

## GENETIC VARIATION STUDIES IN NATURAL POPULATIONS OF NEOZA PINE (*PINUS GERARDIANA*)

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### ABSTRACT

The genetic variation of Neoza pine (*Pinus gerardiana*) populations with restricted distribution confined to arid regions of the temperate Himalayas was studied. These populations located in Kinnaur and Chamba districts of Himachal Pradesh showed difference with regard to both quantitative and qualitative traits. The performance of selected individuals (Plus Trees) within populations showed significant differences for nursery traits. The evaluation of twelve populations of Neoza pine through isozymes using six enzyme systems recorded 14 gene loci of which 6 were polymorphic with 2-3 alleles per locus. Of the 16 alleles found encoded for 6 polymorphic gene loci, SKDH-A3 was rare for its observance in only two populations with one per cent frequency. The differences were observed among populations allelic and genotypic differentiation between and within gene loci among populations, heterozygosity and genetic distance. The mean heterozygosity value varied from 0.17 to 0.21 for population 9 to 1, 5, 6 and 11. Genetic distance values ranged from 0.009 between populations 6 and 10 to 0.039 between populations 3 and 12. The pattern of grouping for nearly the same values of diversity and differentiation among the populations despite altitudinal differences suggest common descent of the populations. The populations with greater genetic diversity values need to be conserved and made use of in future tree improvement programmes of the species.

**Key words:** Neoza-pine, Populations, Heterozygosity, Polymorphic, Genetic distance.

### Introduction

Forest tree species occurring over a wide geographical range generally possess a relatively high level of genetic variation, and as a predominantly out crossing organism, a high degree of individual heterozygosity. As a result, the individuals or populations growing in different locations frequently display differences in one or more traits (Squillace, 1966). The genetic diversity, maintained by plant species and its distribution within and among populations are often associated with certain suites of ecological and life history traits (Hamrick *et al.*, 1979, 1992; Loveless and Hamrick, 1984; Hamrick and Godt, 1989). For example, long-lived woody plants tend to maintain more genetic variation at both the species and population levels, but have less genetic differentiation among populations than non-woody species and these trends are exemplified by the gymnospermous species, especially coniferous trees.

Conifers- the long lived iteroparous species are capable of producing male and female gametophytes for hundred or even thousands of years. Of the three main pine species found in N-W Himalayas, *Pinus gerardiana* Wall is an important conifer occurring in Afghanistan, Baluchistan, Pakistan and India (Jammu & Kashmir and

Himachal Pradesh) between 1600 m and 3300 m elevations (Dogra, 1964). In Himachal Pradesh, it occurs naturally in parts of Kinnaur and Chamba (Pangi and Bharmour) districts covering an area of about 2,844 hectares (Negi, 2002). The forests of Chilgoza pine are open and may grow under very difficult edaphic conditions. Champion and Seth (1968) have classed them as the Neoza pine forests and Dry temperate coniferous forests. Neoza pine is of immense importance much valued for its edible nuts locally called and marketed as "Chilgoza" and is eaten as dry fruits- rich in oil, starch and albumenoids. It is one of the most important cash crops of tribal people supporting their livelihood.

As the local people enjoy the rights of seed collection, they make every possible effort to harvest almost every single cone by lopping the branches for higher returns. The unscientific harvesting, severe biotic interferences, change in climatic conditions and lack of regeneration has threatened this important species. Based on the dwindling population in the wild and extensive collection for trade, the tree has been categorized as "Low Risk /Near Threatened" by the World Conservation Monitoring Centre - Trees database.

As the plantation of neoza pine is being taken up by

Population with greater genetic diversity values needs to be conserved for future tree improvement programmes.

the forest department by collecting seed from the local people, it is important to look for the genetic variation in the species and identify the populations/genotypes with more genetic diversity for use in their afforestation programme. The study of genetic variation in neoza pine which has restricted distribution in arid zone with varied growth of the stands is expected to show genetic diversity. An attempt has been made in this paper to study the genetic variation in different populations of the species in morphometric traits and through isozyme analysis to determine the magnitude and pattern of genetic variation within and among populations of the species for quick decision making process in conservation of genetic resources *in situ*. The distribution of the species in the region was categorised into twelve populations for the following objectives:

- Assessment of population for variation in morphometric traits
- To assess the genetic variation within and between neoza pine populations
- Delineation of populations based on isozyme analysis for their conservation

#### Methodology

The material for the present study comprised of twelve populations of neoza pine as shown in table 1. As the distribution of the species is restricted to arid and semi arid region, the concentrated stands representing different areas were selected. For morphometric traits observations on height, diameter at breast height, crown spread, needle length and needle thickness were recorded on twenty trees in a population. Open pollinated cones from 20 randomly selected trees approximately 50 m apart were harvested from these selected populations during the year 2011-12 (Sept.-Nov.) were taken for genetic variation studies using isozymes. The cones were dried in the sun and seed were separated from cones and kept separately tree wise within the population by

maintaining their identity. Seed lots were then kept at 4°C until used.

Isozyme analysis was carried out with Horizontal Starch Gel Electrophoresis based on the methods given by Shaw and Prasad (1970); Conkle *et al.* (1982) and Cheliak and Pitel (1984) with slight modifications. All the systems were analysed using Tris-Citrate System. These systems were assayed by using 20 trees from each population. While making investigations five samples of the endosperm and 10 samples of embryos per tree were taken for 12 populations which were assayed for 6 enzyme systems. Intra-specific variation was studied to determine the genetic variation between populations.

#### Results and Discussion

##### *Morphometric traits variation*

The twelve populations selected for the study are located in different altitudes varying from 1969 m for Powari to 2925 m Nesang population with varied stand structures. The different growth parameters studied are given in table 2.

The perusal of table depicts variation recorded for the characters assessed. The maximum tree height (18.44 m) was recorded for population Pangi (Chamba) and minimum (10.38 m) for Bharmour. Diameter at breast height (db) varied from a minimum of 46 cm recorded for Moorang population to 60 cm observed for Pangi (Chamba). The variation for crown spread ranged from 4.99 m (Powari) to 6.93 m (Rarang). The needle length was recorded minimum (8.84 cm) for Bharmour to maximum (11.22 cm) recorded for Jangi and needle thickness varied from 0.42 mm (Powari) to 0.57 mm (Pangi).

##### *Selection of plus trees and progeny test*

In addition to the variation recorded for the populations for growth traits, observations for genetic variation in Plus Trees selected in different populations were also recorded and given in the perusal of table 3

Table 1: Geographic location of *Pinus gerardiana* Populations.

| S.No. | Data period    | Altitude (m) | Longitude       | Latitude        |
|-------|----------------|--------------|-----------------|-----------------|
| 1     | Akpa           | 2318         | E78° 23' 17.74" | N31° 35' 08.16" |
| 2     | Pangi (Chamba) | 2710         | E78° 27' 09.21" | N31° 58' 21.18" |
| 3.    | Jangi          | 2728         | E78° 25' 34.52" | N31° 36' 35.91" |
| 4     | Pangi          | 2677         | E78° 16' 42.99" | N31° 35' 32.66" |
| 5     | Moorang        | 2745         | E78° 27' 11.89" | N31° 35' 53.64" |
| 6     | Bharmour       | 2889         | E76° 53' 20.4"  | N32° 44' 56.1"  |
| 7     | Powari         | 1969         | E78° 16' 14.57" | N31° 31' 12.15" |
| 8     | Ribba          | 2566         | E78° 34' 30.88" | N31° 34' 30.88" |
| 9     | Lippa          | 2582         | E78° 22' 57.60" | N31° 39' 33.94" |
| 10    | Nesang         | 2925         | E78° 31' 13.72" | N31° 38' 52.38" |
| 11    | Rispa          | 2331         | E78° 25' 39.35" | N31° 34' 30.11" |
| 12    | Rarang         | 2636         | E78° 21' 18.83" | N31° 36' 07.68" |

Table 2: Variation in morphometric traits amongst different populations.

| Population     | Height<br>(m) | DBH<br>(cm) | Crown spread<br>(m) | Needle length<br>(cm) | Needle thickness<br>(mm) |
|----------------|---------------|-------------|---------------------|-----------------------|--------------------------|
| Akpa           | 18.42         | 59.00       | 6.63                | 10.82                 | 0.52                     |
| Pangi (Chamba) | 18.44         | 60.00       | 5.80                | 10.92                 | 0.54                     |
| Jangi          | 17.71         | 51.00       | 6.43                | 11.22                 | 0.50                     |
| Pangi          | 18.14         | 53.00       | 6.87                | 10.36                 | 0.57                     |
| Moorang        | 12.23         | 46.00       | 5.63                | 10.18                 | 0.47                     |
| Bharmour       | 10.38         | 48.00       | 5.00                | 08.84                 | 0.43                     |
| Powari         | 11.83         | 47.00       | 4.99                | 10.00                 | 0.42                     |
| Ribba          | 16.68         | 50.00       | 6.53                | 10.88                 | 0.53                     |
| Lippa          | 15.56         | 48.00       | 5.32                | 10.94                 | 0.45                     |
| Nesang         | 17.82         | 55.00       | 6.80                | 10.38                 | 0.52                     |
| Rispa          | 14.64         | 49.00       | 6.23                | 09.53                 | 0.46                     |
| Rarang         | 18.14         | 58.00       | 6.93                | 10.36                 | 0.57                     |
| Range          | 10.38-18.44   | 46-60       | 4.99-6.93           | 8.84-11.22            | 0.42-0.57                |
| Mean           | 15.83         | 52.00       | 6.10                | 10.37                 | 0.50                     |
| SE             | 0.837         | 1.420       | 0.208               | 0.195                 | 0.015                    |
| SD             | 3.27          | 4.92        | 0.72                | 0.68                  | 0.05                     |
| CD at 5%       | 1.71          | 2.90        | 0.425               | 0.399                 | 0.031                    |

Table 3: Variation in morphometric traits in plus trees of *Pinus gerardiana*

| Plus<br>Tree<br>No | Height<br>(m) | Diameter<br>(cm) | Cone characters     |                    |                    |                     |                        |                  |               |
|--------------------|---------------|------------------|---------------------|--------------------|--------------------|---------------------|------------------------|------------------|---------------|
|                    |               |                  | Cone length<br>(cm) | Cone width<br>(cm) | Cone weight<br>(g) | No. of<br>seed/cone | 100 seed<br>weight (g) | Germination<br>% | Survival<br>% |
| 1                  | 18.00         | 59.00            | 13.25               | 7.85               | 496.87             | 79.43               | 50.12                  | 69.00            | 72.33         |
| 2                  | 18.50         | 60.00            | 16.48               | 7.86               | 404.17             | 77.57               | 55.10                  | 67.33            | 53.67         |
| 3                  | 17.50         | 51.00            | 14.26               | 10.08              | 681.20             | 81.37               | 40.22                  | 73.33            | 69.33         |
| 4                  | 18.00         | 53.00            | 14.98               | 8.72               | 716.03             | 100.83              | 50.83                  | 67.66            | 65.33         |
| 5                  | 16.50         | 46.00            | 13.42               | 7.82               | 508.18             | 88.33               | 50.36                  | 68.67            | 42.67         |
| 6                  | 12.00         | 48.00            | 14.54               | 7.88               | 375.33             | 61.20               | 39.85                  | 73.67            | 55.67         |
| 7                  | 14.00         | 47.00            | 14.63               | 8.21               | 392.00             | 54.60               | 40.36                  | 71.67            | 68.67         |
| 8                  | 15.50         | 50.00            | 13.70               | 7.36               | 359.00             | 92.00               | 39.29                  | 64.67            | 64.33         |
| 9                  | 17.00         | 48.00            | 13.85               | 8.15               | 458.24             | 101.33              | 39.89                  | 74.67            | 58.67         |
| 10                 | 18.00         | 55.00            | 12.83               | 8.20               | 542.45             | 81.63               | 36.77                  | 70.00            | 58.00         |
| 11                 | 14.00         | 49.00            | 13.75               | 6.90               | 513.56             | 79.85               | 42.64                  | 67.47            | 57.67         |
| 12                 | 17.00         | 48.00            | 14.17               | 7.76               | 628.00             | 84.25               | 41.78                  | 72.67            | 55.67         |
| 13                 | 16.50         | 49.00            | 13.97               | 7.46               | 579.25             | 86.23               | 53.25                  | 70.72            | 48.33         |
| 14                 | 14.50         | 58.00            | 13.27               | 8.12               | 569.47             | 81.20               | 54.84                  | 65.33            | 77.33         |
| 15                 | 16.50         | 61.00            | 14.10               | 8.25               | 614.78             | 79.25               | 52.15                  | 69.33            | 68.67         |
| 16                 | 18.00         | 56.00            | 13.96               | 7.89               | 623.45             | 78.35               | 49.10                  | 69.67            | 61.33         |
| 17                 | 14.50         | 58.00            | 14.19               | 7.65               | 399.33             | 114.83              | 39.78                  | 66.00            | 78.00         |
| 18                 | 12.50         | 54.00            | 13.77               | 8.10               | 423.67             | 101.15              | 40.20                  | 70.00            | 78.67         |
| 19                 | 14.50         | 56.00            | 14.64               | 7.72               | 415.23             | 93.20               | 41.38                  | 74.33            | 69.00         |
| 20                 | 13.00         | 54.00            | 13.80               | 7.65               | 440.87             | 88.80               | 38.75                  | 69.00            | 62.67         |
| 21                 | 18.00         | 48.00            | 14.10               | 7.98               | 398.78             | 98.45               | 50.12                  | 66.33            | 85.00         |
| 22                 | 15.00         | 46.00            | 13.35               | 8.14               | 426.23             | 89.75               | 51.32                  | 73.67            | 75.00         |
| 23                 | 13.50         | 49.00            | 13.26               | 8.10               | 452.10             | 87.35               | 50.48                  | 71.67            | 81.33         |
| 24                 | 14.00         | 52.00            | 12.96               | 7.90               | 525.37             | 79.26               | 48.32                  | 75.00            | 70.33         |
| 25                 | 15.00         | 57.00            | 13.32               | 7.52               | 486.23             | 78.25               | 45.36                  | 69.67            | 53.67         |
| 26                 | 13.00         | 59.00            | 12.98               | 7.60               | 498.14             | 101.10              | 47.12                  | 70.67            | 77.67         |
| 27                 | 15.50         | 61.00            | 14.12               | 7.36               | 416.89             | 98.25               | 42.15                  | 68.67            | 74.00         |
| 28                 | 16.50         | 57.00            | 13.62               | 9.72               | 516.25             | 97.52               | 41.72                  | 75.00            | 71.67         |
| 29                 | 13.00         | 54.00            | 13.65               | 7.60               | 482.30             | 89.50               | 46.32                  | 65.67            | 83.00         |
| 30                 | 12.50         | 56.00            | 13.50               | 8.24               | 467.25             | 84.50               | 56.15                  | 71.00            | 90.00         |
| 31                 | 14.00         | 48.00            | 12.45               | 7.59               | 398.65             | 92.45               | 54.26                  | 73.33            | 53.00         |
| 32                 | 13.50         | 47.00            | 13.75               | 8.52               | 440.28             | 86.30               | 57.89                  | 73.67            | 56.33         |
| Range              | 12.00-18.50   | 46.00-61.00      | 12.83-16.48         | 6.90-10.08         | 359.00-716.03      | 54.00-114.83        | 36.77-55.10            | 64.67- 75.00     | 42.67-90.00   |

reveals that the height for plus trees varied from 12 m (6) to 18.50 m (2) and DBH from 46 cm (5 and 22) to 61 cm (15 and 27). The germination percentage of the seeds collected from plus trees varied between 64.67 for plus tree 8 to 75 for the plus trees 24 and 28. Survival of seedlings is an important aspect and maximum survival of seedlings (90 %) after 90 days was observed for the progenies of plus tree 30 and minimum for the progenies of plus tree 5 (42.67%). Statistical analysis of the data recorded on ten cones per tree for cone and seed characters revealed significant differences ( $p=0.05$ ) among plus trees for cone weight (g), cone length (cm), cone width (cm), no. of seed/cone and seed weight (g) (Table 4) indicating that a significant amount of genetic variation exists among plus trees for these traits.

#### Allele frequency

In total 3600 samples were analysed for six enzyme systems. For these enzyme systems 14 presumable loci were identified, out of which 6 were polymorphic. The frequency distribution of the alleles (Table 5) showed differences among different populations. Out of 16 alleles found encoded for 6 polymorphic gene loci, SKDH-A3 was rare for its observance in only two populations (7 and 11) with 1 % frequency. Differences for existence of different

alleles were also observed for 6PGDH-A wherein allele 6PGDH-A4 was not found in six populations and in the remaining 6 populations with 1 to 2 per cent frequency. Similarly the allele MNR-B3 was scored in six populations with 1 to 2 per cent frequency. Major polymorphism was observed for two alleles of MDH-B, MDH-C, IDH-B and SKDH-A.

#### Allelic differentiation

The gene pool allelic differentiation for individual populations is given in table 6.

In addition to allelic differentiation for each population, the table also shows the allelic differentiation at individual loci and overall means gene pool differentiation which reflects on an average the proportion of the effective number of alleles by which a population differed from the remaining populations over the set of six gene loci.

The gene pool differentiation among the populations varied from 12 % to 15 % with population 10 recording 12 % and population 3 and 5 recording 15 per cent. Large differences were observed for allelic differentiation between and within the gene loci among the populations. The mean differentiation ( $\theta$ ) values among the 6 gene loci varied from 0.16 (MDH-C) to 0.57 (6PGDH-A).

Table 4: Analysis of variance for cone and seed characters in plus trees in *Pinus gerardiana*.

| Sources of variation | Degrees of freedom | MSS         |             |            |                   |             |
|----------------------|--------------------|-------------|-------------|------------|-------------------|-------------|
|                      |                    | Cone weight | Cone length | Cone width | No. of seeds/cone | Seed weight |
| Replication          | 2                  | 35.08       | 0.0047      | 0.089      | 2.17              | 0.13        |
| Treatment            | 32                 | 5082.87**   | 10.08**     | 0.391**    | 412.15**          | 11.30**     |
| Error                | 64                 | 71.90       | 0.084       | 0.047      | 10.78             | 0.18        |

\*\* Significant at 1 % level

Table 5: Allele frequency distribution in 12 populations of *Pinus gerardiana*.

| Gene Loci | Allele | Populations |      |      |      |      |      |      |      |      |      |      |      | Mean  |
|-----------|--------|-------------|------|------|------|------|------|------|------|------|------|------|------|-------|
|           |        | 1           | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |       |
| IDH-B     | 1      | 0.83        | 0.85 | 0.79 | 0.91 | 0.76 | 0.88 | 0.88 | 0.87 | 0.79 | 0.90 | 0.87 | 0.85 | 0.85  |
|           | 2      | 0.17        | 0.15 | 0.21 | 0.09 | 0.24 | 0.12 | 0.12 | 0.13 | 0.21 | 0.10 | 0.13 | 0.15 | 0.15  |
| SKDH-A    | 1      | 0.80        | 0.83 | 0.69 | 0.82 | 0.75 | 0.86 | 0.75 | 0.85 | 0.85 | 0.83 | 0.69 | 0.89 | 0.80  |
|           | 2      | 0.20        | 0.17 | 0.31 | 0.18 | 0.25 | 0.14 | 0.24 | 0.15 | 0.15 | 0.17 | 0.30 | 0.11 | 0.20  |
|           | 3      | -           | -    | -    | -    | -    | -    | 0.01 | -    | -    | -    | 0.01 | -    | 0.001 |
| 6PGDH-A   | 1      | 0.39        | 0.39 | 0.44 | 0.42 | 0.48 | 0.48 | 0.43 | 0.43 | 0.48 | 0.49 | 0.40 | 0.53 | 0.45  |
|           | 2      | 0.53        | 0.44 | 0.48 | 0.50 | 0.42 | 0.48 | 0.54 | 0.48 | 0.47 | 0.47 | 0.51 | 0.42 | 0.48  |
|           | 3      | 0.06        | 0.15 | 0.07 | 0.08 | 0.09 | 0.04 | 0.03 | 0.08 | 0.05 | 0.04 | 0.08 | 0.05 | 0.07  |
|           | 4      | 0.02        | 0.02 | 0.01 | -    | 0.01 | -    | -    | 0.01 | -    | -    | 0.01 | -    | 0.001 |
| MDH-B     | 1      | 0.90        | 0.89 | 0.89 | 0.86 | 0.86 | 0.87 | 0.92 | 0.93 | 0.91 | 0.88 | 0.87 | 0.85 | 0.89  |
|           | 2      | 0.10        | 0.11 | 0.11 | 0.14 | 0.14 | 0.13 | 0.08 | 0.07 | 0.09 | 0.12 | 0.13 | 0.15 | 0.11  |
| MDH-C     | 1      | 0.95        | 0.92 | 0.92 | 0.91 | 0.91 | 0.90 | 0.91 | 0.91 | 0.92 | 0.92 | 0.91 | 0.88 | 0.91  |
|           | 2      | 0.05        | 0.08 | 0.08 | 0.09 | 0.09 | 0.10 | 0.09 | 0.09 | 0.08 | 0.08 | 0.09 | 0.12 | 0.09  |
| MNR-B     | 1      | 0.76        | 0.66 | 0.63 | 0.71 | 0.73 | 0.71 | 0.79 | 0.70 | 0.72 | 0.75 | 0.77 | 0.75 | 72.33 |
|           | 2      | 0.24        | 0.32 | 0.35 | 0.28 | 0.27 | 0.29 | 0.21 | 0.29 | 0.28 | 0.25 | 0.22 | 0.24 | 27.00 |
|           | 3      | -           | 0.02 | 0.02 | 0.01 | -    | -    | -    | 0.01 | -    | -    | 0.01 | 0.01 | 0.67  |

Wide ranges of differentiation values were recorded for different populations within gene loci e.g., SKDH-A (0.20 for population 12 to 0.44 for population 11), MDH-B (0.14 for population 8 to 0.25 for populations 5 and 12).

Overall, a high amount of allelic differentiation among populations was reflected by the mean gene pool value,  $\delta = 14$ , which means on an average, in the gene pool of the six loci, populations differed from their complement populations by 14 % of the effective number of alleles.

#### Heterozygosity

The actual heterozygosity varied for the populations investigated (Table 7). It was found to vary from 0.04 to 0.20 for IDH-B, 0.06 to 0.18 for SKDH-A, 0.23 to 0.38 (6PGDH-A), 0.15 to 0.29 (MDH-B), 0.11 to 0.24 for MDH-C and 0.19 to 0.29 (MNR-B). The mean heterozygosity value varied from 0.17 to 0.21 for population 9 to 1, 5, 6, and 11.

#### Genetic distance

The matrix of the genetic distance calculated for twelve populations is given in Table 8. Genetic distance values ranged from 0.009 between populations 6 and 10 to 0.039 between populations 3 and 12. Out of 66 pair wise combinations 32, i.e., 48.48% showed values of the

genetic distance up to 0.020 whereas 33 i.e., 50 % showed values between 0.020 to 0.030, and 1 showed values more than 0.030.

#### Conclusion

Geographic variation for both morphological and protein characters in trees is more a common observation than is the uniformity of characters. Different forms of natural selection and stochastic forces could cause geographical variation and generate population structures under a broad range of ecological conditions. Due to these reasons, despite of large amount of intrapopulation variation, substantial genetic differences among geographically isolated populations usually exist. A large amount of genetic variation gives a population the necessary potential of adaptation to different environmental conditions.

With regard to morphometric traits variation the population Jangi was found to be uniform in stand structure with average height of the stand with good crown spread was 17 m. The population of Pangri area of Chamba district though found in very small area but have good growth and the local people do not lop trees for collection of cones.

Table 6: Allelic differentiation (value in parenthesis) and Diversity among populations.

| Gene Loci | Populations    |                |                |                |                |                |                |                |                |                |                |                | Mean $\delta$  |
|-----------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|           | 1              | 2              | 3              | 4              | 5              | 6              | 7              | 8              | 9              | 10             | 11             | 12             |                |
| IDH-B     | 1.40<br>(0.29) | 1.34<br>(0.26) | 1.51<br>(0.34) | 1.20<br>(0.17) | 1.58<br>(0.37) | 1.27<br>(0.21) | 1.26<br>(0.21) | 1.28<br>(0.22) | 1.50<br>(0.33) | 1.22<br>(0.18) | 1.30<br>(0.23) | 1.34<br>(0.26) | 1.35<br>(0.26) |
| SKDH-A    | 1.47<br>(0.32) | 1.39<br>(0.28) | 1.74<br>(0.43) | 1.42<br>(0.30) | 1.61<br>(0.38) | 1.32<br>(0.24) | 1.59<br>(0.37) | 1.34<br>(0.26) | 1.34<br>(0.26) | 1.39<br>(0.28) | 1.79<br>(0.44) | 1.24<br>(0.20) | 1.47<br>(0.31) |
| 6PGDH-A   | 2.27<br>(0.56) | 2.70<br>(0.63) | 2.34<br>(0.58) | 2.30<br>(0.57) | 2.41<br>(0.59) | 2.18<br>(0.54) | 2.10<br>(0.53) | 2.37<br>(0.58) | 2.20<br>(0.55) | 2.16<br>(0.54) | 2.34<br>(0.58) | 2.18<br>(0.54) | 2.30<br>(0.57) |
| MDH-B     | 1.21<br>(0.18) | 1.24<br>(0.19) | 1.24<br>(0.20) | 1.31<br>(0.24) | 1.33<br>(0.25) | 1.29<br>(0.23) | 1.18<br>(0.15) | 1.16<br>(0.14) | 1.20<br>(0.16) | 1.27<br>(0.21) | 1.28<br>(0.22) | 1.33<br>(0.25) | 1.25<br>(0.20) |
| MDH-C     | 1.11<br>(0.10) | 1.18<br>(0.15) | 1.17<br>(0.14) | 1.19<br>(0.16) | 1.20<br>(0.17) | 1.21<br>(0.18) | 1.19<br>(0.16) | 1.19<br>(0.16) | 1.17<br>(0.14) | 1.17<br>(0.15) | 1.19<br>(0.16) | 1.27<br>(0.21) | 1.19<br>(0.16) |
| MNR-B     | 2.09<br>(0.53) | 2.45<br>(0.60) | 2.74<br>(0.64) | 2.24<br>(0.56) | 2.16<br>(0.54) | 2.30<br>(0.51) | 1.88<br>(0.47) | 2.41<br>(0.59) | 2.17<br>(0.54) | 2.13<br>(0.53) | 2.03<br>(0.51) | 2.08<br>(0.52) | 2.22<br>(0.55) |
| Gene Pool | 1.15<br>(0.13) | 1.16<br>(0.14) | 1.18<br>(0.15) | 1.15<br>(0.13) | 1.18<br>(0.15) | 1.15<br>(0.13) | 1.14<br>(0.13) | 1.15<br>(0.13) | 1.15<br>(0.13) | 1.14<br>(0.12) | 1.17<br>(0.14) | 1.15<br>(0.13) | 1.16<br>(0.14) |

Table 7: Heterozygosity at 6 polymorphic loci observed for *P. gerardiana* populations.

| Gene Loci      | Populations |      |      |      |      |      |      |      |      |      |      |      | Range     |
|----------------|-------------|------|------|------|------|------|------|------|------|------|------|------|-----------|
|                | 1           | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |           |
| IDH-B          | 0.20        | 0.11 | 0.11 | 0.04 | 0.15 | 0.19 | 0.13 | 0.12 | 0.13 | 0.12 | 0.12 | 0.11 | 0.04-0.20 |
| SKDH-A         | 0.12        | 0.10 | 0.11 | 0.12 | 0.16 | 0.08 | 0.16 | 0.10 | 0.10 | 0.10 | 0.18 | 0.06 | 0.06-0.18 |
| 6PGDH-A        | 0.38        | 0.34 | 0.28 | 0.29 | 0.27 | 0.28 | 0.27 | 0.30 | 0.26 | 0.23 | 0.28 | 0.25 | 0.23-0.38 |
| MDH-B          | 0.19        | 0.21 | 0.22 | 0.27 | 0.29 | 0.26 | 0.17 | 0.15 | 0.18 | 0.24 | 0.25 | 0.29 | 0.15-0.29 |
| MDH-C          | 0.11        | 0.17 | 0.15 | 0.17 | 0.19 | 0.19 | 0.17 | 0.17 | 0.15 | 0.16 | 0.17 | 0.24 | 0.11-0.24 |
| MNR-B          | 0.25        | 0.21 | 0.29 | 0.20 | 0.20 | 0.23 | 0.19 | 0.27 | 0.19 | 0.25 | 0.25 | 0.22 | 0.19-0.29 |
| Mean ( $H_j$ ) | 0.21        | 0.19 | 0.19 | 0.18 | 0.21 | 0.21 | 0.19 | 0.19 | 0.17 | 0.18 | 0.21 | 0.20 | 0.17-0.21 |



Table 8: Gene pool distances for populations

| Populations | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12 |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| 1           | -     |       |       |       |       |       |       |       |       |       |       |    |
| 2           | 0.020 | -     |       |       |       |       |       |       |       |       |       |    |
| 3           | 0.026 | 0.023 | -     |       |       |       |       |       |       |       |       |    |
| 4           | 0.020 | 0.017 | 0.027 | -     |       |       |       |       |       |       |       |    |
| 5           | 0.026 | 0.026 | 0.020 | 0.024 | -     |       |       |       |       |       |       |    |
| 6           | 0.024 | 0.019 | 0.030 | 0.011 | 0.024 | -     |       |       |       |       |       |    |
| 7           | 0.016 | 0.028 | 0.030 | 0.019 | 0.028 | 0.022 | -     |       |       |       |       |    |
| 8           | 0.020 | 0.014 | 0.026 | 0.011 | 0.028 | 0.010 | 0.020 | -     |       |       |       |    |
| 9           | 0.018 | 0.020 | 0.022 | 0.020 | 0.019 | 0.013 | 0.025 | 0.013 | -     |       |       |    |
| 10          | 0.019 | 0.020 | 0.030 | 0.010 | 0.025 | 0.009 | 0.017 | 0.015 | 0.015 | -     |       |    |
| 11          | 0.019 | 0.026 | 0.021 | 0.020 | 0.024 | 0.025 | 0.015 | 0.024 | 0.030 | 0.022 | -     |    |
| 12          | 0.027 | 0.026 | 0.039 | 0.023 | 0.025 | 0.015 | 0.030 | 0.024 | 0.020 | 0.017 | 0.030 | -  |

Based on allelic diversity and differentiation values, population Jangi and Moorang remains separated from the rest of the populations as population 1,4,6,7,8,9, 10 and 12 makes one group and the populations 2 and 11 can be combined into another group. The genetic distance values further supports this grouping as population Jangi and Moorang shows relatively higher genetic distance with the remaining populations whereas the other populations show less genetic distance within the group. The tendency of grouping among different populations despite altitudinal differences suggests common descent of the populations.

Based on morphometric traits and genetic variation in the species, seeds from selected populations/genotypes need to be used in future afforestation programme, besides educating the local people to harvest cones with folding pruners without lopping the branches to help conserve this economically important species.

The education of the local people leaving few cones on the tree at the time of harvest will not only ensure regeneration of the species but also the conservation of this economically important species.

### नीओजा पाइन ( पाइनस जीरार्डियाना ) की प्राकृतिक आबादियों में आनुवंशिक विभिन्नता अध्ययन

राजेश शर्मा

सारांश

शीतोष्ण हिमालयों के शुष्क क्षेत्रों तक सीमित आंशिक वितरण के साथ नीओजा पाइन ( पाइनस जीरार्डियाना ) आबादियों की आनुवंशिक विभिन्नता का अध्ययन किया गया। हिमाचल प्रदेश के किन्नौर और चम्बा जिलों में स्थित इन आबादियों ने मात्रात्मक और गुणात्मक दोनों विशेषकों के संबंध में अन्तर दिखाया। आबादियों के भीतर चयनित एकलों ( घन वृक्षों ) के प्रदर्शन ने पौधशाला विशेषकों के लिए महत्वपूर्ण विभिन्नताओं को दर्शाया छः एन्जाइम प्रणालियों का उपयोग करके आइसोजाइनों के जरिए नीओजा पाइन की बारह आबादियों के मूल्यांकन ने 14 जीन लोसी अभिलिखित किया, जिसमें से 6 प्रति लोकस 2-3 एलीलों के साथ बहुरूपी थी। पाए गए 16 एलीलों में से 6 बहुरूपी जीन लोसी के लिए इन्कोड किया गया, एस के डी एच-ए3 एक प्रतिशत बारम्बारता के साथ केवल दो आबादियों में अपने प्रेक्षणों के लिए दुर्लभ था। आबादियों, विषम युग्मजता और आनुवंशिक दूरी में जीन लोसी के भीतर और के बीच एलीलिक एवं जीन विभेदीकरण आबादियों में विभिन्नता प्रेक्षित की गई। आबादी 9 से 1,5,6, और 11 के लिए औसत विषम युग्मजता मान 0.17 से 0.21 तक था। आनुवंशिक दूरी मान आबादी 6 एवं 10 के बीच 0.009 से आबादी 3 और 12 के बीच 0.039 तक था। उन्नतांशीय विभिन्नताओं के बावजूद आबादियों में विविधता और विभेदीकरण के लगभग समान मानों के लिए समूहन के पैटर्न आबादियों के सामान्य उद्गम का सुझाव देते हैं। वृहत्तर आनुवंशिक विविधता मानों वाली आबादियों को संरक्षित करने तथा प्रजाति के भावी वृक्ष सुधार कार्यक्रमों में उपयोग किए जाने की आवश्यकता है।

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