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Vegetative and reproductive performance of *Caltha palustris* L. in the subalpine region of western Himalaya

Kanak Sahai, Krishna Kumar Rawat* and Dayanidhi Gupta

CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, Uttar Pradesh, India
*Corresponding author's e-mail: drkkrawat@rediffmail.com

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ABSTRACT

Plants growing on mountains are challenged by the variety of environmental factors that affect the performance of plants differently at different altitudinal gradients of mountain ranges. Present study deals with the existence of different response patterns related to vegetative and reproductive performance of medicinally important plant species at varying altitudes of Indian subalpine region, which is not explored so far. The study was carried out at three altitudes, covering the local ranges of *Caltha palustris*. Various vegetative and reproductive traits were studied along the altitudinal gradients and analyzed statistically. The study revealed that plant population became scattered with delayed reproductive phenology at the highest study site. Vegetative and reproductive traits gradually reduced towards the higher altitudes. Though pollinators and their activities were reduced at the highest altitude, maximum visits of dominant 'Syrphid flies' were not influenced by the altitudinal gradients. Post fertilization ovule abortion was observed up to 86% at all the three altitudes of the population.

Key-words: *Caltha palustris*, Vegetative performance, Reproductive performance, Altitudinal variation, Syrphid flies.

INTRODUCTION

Temperate or alpine regions are very susceptible to a rapidly changing environment, therefore to study plant sensitivity or responses towards environmental factors and climate change, elevation gradients are ideal platforms. The environmental variability along altitudinal gradient may cause changes in flowering phenology (Molau et al. 2005), vegetative growth and development and pollinator frequency, which affects the overall performance of alpine plants. Moreover, the sensitivity of plant phenology to the timing of snow melt at varying altitudes directly controls their life cycle patterns. The most commonly reported trends in plant performance with increasing altitude are reduced flowering and growth, longer life span and more intensive clonal growth (Douglas 1981; Hautier et al. 2009). Considering the influence of altitudinal gradient on the vegetative and reproductive performance, an important medicinal plant species *Caltha palustris* L. was selected for the present study.

Caltha palustris L. (Ranunculaceae) is commonly known as 'Marsh Marigold' or 'Kingcup'. It has a circumboreal distribution across much of Europe, Asia and North America (Schuettpelz & Hoot 2004). It is restricted to the sunny side of aquatic, semi aquatic, marshy and damp places of stream side of temperate and arctic regions and commonly associated with melt water, hence it is patchy in distribution. The whole plant especially the older portion contains toxic glycoside protoanemonin having anti-leukemic property (Bonora et al. 1987). Apart from the anti-cancerous property the plant is useful as diuretic, laxative, anti-inflammatory, expectorant and in curing warts, fits and anemia (Lust 1983; Schofield 1989; Aswal & Mehrotra 1994; Moerman 1998; Duke et al. 2002; Kumar & Singhal 2008). In addition, the extract of *Caltha palustris* have been used in traditional Canadian and Asian medicine to treat rheumatism, gonorrhoea, arthritis and various skin diseases (Suszko et al. 2013). A perusal of literature revealed that inspite of detailed medicinal description, studies concentrating mainly on reproductive behaviour are almost lacking on *Caltha palustris*. Moreover, some work related to phenology, growth and embryo development were carried out in other species of *Caltha* (Mani 1978; Ram et al. 1988; Wardlaw et al. 1989; Aydelotte & Diggle 1997; Forbis & Diggle 2001). The present study is the first record on *Caltha palustris* from Indian subalpine region. The aim of the work presented herein was to monitor vegetative and reproductive performance of *Caltha palustris* at subalpine ecosystem of India.

MATERIAL AND METHODS

The study was carried out at varying altitudes of Har ki Doon valley situated at Govind Wildlife Sanctuary, Uttarakhand, India (Text Figure 1a-c). The total area of sanctuary and National park is 958 km, situated between 35°55.00 and 30°17.30 N latitude and 77°47.30 and 78°37.30 E longitudes with its altitudes ranging between 1300-6323 m and receives light to heavy snowfall in winters (Chandra et al. 2012). Snow fall generally begins in October and melt concludes in May to June. The climate varies from tropical to alpine according to altitudinal gradient. Different parts of Govind Wildlife Sanctuary were extensively explored by repeated visits during 2013 to 2015. However, population of *Caltha palustris* was observed only in Har ki Doon valley from 3398m to 3490m altitudes. Ten accessible populations from each of three altitudes were selected for the study. The highest altitude was 3490 m on the flat ground in the shallow stream with the geographical limits 31°09'12.39" N, 78°25'53.23" E, mid altitude was 3434 m in the mountain slope on the edge of the stream with the geographical limits 31°09'00.03" N, 78°25'48.02" E and the lowest altitude was 3398 m in the valley on the edge of stream with the geographical limits 31°08'56.22" N, 78°25'50.08" E (Text Figure 1 d-f).

Caltha palustris is rhizomatous herbaceous perennial plant about 4 cm to 52.5 cm in height along the gradients with rounded to kidney shaped leaves. Basal leaves are produced early in the year while alternate leaves are produced along the stems. The petioles of the basal leaves are longer than the petioles of the alternate leaves that bears small cluster of bright yellow flowers. The flowers are 4 cm to 5.5 cm in diameter with 5-6 bright yellow petal-like sepals surrounding numerous spatulate separate stamens and 9-28 carpel in the centre (Plate 1, figure d). Each carpel had slightly oblique, bi-lobed papillate stigma and small style. Each of the carpel matures into a follicle and the fruit is an aggregate of follicles. Initially all follicles are green and erect which spread out after getting matured and become greenish brown on drying (Plate 1, figure f). Follicles split by terminal pore and release 60-100 minute seeds.

Present study was monitored under the following parameters:

Analysis of vegetative and reproductive traits

Various morphological parameters like average plant height, leaf length (with and without petiole) and width, flower diameter, average number of flowering branch per plant, flowering unit per branch, number of stamen, pistil and their length and follicles (fruits) per flower and ovules per ovary were recorded for vegetative, bud, and flowering and fruiting stages. A total of 25 samples were used for each trait. Young buds, flowers and young fruits were collected and analysed under dissecting microscope for different developmental stages of ovule.

Pollen grain count, pollen viability and stigma receptivity

A total of 100 mature, un-dehisced anthers i.e.10 anthers/flower from each of 10 plants were extracted and macerated in 10 ml of water in an eppendorf tube to release the pollen grains and a homogenous mixture was prepared by shaking well. Pollen counts were recorded with the help of Haemocytometer. A total of 25 readings were taken for each sample. Ten flowers from ten plants were randomly collected daily from first day of their anthesis to wilting of flowers. Observations were made at hourly intervals to find out the time and pattern of flower opening, stigma receptivity and anther dehiscence. Pollen viability and stigma receptivity were tested with 1% Benzedine solution by following Galen et al. (1985).

Nectar analysis, flower life span and flower visitors

Ten freshly opened flowers from 10 randomly selected plants were analyzed for nectar volume. The volume of nectar was measured by the calibrated micropipettes from 1000 hrs to 1600 hrs at the intervals of 2hrs. Two unopened bud was selected from each of ten plants and tagged with coloured thread or marked with ink and observed regularly from onset of flowering to withering of all tepals to analyze the flower life span. Observations on flower visitors and their abundance were carried out during peak flowering period from 0700 hrs to 1700 hrs at the interval of every one hour. The frequent visiting pollinators were caught using sweeping net and images were taken with a digital camera (NIKON P100). Identification of frequent visitors was made using available relevant literature and matching the different angles of the body parts of the insect. Data was statistically tested by the analysis of variance (one-way ANOVA followed by post hoc Tuckey test).

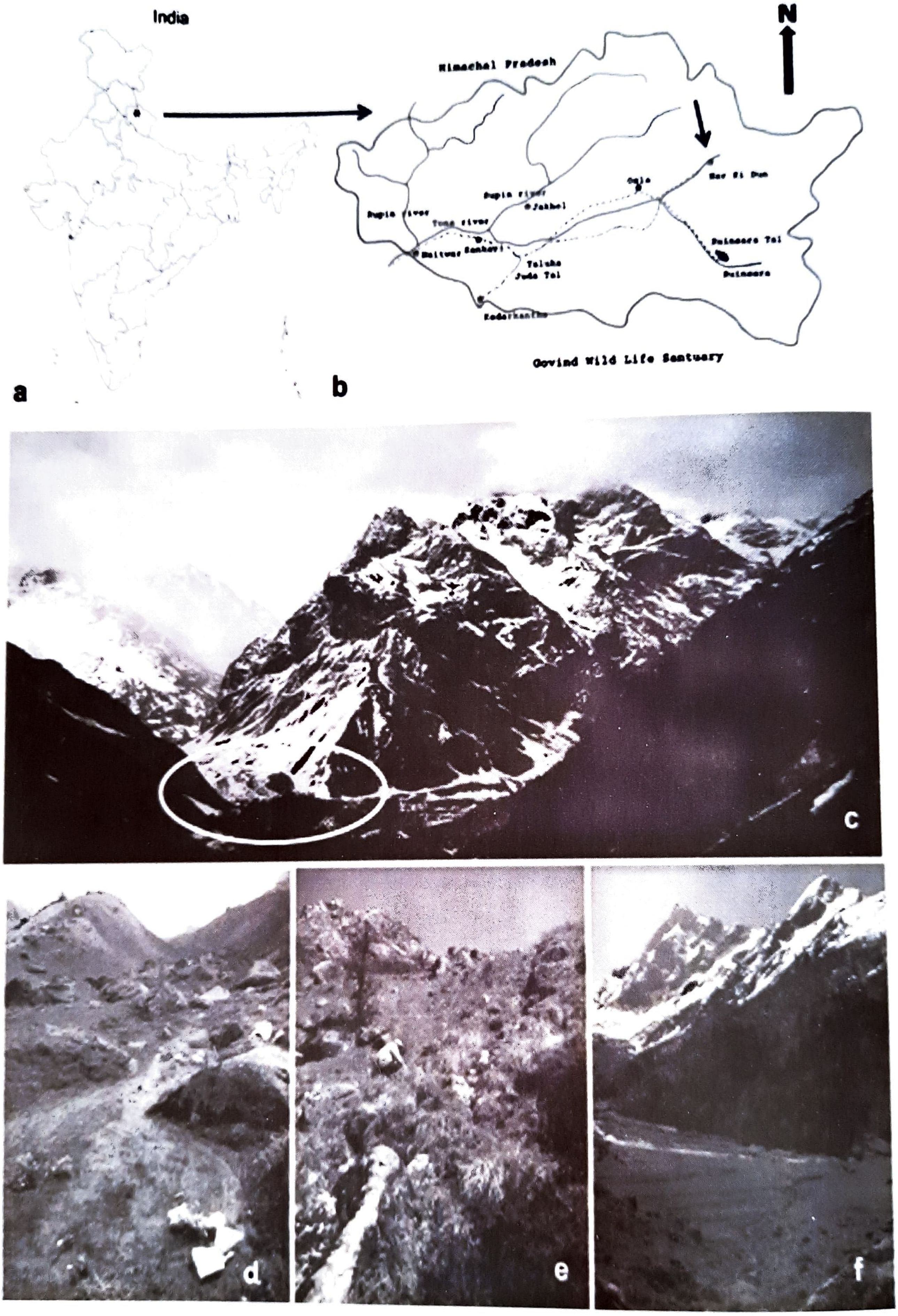
RESULTS

Vegetative and reproductive performance of *Caltha palustris*

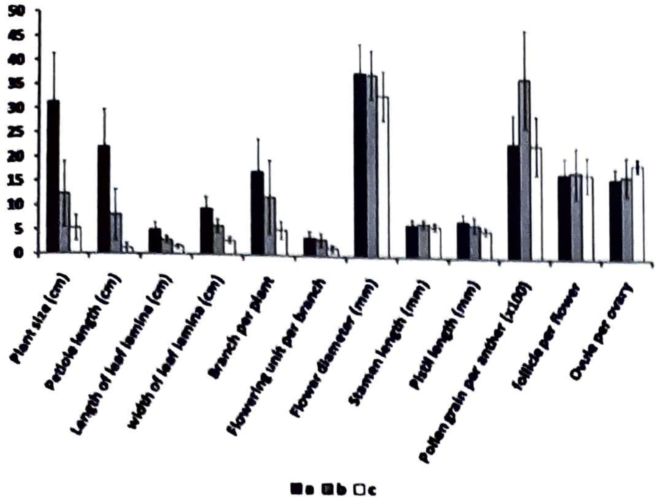
The abundance of *Caltha palustris* decreased with an increase in altitude. The number of plant individuals reduced and became scattered at the highest study site. Plant size gradually reduced towards higher elevation. It ranged 4.1-11.4 cm at highest and 16.4-52.5 cm at lowest altitude of the study area. The flowering stalk became shorter with increasing altitude which reduced plant height significantly [ANOVA $F_{(2,38)} = 61.65$, $p < 0.01$; Tuckey HSD (0.05) = 5.9, HSD (0.01)= 7.5, $p < 0.01$], along with petiole length [ANOVA $F_{(2,96)} = 172.23$, $p < 0.01$; Tuckey HSD (0.05) = 2.31, HSD(0.01) = 2.9, $p<0.01$], leaf lamina length [ANOVA $F_{(2,104)} = 119.268$, $p < 0.01$; Tuckey HSD (0.05) = 0.5, HSD (0.01) = 0.63, $p<0.01$] and width [ANOVA $F_{(2,104)} = 132.88$, $p<0.01$, Tuckey HSD (0.05) = 0.92, HSD (0.01) = 1.5, $p<0.01$] (Text- Figure 2). Though, flowering branches per plant [ANOVA $F_{(2,31)} = 13.754$, $p<0.01$] and flower diameter [ANOVA $F_{(2,33)} = 3.73$, $p<0.05$] were significantly higher in plants of lowest elevation compared to highest elevation plants (Text Figure 2), post hoc Tuckey test for number of flowering branches per plant [HSD (0.05) = 7.43, HSD (0.01) = 9.49] and flower diameter [HSD (0.05) = 0.67, HSD (0.01) = 0.86] showed that means of highest population differed significantly from lowest population at $p<0.1$ and $p<0.05$ respectively. Similarly, a significant variation in number of anther per flower [ANOVA $F_{(2,27)} = 11.326$, $p<0.01$] and pollen grain per anther [ANOVA $F_{(2,27)} = 11.326$, $p<0.01$] were observed along the elevation gradient (Text Figure 2). Post hoc Tuckey test for anther per flower [HSD (0.05) = 13.19, HSD (0.01) = 16.92] showed that mean of the value of highest population differed significantly with that of mid and lowest population at $p<0.01$. However, this parameter was insignificantly variable between mid and lowest population. Similarly, post hoc Tuckey test [HSD (0.05) = 5.47, HSD (0.01) = 6.89] for pollen grain per anther showed that mean value of mid population varied from highest and lowest population at $p<0.01$. However, elevation gradients had no impact on the number of pollen grains per anther. A significant variation in the length of pistil [ANOVA $F_{(2,57)} = 10.066$, $p<0.01$] was also observed among the three different altitudes (Text Figure 2). Post hoc Tuckey test [HSD (0.05) = 1.01, HSD (0.01) = 1.27] showed that mean value of highest population significantly varied with the value of mid and lowest populations at $p<0.05$ and $p<0.01$ respectively.

Reproductive phenology, anthesis and floral life span

The reproductive phenology of *Caltha palustris* varied along the snowmelt gradient. It flowered immediately after snow melt i.e., in the starting of growing season during April last to middle of June along the variable timing of snow melt at different altitudes. Flowering started during end of April to 1st week of May at lowest altitude, during 2nd to 3rd week of May at mid altitude and during 4th week of May to 1st week of June at highest altitude of population. However, flowering, fruiting, seed set and senescence completed



Text Figure 1. a, b. Location of study site in the Indian map (with arrow). c. Photographic view of study area with actual location of study site (in circle). d. Highest altitude of study site. e. Mid altitude of study site. f. Lowest altitude of study site.



Text Figure 2. Different vegetative and reproductive traits of *Caltha palustris* across the three altitudes. a. Lowest. b. Mid. c. Highest.

before the end of growing season i.e. up to the end of July. The flowering was about 20 to 25 days delayed at the highest altitude of population. There was a 7 to 10 days difference between the flowering of mid and lowest altitude.

The flowers of *Caltha palustris* were hermaphrodite, actinomorphic and started to open between 0700 hrs to 0800 hrs and completed their opening up to 1000 hrs. The anther had lateral dehiscence, which initiated in fully bloomed flower. Dehiscence started first in the outer whorl of stamen and gradually proceeded towards the inner whorls at different intervals (Plate 1, figures d-e). Thus, the anthers took irregular intervals to complete their dehiscence. In general, at all the elevations flower bud took 3-4 days from green bud to half-opened flower. This stage of flower was mentioned as Day 1 stage. The flower turned to fully bloomed stage on the next day and remained open for 5-7 days followed by gradual withering of tepals and their fall. The stamens remain attached and gradually fall after the follicles (ovary) begin to enlarge (Plate 1, figures e-f).

Pollen viability and stigma receptivity

The type of dichogamy was caused by protogynae. The stigma was found receptive along the flower opening and remained the same up to 3rd day of flower life. Stigma receptivity gradually decreased along the wilting of tepals, which took 5-7 days. Pollen showed 100% viability during the first day after the complete opening of flower. It was 50-65% at the end of 3rd day of flower life. Like the stigma receptivity, pollen viability also gradually decreased along with the wilting of tepals.

Floral visitors, visitation pattern and rewards

Caltha palustris is a fly-pollinated plant (Myophily). Though various kinds of flies were observed at the study site, maximum visiting flies were identified as "Syrphid flies" (Plate 1, figure c), which were common to all altitudes in spite of their low activity in highest altitude. Pollinator's activity started mostly around 0900 hrs, reached its peak at 1300 hrs and then gradually decreased. However, cloudy and rainy weather adversely affected the visiting activity. The pollen clings to the legs and abdomen of the flies during the visits that could facilitate pollination. Floral reward was nectar, secreted in a small quantity in the small depressions like nectaries present on the base of each side of carpel. A very small quantity i.e., average 1.53 µl of nectar was found only at the highest study site. However, nectar was insufficient to measure at mid and lowest altitudes.

Fruit and seed set

Fruits (follicle) per flower and young ovule per follicle ranged 9-28 and 7-21, respectively. At maturity up to 86% of ovule abortion was observed at different altitudes. Further, field observations on splitting of follicles and seed dispersal could not be carried out due to inaccessibility in the rainy season followed by long winter of snow cover. At maturity seed contained rudimentary embryo, this is a characteristic feature of family Ranunculaceae. Rudimentary embryo occupied about 20% to 60% of the length of the albuminous seed.

DISCUSSION

The reduction in overall size of the plant is most remarkable morphological alternation for the adaptation to increasing altitude that can be observed along elevation gradients. It was also confirmed during the present study as *C. palustris* presented marked variation in growth habit with elevation. In *Caltha palustris*, flowering stalk became shorter with increasing altitude which reduced plant height (Plate 1, figures a-b). Reduction in plant size might allow plant to survive in severe climatic environment with least

Plate 1

Vegetative and reproductive performance of *Caltha palustris* at a. Highest altitude. b. Lowest altitude of study site. c. Visits of syrphid flies. d,e. Pattern of anther dehiscence. f. Young fruits (follicles). g. Rudimentary embryo.

resources. In addition, vegetative growth is expected to reduce the availability of resources for reproduction (Hautier et al. 2009). We found pronounced differences in growth and reproductive performance along elevation gradients. In accordance to Kudo (1991) and Wipf & Rixen (2010), flowering phenology of *Caltha palustris* was also highly associated with the timing of snowmelt. The timing of snow melt and the depth of snow cover was highly variable along the altitudes. Hence, it was observed that reproductive phenology was advanced at lower altitudes. It was 20-25 days delayed at highest altitude of population. At lower elevation snow melting starts earlier and initiates its reproductive phenology accordingly. Because of differences in snowmelt timing, the reproductive phenology was highly variable among the population of different altitudes. Though flower diameter and flowering branches per plant were significantly higher in plants of lowest elevation compared to highest elevation plants, flowering unit per branch, stamen length, follicle per flower and ovule per ovary were varied insignificantly among the population of all the altitudes. Elevation gradients impose severe constrains for reproduction and establishment particularly in high elevation population because of lower temperature, reduced atmospheric pressure, increased light intensity, UV radiation (Körner 2007), shorter growing seasons and longer persistence of snow-cover. At high altitude insect pollination is far more important than wind pollination and out-crossing appears to be the dominant breeding system (Körner 1999). In general, flies tend to be important and principal pollinator in high altitude. However, the plants face pollination problem due to low temperature, which confine insect activities for a short period and even then mainly to the sunny daylight hours (Mani 1962) as observed in the present study. Syrphid flies were dominant over the study sites though their visits decreased from lowest to highest altitude of study sites. Similarly about 80% visits of Syrphid flies along with other insects were reported (Mutin et al. 2009) in *Caltha palustris*. They also reported other 10 genera of flies mostly Hoverflies from Russia visiting the same species. In addition, at high altitudes reduced pollinator abundance, diversity and activity due to unfavourable abiotic conditions and decreased length of growing season are the major constraints, which may promote adaptation for selfing (Bliss 1971; Ellenberg 1988; Heinrick 1993) as observed during the study when *Caltha* was considered for self-pollination experiments and produced few seeds. However, *Caltha palustris* is also able to adapt self-pollination during adverse circumstances. Like most of the alpine plants, *Caltha palustris* also prefers vegetative reproduction towards higher altitudes because of inimical environmental conditions for successful seed set and seedling survival. Rhizomes were the principal mean of vegetative reproduction. Although, above ground portion of the plant decay and disappear after fruiting during July-August, the underground stout portion of plant i.e., rhizome persists for the next growing season. In *Caltha palustris*, flowering was observed immediately after snow melt. These early flowering plants always initiate their flowering primordial in the previous seasons, (Bliss 1971; Bilings 1974) which shoot up early in the next growing season after snowmelt. As a result of which pollination, fruiting, seed setting and dispersal are completed before the end of the growing season.

Though, seed dispersal occurs by wind immediately after the maturation and bursting of follicles, the germination takes place after a long seed dormancy period under the snow. The germination of seed is supported by the moisture provided by the melting water (Whitt 2009) as the alpine soil does not freeze under snow cover (Körner 1988). Like other member of Ranunculaceae, *Caltha palustris* also possessed an embryo type at the time of dispersal that has been referred to as 'rudimentary' (Martin 1946; Atwater 1980) i.e., underdeveloped that must undergo further development to become a complete mature embryo. Seeds of *Caltha palustris* with rudimentary embryo (Plate 1, figure g) had morpho-physiological dormancy (Baskin & Baskin 2002) i.e., morphological dormancy in combination with physiological dormancy, which is characteristic of Ranunculaceae (Baskin & Baskin 1998). As a result of which seeds were unable to germinate at dispersal. However, physiological dormancy appears to be broken first during cold stratification period and then the embryo must also overcome morphological dormancy by its full development as observed by Forbis & Diggle (2001) in *Caltha leptosepala*. However, the ability of such embryo to develop and germinate at low temperature may be an adaptation to its environment (Forbis & Diggle 2001).

In conclusion, the findings highlighted the existence of different response pattern to climate change at varying altitudes. Like other alpine plants, *Caltha palustris* exhibited delayed reproductive phenology, less flowering units, reduced growth and development with increasing altitude of population, which was a marked adaptation against the climate change. Moreover, *C. palustris* is also capable to show important adaptive features i.e., pre-formation of vegetative and flowering primordial to cope with short growing season and prolong winter as the flowers along with vegetative parts sprout out just after the snow melt.

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