Genetic Polymorphism in Inter Population Variation of *Podophyllum hexandrum* Royle- an Endangered Medicinal Plant of Himalaya, India

Vinay Prakash[1] and Hemlata Bisht[2],
Department of Seed Science and Technology[1],
Department of Botany[2]
Chauras Campu, HNBG (A Central University) Srinagar Garhwal, 246 174, Uttarakhand, India
hvnautiyal@gmail.com

Abstract: Variation in respect of isoenzyme and polypeptides were analysed in seeds of *Podophyllum hexandrum* having two and three leaves. The material was collected from two populations one growing at Harkidun (3000m) Distt. Uttarakashi and the other in Valley of Flowers (3300m) Distt. Chamoli] and cultivated at lower altitude Pothivasa (2200m) Distt. Rudraprayag of Uttarakhand. Several low molecular polypeptides were prominent in all the populations. While the presence of high molecular weight polypeptides were observed only in naturally grown population having three leaves. Some specific bands of isoenzyme were observed in population cultivated at lower altitude. It may reflect the presence of some thermolabile proteins. To overcome the harmful effect of some toxic compounds liberated during the acclimation at lower altitude, plants may increase the production of an enzyme and protein. [Report and Opinion 2010;2(7):55-58]. (ISSN: 1553-9873).

Keyword: Podophyllum hexandrum, population, genetic variation, polypeptide, isoenzyme

Introduction

Podophyllum hexandrum Royle vernacular name Bankakri commonly known as Himalayan May apple of family Podophylaceae is one of the critically endangered (Gupta and Sethi, 1983) medicinal species of Himalayan highlands. It is a perennial, rhizomatous herb distributed widely in the Himalayan zone ranging from 2000 to 4000m altitudes. The underground parts of this species contain Podophyllotoxin, which is used for the treatment of lung cancer and tumors (Jackson and Dewic, 1984 a and b). Indian species of *Podophyllum* contains higher levels of Podophyllotoxins as compared to the American species *P. peltatum* (Fay and Ziegler, 1985, Jackson and Dewic, 1984 a & b). This species has greatly declined in nature due to its high demand and unskilled overexploitation. Seed germination and seedling establishment is very poor under natural conditions. Some populations of this species have disappeared from nature (Bhadula et al., 1996). Out of 214 Himalayan endangered species, *P. hexandrum* is also included in a list of 37 requiring priority attention for conservation (Khoshoo, 1993). Populations of this plant show considerable variation in morphological characteristics, i.e. plant height, number, size and shape of leaves, size of fruits, number of seeds per fruit and seed size and colour etc.. Pattern of isoenzymes and polypeptides gave a reproducible pattern and showed qualitative and quantitative differences among and within populations, respectively (Lata, 1997). As reported earlier, both resin and toxin contents were highest in plants with one leaf and followed by two, three and least in four leaf plants (Purohit et al., 1999).

In view of the above, the present investigation was carried out to assess the genetic polymorphism in seeds of *P. hexandrum* having two and three leaves. Such studies may be of use in finding out the correlation in molecular configuration of proteins and isoenzymes with changes in leaf number growing naturally at different sites and during acclimatization at lower altitude. The resultant information might prove useful in understanding the mechanism of adaptation in plants at lower altitude. Since in general, endangered and rare species show low levels of morphological or genetic diversity, it is important to score variations among the populations to determine polymorphism at the biochemical or genetic level to develop appropriate conservation strategies.

Material and Methods

Seeds of *Podophyllum hexandrum* showing variability in leaf number viz. two and three leaves were collected from their natural habitat viz. Herkidun (3000 m) district Uttarkashi and Valley of Flowers (3300 m) district Chamoli and from cultivated population in field nursery at Pothivasa (2200 m) district Rudraprayag of Uttaranchal, India. Seeds of one and four leaved *P. hexandrum* could not be collected due to rare presence of such plants. Seeds

were washed properly, dried and homogenized with 0.1 M Tris-HCl [{2- amino-2- (hydroxymethyl) propane-1, 3-diol}- hydrochloric acid] buffer pH 7.5 with 0.5% β-mercaptoethanol and 1% PVP (Polyvinylpyrollidone). To minimize proteolytic activity a pinch of protease inhibitor PMSF (Phenylmethylsulphonyl fluoride) was used at the time of grinding. Homogenates were centrifuged at 10,000 rpm for 30 min. and Supernatant was used subsequent analysis.

The method described by Bradford (1976) was adopted for quantitative estimation of proteins to load the equal amount of protein in PAGE (Polyacrylamide Gel Electrophoresis). Polypeptides were separated by SDS-electrophoresis using the method of Laemmli (1970). Standard proteins (SIGMA) of molecular weight ranging from 94.0 KD to 14.3 KD were loaded in the gel along with the sample (5μg in each). Gels were stained with silver nitrate by following the method of Wray et al. (1981).

To observe the isoenzyme variation Native electrophoresis (7.5% Polyacrylamide slab gel) was used. The gels were run at constant current of 10 mA and after completion of run of the gel, stained individually for peroxidase, esterase and acid phosphatase isoenzymes. Peroxidase isoenzyme appeared by incubating the gel in 0.2 M acetate buffer pH 5.0 for ten minutes and then kept the gel in 50 ml 0.2 M acetate buffer pH 5.0 having O-diansidine (8 mg) dissolved with one ml N-N dimethylformamide and 0.02 ml H_2O_2 (30%). H_2O_2 (30%) was added in staining solution just prior to use. Esterase bands appeared by incubating the gels in 100 ml of 0.1M phosphate buffer (pH 7.4) containing 80 mg fast blue RR salt, 40 mg α-nepthyl acetate and incubated at 25^0C till the resolution of the bands. Acid phosphatase showed the band after incubating the gel in 100 ml acetate buffer pH 5.0 containing 100 mg fastblue RR salt and 250 mg polyvinylpyrolidone, 1 gm NaCl, 100 mg α-nephthyl phosphate, 10 drops of 10% $MgCl_2$ were added just prior to use and incubated at 37^0C (Brewbaker et al., 1968).

Results and Discussion

Several isoenzyme/alloenzyme systems have long been used in the analysis of genetic diversity and identification of species, populations, cultivars and mutants (Godt & Hamrick, 1995). Polypeptide patterns and isoenzymes analysis have been very useful in crop improvement and conservation of genetic diversity. The degree to which genetic variations allow a species to tolerate or to adapt to environmental changes depends on positive associations between natural and adaptive genetic variation.

The extent of isoenzyme polymorphism and its genetic basis were analyzed in three populations of *Podophyllum hexandrum* by selective enzyme staining following PAGE. Distinct zones of isoenzyme activity were detected on the gels based on the patterns of electrophoretic variations. In some cases the patterns of isoenzyme were specific in respect to population and number of leaves.

Several bands of polypeptide were commonly present in all the populations, some polypeptides however, appeared to be specific to a population. The polypeptide of higher molecular weight showed low intensity of stain in comparison to low molecular weight polypeptides in all the populations. All the populations showed the supremacy of bands of low molecular weight in contrast to bands of other molecular weight. Maximum number of high molecular weight bands were only appeared in naturally grown population having three leaves as compared to two leaves and even in both the variants, cultivated at lower altitude.

Population having two or three leaves was similar with respect to their electrophoretic mobility and intensity of staining of peroxidase isoenzyme bands except in the population of three leaved from Pothivasa (2200 m). It may possibly due to the presence of some thermolabile enzymes that disappear during the acclimation at lower altitude.

Population of *P. hexandrum* from Valley of Flowers having two and three leaves showed dark intensity of bands of esterase followed by the population from Herkidun and those cultivated at Pothivasa. Population from their natural habitat showed some specific esterase bands, which did not appear in population cultivated at lower altitude (2200 m). It is concluded that the intensity of esterase bands decreased with the decreasing altitude and presence of some specific bands might be a result of microclimatic changes at lower altitude. Esterase isoenzymes are excellent markers of different populations and in most cases indicate intrapopulation variants (Bhadula et al. 1996)

Bands of acid phosphatase showed similarity with respect to their electrophoretic mobility in all the population but showed dark intensity of staining in the population having two leaves. Some specific high molecular acid phosphatase bands were appeared in all the two leaved populations. In the seed sample from low altitude, this isoenzyme showed similarity with the sample from high altitudes and has shown thermostability of acid phosphatase and thus reflects an adaptive step towards high temperature acclimation at lower altitude. This support the conclusion arrived at by Bhadula et al., 1986. Thermostability is an

indirect indicator of conformational flexibility of molecules and increase in thermostability during high temperature acclimation has been suggested to be an adaptive shift has also been reported by Bhadula et al., 1986.

Isoenzyme characterization is useful for examining the degree of genetic diversity and relationship within and among species. Barone et al. (1996) used esterase, peroxidase and acid phosphatase isoenzymes to identify *Pistacia vera* L. germplasm. Thus, unlike other rare and endangered species which show a low level of genetic diversity (Karron, 1991), *P. hexandrum* may have atleast an average level of genetic diversity as appeared from its considerable polymorphism, also support the data of Lata (1997).

The study of protein profile and isoenzymes revealed substantial information on genetic and phytogeographical variability among many closely related species (Bhadula et al. 1981 and Purohit et al. 1983). In Western Himalaya, two population of *P. hexandrum* have been charecterised for their inter-intra population genetic variation using RAPD analysis (Sharma et al, 2000). Lata et al. (2002) studied to find out the suitable genetic marker for the assessment of genetic diversity of *Podophyllum peltatum.* Plants have the capacity to produce several or many isoenzymes and it seems likely that these have different selective advantages in different environments. It is commonly stated that protein characters are by their complexity and close relationship with the genetic material, immune, either partially or wholly, to environmental modifications. Protein characters are probably exceptionally sensitive to selection pressure. Some polypeptides and isoenzymes appeared to be specific to a population and it may point towards genetic polymorphism. Several isoenzymes including esterase have also been used in the analysis of genetic diversity of endangered species (Godt and Hamrick, 1995). Genetic diversity among the 28 genotypes of *P. hexandrum* distributed in 11 geographical region from Himachal Pradesh, India was analyzed using RAPD markers (Alam et al, 2009).

Conclusion

Adaptation to change environment is a characteristic feature of alpine plants. The evolutionary history, power of dispersal, interaction with other species and the abiotic features in its environment are the source data to know about the distribution of a species. Organisms may alter their behaviour, morphology and physiology to accommodate the new conditions during their adaptation. Gray (1989) has suggested that communities with a high proportion of ecotypes may be characteristic of disturbed habitats and an identification of population facing long term stress. High temperature during acclimation at lower altitude may denature the structure and function of protein that may become the dangerous for metabolic activity. The appearance or disappearance of some polypeptides and enzymes at a specific habitat observed in the present study may be due to prolonged stress as a result of habitat disturbance. It can be concluded that the reduction of its population size in nature is mainly due to overexploitation. By having a considerable degree of polymorphism in this species, can grow in *in-situ* condition, which will help in conservation of gene pool resources of this species and also protect the natural habitat. The population that did not show genetic diversity among or within population may be more sensitive to extinction due to reduction of population growth rates.

Corresponding Author:
Dr. Vinay Prakash
Seed Science and Technology Department
HNBG (A Central University), Srinagar Garhwal
Uttarakhand, 246174, India
Phone:91-9456156768
E-mail: hvnautiyal@gmail.com

References

[1] Alam Md A, Gulati P, Gulati A K, Mishra G P and Naik P K. Assessment of genetic diversity among *P. hexandrum* genotypes of the North-Western Himalayan region for podophyllotoxin production. Indian Journal of Biotechnology. 2009; 8(4): 391-399.

[2] Barone E, Marco L D, Marra F P and Sidari M. Isoenzyme and canonical discriminate analysis to identify Pistachio (*Pistacia vera* L.) germplasm. Horti. Sci. 1996;31 (1): 134-138.

[3] Bhadula S K, Pandey O P and Purohit A N. Thermostability of acid phosphatase in *Selinum vaginatum* Clarke and *Acer caesium* Wall. grown at low and high altitudes. Biologia Plantarum. 1986; 28(6): 465-469.

[4] Bhadula S K, Singh A, Lata H, Kuniyal C P and Purohit A N. Genetic resources of *Podophyllum hexandrum* Royle, an endangered medicinal species from Garhwal Himalaya, India. Plant Genetic Resources News Letter. 1996; 106: 26-29.

[5] Bhadula S K, Thapliyal A P and Purohit A N. Seed proteins, esterases and acid phosphatases in *Rhododendron* species from different altitudes in Garhwal Himalaya. Indian J. Exp. Biol. 1981; 19: 199.

[6] Bhadula S K, Khanduri S K, Pandey O P and Purohit A N. Some metabolic modifications in plant species when grown above the timber line. Plant Physiol. Biochem.1982; 9(2): 88-93.

[7] Bradford M M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Ann. of Biochemistry. 1976; 72**:** 248-254.

[8] Brewbaker J L, Upadhyay M D, Makinam Y and Mac Donald T. Physiol. Plant. 1968; 21**:** 930.

[9] Fay D A and Ziegler H W. Botanical source differentiation of *Podophyllum* resin by high performance liquid chromatography. J. Liq. Chrom. 1985; 8**:** 1501-1506.

[10] Godt M J W and Hamrick J L. Allozyme variation in two great Smoky Mountain endemics**:** *Glyceria nubigena* and *Rugelia nudicaulis*. J. Hered. 1995; 86: 194-198.

[11] Gray J S. Effect of environmental stress on species rich assemblages. In: Evolution, ecology, and environmental stress. eds. P. Calow and R. J. Berry. Academic press, London. 1989; 19-32.

[12] Gupta R. and Sethi K L. Conservation of medicinal plant resources in the Himalayan region. In: Conservation of tropical plant resources. Eds. S. K. Jain and K. L. Mehra. Botanical survey of India, Howarh. 1983; 101-107.

[13] Jackson D E and Dewic P M. Biosynthesis of *Podophyllum* lignans-I. Cinnamic acid precursors of podophyllotoxin in *Podophyllum hexandrum*. Phytochemistry. 1984 a; 23**:** 1029-1035.

[14] Jackson D E and Dewic P M. Arylation lignans from *Podophyllum hexandrum* and *Podophyllum peltatum*. Lignans-II. Phytochemistry. 1984 b; 23**:** 1147-1152.

[15] Karron J D. Patterns of genetic variation and breeding systems in rare plant species. In: Genetics and conservation of rare plants. eds. D A Falk and K E Holsinger. Oxford University Press, New York. 1991; 87-98.

[16] Khoshoo T N. Himalayan biodiversity conservation- an overview. In: Himalayan Biodiversity: Conservation strategies. ed. U Dhar. Himvikash Publication no. 3, Gynodaya Prakashan, Nainital. 1993; 5-35.

[17] Laemmli U K. Cleavage of structural proteins during the assembly of head of Bacteriophage T4. Nature. 1970; 227**:** 680-685.

[18] Lata H. Biochemical characterization and micropropagation of different populations of an endangered medicinal herb *Podophyllum hexandrum* Royle. D. Phil. thesis, H. N. B. Garhwal University, Srinagar Garhwal, Uttaranchal. 1997.

[19] Lata H, Moraes R M, Douglas A and Scheffler B E. Assessment of genetic diversity in *P. peltatum* by molecular markerp. 537-544. In: J. Janick and A. Whipkey. eds. Trends in new crops and new uses. ASHS Press, Alexandria VA. 2002.

[20] Purohit A N, Raturi R P, Thapliyal A P and Gaur R D. Seed proteins, esterases and acid phosphatases in alpine and temperate *Potentilla* species. Plant Physiology and Biochemistry. 1983; 10(1): 52-59.

[21] Purohit M C, Bahuguna R, Maithani UC, Purohit A N and Rawat M S M. Variation in podophylloresin and podophyllotoxin contents in different populations of *Podophyllum hexandrum*. Curr. Sci. 1999; 77(8): 1078-1080.

[22] Sharma K D, Singh B M, Sharma T R, Katoch M and Guleria S. Molecular analysis of variability in *P. hexandrum* Royle-an endangered medicinal herb of north Western Himalaya. Plant Genetic Resources News Letter. 2000; 124: 57-61.

[23] Wray W T, Boulicas T, Wray V P and Hancock R. Silver staining of proteins in polyacrylamide gels. Annals of Biochemistry. 1981; 118**:** 197-203.

Submission: 7th July 2010