# Cross-transferability-based identification and validation of simple sequence repeat (SSR) markers in oaks of western Himalayas

Chander Shekhar, Anita Rawat, Maneesh S. Bhandari, Santan Barthwal, Harish S. Ginwal, Rajendra K. Meena*

Division of Genetics & Tree Improvement, Forest Research Institute, Dehradun-248 195, Uttarakhand, India.

* Corresponding author: Rajendra K. Meena, E-mail: rajnrcpb@gmail.com

## Abstract

Cross-amplification is a cost-effective method to extend the applicability of SSR markers to closely related taxa which lack their own sequence information. In the present study, 35 SSR markers developed in four oak species of Europe, North America and Asia were selected and screened in five species of the western Himalayas. Fifteen markers were successfully amplified in *Quercus semecarpifolia*, followed by 11 each in *Q. floribunda* and *Q. leucotrichophora*, 10 in *Q. glauca*, and 9 in *Q. lanata*. Except two primer pairs in *Q. semecarpifolia*, all were found to be polymorphic. Most of the positively cross-amplified SSRs were derived from the Asian oak, *Q. mongolica*. The genotyping of 10 individuals of each species with positively cross-amplified SSRs displayed varied levels of polymorphism in the five target oak species, viz., QmC00419 was most polymorphic in *Q. floribunda*, QmC00716 in *Q. glauca* and *Q. lanata*, QmC01368 in *Q. leucotrichophora*, and QmC02269 in *Q. semecarpifolia*. Among five oak species, the highest gene diversity was depicted in *Q. lanata* and *Q. semecarpifolia* with expected heterozygosity (He = 0.72), while the minimum was recorded for *Q. leucotrichophora* and *Q. glauca* (He = 0.65). The SSRs validated here provide a valuable resource to carry out further population genetic analysis in oaks of the western Himalayas.

Keywords: *Cross-transferability, Genetic diversity, SSRs, Western Himalayan oaks, Quercus semecarpifolia, Quercus floribunda, Quercus leucotrichophora, Quercus glauca, Quercus lanata*

## Introduction

*Quercus* (Oak) (Fagaceae), is a large group of hardwood trees of about 600 species principally distributed across the northern temperate zone, and south into areas of subtropical and tropical Asia, and the northern South America (Keator and Bazel, 1998). In India, 35 oak species have been reported to occur in the Indian Himalayan Region; Jammu and Kashmir in northwestern, Uttarakhand and Himachal Pradesh in the central, and Manipur and Arunachal Pradesh in the northeastern zone (Negi and Naithani, 1995; Pandey and Tamta, 2014). Indian oaks are evergreen and medium to large sized trees, and distributed throughout the Himalayas between 800 to 3800 meter above mean seal level (m amsl). Five oak species are naturally occurring in the western Himalayas, namely *Q. floribunda* Rehder, *Q. glauca* Thunb., *Q. leucotrichophora* A. Camus, *Q. lanata* J. E. Sm., and *Q. semecarpifolia* Smith (Troup, 1921). In this region, oak forest is an indicator of climax vegetation at elevations between 1000 to 3500 m amsl and plays a crucial role in conservation of soil, water, native flora and fauna (Singh and Singh, 1992; Upreti et al., 1985).

Due to the inaccessible terrain, inhabitants of the Himalayas have been dependent upon native natural resources for thousands of years. In the recent time, the dependence on the resources further increased due to easy accessibility of the global market and growing demands of socio-economic development. A major challenge for resource managers is the conflict between forests and communities, which led to the continuous degradation of forests. As per the recent assessment and studies, a noticeable degradation was observed in the oak forest, and only some patches of oak forests are remained intact (Singh et al., 1984). Deterioration of oak forest in the

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western Himalayas is mainly associated with the lopping of tender branches for fodder and intrusion of alien invasive species, such as *Eupatorium adenophorum* Spreng., *Lantana camara* L. and aggressively spreading *Pinus roxburghii* Sargent. Selective removal of oaks through cutting and burning in many parts of western Himalaya have changed the soil properties and recruited the invasion of other species. The replacement of oak by *P. roxburghii* is an ongoing pattern seen across the region (Saxena and Singh, 1982; Singh et al., 1984; Nautiyal, 2015). Apart from these ecological changes, oak forests in the western Himalayas are dwindling due to lack of regeneration, irregular seed production, defoliation, acorn predation and an increased fire frequency (Shrestha, 2003). Altogether, there is continuous degradation and fragmentation of oak habitats in the western Himalayan region which increases their risk of genetic erosion. Therefore, the evaluation and conservation of genetic diversity is of utmost importance for the key oak species based upon the degree of disturbances and exploitation experienced by them.

Molecular marker-based population genetic analysis provides the crucial information required for conservation and management. Among several marker technologies, simple sequence repeats (SSRs) are preferentially used for genetic studies in plants due to their abundance in the genome, high polymorphism, reproducibility and co-dominance (Jarne and Lagoda, 1996). Unlike other random primer-based marker techniques, SSRs are developed from the unique flanking sequence of microsatellite repeat loci, which therefore restrict their utilization to the species having their own sequence information (Zane et al., 2002; Squirrell et al., 2003). It has been well established that the flanking regions of microsatellite marker loci share a high level of homology among taxonomically related taxa (Saha et al., 2004; Sharma et al., 2008), and thus, SSR markers developed in one species can often be used to cross-amplify in other related species (Sharma et al., 2009; Mason, 2015; Cai et al., 2019; Aiello et al., 2020). Hence, cross transferability is the rapid and cost-effective approach for developing locus specific SSR markers in related species (Barbara et al., 2007) and has been successfully utilized in several oak species (Steinkellner et al., 1997a; Isagi and Suhandono, 1997; Aldrich et al., 2003). The SSR markers have been used for numerous analyses in oaks of Europe, North America and Asia, but limited studies have been conducted in Himalayan oaks (Kumar and Kumar, 2014; Saha, 2018) and none of these were based on SSRs. Here we test 35 SSR markers developed in four other *Quercus* species for their cross-transferability to five oak species of western Himalayas, and characterized them for their level of polymorphism.

## Materials and Methods

### *Sampling of plant material and extraction of genomic DNA*

Leaf samples were collected for five oak species, namely *Q. floribunda, Q. lanata, Q. leucotrichophora, Q. glauca,* and *Q. semecarpifolia* from their natural range in the western Himalayan region within the state of Uttarakhand, India (Figure 1). Random sampling was performed from ten distantly located individuals of each species, and every sampled individual was more than 100 m apart at each location. The samples were desiccated using silica gel and stored at -80°C. Total genomic DNA was extracted using standard cetyl trimethyl ammonium bromide (CTAB) method of Doyle and Doyle (1987) with polyvinyl pyrrolidone (Pandey and Tamta, 2015).

### *Screening of SSR markers for cross-transferability*

For cross-amplification in Himalayan oaks, 35 SSR markers developed in oaks of Europe, North America and Asia were selected from the published literature (Table 1). Thirteen SSRs were developed in Japanese oak *Q. mongolica* Fisch; two of these were genomic (Mishima et al., 2006) and 11 were genic (Ueno and Tsumura, 2008; Ueno et al., 2008). In addition, the genomic SSRs developed through random genome sequencing were also selected, viz., five from the North American oak *Q. rubra* L. (Aldrich et al., 2002 and 2003), eight from the European oak *Q. petrea* Matt. (Steinkellner et al., 1997b), and nine from the Japanese oak *Q. myrinsifolia* Blume (Isagi and Suhandonos, 1997). Each primer pair was screened through polymerase chain reaction (PCR) amplification in our target oak species. The PCR was performed in a 15 µL reaction volume containing 15 ng template DNA, 1× *Taq* buffer, 2.4 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM each forward and reverse primer and 0.65 U Taq DNA polymerase (Genei, India). The PCR amplification was performed with the following cycling parameters; a denaturation step at 94°C for 3 min, followed by 40 cycles of 94°C for 45 sec, 48-55°C for 45 sec, 72°C for 45 sec and a final extension at 72°C for 7 min. The PCR products were separated on 2 % agarose (Sigma-Aldrich) gel stained with ethidium bromide. Based upon the failure of PCR amplification with some primer pairs, they were again attempted to amplify after optimizing the PCR reactions and annealing temperature.

### *Testing and characterization of selected microsatellite loci for genotyping*

The positively amplified SSR loci were first tested for polymorphism by subjecting to PCR reactions with five randomly collected individuals of each species, and separated in a 4 % high resolution agarose (Sigma-Aldrich) gel. Subsequently, polymorphic SSRs were used for genotyping the 10 sample individuals of each species and PCR product were separated using singleplex capillary electrophoresis in Nucleic Acid Analyzer (LabChip GX Touch 24, PerkinElmer, USA) along with an internal size standard. The allelic data were further viewed and analysed using Gene Reviewer software (PerkinElmer, USA). The fractional allele sizes generated by machine were first transformed into integers and binned as per the periodicity of the repeat motifs using the software TANDEM version 1.07 (Matschiner and Salzburger, 2009). The marker attributes and population diversity measures, including number of alleles per locus (Na), effective number of alleles per locus (Ne), observed heterozygosity (Ho) and expected heterozygosity (He), were estimated using the software GenAlEx version 6.3 (Peakall and

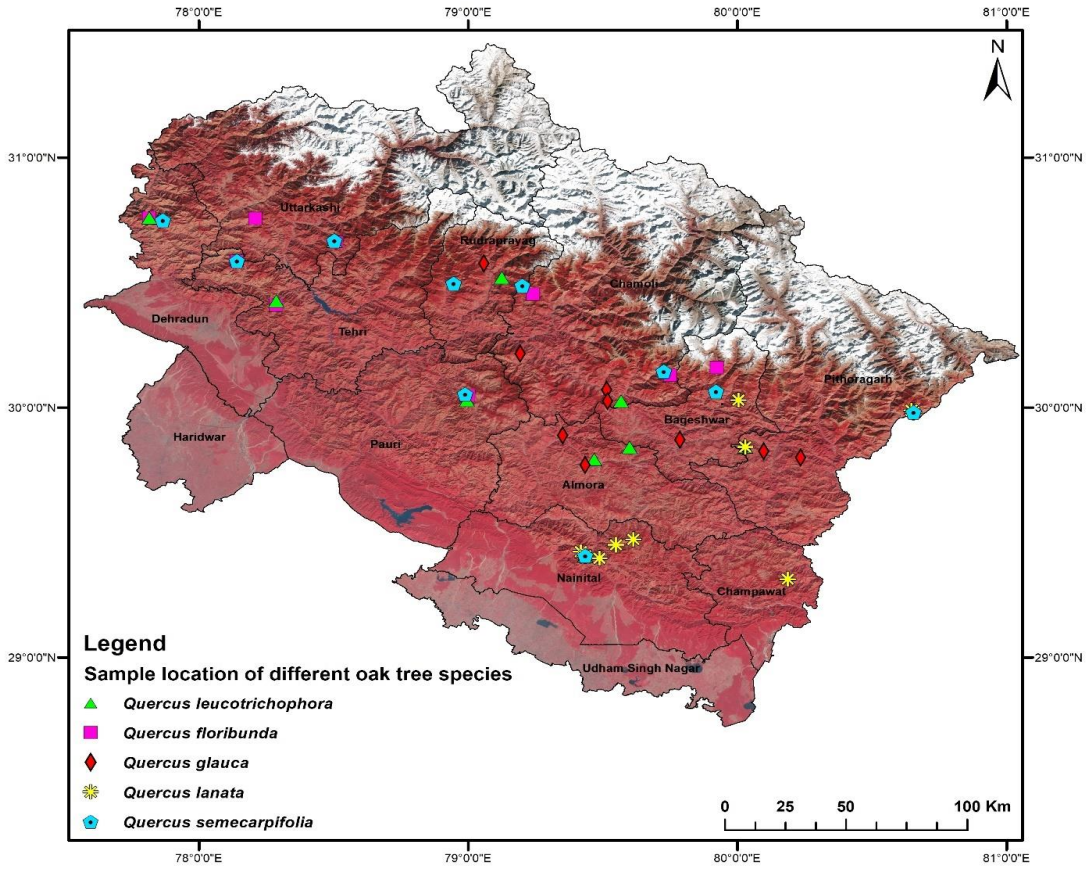



Figure 1
Geo-spatial map of sampled individuals of five oak species in the western Himalayan region under the state Uttarakhand, India

Smouse, 2006). Polymorphism information content (PIC) of the marker loci was calculated using the software PowerMarker version 3.25 (Liu and Muse, 2005). In order to understand the genetic relationship among studied oak species, the pairwise genetic distance was further used to construct a dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) using the software POPGENE version 1.32 (Yeh et al., 1999).

## Results

### *SSR cross-transferability*

The SSR primer pairs producing PCR amplicon(s) within the expected size range were considered to be positively transferred (Table 1). Among 35 tested marker loci, 15 were successfully amplified in *Q. semecarpifolia*, followed by 11 in *Q. floribunda* and *Q. leucotrichophora*, 10 in *Q. glauca*, and 9 in *Q. lanata*. Except two primer pairs in *Q. semecarpifolia*, all the cross-amplified SSR markers were polymorphic among the individuals of four tested Himalayan oaks. The success of cross-transferability was highly varied among SSRs of the four species. The EST-based SSRs derived from the Asian oak, *Q. mongolica* displayed the highest transferability and polymorphism in Himalayan oaks; whereas, the genomic SSRs derived from other oaks of Asia (*Q. myrinsifolia*), Europe (*Q. petrea*) and North America (*Q. rubra*) showed very low cross- transferability.

### *Characterization of selected microsatellite loci*

The polymorphic SSR markers were amplified across ten individuals of each species. The allelic data were analyzed to determine the key marker characteristics and population diversity measures. However, the marker loci which were common across all five oak species, analyzed together to determine the phylogenetic relationship among them. The average number of alleles detected for the positively tested markers varied between 4.4 in *Q. leucotrichophora* to 7.16 in *Q. semecarpifolia*. The tested SSR loci displayed varied levels of polymorphism in the five target oak species. Marker QmC00419 was most polymorphic in *Q. floribunda* (Na = 6; He = 0.78; PIC = 0.75), QmC00716 in *Q. glauca* (Na = 9; He = 0.88; PIC = 0.87) and *Q. lanata* (Na = 11; He = 0.82; PIC = 0.81), QmC01368 (Na = 9; He = 0.87; PIC = 0.86) in *Q. leucotrichophora*, and QmC02269 in *Q. semecarpifolia* (Na = 13; He = 0.91; PIC = 0.91). Among the five oak species, the highest gene diversity (Ho = 0.52-0.60; He = 0.72) was depicted in *Q. lanata* and *Q. semecarpifolia* and lowest (Ho = 0.50-0.58; He = 0.65) in *Q. leucotrichophora* and *Q. glauca* (Table 2).

Table1
SSR marker details along with their product size. P: Polymorphic, M: Monomorphic, Ta: annealing temperature, bp: base pairs, NA: Not amplified, *Qf*: *Q. floribunda*, *Qg*: *Q. glauca*, *Ql*: *Q. lanata*, *Qle*: *Q. leucotrichophora*, and *Qs*: *Q. semecarpifolia*

| Sl. No. | locus name | Repeat motif | Primer sequences (5'-3') | Ta (°C) | SSR location | Expected product size (bp) | Observed product size (bp) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Qf* | *Qg* | *Ql* | *Qle* | *Qs* |
| 1. | CR627959 | (GCA)7 | F-GCTCCCTGGTAGTCGGCTAAAGA<br>R- CAATTGGGACAACATGGAAGCAT | 55 | Coding | 243–251 | 234-253 (P) | 246-291 (P) | 235-244 (P) | 253-268 (P) | 243-252 (P) |
| 2. | DN949770 | (TC)9 | F-TCTCCTTCTGCACTCACTCACGA<br>R-AACGTAAAGGCAAATTGATCGGG | 55 | Non-coding | 277–337 | NA | NA | NA | NA | 277-377 (P) |
| 3. | DN950634 | (TC)10 | F-TCAAACAATCTCAAGGCTCCCAA<br>R-GCTTTTGAGAAACTTTGGCCACC | 55 | Non-coding | 361-381 | NA | NA | NA | NA | 390 (M) |
| 4. | DN950717 | (GGA)6 | F-TAGTTTTCCCAGCGAATCCAACA<br>R- CTTCTTGAAGGGACTGACCCCAT | 55 | Coding | 242-260 | 227-260 (P) | 232-244 (P) | 239-296 (P) | 227-242 (P) | 229-241 (P) |
| 5. | DN950726 | (GAT)6 | F-GCAAGAAGCATGCAGATGGAGAT<br>R- GCATGGCCGTCATTAGCATTAAG | 55 | Coding | 314-319 | 383-395 (P) | 333-336 (P) | 332-344 (P) | 335-344 (P) | 341-350 (P) |
| 6. | QmC00141 | (TCT)6 | F-ACACTACACTTAGCACCCCGCAA<br>R- CAATGGTAATCACCGCCTCCATA | 55 | Coding | 172 | 257-272 (P) | 257-273 (P) | 257-274 (P) | 257-275 (P) | 280-292 (P) |
| 7. | QmC00419 | (AG)3(TC)7 | F-TTTTTGGTGTTTCGGTGGTTTGG<br>R-AAGGATGAATTGAGGGTTTCGGA | 55 | 5' UTR | 171 | 164-192 (P) | 162-196 (P) | 164-190 (P) | 179-209 (P) | 166-218 (P) |
| 8. | QmC00716 | (TC)15 | F-AAGAGAACCCATTCCATCCCTGA<br>R- TTTCCCGAACAGTGGTTTCTTGA | 55 | 5' UTR | 270 | 237-251 (P) | 239-307 (P) | 238-286 (P) | 241-267 (P) | 270-300 (P) |
| 9. | QmC00898 | (AG)14 | F-AGGCAGCCGTAAGAGTTGAGCTT<br>R-CTTTCTTCCTTTGCTGCCCTTGT | 55 | 5' UTR | 95 | 72-108 (P) | 70-107 (P) | NA | 70-91 (P) | 81-96 (P) |
| 10. | QmC01368 | (CCA)7 | F-TAGCAGAATCATCGGAAGCGAAC<br>R-CCGAGCTTGATGAACTGCGTACT | 55 | Coding | 155 | 142-164 (P) | 141-155 (P) | 144-160 (P) | 141-203 (P) | 147-155 (P) |
| 11. | QmC02269 | (TC)13 | F-CGGGTTCTAGCTGCTGTTGGTTT<br>R-GATTATGGGTGCGTTCATGGTGT | 55 | 5' UTR | 184 | 186-204 (P) | 151-195 (P) | NA | 190-196 (P) | 151-201 (P) |
| 12. | Bcqm76 | (GT)7 | F-ATTAGTTTGCCTAGCTCTACCATG<br>R- AGTCGCAGTCCCAATAGTAAAG | 57 | Genomic | 180-223 | 170-218 (P) | NA | 173-189 (P) | 174-186 (P) | 164-218 (P) |
| 13. | bcqm 96 | (AGT)2(GT)8 | F-CATATCTCAATTTCTGTGTTCTTAGTAG<br>R-TATGTTAATGTGTTGGCAATCC | 57 | Genomic | 121-31 | 74-114 (P) | 72-132 (P) | 73-131 (P) | 73-137 (P) | 81-167 (P) |
| 14. | QM 33 GA1 | (GA)33 | F- TGGGTTGACATTTCATTTCG<br>R- GAAACGAATCATAAGGAGAG | 48 | Genomic | 103 | NA | NA | NA | NA | NA |
| 15. | QM 51 GA1 | (GA)17 | F-ATCACCTCAAAAACCTAAAC<br>R- TAGTGGGTCAGTGTGGTTAG | 48 | Genomic | 229 | NA | NA | NA | NA | NA |
| 16. | QM 55 GA | (GA)26 | F- ATTTGATATTTGATGTTGTTT<br>R- TGATGTAACATTGCTTAGAGT | 47 | Genomic | 306 | NA | NA | NA | NA | NA |
| 17. | QM 63 -2M3 | (TG)5 | F- TTATTGTATATGCTGGTTCT<br>R- GATTGAATAGGAAAAGAGTG | 47 | Genomic | 155 | NA | NA | NA | NA | NA |
| 18. | QM 58TGT | (GCC)5 | F- GGTCAGTGTATTTTGTTGGT<br>R- AAATGTATTTTGCTTGCTCA | 51 | Genomic | 212 | NA | NA | NA | NA | NA |
| 19. | QM 69-2M1 | (TGG)6 (CGG)(TGC)2 | F-CACAATCTGCCCACATCATC<br>R-GGATGGACGAAGAGAAAGAT | 53 | Genomic | 217 | NA | NA | NA | NA | NA |
| 20. | QM 67-3M1 | (CCA)3 (GGC)4(GGT)4 (CCC)(GGA) | F-TGGCTTATCCAATGTTTGTGATT<br>R-GCGTCGGTGGCGGCTTAGAGATT | 56 | Genomic | 164 | NA | NA | NA | NA | NA |

Table 1
Continued

| Sl. No. | locus name | Repeat motif | Primer sequences (5'-3') | Ta (°C) | SSR location | Expected product size (bp) | Observed product size (bp) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Qf* | *Qg* | *Ql* | *Qle* | *Qs* |
| 21. | QM 50-3M | (CCT)3 (CCG)(CCT)2 (CCA)(CCI)2 + (CCA)7 | F-CCCGATTTCCCTTCCCTGCT R-CGGGCTTTGGATACGGATTC | 58 | Genomic | 253 | NA | NA | NA | NA | NA |
| 22. | QM 57-3M | (GCC)5 | F-TGAGGAGGTTGGTGGAGAA R- ATGTGGAGGGCTAAGATTT | 53 | Genomic | 235 | NA | NA | NA | NA | NA |
| 23. | ssrQpZAG1/5 | (GT)5(GA)9 | F-GCTTGAGAGTTGAGATTTGT R-GCAACACCCTTTAACTACCA | 55 | Genomic | 160-190 | NA | NA | NA | NA | 180-210 (P) |
| 24. | ssrQpZAG 9 | (AG)12 | F-GCAATTACAGGCTAGGCTGG R-GTCTGGACCTAGCCCTCATG | 50 | Genomic | 182-210 | NA | NA | NA | NA | NA |
| 25. | ssrQpZAG 15 | (AG)23 | F-CGATTTGATAATGACACTATGG R-CATCGACTCATTGTTAAGCAC | 50 | Genomic | 108-152 | NA | NA | NA | NA | NA |
| 26. | ssrQpZAG 36 | (AG)19 | F-GATCAAAATTTGGAATATTAAGAGAG R-ACTGTGGTGGTGAGTCTAACATGTAG | 50 | Genomic | 210-236 | NA | NA | NA | NA | NA |
| 27. | ssrQpZAG 46 | (AG)13 | F-CCCCTATTGAAGTCCTAGCCG R-TCTCCCATGTAAGTAGCTCTG | 48 | Genomic | 192-222 | NA | NA | NA | NA | NA |
| 28. | ssrQpZAG 58 | (GA)34 | F-CTGCAAGATTCGGACAAGCAA R-TCTTTTTCCTAATCTCACCTG | 50 | Genomic | 150-210 | NA | NA | NA | NA | NA |
| 29. | ssrQpZAG 104 | (AG)16AT(GA)3 | F-ATAGGGAGTGAGGACTGAATG R-GATGGTACAGTAGCAACATTC | 50 | Genomic | 176-196 | NA | NA | NA | NA | NA |
| 30. | ssrQpZAG 110 | (AG)15 | F-GGAGGCTTCCTTCAACCTACT R-GATCTCTTGTGTGCTGTATTT | 48 | Genomic | 206-262 | NA | NA | NA | NA | 225 (M) |
| 31. | quru-GA-0C11 | (GA)15 | F-ATACCCAGCTCCCATGACCA R- TCCCCAAATTCAGGTAGTGT | 53 | Genomic | 190-222 | NA | NA | NA | NA | NA |
| 32. | quru-GA-0M07 | (GA)19 | F-TTTAGCATCACATTTCCGTT R-GCATCACATTTCCGTT | 45 | Genomic | 181-209 | NA | NA | NA | NA | NA |
| 33. | quru-GA-1C08 | (GA)29 | F-TCCCAATCGATGTTTGATAAGG R-GGGCTCTTGAGAGGATGTAGG | 50 | Genomic | 224-276 | NA | NA | NA | NA | NA |
| 34. | quru-GA-1M17 | (GA)19 | F-GTTTGTGCTTGCTGGGAGG R-TTCTTCTTAGCTTCCCAACTGAA | 53 | Genomic | 117-123 | NA | NA | NA | NA | NA |
| 35. | quru-GA-1i06 | (GA)23 | F-CAAGCTTCCACTGAGTCGTCGGT R-CTCTCGCTTTGATTTCACTCCCA | 58 | Genomic | 171-253 | NA | NA | NA | NA | NA |

Primers 1-11 derived from *Q. mongolica* (Ueno and tsumura, 2008; Ueno et al. 2008; Mishima et al. 2006); Primers 14-22 derived from *Q. myrinsifolia* (Isagi and suhandono, 1997); Primers 23-30 derived from *Q. petrea* (Steinkellner et al., 1997); and Primers 31-35 derived from *Q. rubra* (Aldrich et al., 2002 and 2003).

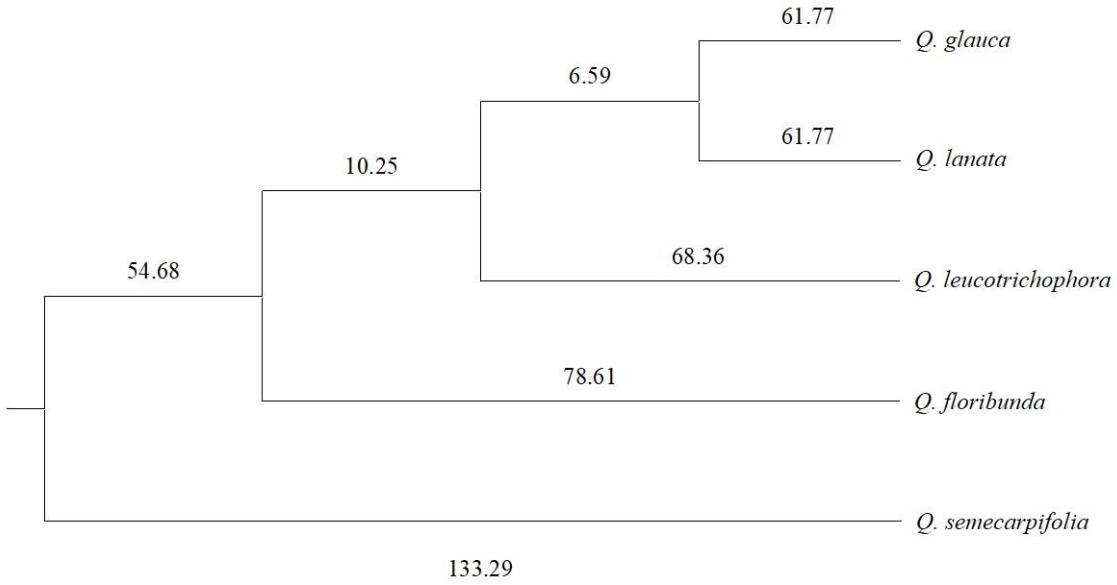



Figure 2
UPGMA method based phylogenetic relationship among five oak species of western Himalayan. Genetic distance is indicated by branch length for each species.

Table 2
Diversity measures calculated with polymorphic SSR markers in oaks of western Himalayas.

| Locus name | *Q. floribunda* | | | | *Q. glauca* | | | | *Q. lanata* | | | | *Q. leucotrichophora* | | | | *Q. semecarpifolia* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Na | Ho | He | PIC | Na | Ho | He | PIC | Na | Ho | He | PIC | Na | Ho | He | PIC | Na | Ho | He | PIC |
| CR627959 | 3 | 0.5 | 0.54 | 0.47 | 2 | 0.6 | 0.42 | 0.33 | 3 | 0.5 | 0.64 | 0.56 | 3 | 0.3 | 0.4 | 0.37 | 4 | 0.5 | 0.5 | 0.50 |
| DN950717 | 5 | 0.4 | 0.64 | 0.60 | 3 | 0.4 | 0.51 | 0.46 | 5 | 0.4 | 0.59 | 0.55 | 3 | 0.5 | 0.66 | 0.59 | 5 | 0.3 | 0.42 | 0.40 |
| DN950726 | 5 | 0.5 | 0.75 | 0.67 | 2 | 0.3 | 0.45 | 0.35 | 4 | 0.5 | 0.67 | 0.61 | 3 | 0.3 | 0.62 | 0.55 | 5 | 0.3 | 0.7 | 0.66 |
| bcqm76 | 6 | 0.6 | 0.81 | 0.78 | - | - | - | - | 4 | 0.5 | 0.73 | 0.68 | 3 | 0.6 | 0.66 | 0.59 | 10 | 1.0 | 0.87 | 0.86 |
| QmC00141 | 2 | 0.4 | 0.48 | 0.36 | 3 | 0.4 | 0.61 | 0.53 | - | - | - | - | 3 | 0.4 | 0.64 | 0.56 | 4 | 0.4 | 0.69 | 0.64 |
| QmC00419 | 6 | 0.6 | 0.78 | 0.75 | 7 | 0.6 | 0.79 | 0.77 | 4 | 0.5 | 0.68 | 0.63 | 8 | 0.6 | 0.84 | 0.82 | 10 | 0.7 | 0.84 | 0.82 |
| QmC00716 | 4 | 0.5 | 0.73 | 0.69 | 9 | 0.7 | 0.88 | 0.87 | 11 | 0.7 | 0.82 | 0.81 | 4 | 0.6 | 0.71 | 0.66 | 4 | 0.5 | 0.71 | 0.66 |
| QmC00898 | 6 | 0.7 | 0.77 | 0.74 | 5 | 0.7 | 0.62 | 0.57 | - | - | - | - | 4 | 0.6 | 0.53 | 0.49 | 6 | 0.9 | 0.74 | 0.70 |
| QmC01368 | 5 | 0.4 | 0.77 | 0.74 | 7 | 0.6 | 0.79 | 0.77 | 6 | 0.5 | 0.8 | 0.77 | 9 | 0.4 | 0.87 | 0.86 | 4 | 0.3 | 0.53 | 0.50 |
| QmC02269 | 5 | 0.7 | 0.71 | 0.67 | 4 | 0.7 | 0.69 | 0.65 | - | - | - | - | 3 | 0.6 | 0.5 | 0.44 | 13 | 0.8 | 0.91 | 0.91 |
| bcqm 96 | 4 | 0.7 | 0.51 | 0.45 | 7 | 0.8 | 0.74 | 0.71 | 7 | 0.6 | 0.81 | 0.79 | 6 | 0.6 | 0.72 | 0.68 | 12 | 1.0 | 0.88 | 0.87 |
| DN949770 | - | - | - | - | - | - | - | | - | - | - | | - | - | - | - | 9 | 0.6 | 0.87 | 0.86 |
| Mean | 4.6 | 0.54 | 0.68 | 0.63 | 4.9 | 0.58 | 0.65 | 0.60 | 5.5 | 0.52 | 0.72 | 0.68 | 4.4 | 0.5 | 0.65 | 0.60 | 7.16 | 0.6 | 0.72 | 0.70 |

The genetic relationship among five Himalayan oak species derived through the UPGMA clustering showed a pattern in accordance to their occurrence in the altitudinal gradient, where species sharing altitudinal ranges showed more genetic similarity (Figure 2). Two oak species, namely *Q. semecarpifolia* (2400 m to 3700 m amsl) and *Q. floribunda* (1800 m to 2700 m amsl) were clustered together which generally occupied the highest altitudinal range in the western Himalayas. While the other three oak species are dominant at the medium hills, in which *Q. glauca* is the first to be encountered (600-1500 m), which is followed by *Q. leucotrichophora* (1200 m to 2400 m amsl) and *Q. lanata* (1800 m to 2400 m amsl) (Negi and Naithani, 1995).

## Discussion

The Himalayas is a one of the key biodiversity hotspot of India encompassing about 10,000 plant species but still it is the most data-deficient regions of the world (Singh, 2018). Various studies have demonstrated its vulnerability against the changing climate (Srinivasan, 2006; Saran et al., 2010; Joshi et al., 2018). Despite the key components of Himalayan forest ecosystem, high socio-economic and ecological importance, Himalayan oaks are least investigated. The present study unveiled the first evidence for identification and validation of polymorphic SSR markers in Himalayan oaks. The markers identified herein offer an important resource for future genetic and phylogenetic analysis in Himalayan oaks. In total, 35 SSRs of European and Asian oaks were screened for their cross-amplification in five oak species of western Himalayas. Noticeably, the cross-transferability was very high for the genic EST-SSRs than the genomic SSRs developed through random genome sequencing. Vitally, the SSR transferability is mainly dependent on their genomic locations, i.e., genic (derived from transcribed region) or genomic (derived by random genome sequencing). Genic SSRs have more sequence conservation across the taxonomically related species than the anonymous genomic SSRs, and hence, these have more transferability (Zhang et al., 2005; Ellis and Burke, 2007) but are less polymorphic (Varshney et al., 2005). In contrary, the transferability of genomic SSRs is low but displayed high level of polymorphism. However, the markers located in genic-noncoding regions, viz., introns and untranslated regions (UTRs), could combine advantages of both genic and genomic SSRs and reveal good level of transferability and polymorphism (Lebedev et al., 2020). Out of the 35 SSR markers screened in the present study, 11 were derived from the ESTs and 24 from random genome sequencing. Consequently, the higher cross-transferability detected for the SSRs of *Q. mongolica* may be owed to their EST-based origin. Due to high sequence homology, the genic SSRs were suggested as best marker of choice to be used for comparative, phylogenetic and evolutionary studies (Varshney et al., 2005). In concurrence to the present study, the high cross-transferability of the EST-SSRs of *Q. mongolica* has also been demonstrated in *Q. serrata* Murray (90 %) and *Q. dentata* Thunb. (100 %) (Ueno et al., 2008). As expected, poor cross-transferability of genomic SSRs is also evident in the present study, though these markers were demonstrated to manifest varied level of transferability in other oaks. The genomic SSRs of *Q. petrea* were successfully transferred in five other *Quercus* spp., namely, *Q. cerris* L. (64 %), *Q. palustris* Münchh. (47 %), *Q. pubescens* Willd. (100%), *Q. robur* L. (100 %) and *Q. rubra* (47 %) (Steinkellner et al., 1997a). Out of the eight tested SSRs of *Q. petrea*, only two could be successfully amplified in one of the five studied oak species, namely *Q. semecarpifolia*, while no amplification was detected in other four species. Similarly, a varied level of transferability was recorded for nine genomic SSRs of *Q. myrinsifolia* in 8 *Quercus* spp., namely *Q. salicina* Blume (100 %), *Q. glauca* (100%), *Q. suber* L. (44.5 %), *Q. serrata* (77.8 %), *Q. aliena* Blume (22.3 %), *Q. acutissima* Carruth. (77.8 %), and *Q. phillyreoides* A. Gray (22.3 %) (Isagi and Suhandono, 1997), but the results could not be reproduced in our Himalayan oaks including *Q. glauca.* Besides, 30 genomic SSRs of *Q. rubra* were also tested for their cross-transferability in other related species, but only 12 (40 %) could be successfully amplified in the two *Quercus* spp. (*Q. petraea* and *Q. robur*), and five (16.7 %) were amplified in phylogenetically distant Chinese chestnut (*Castanea mollissima* Blume) (Aldrich et al., 2003); nevertheless, none of these tested markers could be amplified in Himalayan oaks.

The present study revealed high genetic diversity in the all five Himalayan oak species, which is either more or comparable to other associated tree species, viz. *Pinus roxburghii* (Ht = 0.746; Ginwal et al., 2010); *Taxus wallichiana* Zucc. (Ht = 0.419; Poudel et al., 2014), *P. kesiya* Royle ex Gordon (Ht= 0.638; Rai and Ginwal, 2018); *C. deodara* (Roxb.) G.Don (Ht = 0.66; Ginwal et al., 2020). The genetic diversity recorded in the Himalayan oaks (He = 0.65 to 0.72) was also similar to other oaks of the world, viz., 0.16 to 0.92 in *Q. myrsinifolia* (Isagi and Suhandono, 1997), 0.54 to 0.88 in *Q. mongolica* (Mishima et al., 2006), 0.643 to 0.82 in *Q. glauca* (Lee et al., 2006), 0.63 to 0.72 in *Q. serrata* (Kitamura et al., 2017), 0.575 to 0.834 in *Q. petraea* (Lupini et al., 2019), and 0.62 to 0.66 in *Q. rubra* (Pettenkofer et al., 2020). Overall, the high genetic diversity recorded herein indicates the ample evolutionary and adaptive potential of the Himalayan oaks against the environmental and ecological changes, which need to be maintained and conserved by minimizing ecological and anthropogenic factors causing population deterioration. Since, the measures estimated here were based on a limited number of samples per species, these could not be utilized for conservation implications, but are sufficient to get an approximate clue of the level of genetic diversity existed in studied oak species. Hence, the comprehensive population genetic analysis needs to be carried out in each individual species to demarcate the potential diversity hotspots and apply the appropriate conservation measures. All the five oak species are dominantly growing as pure stands in their altitudinal range, but the habitats are often overlapping at the transitional zone. Furthermore, oaks are wind-pollinated and the chances of interspecific hybridization are more at the overlapping zone (Bacilieri et al., 1996; Borazan and Babaç, 2003; Petit et al., 2004; Charalambos et al., 2011). Also, the natural recombinants

were reported in the populations of *Q. leucorichophora* and *Q. floribunda* at Dalhousie hills of Himachal Pradesh in western Himalayan region (Parker, 1939) and in the populations of *Q. semecarpifolia* and *Q. baloot* at Sarswag, Kurram, Pakistan (Nasir, 1976). In such cases, EST-SSR markers identified in the present study could be highly valuable for analyzing genetic structures of the populations located at transition zones and delineation of putative hybrids.

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