Phenological Episodes and Reproductive Strategies of *Inula racemosa* (Asteraceae)-a Critically Endangered Medicinal Herb of North West Himalaya

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**Abstract:** The phenological episodes of a plant are governed by the eco-edaphic conditions it grows in, which in turn direct and dictate the strategies of the plant in general and alpine herbs in particular. The structure of such communities thus determines the behaviour and functioning of a particular species on a particular habitat. The present study was carried out to unravel the phenological behaviour and strategies of *Inula racemosa* (Asteraceae), a critically endangered medicinal herb of North West Himalaya. The study was carried out for a period of 36 months to monitor the various developmental features in four different geographical areas of Kashmir Himalaya, which is highly useful in terms of its prescription and panacea for development of conservation strategies, multiplication, domestication and genetic improvement of the species.

**Key words:** Phenology, alpine plant, pollination, self-incompatibility, competition, strategies

**INTRODUCTION**

Reproductive biology is one of the fundamental fields for development of productive conservation protocol for elite and threatened plants. Very little is known about the reproductive biology of rare and threatened medicinal herbs. There is always a buzz all over the globe to unravel the basic and detailed information about the reproductive biology of important species in general and of endemics in particular preferably for their genetic improvement through hybridization as well as for development of conservation protocol. Phenology in general and reproductive phenology in particular is a critical and important trait of a plant because it determines the growth, developmental pattern and number of potential mates thus providing a mechanism for reproductive isolation or speciation over time (Rathcke, 1983; Bronstein *et al.*, 1990). Flowers essentially act as a food source for pollinators and other visitors thereby giving the study of flowering phenology both ecological and evolutionary significance. Flowering phenology can also be viewed from eco-edapho-physiological perspective.

Researchers always and continuously try to identify environmental factors that correlate with phenological events such as initiation of flowering, the synchronization of flowering, the length of the flowering phase and variation in flower abundance (Opler *et al.*, 1980; Borchert, 1983; Inouye and McGuire, 1991; Beaubien and Johnson, 1994; Inouye *et al.*, 2002). Environmental factors that initiate the onset of a particular phenophase including flowering, photoperiod, temperature and precipitation (Rathcke and Lacey, 1985) The same environmental factors can delimit a particular phenophase including flowering season in some specific eco-edaphic conditions and/or environments such as rain in a desert and/or snowmelt in alpines, which normally serve as a cue to trigger flowering (Borchert, 1980; Inouye and McGuire, 1991).

*Inula racemosa* (Asteraceae), a critically endangered North West Himalayan alpine herb (Anonymous, 1998), is a commercially useful plant mainly used as an anti-spasmodic, hypotensive and for treatment of cardiovascular and liver troubles. It is also used for treatment of respiratory tract disorders, foul ulcers and chronic bronchitis and as an antiseptic (Kaul, 1997). Due to the fragile nature of its habitat and its exploitation due to commercial medicinal properties, the species is facing the onslaught of indiscriminate over-exploitation, habitat destruction and competition. The populations of the species in the entire North West Himalayan range are witnessing a speedy decline in density and diversity thus dwindling both in size and number. Unabated as the plant extraction and habitat destruction continues to be, far are not days when these herbal gems will become extinct from the globe. It indeed is a critical situation for all such species, calling for salvage of whatever is left and if not rescued now, irrevocable loss of this precious legacy from the globe will be the eventual and inevitable consequence.

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To develop a suitable prescription and panacea for this malady, the understanding of reproductive biology, breeding behavior and seed biology is regarded to be of nuclear importance as well as a central element (Primack, 1980; Jain, 1995; Ayensu, 1981; Henriquin et al., 1998; Wafa and Nawehoo, 2001). The present study is aimed to unravel these critical events which in turn will help in planning strategies for the effective and efficient conservation, validation and management and also pave the way to a sustained generation and regeneration of raw material for reintroduction into natural habitats and for the commercial usage in pharmaceutical industries in future.

MATERIALS AND METHODS

Exploration: Exploration of several N. W. Himalayan natural habitats was carried out and several specific sites identified in different regions of the valley where the plant (Inula racemosa) was growing. The sites have been identified on the basis of the following features: Habitat requirements of the plant, Adaptability of the plant to the specific habitat, Population size and dominance and Accessibility of the selected sites. The selected sites were surveyed from June-2003 to October 2005.

Phenology: For phenological investigation several plants were tagged in different populations and monitored throughout the growing season. The phenological parameters studied include: Sprouting of the rhizomes, Initiation and duration of vegetative and reproductive phases, Flowering development and behavior, Seed development and seed dispersal, Senescence of the aerial shoots.

Breeding system: To investigate the nature of breeding system operative in the species the following aspects were studied: Pollen emission and stigma receptivity, pollen viability and pollen ovule ratio, pollination system, breeding behavior, seed set and odes of propagation.

Stigma receptivity: Stigmas at different developmental stages were fixed in 1:3 acetic alcohol. Subsequently they were stained in a mixture of 2 mL of 1% aqueous light green, 2 mL of 1% aqueous acid fuchsir, 40 mL lactic acid and 60 mL distilled water. The stigmas were allowed to remain in the stain for about an hour. The stigmas carrying the germinating pollen grains were recorded as receptive.

Pollen viability and pollen ovule ratio: To test the pollen viability, pollen grains from ready to dehisce anthers were stained in 2, 3, 5 triphenyl tetrazolium chloride solution and 1% acetoearmine and in 1% aniline blue-lactophenol. The healthy and plump stained pollen were recorded as viable.

The pollen ovule ratio was calculated following Cruden's, (1977) method as follows:

\[
P/O = \frac{\text{Pollen count per anther} \times \text{No. of anthers}}{\text{No. of ovules per flower} \times 100}
\]

Pollination mechanism: Pollination mechanisms were studied by observing the foraging behavior of various insects visiting the flowers for pollen and nectar. The frequency of insect visitation was observed during different periods of a day as well as under different habitat conditions. The insect visiting frequency was calculated as follows:

\[
\text{IVF} = \frac{\text{No. of flowers visited by the insects in one turn}}{\text{Total No. of flowers available}}
\]

The insects visitors were collected, preserved and identified in the Department of Zoology, University of Kashmir, Srinagar-190006, India.

Breeding behavior: In order to unravel the nature of the breeding system operative in the species, the following bagging experiments were carried out:

Expt. 1: Unemasculated flower heads were tagged and allowed to open pollinate

Expt. 2: Unemasculated flower heads were bagged to avoid foreign pollen deposition and allowed to self pollination

Expt. 3: Emasculated florets/capitulum were bagged to check the apomictic development of seeds

Expt. 4: Stigmas were hand pollinated with the pollen from the anthers of same head and bagged to check the gynogamy

Seed set: To workout the percentage seed set Lubber’s and Christenson’s, 1986 formula was followed:

\[
\% \text{age seed set} = \frac{\text{Total No. of seeds produced per plant}}{\text{Total No. of ovules borne by the plant}} \times 100
\]

Observations: The geographical range of the habitat of Inula racemosa varies from 34°4'N/74°20'E to 34°30'N/75°30'E inhabiting the sub-alpine to alpine
regions (3000-3600 m amsl) of Kashmir Himalaya. The altitude and eco-edaphic conditions seem to play an important role in the phenological behavior of the species. It is evident by the fact that at higher altitudes with low temperature and late melting of snow cover, the plants enter into the vegetative and reproductive phases of life cycle relatively later than the plants grown at lower altitudes (Table 1).

The rhizomes remain dormant throughout the winter months owing to the sub-zero temperatures (November to March/April). Sprouting of the ones at different altitudes varies as is revealed by sprouting in the last week of May at Panchal (3150-3250 m), first week of June at Thajwass (3050-3300 m) and third week of March at Herbal Garden Kashmir University, Srinagar (1580 m, Transplanted population) and continues up to second week of June, third week of June and forth week of March at these sites, respectively. The time taken by a plant to shift from vegetative to sexual mode varies from 15-20 days in natural populations and 7-10 days under transplanted conditions. Concomitantly with the vegetative phase, sexual phase initiates in the 2nd week of June at Panchal. Third week of June at Thajwass and last week of April in transplanted population. The florets begin to open in the 2nd week of July at Panchal, 3rd week of July at Thajwass and 1st week of June in transplanted population. Within the heads the peripheral florets open first and the anthesis gradually proceeds towards the centre (centrepetal). All the florets of a capitulum open within 4 days and all the capitula (at the most two) of a plant open within 14 days. After the completion of the process of pollen transfer to the stigma, the initiation of seed development starts from 3rd week of September at Panchal, 1st week of September at Thajwass, and seeds were not produced in transplanted population, possibly due to the absence of the pollinators. Seed development lasts up to 4th week of September at Panchal and 3rd week of September at Thajwass. Finally, the senescence gets initiated in 2nd week of October at Panchal, 1st week of October at Thajwass and in 3rd week of October in transplanted population and enters into a dormant premating phase.

**Bagging experiments:** The results obtained showed that the unemusbated hermaphrodite florets allowed to open pollinate (Exp. 1) culminated in seed production (490.66±61.329), while neither those allowed to self pollinate nor those checked for apomictic development produced seeds. The nature of self incompatibility was confirmed by dusting the stigmas with the pollens of same capitulum. The self pollinated stigmas were observed under the microscope after 1, 2, 3 and 4 days of pollination. Not even a single pollen was found with germinating pollen tube, suggesting and confirming the sporephytic self incompatibility nature of the species. The results are summarized in the Table 2.

**Pollen emission and stigma, receptivity:** The sexual tracks (sexual dimorphism) in *Imula racemosa* is temporarily separated. To begin with the hermaphrodite florets elongate and emerge out of the capitulum. Two days after the anthesis, the pollen mass starts to ooze out through the tip of florets in the form of white dust. The stigma still remains concealed within the floret till all the pollen grains are completely thrown out of the anther column. As the anther dehiscence completes (protandrous nature) the style elongates and emerges out in the form of a rod. Within 5 h of complete emergence the stigmas open into a bilobed structure and expose their receptive surfaces. The receptive surface is papillate and continues to be receptive for about 3-4 days. After 4 days the stigmatic surface begins to shrivel marking the end of receptivity at the end of 5th day.

**Pollen viability and pollen to ovule ratio:** *Imula racemosa* produces a large number of healthy, plump and stainable tricolute pollen grains. The pollen grains of *Imula racemosa* did not respond to the 2, 3, 5 tetrazolium chloride test but did so in Acetocarmine 1%. The pollen viability in different sites revealed that in each population pollen viability by and large remains the same (Table 3).

**Pollen to ovule ratio:** The species produces a large number of pollen grains compared to the ovules. Each floret of the species possess a single ovule except the peripheral ones which gets modified into petal like structures to help and support the plant to attract pollinators. These ray florets are usually neutral and/or with male sex. Pollen ovule ratio on an average works out to be 2792.5±78.503 per floret and per capitulum/plant 2839.147±480.188 (Table 4).

**Pollination system:** The bright yellow color of the heads of *Imula racemosa* together with the large quantities of scented and smelly pollen grains produced by the species in the form of white mass attracts the insects from distance. The insects regularly visit the flowering heads inserting their mouth parts into the anther collar in search of nectar, concomitantly and/or accidentally get heavily loaded with the pollen grains. Anthesis proceeds in an asynchronous manner from floret to floret within the capitulum and from capitulum to capitulum between the plants of a population. Asynchronous nature of the anthesis facilitates the long term availability of flowers and pollen thereby attracting the insects regularly for longer durations thus maximizing the destiny of sexual aim.

Foraging on a sunny day usually starts early in the morning (09.00 h) reaching its peak around 12.30 h followed by a decrease up to 15.00 h. Thereafter it gradually increases again up to 17.30 h followed by a
gradual decline up to 18.30 h which is the time for the bees to leave the field. Enjoing the sweet and rich source of energy, the pollinators start to dance like cabare dancers, distributing the already deposited pollen from other plants on maximum number of stigmas.

Weather conditions have a profound effect on the insect visitation which is altogether absent on a rainy day (Fig. 1). The insects identified as the pollinator of the species belongs to order Hymenoptera. The Hymenoptera is represented by two species of genus Bombus belonging to family Bombidae and one species of genus Bremus belonging to family Bremidae. The Bombus species is the most frequent visitor and starts day to day visitation earlier than other visitors and continues till late evening. The frequency of visitation was found to follow a positive correlation with the number of flowering heads available (Table 5 and 6). In one of the natural population
(Gulmarg), there were 338 individuals with 22 maximum number of flower heads available at a particular time with frequency of visitation (Table 5). At Panchal population, 9 flower heads were present at a particular time. Another population at Gulmarg with 33 individuals, 6 seedlings, 7 reproductive and 20 sprouted was observed with extremely low visitation due to the least number of flower heads available at a particular time with zero seed set.

Seed set: The destiny of sexual phase in this plant shows a positive correlation both with the size of a population particularly the number of reproductive individuals and the size of the flower heads apart from other ecological requirements. Highest seed set was observed in the populations of Gulmarg (67.873%) and lowest at Panchal (58.387%) Table 7.

**DISCUSSION**

The phenological studies in general and flowering phenology in particular are useful in planning out the conservational strategies as well as formulating measures for cultivating them on large scale (Schemake et al., 1994; Wafai et al., 1996; Bernardello et al., 2001). The phenology of vegetative phases is important, as cycles of leaf flush and leaf fall are intimately related to process such as growth, plant water status and gas exchange (Reich, 1995). Anthesis is fairly asynchronous from floret to floret, capitulum to capitulum, plant to plant and population to population. This asynchrony prevails in subsequent developmental stages as well. However, the plant shows an excellent example of synchrony between conspecifics, were male-female phases overlap between functionally male and functionally female florets of different heads of different plants. Nevertheless, the protracted asynchronous mode of pollen presentation by the species guarantees the long term availability of the pollen to ensure effective pollination, a view supported by Wyatt (1982). The asynchrony within the florets of a capitulum/plant together with high degree of synchrony between conspecific mates is a mechanism to enforce the out breeding potential of the species for which the members of Asteraceae are well known (Lawrence, 1985).

The present study confirms the earlier reports on pollen ovule ratio of certain allogamous species including tribe Inuleae (Asteraceae) and genus Senecio (Short, 1981; Lawrence, 1985). The higher pollen ovule ratio observed in the species suggests its outbreeding nature as suggested and supported by Cruden (1976).

The species together with vegetative mode of reproduction qualifies for semelpary. However the sexual mode is much more important from genetic and economic
perspective. The species produces a fairly normal seed set. The species in some populations, due to habitat degradation and extraction, is subjected to Allee effect, were the decreasing size of a population and more importantly the number of reproductive individuals of a population usually decreases the insect visitations and subsequently seed set. Clearly, the Table 5 and 6, suggests that the abundance and/or density of reproductive individuals have a strong influence over the plant-pollinator interactions and reproductive success, therefore affecting both the quantity and quality of pollen and pollination services received. Lower visitation rates may lead to insufficient pollen transfer therefore to a decreased seed set. Even if the pollinator visitation is not affected by population size or density, still small populations may suffer reduced seed set due to declines in pollen quality (Kunin, 1993; Wolf and Harrison, 2001).

In addition the flowering season of *Imula racemosa* overlaps with other North West Himalayan species like, *Acnistum chasmalum*, *Arnebia benthamii*, *Angelica glaca*, *Bergeria ligulata*, *Imula raylea*, *Rheum emodi*, *Picrodora kurrooa* etc. Thereby maximizing the chances of pollinator attraction from distance. Once the pollinator visits the particular population, *Imula* with its showy bright yellow color flower heads, is being preferentially visited by the visitors.

From evolutionary and ecological perspective, the species does possess some advanced traits with efficient and economical strategies, smelling the smell of competition both at intra and inter population levels, came with the prescription for its unborn ones by developing the parachute like structures on its seeds which aid in dispersing them efficiently and elegantly to a longer distance thereby minimizing their competition, maximizing survival and at the same time extending its coverage.

Density dependent seed set in *Imula racemosa* has also important implications for restoration efforts on other self incompatible and critically endangered taxa. Out planting individuals at higher densities may serve to alleviate the negative effects of density-dependent seed set. Further as the plant population/size decreases, due to factors such as extraction, habitat degradation, invasive species etc., extinction risks may be heightened due, in part to Allee effects. This suggests and promotes the need to unravel the reproductive biology particularly for rare and threatened species and the factors effecting the reproductive success which undoubtedly will be a critical step as far as the management and conservation of rare and threatened species is concerned.

REFERENCES


