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Habitat Preference, Ecological Parameters and Conservation of *Fritillaria roylei* Hook., an Endangered Medicinal Herb of the Astavarga Group

Rajendra S. Chauhan[1*] • Mohan C. Nautiyal[1] • Jaime A. Teixeira da Silva[2] • Pratti Prasad[1] • Harish Purohit[1]

[1] High Altitude Plant Physiology Research Centre, HNB Garhwal University, Srinagar (Garhwal) - 246 174, Uttarakhand, India
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan

Corresponding author: * rchauhanua@gmail.com

ABSTRACT

Fritillaria roylei is a perennial, threatened medicinal plant of the Astavarga group growing in open sunny slopes of temperate to alpine regions of the Himalayas. During the last few years a decrease was felt in natural populations of this species. The present study deals with habitat preference, multiplication and conservation methods. Open sunny meadows with moderate slope, rich in humus are the preferred habitat of *F. roylei*. The density of species was between 0.40-4.20 plant m^{-2} among studied populations. Heavy harvesting pressure, anthropogenic activities, competition with other associated species and low seedling establishment are the major threats. A 58-77% population reduction was recorded during the last 20-30 years. Being a medicinally important plant, such species should be given priority for conservation through both *in situ* and *ex situ* methods.

Keywords: Astavarga, Liliaceae, medicinal plant, population decline

INTRODUCTION

The genus *Fritillaria* (family Liliaceae) is represented by 100 species distributed in the northern temperate zone (Mabberley 1987). In India, it is represented by approximately 6 species (Anon. 1956). *Fritillaria roylei* Hook., commonly known as Kakoli, grows in sunny meadows of temperate to alpine regions between 3000-4200 msl. The bulbs of some other species of this genus contain peimine ($C_{26}H_{43}O_3N$), peiminine ($C_{26}H_{41}O_3N$), fritimine, fritillarin, verticin and verticillin along with some minor alkaloids, due to which plants of this genus are well known for their medicinal properties (Anon. 1956). Bulbs form important constituents of many medicines in ISM (Indian System of Medicine) (Kaul 2010), and are used as a health tonic, a member of the Astavarga Group (a combination of eight rejuvenating drugs) and used in the preparation of the Ayurvedic tonic '*Chyawanprash*' (Anon. 1956). The bulbs are useful in the treatment of fever, cough, hemorrhage, milk deficiency, rheumatism, and as an antipyretic, expectorant and lactagouge (Kirtikar and Basu 1984). *F. roylei* is useful as an aphrodisiac and against asthma in TSM (Traditional System of Medicine) in India (Kaul 2010). The market demand of this high altitude medicinal plant species is increasing while supply is gradually decreasing (Ved and Goraya 2008). The bulbs extracted every year for medicinal use from natural populations create heavy harvesting pressure. The species became endangered (Anon. 2003) due to overexploitation of bulbs for medicinal value, habitat degradation and other biotic interferences in its distribution ranges.

The rarity of the species can be justified on the basis of its populations in nature. The IUCN categorized the species as CR (critically endangered) for Uttarakhand and EN (endangered) for other natural populations (Anon. 2003). Demographic studies are essential for a better understanding of the relationship between natural dependent plants and the community in which they are found (Zotz and Schmidt 2006). The species is very difficult to cultivate based on existing information (Anon. 2003) and needs a study of the life history prior to domestication.

Little general information is available in other species of this genus (Shimizu *et al.* 1998; Wang *et al.* 2007) and is completely lacking for *F. roylei*. Being an endemic, endangered and medicinally important species, a conservation strategy needs to be initiated. Therefore, it is important to study the distribution pattern, ecological behaviour, and regeneration methods for effective conservation management. In view of the above, this paper presents a quantitative estimation, ecological and regeneration potential of the species. Such a study will be helpful to understand the ecological complexity of *F. roylei* along with other threatened species.

Table 1 Sites' characteristics of the selected populations of *F. roylei*.

Location of study sites	Code	Altitude (msl)	Habitat	No. of associated species	Dominant associate
Dayara	DR	3000-3400	Open meadow	12	*Anaphalis* spp., *Phlomis* spp.
Dronagiri	DRO	3200-3500	Partial shade	12	*Anaphelis* spp., *Pteridium* spp.
Kedarnath	KN	2900-3500	Open meadow	12	*Anaphelis* spp., *Pteridium* spp.
Kunwari Pass	KP	3000-3400	Open meadow	9	*Danthonia* spp., *Caltha* spp.
Rudranath	RN	3000-3200	Partial shade	12	*Anaphelis* spp., *Pteridium* spp.
Tungnath	TN	3200-3600	Partial shade	14	*Danthonia* spp., *Fragaria* spp.
The Valley of Flowers	VF	3000-4200	Open meadow	11	*Danthonia* spp., *Polygonatum* spp.

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Research Note

MATERIALS AND METHODS

Study sites

The present study was carried out in 7 natural populations of western Himalaya (Uttarakhand), India (29°26'-31°28'N Lat. and 77°49'-80°6'E long.) known for the occurrence of this species. To analyze phenological details 2-3 visits of selected areas were carried out throughout the active growth season from June to October. A detailed description of each site is given in **Table 1**. Generally these sites are under heavy snow cover during winter, where maximum air temperature during the day reaches up to 25°C followed by near-freezing at night in summer. The soil of these sites is generally clay type in texture, whereas the substrate soil of the species is slightly acidic in nature (pH 6.0-6.65, unpublished data).

Phytosociological analysis

A general survey was carried out to get an idea of the entire study area. Vegetation sampling was conducted through a vertical belt transects method (Michel 1990). Three stands were randomly selected (10 m^2 size) within each transect and the study was conducted with a species area curve. Analytical features such as percentage frequency (%F), density (D, plants m^{-2}) and total basal cover (TBC, cm^2 m^{-2}) was calculated according to Mishra (1968). The distribution pattern was analyzed on the basis of abundance (Kershaw 1973).

Multiplication behaviour

Seeds were collected in September-October, dried for 2 weeks in the shade and stored at room temperature until the start of the germination experiment. Only healthy seeds (i.e., that settled when soaked with distilled water) were used for experiments as a means to ensure uniform seed viability, germination and seedling vigour. The moisture content of seeds was determined with the oven dry method (i.e. seeds were oven dried at 103°C for 17 hrs) (ISTA 1985) and seed viability with the methods of Moor (1962). Seeds were surface sterilized in a 0.5% aqueous solution of $HgCl_2$ to discourage fungal infection, washed with 10-15 ml of distilled water three times and kept in glass Petri dishes (90 mm diam.) on a single layer of Whatman No.1 filter paper. These seeds were kept inside a seed germinator (Calton®) at 15°C with a 16-h photoperiod (illuminated with four 40-W tubes) under laboratory conditions in triplicate (20 seeds replicate^{-1}). Seeds were kept moist and considered germinated when a 0.5 cm-long radical emerged. Seedlings were pooled at the end of the germination experiment (20 days) and transplanted to a glasshouse (house covered with glass to allow sunlight to enter) to observe seedling survival. Each replicate contained 20 uniform seedlings in triplicate. Survival percentage of transplanted seedlings was observed after 1 month of transplantation.

RESULTS

Growth phase

In their natural habitats, new leaves from buds of *F. roylei* started to emerge a few days after snow had melted, when temperature had increased slightly and one phenophase (vegetative or reproductive) of the lifecycle was complete (mid-April to September depending on the microhabitat, **Fig. 1**). Even buds were produced from bulbs at the time of senescence, which remained dormant under the soil throughout winter. The underground buds produced leaves in the next growing season when sufficient moisture provided by melting snow became available to dormant bulbs. Radical leaf expansion or shoot elongation took place soon after and mature plants reached up to 30 cm in height within 8-10 weeks. *F. roylei* bears attractive yellow flowers in May-June and fruit in September. With the onset of winter in October, yellowing of aerial parts indicate the onset of senescence with maturation and the dispersal of seeds in their natural habitat. Aerial parts of the plants dried and became buried under the snow while underground bulbs with newly emerged buds entered a dormant phase until the return of a favourable climate.

Fig. 1 Different stages of *Fritillaria roylei*. Mature plants growing in natural habitat (**A, B**). Seed germination in laboratory (**C**). Seedling development in seedling tray (**D**). Plants grown in nursery (**E**).

Phytosociological analysis

Seven populations were identified in the study areas, most of which were represented by two pockets. The number of species associated with *F. roylei*, habitat type and dominant associates are presented in **Table 1**. The frequency of *F. roylei* varied between 40-80%, whereas plant density varied

Table 2 Ecological parameters of different populations of *F. roylei*.

Populations*	Frequency (%)	Density (plant m^{-2})	Community TBC (cm^2 m^{-2})	% Share of *F. roylei*	IVI
DR	80	3.8	32.37	2.10	13.38
DRO	80	2.4	33.79	0.82	10.92
KN	60	4.20	36.15	1.74	11.28
KP	70	1.1	11.63	1.11	13.06
RN	60	3.8	36.41	1.56	10.68
TN	40	0.40	6.50	1.23	6.42
VF	80	1.8	6.18	0.80	13.50

* Codes (see **Table 1**); TBC = Total Basal Cover; IVI = Importance Value Index.

Table 3 Seed viability and seed germination among different populations of *F. roylei*

Populations*	Percentage seed viability	Germination percentage	Days required for the onset of germination	Days required for final germination
DR	95.00 ± 5.00	91.67 ± 7.64	12	20
KN	96.67 ± 2.89	93.33 ± 5.77	12	20
TN	97.33 ± 2.66	93.33 ± 5.77	13	19
VF	91.67 ± 7.64	88.33 ± 2.89	14	20

* Codes (see **Table 1**)

between 0.40-4.20 plants m^{-2} among the studied populations (**Table 2**). The percentage share of *F. roylei* in these populations varied between 0.80-2.10% (**Table 2**). The Importance Value Index (IVI) of *F. roylei* was lowest (6.42) in Tungnath and almost similar (10.68-13.50) in other populations. In general, open sunny meadows with a moderate slope and rich in humus were found to be the preferred habitat of *F. roylei* in terms of growth performance and distribution.

Multiplication methods

The species multiply by vegetative and sexual multiplication. Once the size of bulbs (underground food storage organs) increase sufficiently (> 1 cm diameter), 2-3 new bulbs formed at the vertical axis and as older bulbs started to decompose, new bulbs became separated and produced daughter plants. Low seed germination is another way in which *F. roylei* multiplies in its natural habitat. Seeds collected from different populations showed good seed viability (91.67-97.33%), whereas seed germination under laboratory conditions was 88.33-93.33% (**Table 3**). 12-14 days were required for the onset of germination and almost 20 days for its completion. The presence of very few seedlings in nature confirms that seed germination and seedling establishment are very poor (unpublished data). Seedlings can be easily identified by a single radical leaf and the testa remains present on the leaf apex for a few weeks after germination (**Fig. 1C**). Seedlings transplanted to a glasshouse showed only 54.66 ± 18.90% survival after one month of transplantation.

DISCUSSION

One might expect the abundance data to be superior under all circumstances because more information is collected at each site (Lina *et al.* 2006). The present study revealed that open sunny meadows with a moderate slope rich in humus are the preferred habitat of *F. roylei*. Another species of the same genus i.e. *F. camtschatcensis* also prefers such habitat at 2000 msl on Mt. Hakusan, Japan (Shimzu *et al.* 1998). The low density of *F. roylei* among all the studied populations may be due to overexploitation for medicinal purposes, poor regeneration potential and seedling establishment in nature, competition with other dominant species of the community, etc. Overgrazing, forest clearing or dense invasion by exotic species may reduce the species population by 40-60% (Scholes and Biggs 2005). Seedling establishment in these habitats is episodic (Billings and Mooney 1968) whereas habitat specificity, climate change, heavy grazing pressure, natural enemies, etc. may be other causes of rarity. A random and contagious pattern of distribution showed that species grow in uniform environmental conditions as suggested for other alpine herbs (Kershaw 1973). The high variation in IVI of *F. roylei* in Tungnath compared to other populations may be due to the steep slope, variation in associated species and other biotic interferences. IVI and the percentage share of species in the community show that *F. roylei* is facing competition with other associated species. A few associated species i.e. *Danthonia cachemyriana*, *Anaphalis nepalensis*, *Polygonatum verticillatum*, *Pteridium* spp., among others become larger than *F. roylei* and compete for light, moisture, minerals, etc. This may explain

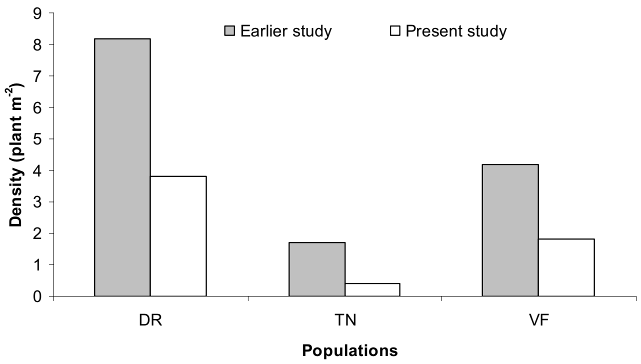

Fig. 2 Decrease in population density of *F. roylei* as compared to earlier studies.

the low regeneration potential and low seedling establishment.

The density of *F. roylei*, when compared with past studies (**Fig. 2**), revealed a continuous decline in the population (58-77%) during the last few years. Such a decrease in population may be due to heavy harvesting pressure for market demand, low regeneration potential, long-term change in climate, habitat degradation, change in herbivore activity, pollinator activity, etc. The CR status of *F. roylei* in the Tungnath population seems mostly due to of the steep slope and heavy grazing pressure. Grazing pressure is much higher in this site as suggested by Nautiyal *et al.* (2004). Due to man-induced changes caused by over- and illegal exploitation of plants in this area of high conservation value, a centre of endemism and species diversity, the original habitat has now been fragmented into isolated patches, leading to fragmentation of species population, as occurs in the Himalayas (Tandon 1998).

F. roylei showed clonal as well as sexual multiplication. Despite good seed germination (93.33%) in the laboratory and seedling establishment in the glasshouse (54.66%), the population density remains low in natural habitats. Seed germination and seedling establishment were also better in a high-altitude endangered medicinal plant *Nardostachys jatamansi* when placed under controlled temperature (80%), but much lower (11.16%) in its natural habitat (Chauhan and Nautiyal 2007). Adults are numerically dominant in populations of perennial species with clonal growth (Cook 1985), and seedling recruitment is often greatly reduced in populations growing in harsh environments (Baskin and Baskin 1998). Obstruction of the reproductive phase by early snowfall and other biotic factors may have prevented seed maturation and reduced the plant population in most alpine vegetation in the western Himalayas (Nautiyal *et al.* 2001). Hence plants adapt themselves to produce underground perennating organs. These two modes of reproduction have a short- and long-term advantage (Silander 1985). Sexual reproduction has been thought to create a new combination of genomes (Maynard 1978) whereas clonal propagation is assumed to ensure the maintenance of a local population even when recruitment by seedlings is difficult (Nault and Gagnon 1993). Vegetatively reproducing species form a clone comprising many more-or-less independent ramets (Cook 1985).

CONSERVATION

Although *F. roylei* has been declared endangered there has been no management plan for its conservation to date except for a ban on collection from natural populations. However, illegal and destructive harvesting is reported for few alpine medicinal plants (Kala 2005; Ved and Goraya 2008). Even after collection of this species from the wild was banned, destructive harvesting continues illegally because collection of medicinal and aromatic plants (MAPs) is associated with the socioeconomy of this region (Chauhan 2004).

Cultivation of such MAPs should be initiated to improve the socioeconomy because *F. roylei* showed good seed germination under laboratory conditions. The sale of medicinal herbs generates more income than traditional crops in Uttarakhand (Silori and Badola 2000). Although cultivation can not substitute the urgency of conservation of natural populations of MAPs, it can help in meeting the industrial demand and reduce pressure on wild stocks (Tyagi 2005). *In situ* and *ex situ* conservation methods with systematic planning will also be useful for such economically important endangered MAPs. On the basis of such multifaceted information on specific species, future conservation strategies and cultivation of wild MAPs can be initiated (Airi *et al.* 2000). It can be concluded that priority should be given for restoration of *F. roylei* to protect it urgently in its natural habitat. Otherwise, rising demand with shrinking habitats may lead to local extinction of many MAPs, including *F. roylei* (Kala *et al.* 2006).

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