Genetic variation in seabuckthorn (*Hippophae rhamnoides* L.) populations of cold arid Ladakh (India) using RAPD markers

Seabuckthorn is one of the major plants of the Ladakh region, found in four of the five valleys. Seabuckthorn is locally called as 'Tsermang' and the fruits are called as 'Tsestalullu'. It is generally found in the altitude range of 8000–13500 ft amsl. Ladakh is a old desert in the Trans Himalayan range of 32°N to 76°E. The region is characterized by extreme climatic conditions like high wind velocity, high rate of soil erosion, and extreme temperatures, i.e. –30°C in winter to +35°C in summer. Seabuckthorn is generally found in the cold and hilly areas of Ladakh in Jammu & Kashmir, Lahaul-Spiti in Himachal Pradesh and parts of Arunachal Pradesh and Sikkim. Fruits of seabuckthorn are a rich source of vitamins like A, B_1, B_2, C, D, E and K. Its leaves are also a rich source of protein and antioxidants. Its fruits are now utilized for making juices and other food products. Its oil has anti-radioactive and UV-protective effect. Thus seabuckthorn is a multipurpose plant of Ladakh. Its natural population varies in colour, shape, size of fruits, leaves, thorns, etc. and thus provoked the present study. We report in the present study genetic variability as revealed by RAPD in 17 morphotypes found in Nubra valley of Ladakh.

Documenting the nature of genetic variability and its magnitude among natural populations of seabuckthorn (*Hippophae rhamnoides*) is necessary to know its status in Ladakh. Availability of reliable polymorphic markers often limits accurate estimation of genetic variation among individuals or different populations. The extent of genetic variation among geographical populations depends upon several factors[1,2], including gene flow between populations and various other factors. Usual DNA-based techniques such as RFLP and PCR-based Random Amplified Polymorphic DNA (RAPD) have been handy and convenient alternative techniques for investigations of genetic variation and genome mapping[3–5]. Because of the nature of the primer sequences, RAPD analysis samples the genome more randomly than other methods and has been successfully employed in the construction of linkage maps[6–9]. Being simple and non-radioactive, the technique is quite sensitive and used to detect genetic variation in several living things. It has been extensively used for molecular fingerprinting and population diversity analysis[10–12]. The variations that can be accounted for, between and within populations through RAPD, appear to be numerous. Yet, the dominant nature of these markers is a greater leveller and introduces subjectivity in understanding the structure of populations, where allelic frequencies of the gene matter. Further, cyclic amplification of DNA being an extremely powerful technique, RAPD patterns are protocol-sensitive, which limits cross-comparison of information generated by this method with others.

Seabuckthorn is one of the major plants of Ladakh region, found in four of the five valleys. Its natural population varies in colour, shape, size of fruits, leaves, thorns, etc.[13]. We report here genetic variability as revealed by RAPD in 17 morphotypes found in Nubra valley. Young leaves were collected during August and genomic DNA was isolated using C-TAB method[14]. DNA was further purified by phenol–chloroform–isoamyl alcohol treatment. DNA was diluted to 40 ng/µl before being utilized in PCR reactions. A set of 20 random decamer primers selected randomly from OPA and OPD kits were tested across all 17 morphotypes. PCR was carried out with each primer in 25 µl standard reaction mixture consisting of 40 ng of template DNA, 0.2 mM primer, 3.5 mM magnesium chloride, 1X Bangalore Genei buffer. PCR amplification was carried out on Eppendorf master gradient cycler with hot lid facility. PCR started with a 2 min initial denaturation at 94°C followed by 40 cycles of 1 min at 94°C for denaturation, 1 min at 36°C for annealing, 2 min at 72°C for extension and ended with a final 10 min extension at 72°C. These reaction products were kept overnight at 4°C prior to electrophoresis on 1.2% agarose gel at 5 V/cm for 3 h, which resolved DNA fragments ranging from 100 bp to 1 kbp. All the 20 primers were tested at least twice for reproducibility of banding pattern. Out of 20 decamer primers used for RAPD analysis, 13 primers, viz. OPA-01, OPA-02, OPA-04, OPA-05, OPD-01, OPD-02, OPD-04, OPD-05, OPD-06, OPD-08, OPD-10, OPD-13, and OPD-15 producing reproducible banding patterns were selected for the present study. All the bands in the range of resolution were scored, except a few faint and ghost bands. The gel pictures acquired through gel documentation system into the computer were processed and scored to obtain binary data. The presence/absence data (1, 0) matrix was analysed using the standard procedure in NTSYS Pc2 package. The genetic distance[15] or similarity was determined by Jacquard similarity[16,17].

All the 13 gels resulting from short-listed primers had maximum number of clear and scorable amplicons in each DNA sample with few ghost or minor bands, which were ignored. A sample gel resulting from OPA-04 primer showing the banding pattern of all 17 morphotypes in the natural population of seabuckthorn is presented in Figure 1. A total of 265 amplicon levels were produced by 13 primers available for analysis. The highest number of 32 amplicon levels was produced by OPA-5 followed by 28 in OPD-

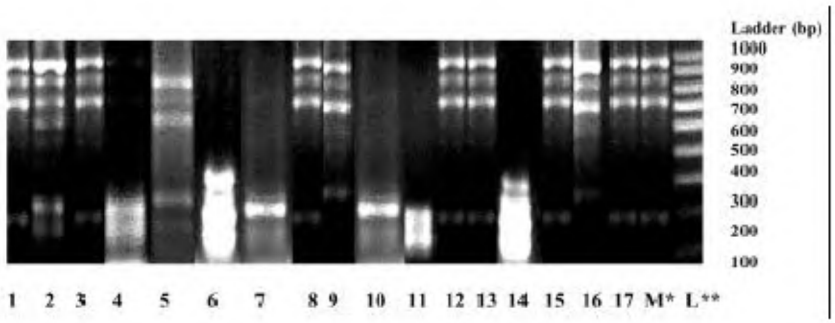

Figure 1. PCR-amplified genomic DNA of seabuckthorn morphotypes using OPA-04 random primer. *Male plant; **Ladder (100 bp).

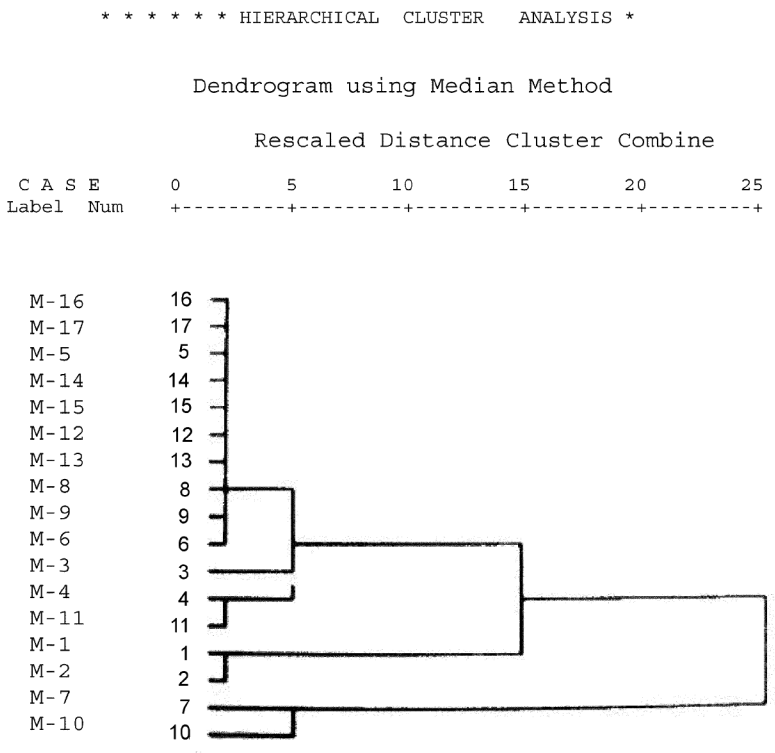

Figure 2. Dendrogram showing diversity of natural populations of *Hippophae rhamnoides* in Ladakh.

10 and the least of eight marker levels was produced by OPD-13. There were 18.86 amplicon levels per primer, of which 16.80 were polymorphic indicating higher variability among *H. rhamnoides* population. Out of 20 primers selected for the study, 13 produced banding pattern that could differentiate all the morphotypes growing naturally. Polymorphism revealed by RAPD markers serves as a dominant Mendelian marker. Population similarity coefficient matrix based on shared banding pattern shows wide differences. RAPD banding pattern was compared to assess genetic variability at DNA level. Considerable differences in banding pattern were observed. Germplasm characterization using RAPD will be useful in assessing genetic diversity among natural populations of seabuckthorn.

In the present study RAPD polymorphisms were analysed with a phenatic distance measure (Jacquard coefficient) from which a dendrogram was constructed using median method, providing an indication of diversity present within the *H. rhamnoides* population (Figure 2). Clustering analysis clearly showed five major groups in the natural population of *H. rhamnoides*. Overall within a similarity coefficient in the range of 0.0 to 0.25, sub-clustering is in agreement with the geographical proximity. One of the important observations of the study is that none of the morphotypes showed a similarity more than 25%, indicating high level of genetic difference between morphotypes. Genetic similarity among natural populations from the data was within a similarity coefficient ranging from 0.0 to 0.25. None of the population compared to one another lying on a similarity coefficient equivalent to 0.25. Population similarity coefficient matrix based on shared banding pattern shows wide differences. The RAPD analysis of all the 17 morphotypes revealed low level of genetic distance suggesting high level of gene flow. The high genetic variability will help species to evolve. Genetic variability in the natural seabuckthorn population indicates the presence of subspecies in the region, which needs to be confirmed by taxonomists.

1. Hart, D. L., In *Principles of Population Genetics*, Associates Inc, Sunderland, MA, USA, 1980.
2. Templeton, A. R., Shaw, K., Routman, E. and Davis, S. K., *Ann. Mo. Bot. Gard.*, 1990. **77**, 13–27.
3. Baker, H. G. and Stebbins, G. L. (eds), *The Genetics of Colonizing Species*, Academic Press, New York, 1965.
4. Welsh, J. and McClelland, M., *Nucleic Acids Res.*, 1990, **18**, 7213–7218.
5. Williums, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
6. Grattapaglia, D. and Sederoff, R., *Genetics*, 1994, **137**, 1121–1137.
7. Sobral, B. W. and Honeycutt, R. J., *Theor. Appl. Genet.*, 1993, **86**, 105–112.
8. Postlethwait, J. H. *et al.*, *Science*, 1994, **264**, 699–703.
9. Reiter, R. S., Williums, J. G. K., Fieldman, K. A., Rafalski, J. A., Tingey, S. V. and Scolink, P. A., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 1477–1481.
10. Dawson, I. K., Chalmers, K. J., Waugh, R. and Powell, W., *Mol. Ecol.*, 1993, **2**, 151–159.
11. Chapco, W., Ashton, N. W., Martel, R. K. B., Antonishyn, N. and Crosby, W. L., *Genome*, 1992, **35**, 569–574.
12. Kambhampati, S., Black, IV W. C. and Rai, K. S., *J. Med. Entomol.*, 1992, **29**, 939–945.
13. Singh, R., Dwivedi, S. K. and Mishra, S. N., *Phytomorphology*, **55**, 123–132.
14. Doyle, J. J. and Doyle, J. L., *Phytochem. Bull.*, 1987, **19**, 11–15.
15. Lynch, M. and Milligan, B. G., *Mol. Ecol.*, 1994, **3**, 91–99.
16. Virk, P. S., Brain, V. F. L., Jackson, M. T. and Newbury, J., *Heredity*, 1995, **74**, 170–179.
17. Hedrick, P., In *Genetics of Population*, Jones and Bartlett, CA, USA, 1985, pp. 72–73.

Received 5 December 2005; accepted 21 August 2006

RANJIT SINGH[1,*]
S. N. MISHRA[2]
S. K. DWIVEDI[1]
Z. AHMED[1]

[1]*Field Research Laboratory (DRDO), C/O 56 APO, Leh, Ladakh 194 101, India*
[2]*Department of Biosciences, M. D. University, Rohtak 124 001, India*
**For correspondence.*
e-mail: sangwanranjit@rediffmail.com