Bhatwari-Taknor Range of Uttarakhand ranged from 1.2857 to 2.0000 with the mean 1.7031 and the effective number of alleles ranged from 1.0454 to 1.4317 with the mean 1.2067. The number of polymorphic loci was 45 and the percentage of polymorphic loci was 70.31%.

Table 6: Single Population Descriptive Analysis of Population 3

Locus	na*	ne*	h*
M-119	1.8462	1.2085	0.1448
M-156	2.0000	1.2913	0.2077
M-184	2.0000	1.4317	0.2841
M-198	1.4615	1.0939	0.2841
OPA-2	1.2857	1.0454	0.0392
Mean	1.7031	1.20669	0.0392

** na = Observed number of alleles; * ne = Effective number of alleles (Kimura and Crow, 1964); * h = Nei's (1973) gene diversity.*

The number of polymorphic loci: 45

The percentage of polymorphic loci: 70.31 %

Population 4

In a single descriptive analysis, the number of alleles observed in the population of Nohra Shimla located in Himachal Pradesh ranged from 1.3846 to 2.0000 with the mean 1.7031 and the effective number of alleles ranged from 1.1098 to 1.2099 with the mean 1.1830. The number of polymorphic loci was 45 and the percentage of polymorphic loci was 70.31%.

Table 7: Single Population Descriptive Analysis of Population 4

Locus	na*	ne*	h*
M-119	1.3846	1.1635	0.1119
M-156	2.0000	1.2099	0.1729
M-184	1.5000	1.1098	0.0900
M-198	1.6154	1.1612	0.07431
OPA-2	2.0000	1.1261	0.2030
Mean	1.7031	1.1830	0.1424

** na = Observed number of alleles; * ne = Effective number of alleles (Kimura and Crow, 1964); * h = Nei's (1973) gene diversity.*

The number of polymorphic loci: 45

The percentage of polymorphic loci: 70.31 %

Table 8: Single Population Descriptive Analysis of all the populations

Locus	na*	ne*	h*
Population 1	1.8906	1.0443	0.0414
Population 2	1.4375	1.1129	0.0874
Population 3	1.7031	1.20669	0.0392
Population 4	1.7031	1.1830	0.1424

SUMMARY

In a multi-population descriptive analysis, the total diversity (Ht) in the four populations of *Quercus leucotrichophora* ranged from 0.1620 to 0.3215 with the mean 0.2253 (Table 3). The within population gene diversity (Hs) ranged from 0.0928 to 0.2204 with the mean 0.1542. The among-population differentiation (Gst) ranged from 0.0304 to 0.3177 with the mean 0.3155. The gene flow observed among the populations was 1.0846, which is greater than 1 indicating higher levels of gene flow in the populations studied.

In a single descriptive analysis, the number of alleles observed in the population of Jhungi Suket located in Himachal Pradesh ranged from 1.6429 to 2.0000 with the mean 1.8906 and the effective number of alleles ranged from 1.0443 to 1.3574 with the mean 1.0443. The number of polymorphic loci was 57 and the percentage of polymorphic loci was 89.06% (Table 4).

Similarly, the number of alleles observed the number the number of alleles observed in the population of Taradevi Shimla located in Himachal Pradesh ranged from 1.0000 to 2.0000 with the mean 1.4375 and the effective number of alleles ranged from 1.0000 to 1.6482 with the mean 1.1129. The number of polymorphic loci was 28 and the percentage of polymorphic loci was 43.75% (Table 5).

In a single descriptive analysis, the number of alleles observed the number the number of alleles observed in the population of Bhatwari-Taknor Range located in Uttarakhand ranged from 1.2857 to 2.0000 with the mean 1.7031 and the effective number of alleles ranged from 1.0454 to 1.4317 with the mean 1.2067. The number of polymorphic loci was 45 and the percentage of polymorphic loci was 70.31% (Table 6).

In a single descriptive analysis, the number of alleles observed the number the number of alleles observed in the population of Nohra Shimla located in Himachal Pradesh ranged from 1.3846 to 2.0000 with the mean 1.7031 and the effective number of alleles ranged from 1.1098 to 1.2099 with the mean 1.1830. The number of polymorphic loci was 45 and the percentage of polymorphic loci was 70.31% (Table 7).

CONCLUSION

In *Quercus leucotrichophora* genetic diversity was studied using five polymorphic RAPD markers. The total diversity (Ht) in the four populations of *Quercus leucotrichophora* ranged from 0.1620 to 0.3215 with the mean 0.2253. The within population gene diversity (Hs) ranged from 0.0928 to 0.2204 with the mean 0.1542. The among-population differentiation (Gst) ranged from 0.0304 to 0.3177 with the mean 0.3155. The gene flow observed among the populations was 7.9677, which is greater than 1 indicating higher levels of gene flow in the populations studied. Highest similarity coefficient of 0.1793 was observed between populations from Jhungi Suket located in Himachal Pradesh and Nohra Shimla located in Himachal Pradesh. The highest percentage of polymorphic loci (89.06%)

was found in Population 1. The genetic diversity of all the four populations of the same species from different regions were found to be low, so if the trees of one place are damaged by any means the trees of similar genetic diversity from other places could be planted. In conclusion, molecular characterization of selective populations of *Quercus leucotrichophora* was studied through DNA-based molecular markers that showed high gene flow and lead to low genetic diversity among the four populations from different places. This information obtained through statistical analysis can be used to identify the diverse population used for better management and conservation of germplasm.

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