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Full Length Research Paper

Morpho-biochemical variability and selection strategies for the germplasm of *Fritillaria roylei* Hook (Liliaceae) - an endangered medicinal herb of western Himalaya, India

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***Fritillaria roylei* Hook. (Liliacece) is an important medicinal herb of the Astavarga group, distributed in sub-alpine to alpine regions of the Himalayas. Its bulbs are an important constituent of many medicines and health tonics. Over exploitation for medicinal uses has decreased the availability of *F. roylei* in natural habitats and brought this species into endangered, making conservation and cultivation studies necessary. Variability studies may serve as an important tool for effective conservation and for a crop improvement program. Therefore, natural populations of Garhwal Himalaya were analyzed for variability on the basis of morphological, biochemical and isoenzyme patterns. The studied populations were grouped into three clusters based on D^2 values. Geographical isolation is responsible for variability among these populations. Existing variability among different populations opens up new areas for conservation and perspectives for a genetic improvement program of *F. roylei*.**

Key words: Astavarga group, cluster composition, crop improvement, D^2 statistics, endangered plant, isoenzyme.

INTRODUCTION

The genus *Fritillaria* (Liliacece) is represented by 100 species distributed in the northern temperate zone (Mabberley, 1987), six of which are distributed in India (Anon., 1956). The bulbs are important constituent of the Indian System of Medicine (ISM), health tonic, Astavarga Group (a combination of eight rejuvenating drugs) and auyrvedic medicines (Anon., 1956). *Fritillaria roylei* Hook., commonly known as Kakoli (Anon., 1956), grows in sunny meadows of sub-alpine to alpine regions between 3000 to 4200 m asl in Garhwal Himalaya (Chauhan et al., 2011). The International Union for Conservation of Nature (IUCN) categorized the species as critically endangered (CR) for Uttarakhand and endangered (EN) for Himachal Pradesh and Jammu and Kashmir (Anon., 2003). The market demand of this species is increasing while supply is gradually decreasing (Ved and Goraya, 2008). This calls for conservation as well as cultivation measures to be implemented. However, multiplication behaviour has been published; via *in vitro* bulblet regeneration (Joshi et al., 2007) and seed germination methods (Chauhan et al., 2011).

Effective conservation, management and recovery of rare and endangered species may be deliberated through variability analysis. Variations in morphological appearance among the members of a species become

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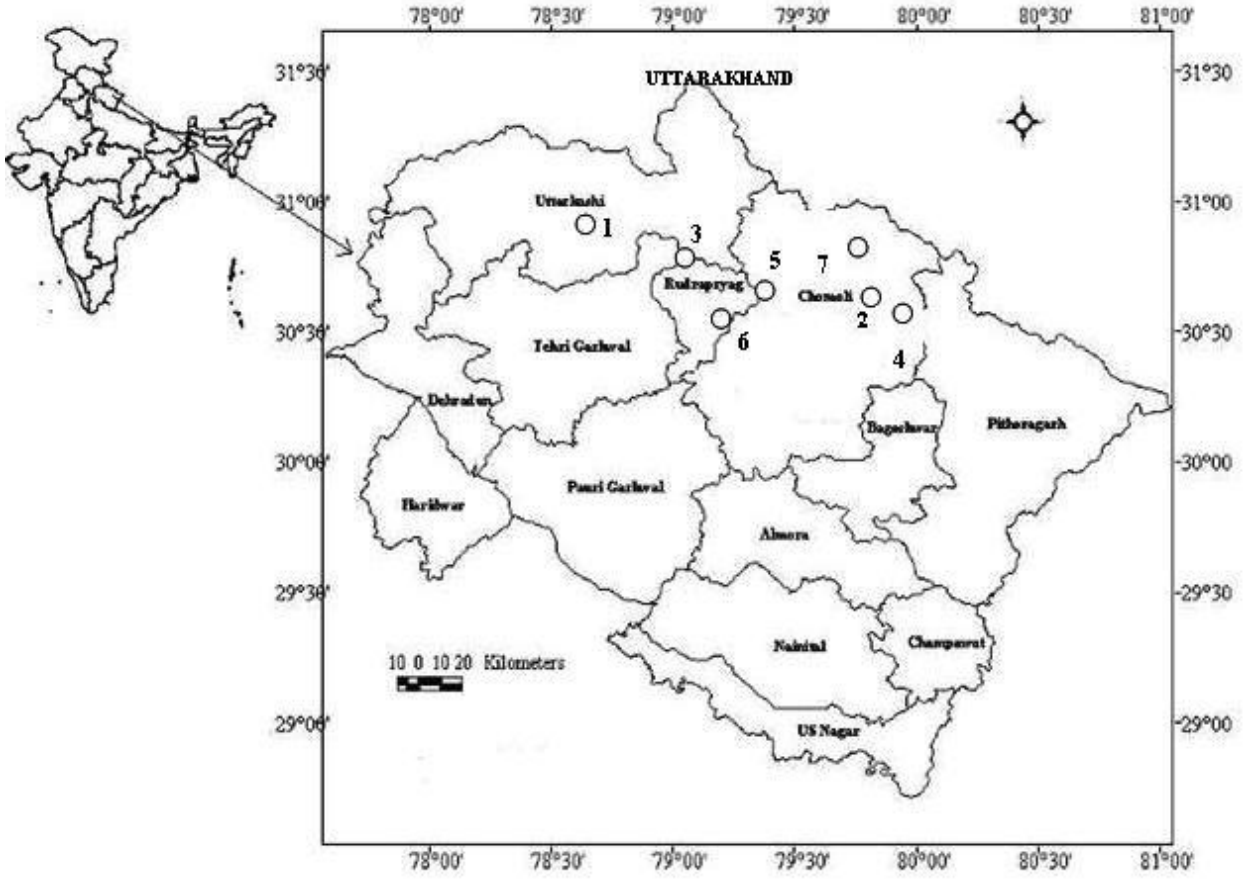

Figure 1. Map showing locations of selected sites of *F. roylei* (1-FR1, 2-FR2, 3-FR3, 4-FR4, 5-FR5, 6-FR6 and 7-FR7)

important to biologists solely because they indicate the presence or absence of physiological attributes that are of ecological significance. Natural selection plays an important role in structuring the observed patterns of variation, more than other factors (Dawson et al., 2009). Statistical methods, including principle components or cluster analysis, can be used as useful tools for screening such variations (Karimi et al., 2009). Adequate information is available about the distribution pattern (Mabberley, 1987; Chauhan et al., 2011) and medicinal properties (Anon., 1956; Kirtikar and Basu, 1984; Kaul, 2010) of *F. roylei*, but there is a complete lacking of knowledge on the nature of variability. In view of this gap, the present paper aims to reveal variability and selection studies among natural populations of *F. roylei* in the Garhwal Himalaya. Such studies will be helpful for effective conservation management and genetic improvement of *F. roylei*.

MATERIALS AND METHODS

Extensive surveys were conducted in several parts of Uttarakhand, Western Himalaya, India known for occurrence of *F. roylei*. Seven different populations were identified and selected for detailed investigations. These seven sites cover the most distribution area of *F. roylei*. A detail description of the selected study sites is presented in Figure 1 and Table 1. Considering critically endangered status of the species in Uttarakhand, experiments were design in such a way that minimum number of samples may provide optimum information's. Ten mature plants from each study site were sampled randomly for morphological traits (plant height and number of leaves/plant) during September. These plants were dug out to estimate the economic yield of bulbs, which were separated, washed with running water and dried at 40°C until constant weight. One set of fresh bulbs were immediately crushed in liquid nitrogen to seize enzymatic activities and then stored at (-80°C) in a deep freezer (Iishin Laboratory Company, Korea) until analysis.

These samples were used to estimate variations in soluble sugars, protein content and isoenzymes. Soluble sugar content was estimated using the Anthrone method (McCready et al., 1950) and soluble protein content was determined using the Bradford method (1976). The absorbance of the reaction mixture was read in a spectrophotometer (Beckman DU-640). Isoenzyme variation was analyzed on 10% polyacrylamide slab gels in a discontinuous gel electrophoretic system at a constant current of 20 mA (Davis, 1964). Esterase (EC 3.1.1.1) and peroxidase (EC 3.4.11) isoenzymes were detected on gels using the methods of Bhadula and Sawhney (1987) and Welter (1982), respectively. Collected data were subjected to analysis of variance (ANOVA) and only significant variations were used for multivariate analysis of D^2 statistics (Mahalanribis, 1936; Rao, 1952). The analysis was performed using indostat statistical package and the cluster formation was confirmed by the Tocher method (Rao, 1952).

Table 1. A detail of selected natural habitats of *F. roylei.*

Study site	Code	Distribution range (m asl)	Habitat	Latitude	Longitude
Dayara (Uttarakashi)	FR1	3000-3400	Open meadow	30°50' N	78°33' E
Dronagiri (Chamoli)	FR2	3200-3500	Partial shade	30°30' N	79°52' E
Kedarnath (Rudraprayag)	FR3	2900-3500	Open meadow	30°44' N	79°03' E
Kunwari pass (Chamoli)	FR4	3000-3400	Open meadow	30°07' N	79°68'E
Rudranath (Chamoli)	FR5	3000-3200	Partial shade	30°11' N	79°28' E
Tungnath (Rudraprayag)	FR6	3200-3600	Partial shade	30°14' N	79°22' E
The valley of flowers (Chamoli)	FR7	3000-4200	Open meadow	30°10' N	79°70' E

Table 2. Mean growth performance and biochemical parameters of different natural populations of *F. roylei*.

Study site	Plant height (cm)	No. of leaves/plant	Bulb DW (g/plant)	Soluble sugar (mg/g)	Soluble protein (mg/g)
FR1	15.67	6.33	0.84	20.75	30.60
FR2	19.42	12.22	1.15	14.11	27.41
FR3	7.13	12.11	1.64	17.96	30.10
FR4	10.49	11.22	0.96	10.09	31.46
FR5	18.11	11.56	1.00	13.50	29.54
FR6	15.70	11.33	0.99	12.11	37.60
FR7	22.50	11.33	1.30	11.74	32.49
Mean	15.57	10.87	1.12	14.32	31.32
SD*	5.27	2.04	0.27	3.76	3.20
Range	7.13-22.50	6.33-12.22	0.84-1.64	10.09-20.75	27.41-37.60
CD** 5%	4.53	1.93	0.58	4.61	7.04

*SD, Standard deviation; **CD, critical difference.

RESULTS

The germplasm collected from different populations showed considerable variation in plant height, number of leaves/plant, economic yield, biochemical parameters (Table 2) and Esterase and peroxidase isoenzymes banding pattern (Figure 2). There were significant differences among the studied parameters, indicating variability among studied populations. The range of variability was 7.13 to 22.50 cm for plant height, 6.33 to 12.22 leaves/plant, 0.84 to 1.64 g bulb weight/plant, 10.09 to 20.75 mg/g soluble sugar and 27.41 to 37.60 mg/g soluble protein content. In view of such considerable variability, D^2 values were computed for all possible pairs of populations. Based on D^2 values these populations could be grouped into three clusters (Figure 3). Plant height (47%), number of leaves/plant (23%) and soluble protein (19%) contributed most variability while other characters, namely bulb weight and soluble sugar, contributed the remaining share, 4.76% each. Mean growth performance of the three clusters is presented separately in Table 3. Cluster 1 contains a maximum of five populations that is, FR2, FR5, FR7, FR6 and FR4, whereas cluster 2 and 3 contain a single population each, FR3 and FR1, respectively.

The average intra-cluster distance ranged from 0.0 to 3.39 whereas the inter-cluster distance ranged from 6.15 to 9.42. This study also revealed wide variation in the esterase isoenzyme banding pattern (Figure 2A). Total number, intensity and position of bands varied among studied populations. FR1, FR3 and FR4 populations emerged as a distinct one by showing a specific banding pattern compared to the other populations. These three populations showed more compact and darkly stained bands. The peroxidase pattern (Figure 2B) in FR1 and FR4 populations emerged as being distinct one by showing a specific banding pattern as compared to other populations. The intensity of bands was very dark in FR5 followed by FR7 populations. Bands of light intensity were resolved in FR3 and FR6 populations.

DISCUSSION

Variation in the morphological and biochemical parameters as well as isoenzyme patterns may be due to the particular ecological niche, altitude and micro-environment of different populations and genetic variability contained in each. Geographical isolation of these populations may be an important factor that may be responsible for variability. Variation in plant height is related to soil and climatic factors that vary seasonally

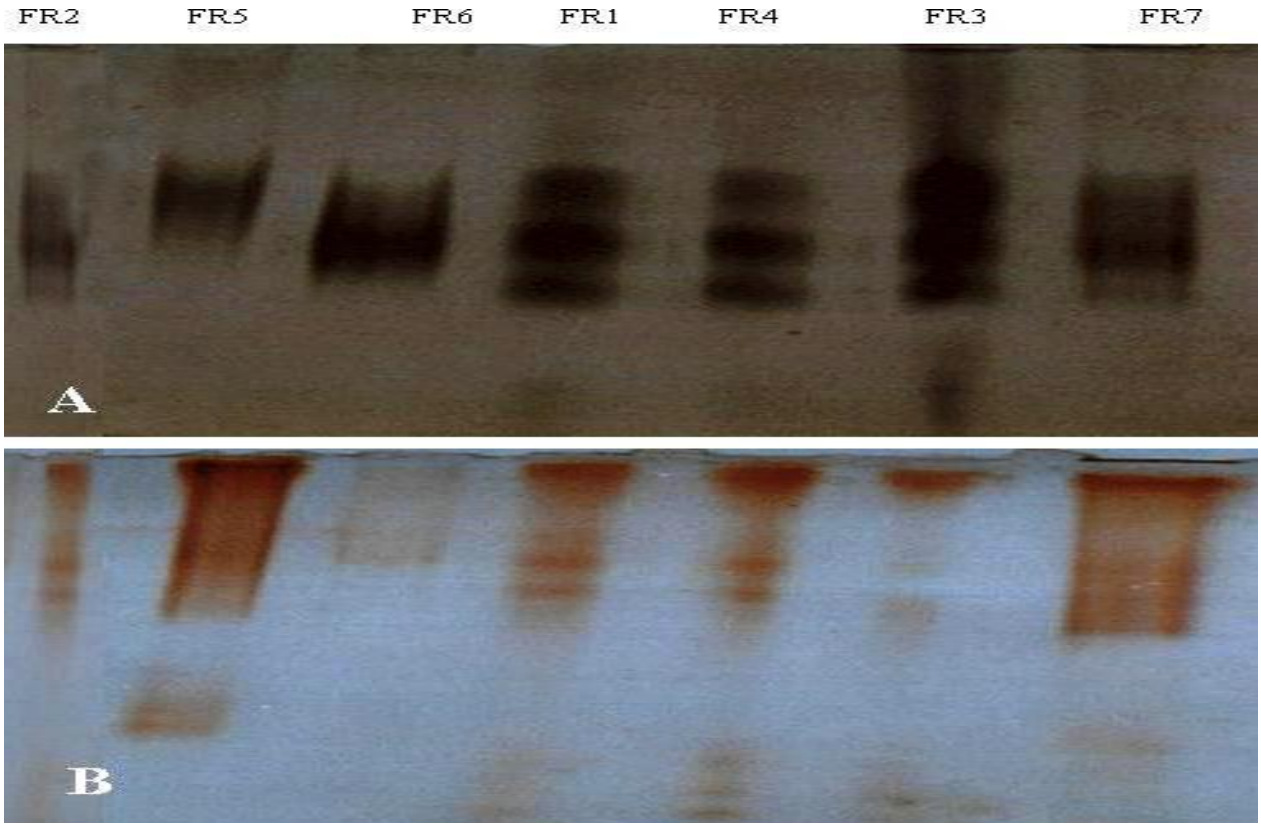

Figure 2. Variations in isoenzyme pattern among different populations of *F. roylei*-; (A) Esterase and; (B) peroxidase.

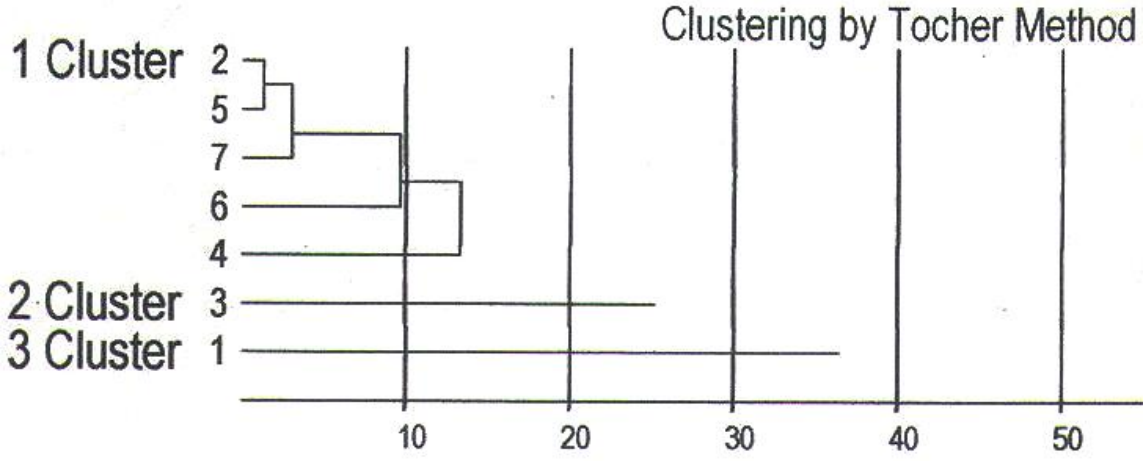

Figure 3. Dendrogram of *F. roylei* by clustering method (1-FR1, 2-FR2, 3-FR3, 4-FR4, 5-FR5, 6-FR6 and 7-FR7).

Table 3. Cluster mean of seven populations of *F. roylei.*

Cluster	No. of populations	Plant height (cm)	No. of leaves/plant	Bulb DW (g/plant)	Soluble sugar (mg/g)	Soluble protein (mg/g)
1	5	17.24	11.53	1.08	12.31	31.70
2	1	7.13	12.11	1.64	17.96	30.10
3	1	15.67	6.33	0.84	20.75	30.60

and altitudinally (Krishnan et al., 2000). Leaf characteristics in plants are sensitive to varying environmental conditions (Lynn and Waldren, 2001). Morphological and biochemical variation among natural populations were also reported in *Aconitum atrox* (Bruhl) Muk. (Kuniyal et al., 2002) and *Nardostachys jatamansi* (Chauhan et al., 2011). Highest economic yield in the FR3 population was positively correlated with the number of leaves and soluble sugar content.

Increase in the number of leaf may be responsible for increased photosynthetic produce that could store in bulbs. Such information on morphometric, primary metabolites and isoenzyme pattern suggest that secondary metabolites may also vary. As increase in primary metabolites showed significant increase in active constituents in ferns (Guha et al., 2006). Isoenzyme studies also supported variability among studied populations. Germplasm diversity based on standard morphological marker has proved to be inadequate because of wide spectrum of phenotypic variation and their interaction with environment (Mannetji, 1984), whereas, esterase isoenzyme is excellent marker in variability studies which indicate intra population variations (Bhadula et al., 1996). Variability existing in these populations opens a new area for conservation and genetic improvement program through plant breeding as well as biotechnological tools. FR3 (Kedarnath) population showed better performance among the studied populations, which can be used as a source of elite germplasm for mass multiplication. On the basis of such multifaceted information on specific species, future conservation strategies and cultivation of wild medicinal species can be initiated (Airi et al., 2000).

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