*Indian J. Plant Physiol.*, Vol. 12, No. 3, (N.S.) pp. 246-252 (July-Sept., 2007)

# BIOCHEMICAL AND ELECTROPHORETIC VARIABILITY AT POPULATION LEVEL IN TWO *ANGELICA* SPECIES FROM GARHWAL HIMALAYA

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Received on 25 Sept., 2006, Revised on 7 Sept., 2007

## SUMMARY

**Biochemical and isoenzyme patterns, in rhizomes of *Angelica glauca* Edgew. and *Angelica archangelica* Linn. were studied in different populations collected from Garhwal Himalaya. In general both species showed much variation in soluble protein, carbohydrate and total free amino acid content. Isoenzyme pattern for different enzymes also varied greatly among different populations of both *Angelica* species. In *Angelica glauca* populations such as VF, KN, RN and GH showed dark intensity bands while in *Angelica archangelica*, RN and PK populations showed dark intensity bands in all the enzymes studied.**

***Key words:*** **Electrophoresis, genetic diversity, isoenzymes**

## INTRODUCTION

Genetic diversity among the populations can also be assessed by using the isoenzyme variability. Management studies and strategies for conservation of endemic species are enhanced by the proper understanding of level and distribution of genetic variation within the species (Templeton 1991, Godt and Hamriek 1995). In many cases the correct identification of the plant species may be difficult, so for over coming this problem electrophoretic techniques may help in identifying the cultivars properly. Barone *et al.* (1996) used the electrophoretic techniques for identifying the different germplasm of Pistachio. Electrophoretic analysis of proteins and isoenzymes offers an efficient and cost effective method towards evaluation of geographical and taxonomic distribution of genetic variation for sampling strategies in germplasm conservation (Brown 1978).

*Angelica glauca* Edgew. (Choru, Gandhrayan) belonging to family Apiaceae is a glabrous rhizomatous, aromatic herb found between 1,800-3,700 m in Western Himalaya. Roots of *A. glauca* are used as a spice or condiment, and also as a drug. The odour is characteristically aromatic and pungent with a sweet bitter taste (Anonymous 1985). Roots can be collected and dried during Sept. - October. The root is considered as cardio active and stimulant, carminative, expectorant, diaphoretic and cordial; it is also useful in constipation. The powdered root is administered with warm water in stomach ailments of children; it also checks vomiting. On steam distillation the roots yield a pale to brownish yellow essential oil.

*Angelica archangilica*, Linn. (Rickchoru) is an aromatic, stout, perennial herb found at an altitude of 2600-3900 m in Garhwal Himalaya. The herb, including the fruits and roots, is used for flavoring, and is reported to posses carminative properties. The leaf stalks are employed in confectionery. The root is aromatic and is reported to posses diaphoretic and diuretic properties, and is used in flatulent colic. It is sometimes applied externally as a counter-irritant. On steam distillation, the roots yield a light greenish yellow essential oil (0.35-1.0%, dry basis) having strong aromatic odour. The oil has not yet been commercially exploited in India. In Europe, the

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essential oil from *A. archangelica* is employed in liquors, dental preparations and in high-grade perfumery to impart a musky note, which cannot be distinguished easily from that of true musk. The root and seeds are reported to have mild antiseptic properties and depressant action on the central nervous system (Anonymous 1985). Despite having such importance, studies on the variability of these species at population level are scanty. Therefore, the present work is aimed at to study the variation at biochemical and electrophoretic level among different populations of *Angelica glauca* and *A. archangelica* from Garhwal Himalaya**.**

## MATERIALS AND METHODS

Rhizomes of *Angelica glauca* and *A. archangelica* used for analysis were collected from different alpine and sub-alpine habitats namely Tungnath - TN **(**3010 m**),** Panwalikantha - PK (3110 m), Rudranath - RN (3490 m), Kedarnath - KN (3850 m), Valley of Flowers – VF (3010 m), Dayara - DR (3380 m), Kuwaripass - KP (2950 m), Bharnala -BH (2650 m) and Ghese - GH (2450 m). However, *A. archangelica* was not found in last two habitats. Fresh rhizomes of different populations were used for biochemical and isoenzyme analysis.

***Biochemical analysis:*** The method described by Bradford (1976) was used for quantitative estimation of proteins. Soluble sugars and starch were estimated following the method described by Mc Cready *et al*. (1950). Total free amino acids were estimated according to the method of Moore and Stein (1954).

***Isoenzyme analysis:*** Isoenzymes were characterised on 10% poly acrylamide gels. The gels were run at constant current of 40 mA in a BIO-RAD Mini PROTEAN II electophoresis appratus at $4^0$C. Isoenzymes of esterase (Bhadula and Sawhney 1987), acid phosphatase and peroxidase (Davis 1964) are seperated electrophoretically and detected by method of Welter (1982).

***Cluster analysis:*** The banding patterns observed after enzyme electrophoresis were compared among the different populations of both the species. Different patterns occurring in each zone of activity were scored as discrete variables using "1" to indicate the presence and "0" to indicate the absence of a unique pattern. A dendrogram depicting the degree of relationships among the populations were produced on the basis of cluster analysis based on Nei's (1978) unbiased genetic distance identity using unweighted pair group mean analysis (UPGMA).

## RESULTS

***Biochemical variability:*** The total soluble protein content was maximum (63.80mg/g fm) in the rhizomes of GH population of *A. glauca*, followed by VF (59.23mg/g fm), RN (56.39mg/g fm), KN (44.97mg/g fm), DR (40.24mg/g fm), TN (31.86 mg/g fm), PK (31.28mg/g fm), BH (29.62 mg/g fm) and minimum in KP (20.80mg/g fm) population (Fig. 1). In *A. archangelica* the total soluble protein content was observed maximum (46.36mg/g fm) in RN followed by KP (36.26mg/g fm), KN (26.40mg/g fm), PK (23.13mg/g fm), TN (22.76mg/g fm), VF (20.53mg/g fm) and minimum in DR (19.80mg/g fm) population.

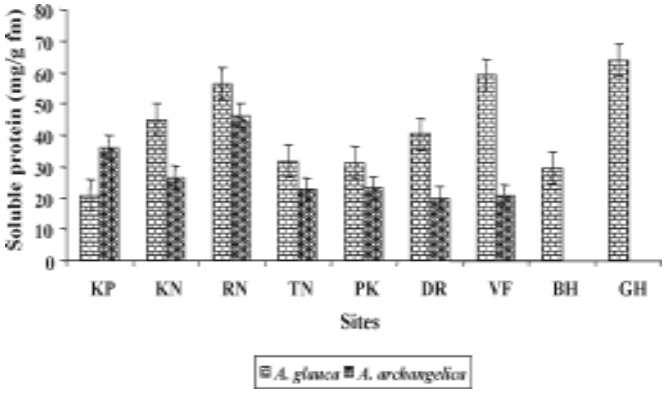


**Fig. 1. Soluble protein content in different populations of *Angelica glauca* and *A. archangelica* (Details of sites given in text)**

The total soluble sugar content in the rhizomes of *A. glauca* and *A. archangelica* are shown in Fig. 2. Maximum soluble sugar content was in GH (74.00mg/g fm), followed by VF (71.08mg/g fm), TN (62.72mg/g fm), DR (49.36mg/g fm), PK (43.31mg/g fm), RN (37.38mg/g fm), BH (34.47mg/g fm), KP (24.30mg/g fm) and minimum in KN (19.90 mg/g fm) population of *A. glauca.* Whereas, in *A. archangelica* the total soluble sugar content was maximum in PK (51.62mg/g fm), followed by the VF (35.73mg/g fm), KP (30.34mg/g fm), KN

(29.4mg/g fm), TN (25.99mg/g fm), DR (20.82mg/g fm) and minimum in RN (20.68mg/g fm) population.

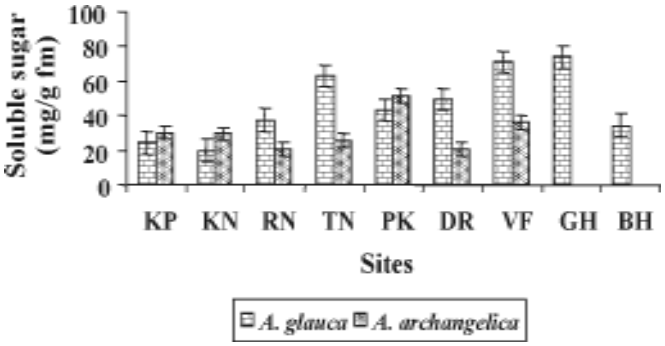


**Fig. 2. Soluble sugar content in different populations of *Angelica glauca* and *A. archangelica* (Details of sites given in text)**

Maximum starch content in the rhizomes of *A. glauca* was recorded in VF (33.32mg/g fm), followed by BH (32.27mg/g fm), PK (28.09mg/g fm), KN (22.65mg/g fm), RN (20.28mg/g fm), GH (18.46mg/g fm), TN (18.26mg/g fm) DR (16.45 mg/g fm) and minimum in KP (16.34mg/g fm) population (Fig. 3). Whereas, in *A. archangelica* the starch content was maximum in KP (68.27 mg/g fm), followed by KN (65.33mg/g fm), PK (61.48mg/g fm), RN (47.26mg/g fm), DR (44.98mg/g fm), VF (30.90mg/g fm) and minimum in TN (20.30mg/g fm) population.

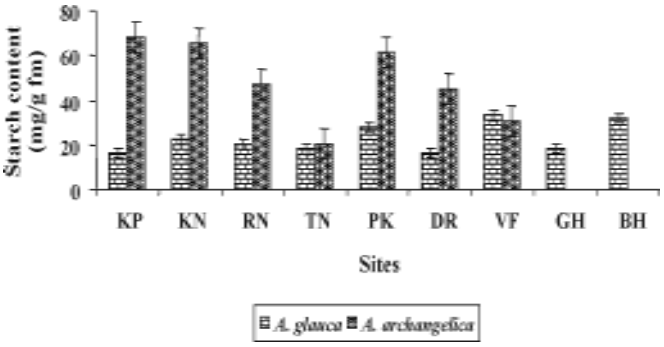


**Fig. 3. Starch content in different populations of *Angelica glauca* and *A. archangelica* (Details of sites given in text)**

Total free amino acid content in the rhizomes of *A. glauca* and *A. archangelica* was maximum in KP (16.66mg/g fm), followed by DR (12.83mg/g fm), PK (12.14mg/g fm), BH (9.76mg/g fm), VF (8.95mg/g fm), GH (8.79mg/g fm), RN (8.78 mg/g fm), TN (8.35mg/g fm) and minimum in KN (3.46mg/g fm) population of *A. glauca* (Fig. 4). Whereas, in *A. archangelica* the total free amino acid was maximum (7.60mg/g fm) in RN , followed by VF (6.29mg/g fm), KN (6.14mg/g fm), PK (5.78mg/g fm), KP (5.62mg/g fm), TN (5.34mg/g fm) and minimum (4.72mg/g fm) in DR population.

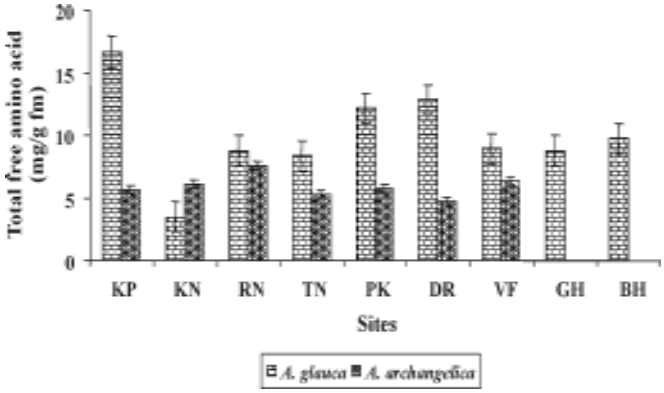


**Fig. 4. Total free amino acid content in different populations of *Angelica glauca* and *A. archangelica* (Details of sites given in text)**

***Isoenzymes:*** The peroxidase pattern in *A. glauca* from VF, KN, RN and GH populations was very similar. Intensity of bands was very dark in KN population followed by VF, RN and GH populations. Bands of light intensity were found in TN, DR, PK and BH populations. Peroxidase isoenzyme bands in *A. archangelica* were dark in TN and DR populations followed by VF and KP populations. While, poor banding pattern was resolved for KN population, other populations, i.e. RN and PK showed similar band pattern (Plate 1A & B).

The esterase band patterns in *A. glauca* from VF, KN, RN and GH populations was very dark while KP showed poor band pattern and rest of the populations such as TN, DR, PK and BH showed a similar band pattern. The esterase band patterns in *A. archangelica* from PK population showed a dark band followed by KP and KN population. Band was poorly resolved for RN population. Rest of the populations such as TN, DR and RN showed a similar band pattern (Plate 1C & D).

Appearance of acid phosphatase bands was dark in GH population followed by RN and VF while KP population showed a poor band pattern. Rest of the populations such as TN, DR, BH and PK showed a

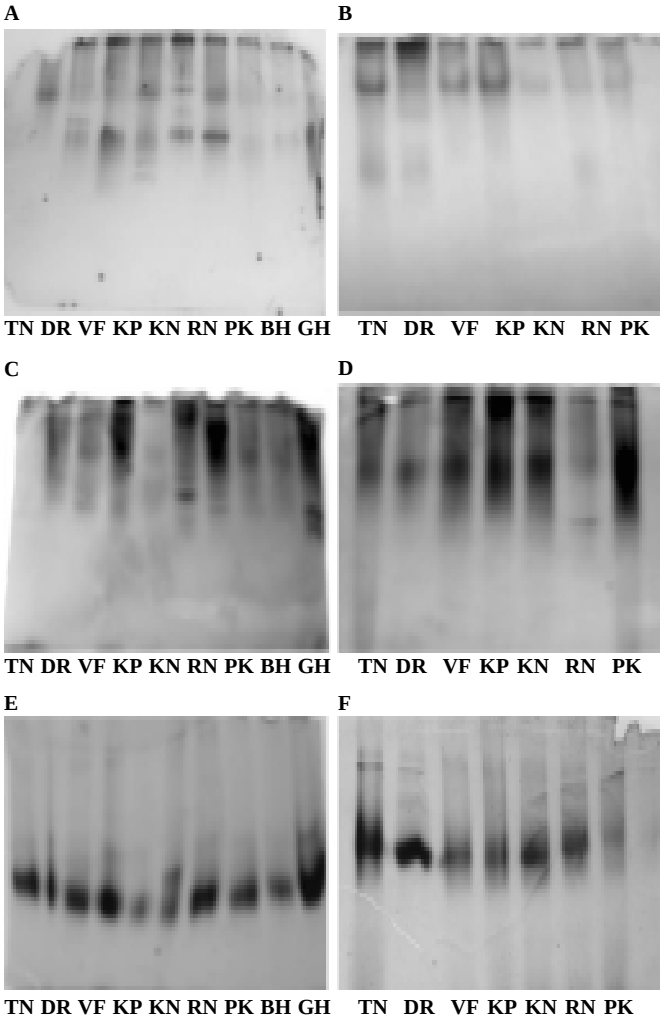


**Plate 1. Peroxidase activity in different populations of *Angelica glauca* (A) *and A. archangelica* (B), Esterase activity in different populations of *Angelica glauca* (C) and *A.archangelica* (D), Acid phosphatase activity in different populations of *Angelica glauca* (E) and *A. archangelica* (F)**

similar band pattern in *A. glauca*. Appearance of acid phosphatase bands in TN and DR population of *A. archangelica* was dark followed by KN and RN while PK population showed a poor band pattern. Rest of the populations such as VF and KP showed a similar band pattern (Plate 1E & F).

When these two species were compared to each other, peroxidase band was dark in KN population of *A. glauca*, while in *A. archangelica* RN population showed dark band. Light band in *A. glauca* appeared in TN population, while it was VF in *A. archangelica*. Esterase band was dark in *A. glauca* in the VF population, while in *A. archangelica* bands appeared dark in PK population. Band was lighter in *A. glauca* in KP, while in *A. archangelica* it was in RN population. Acid phosphatase band was dark in *A. glauca* at GH while in *A. archangelica* it was in PK population. Band was lighter in KP in *A. glauca*, while in *A. archangelica* it was in TN population (Plate 2).

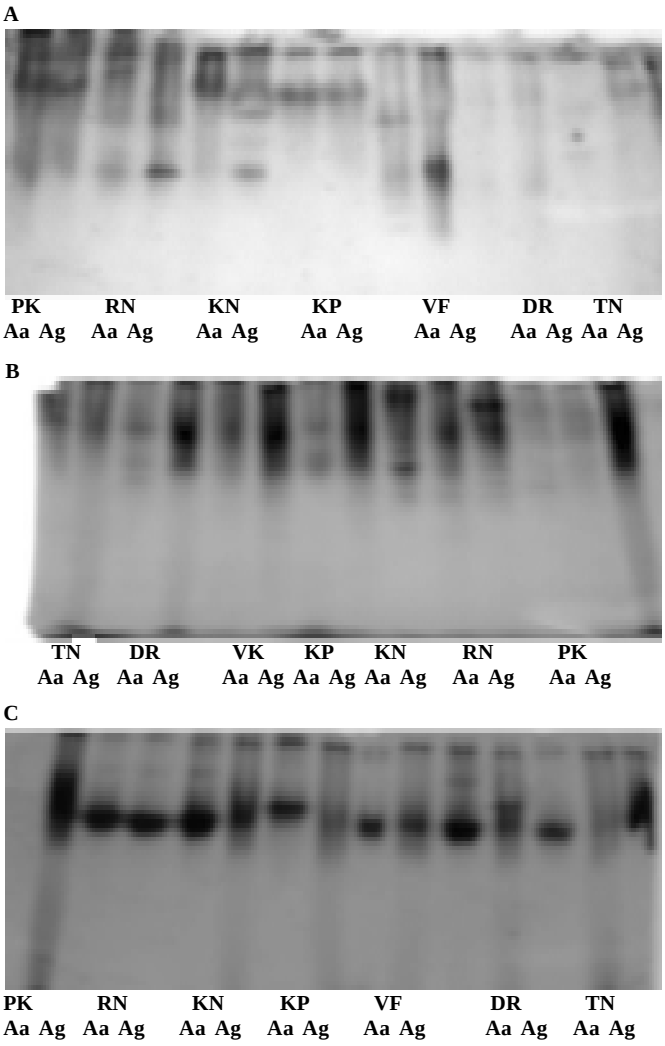


**Plate 2. Peroxidase activity (A), Esterase activity (B) and Acid phosphatase activity (C) in interpopulation of *Angelica glauca* and *A. archangelica***

Cluster analysis of isoenzymes for different populations of *A. gluaca* clearly showed three main clusters, first one comprised of RN, and KN, second one comprised of GH and sub-cluster of PK, TN, BH

populations. The third one with DR and a sub-cluster of VF and KP populations (Fig. 5). However, cluster analysis of isoenzymes in different populations of *A. archangelica* showed a different pattern. Here all the

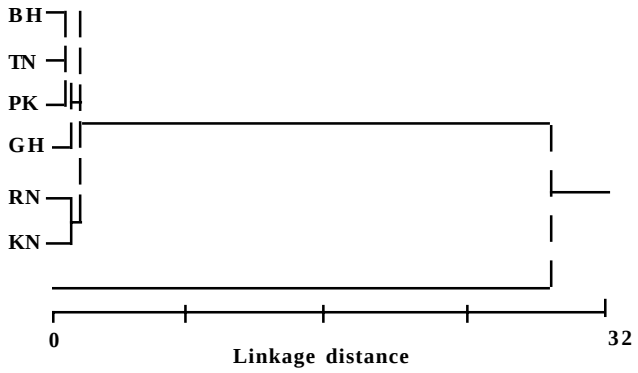


**Fig. 5. Dendrogram of isoenzyme clustering of different populations of *A. glauca***

populations were clustered into two groups. The first group comprises two sub-clusters, first sub-cluster comprises VF, KP and second one with RN, KN, and PK populations. The second major group comprises TN and DR populations (Fig. 6).

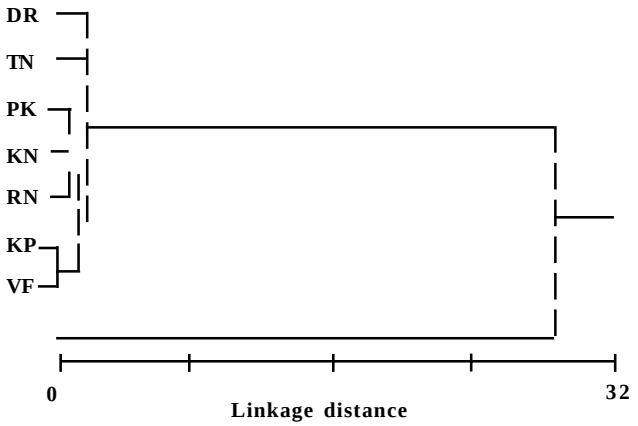


**Fig. 6. Dendrogram of isoenzyme clustering of different populations of *A. archangelica***

## DISCUSSION

Variations in soluble protein, total free amino acid, total soluble sugar and starch contents were observed in nine populations of *A. glauca* and seven populations of *A. archangelica*. Earlier, variations in the biochemical features were also recorded by Maithani, (2000) in two *Rheum* species. While morphological variability in plants and biochemical differences in protein and sugar levels were important parameters to study natural populations of plants, these parameters may also reflect a variation due to environmental conditions rather than genetic variation.

Isoenzyme analysis has been very useful in improvement and conservation of genetic diversity. Such an isoenzyme examination of species may provide useful information on the genetic diversity. The degree to which genetic variations allow a species to tolerance or to adapt to environmental changes depends on a positive association between natural and adaptive genetic variation.

Peroxidase, esterase and acid phosphatase isoenzymes showed considerable variability in different populations of *Angelica* species (Plate 1). Although several common bands of esterase, peroxidase and acid phosphatase were present in all populations of both the *Angelica* species, some of the peroxidase, esterase and acid phosphatase bands appeared to be site specific. Several common bands of esterase isoenzyme showed the adaptation of these plants in different regions. This type of isoenzyme variation was observed among different populations of *Aconitum heterophyllum* (Bahuguna *et al.* 2003). Several isoenzymes including esterase have also been used in the analysis of genetic diversity of endangered species (Bousquenet *et al.* 1986, Godt and Hamrick 1995). Isoenzyme variation is only one measure of genetic diversity that has been used frequently to characterize germplasm collections (Brown, 1978; Goodman and Stuber, 1983; Souza and Sorrels, 1989). The study of protein profile and isozymes has revealed substantial information on genetic and phytogeographical variability among many closely related species (Pandey and Purohit 1979, Bhadula *et al.* 1981, Purohit *et al.* 1983). In the present study also cluster analysis of isoenzymes studied clearly showed the variation among different populations of both the species.

Acid phosphates have been shown to increase during heat hardening and an adaptive significance of this increased thermo stability has also been reported (Feldman *et al.* 1966, Bhadula *et al.* 1986). Acid phosphatase is a common non-specific phosphatase and its role in several processes has been discussed (Sacher

1975, Ribas 1977). Barone *et al*. (1996) used esterase, peroxidase and acid phosphates for identification of *Pistacia vera* germplasm.

Isoenzyme patterns showed variation in intensity of bands of different populations, that may be an adaptive feature of these populations in varying habitat such as that population which showed very light intensity of bands in comparison to other population may reflect their low presence in varying geographical regions.

## ACKNOWLEDGEMENTS

Authors gratefully acknowledge financial assistance from MH&FW, DBT and CSIR, Govt. of India. Former Director, HAPPRC Prof. A. N. Purohit and present Director Prof. A. R. Nautiyal are thanked for providing necessary facilities and constructive criticisms.

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