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Phenology of *Berberis lycium* Royle- an important medicinal plant of NW Himalayas

Seema Devi* and Namrata Sharma
Department of Botany, University of Jammu, Jammu - 180006.

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***Corresponding Author Email:** dseema254@gmail.com

Abstract
Berberis lycium Royle is a perennial, semi-deciduous shrub of great medicinal virtues. Rasout, an important drug obtained from the plant is rich in alkaloid berberine, due to which it is employed to cure many human ailments and is a part of several ayurvedic formulations. Present communication deals with phenology of the species in the sub-tropical climate of Jammu, J&K UT, India, based on observations in two populations in the area. The species undergoes semi deciduous phase in autumn, with the reappearance of leaves occuring in March, after the winters pass away. Flowering season commences in the end of March or in the beginning of April. Flowers are arranged in raceme inflorescences and have a long-life span of five days. These undergo several tactile movements during this period. Flowering ends in the last week of April or in the first week of May. Fruiting period spans the month of May and June. Fruit is a berry.

Keywords
Berberis lycium, perennial, phenology, rasout, semi-deciduous.

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INTRODUCTION:
Berberidaceae consists of 19 genera and 755 species worldwide (www.the plantlist.org). It is represented by 3 genera and 68 species in India [8]. The three genera are *Berberis*, *Mahonia* and *Epimedium* [3]. *Berberis* is one of the most important genus in the family and has the largest diversity in Asian continent with centre in the Himalayan region [1,5]. It is the largest genus of family Berberidaceae having 400 species globally and 55 species in India. *Berberis lycium* Royle is an important medicinal plant of NW himalayas that is valued for the crude drug 'Rasout' or 'Rasanjan' extracted from its roots. The bark of the root and lower part of the stem is characteristic yellow in colour and yields berberine- a medicinally important alkaloid [4,6]. The plant is, thus, mercilessly uprooted in nature which puts pressure on its natural population. Barring reports from Sharma and Verma (2016) [7] and Verma et al. 2021 [10], data on plant and flower phenology is meagre for the species in the area of study. Present communication deals with the phenological events in the species.

MATERIAL AND METHODS:
The species is semi-decidous and grows luxirantly in the outer Himalayas. For the present study, two sites, Billawar (1010masl, N32˚38.134' E075˚35.362') and Ramnagar (916masl, N32˚53.747' E075˚13.869') were tagged in Jammu area of J&K UT, India where it is locally called 'Kemblu'.

Methods:
Plant morphology and phenology: Data on the time of leaf shedding, reappearance of leaves, flowering and fruiting phase was collected during regular field visits from 2019-2022. Detailed morphology of the plants was studied in field and data was collected on various aspects like height of plant, number of

branches per plant, number of inflorescence per plant, length of inflorescence, number of flower per inflorescence, number of leaves per whorl, number of spines present on leaf margin.

Floral morphology and phenology: Finer details of floral morphology were worked out in the laboratory. Morphometric data on floral characters viz. length of flower, length of stamen, length of pistil and size of accessory whorls like sepals and petals were noted. All these measurements were carried out using a scale. Observations on anthesis and anther dehiscence were made in the field at regular intervals of time. Stigma receptivity was checked by observing the carpels of different ages under the microscope for pollen germination. Pollinated pistils were fixed in carnoy's fixative for 6-8 hours and stored in 70% ethyl alcohol. These were later stained in a mixture consisting of 2ml of 1% aqueous acid fuchsin, 2ml of 1% aqueous light green, 40ml lactic acid and 46ml distilled water and observed under microscope [9]. Mature buds were tagged (n=30) and observed regularly in the field till their senescence to determine flower longevity. Movement of petal, sepals and stamens vis-a-vis the stage of flower development was noted continuously in the field on different days in both the populations.

RESULTS AND DISCUSSION:

The species is a perennial, semi-erect shrub (Fig.1a) and attains an average height of 2.70 $\pm$ 0.26 m in Billawar and 3.08 $\pm$ 0.24 m in Ramnagar populations. Stem is branched and bears whitish angular branches which are 1 – 4 in number and arise from the base of the plant. These further divide into secondary branches and then into fine tertiary branches and form the shrubby structure of the plant. All these branches are branches of limited growth or long shoots. These long shoots bear dwarf shoots at their node which are 1-2 mm in length (Fig.1b). At the tip of dwarf shoots arise 2 – 9 foliose leaves that are alternately arranged in clusters. Below the tuft of foliose leaves, trifid spines are present which are modification of primary leaves. The leaves are sub-sessile, obovate in shape with margins either entire or spinous. They are thinly coriaceous, dark-green above and have pale green and glaucous dorsal surface with inconspicuous veins. From the centre of tuft of leaves, arises an inflorescence which is longer than the leaves (Fig.1b) (Table 1). Number of inflorescences per plant is high and averages 1043-1400. Each inflorescence on an average bears 20-25 flowers (Table 1). It bears beautiful yellow colour flowers arranged in corymbose raceme. The species comes in flowering in the last week of March in Ramnagar populations. However, flowering is slightly late in Billawar population where it commences in the first week of April. Temperature during this period fluctuates between 14- 34 ˚C. Peak flowering occurs in second and third week in Ramnagar and third and fourth week of April in Billawar populations.

Individual flower is bracteate, actinomorphic, hermaphrodite and have petals and sepals arranged in a series of 3+3+3+3. It is 1.59 $\pm$ 0.09 (1.1 – 2.4) cm long and 5.72 $\pm$ 0.34 (3 – 9) mm in diameter. Petals have long ovoid nectaries at their bases (Fig.1d). Sepals of inner whorl are larger in size [(l $\times$ b) (n = 30), [5.05 $\pm$ 0.08 (4 – 6) $\times$ 3.37 $\pm$ 0.08 (3 – 4) mm] than that of the sepals in outer whorl [(l $\times$ b), 3.62 $\pm$ 0.14 (2.5 – 5) $\times$ 2.28 $\pm$ 0.10 (1.5 – 4) mm] and petals [(l $\times$ b), 5 $\pm$ 0.00 (5) $\times$ 3.23 $\pm$ 0.06 (3 – 4) mm] (Table 2). The aestivation is imbricate. Stamens, 3.64 $\pm$ 0.08 mm in length, are bithecous, adnate and are aligned opposite to petals in two whorls of 3 each. Each lobe is further divided into a large and a small pollen sac (Fig.1e). Pistil is monocarpellary and 3.96 $\pm$ 0.03 mm long. Stigma is round, have a recurved marginal band with a hole in the centre. Style is hollow and reduced. Ovary is oblong with ovules arranged in basal placentation.

Anthesis begins in the morning around 0700h and continue till 1500h in the afternoon. Single flower has an average life of 5.4 $\pm$ 0.10 days. The orientation of sepals and petals undergoes considerable change during and after anthesis. Both of these are at an angle of 30˚ w.r.t pistil during first five to six hours of flower opening (Fig. 2b). Thereafter petals remain in their original position while the sepals, both large and small, attain perpendicular position w.r.t central pistil exposing abaxial surface of obovate petals (Fig. 2c). After 24 hours the large sepals reflex towards pedicel reaching at an angle of 123˚ w.r.t pistil, with the apex pointing down thus exposing the petals fully. However outer whorl of small sepals remains at perpendicular position w.r.t pistil (Fig. 2e). After 24 hours, petal senescence starts. This marks the end of anthesis. It is followed by the senescence of sepals (Fig.2f) (Table 2). As the flower opens up, anther dehiscence starts and completes in 3 to 4 hours of flower opening. Flowers are weakly protogynous as the stigma becomes receptive in the bud stage 10 to 12 h before flower opening. It remains receptive for 87- 96 h.

The fruiting period spans the month of May and June. The fruit is a berry (Fig.1f). It is green in colour when unripe and turns dark purple on ripening. It is edible and is a rich source of vitamin C. In the month of July on the arrival of monsoon, the fruits are shed off. The seed germination takes place in January after these

receive chilling temperatures. During the month of September- October, partial shedding of leaves occurs; and after the winters passes away, leaves start reappearing in the month of March. Detailed phenological events in the life-cycle of the species are shown in Fig.3, Table 3.

Table 1: Mormhometric details of *Berberis lycium* in the two populations.

S.No.	Character	Billawar population	Ramnagar population
1	Plant height (metres) (n = 30)	2.85 ± 0.21* (1.4 - 5.1)**	3.11 ± 0.19* (1.2 - 4.7)**
2	Number of primary branches per plant (n = 30)	2 ± 0.22 (1 – 5)	2.13 ± 0.23 (1 - 5)
3	Number of secondary branches per plant (n = 30)	5.2 ± 0.57 (1 - 12)	7 ± 0.90 (2 – 25)
4	Number of leaves per whorl (n = 30)	6.03 ± 0.26 (4 – 9)	5.87 ± 0.37 (2 – 9)
5	Size of leaf (cm) (l × b) (n = 30)	4.50 ± 0.15 (3 – 6) × 1.16 ± 0.07 (0.4 – 1.9)	4.59 ± 0.18 (3.4 – 6.9) × 1.21 ± 0.05 (0.6 – 1.8)
6	Number of spines on leaf margin (n = 30)	2.87 ± 0.35 (0 – 6)	3.7 ± 0.41 (0 – 7)
7	Number of inflorescence per plant (n = 30)	1412.53 ± 299.70 (60 – 5610)	1043.60 ± 315.10 (12 – 9200)
8	length of inflorescence (cm) (n = 30)	5.21 ± 0.35 (2.2 – 9.5)	5.33 ± 0.60 (2.6 – 15.4)
9	Number of flowers per inflorescence (n = 30)	21.7 ± 0.95 (11 – 33)	25.28 ± 1.88 (12 – 53)

*Mean ± standard error
**Range

Table 2: Flower longevity

S.NO.	Time	Phenological event
1	Day 1	Buds open up at top and after 5 to 6 h of opening, petals and sepals come to lie at an angle of 30˚ with respect to central pistil. Thereafter petal remain at the same position and sepals (both large and small) align themselves at perpendicular position with respect to central pistil in 2-3 hours.
2	Day 2	After remaining in perpendicular position for 5 to 6 h, large sepals begin to show reflex movement towards the pedicel making an angle of 120˚ -125˚ with central axis.
3	Day 3	The flower keeps its sepals reflexed in the same position for the whole day.
4	Day 4	The colour of petals begins to change from pale yellow to dull yellow and the surface of stigma turns black due to drying of exudates. The senescence of stamens sets in.
5	Day 5	The petal senescence begins with their shrivelling and turning slight brownish in colour. However sepals retain their size and colour. The senescence of former marks the end of anthesis.
6	Day 6 & 7	Complete senescence of petals continues for days 6 and 7. This is followed by the senescence of sepals.

Table 3: Details of flowering and anthesis in two populations of *Berberis lycium*

S.No.	Character	Billawar population	Ramnagar population
1	Onset of flowering	Ist week of April	last week of March
2	Bud start opening	2nd week of April	Ist week of April
4	Full blooming	3rd and 4th week of April	2nd and 3rd week of April
5	Days taken by an inflorescence to complete anthesis	14-16 days	14-16 days
6	Time taken by a bud to transform into a flower	23-26 days	23-26 days
7	Peak hours of anthesis	0008-1200 hours	0008-1200 hours
8	Decline in blooming	Ist week of May	last week of April
9	Duration of stigma receptivity	87-96 hours	87-96 hours

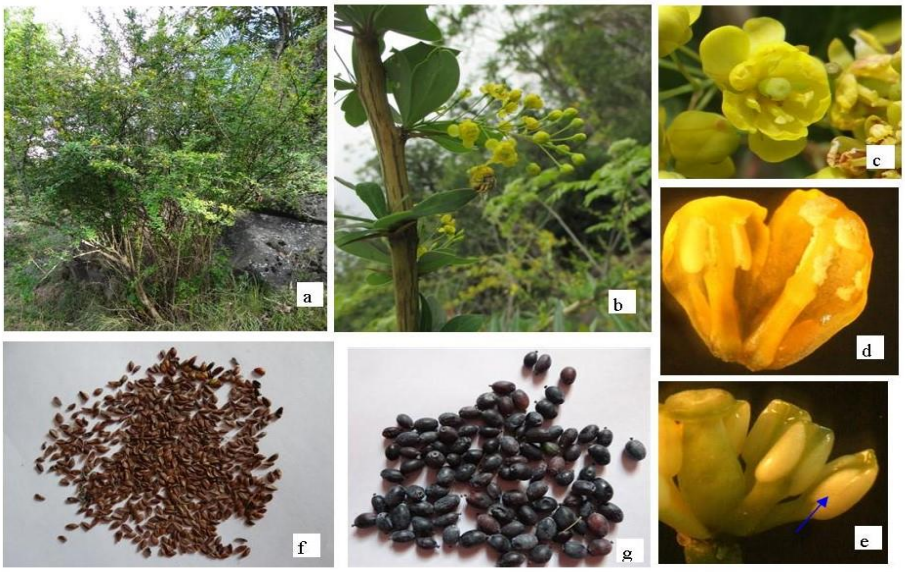

Fig.1: (*a*) Plants of *Berberis lycium* in flowering stage, (*b*) A portion enlarged to show long and dwarf shoot; the latter terminating in an inflorescence, (*c*) Individual flower (X3.32), (*d*) Antipetalous stamens positioned between two nectaries at the petal base (X7.4), (*e*) A pistil surrounded by stamens; arrow indicating anther lobe divided into large and small pollen sac (X7.69), (*f*) seeds and (*g*) fruits

Fig.2: Flower phenology (*a*) flowering opening at top (X3.15) (*b*) A fully opened flower (X5.59) (*c*) sepals aligned perpendicular to the pistil; while petals are at an angle of 30˚ (X4.37) (*d*) abaxial side of petals exposed fully by the reflex movement of sepals (X4.15) (*e*) sepals reflexed (X5.37) (*f*) petal senescence (X3.57)

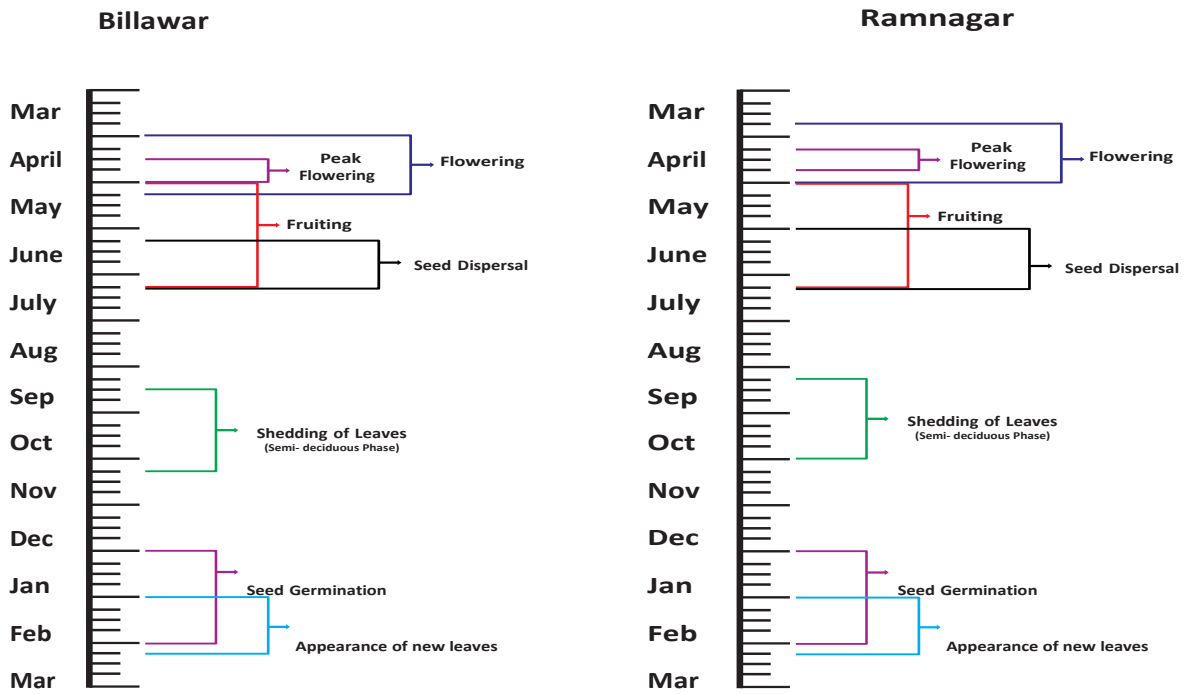

Fig.3: Plant phenophases in both the populations

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