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Phenological growth stages of *Meconopsis aculeata* Royle, an endangered medicinal plant of western Himalaya, using BBCH scale

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Abstract

Alpine plant species are perennial but have a shorter growing period according to prevailing habitat conditions that remain snow-covered during winter. In these areas, many of the species are important sources of valuable drugs. Phenological observations on *Meconopsis aculeata* were recorded at a weekly interval in Tungnath region of western Himalaya. Phenological studies in alpine plants are minimal, especially in Indian Himalayas thus, the present study was carried out to describe detailed phenological stages of *M. aculeata* in Tungnath region of the Indian Himalaya. The BBCH (Biologische Bundesantalt, Bundessortenamt, and Chemische Industrie) scale was used to characterize seven primary growth stages from seed germination to senescence, as well as secondary growth stages. These stages include stage 0 (germination phase), stage 1 (leaf development), stage 5 (Inflorescence emergence), stage 6 (flowering), stage 7 (fruit development), stage 8 (Fruit ripening) and stage 9 (Senescence). Due to the high market demand of medicinal plants in local and international markets, work on the various aspects of domestication and crop improvement is a dire need, therefore the detailed phenological growth stages of *M. aculeata* could be helpful and complimentary for local growers, breeders and researchers.

Key words: Phenology, BBCH scale, *Meconopsis*, Garhwal Himalaya

Introduction

India is rich in terms of topography and plant biodiversity, with more than 17,000 angiosperm species, 64 gymnosperms, 1,200 pteridophytes, 2,850 bryophytes, and 2,021 lichens (Joshi and Pant, 2012; Singh *et al.*, 2019). Out of the total plant species, about 45-50 % of species are used as medicinal and aromatic plants. The diverse topography and climatic conditions provide rich medicinal plants diversity in Indian Himalaya, especially alpine region are the major source of important medicinal plants (Singh *et al.*, 2019). Himalaya is one of the major hot spot of the world having considerable diversity with many endemic plant species.

Phenological studies are generally related to recording the life cycle events of plants in relation to climatic factor *i.e.,* temperature, rainfall and humidity. Prehistoric phenological data are helpful to understand the impact of climate on the life cycle *viz.,* phenology, reproductive biology, and regeneration. Clear understanding regarding phenology, reproductive biology and regeneration of medicinal plants is a perquisite for their protection and management (Padmavathi *et al.*, 2012).

In nature, every species has a certain period for sprouting, seedling development, flowering and fruiting and in the existing pattern of a plant life cycle, every single stage is significantly affected by various ecological variables *i.e.* temperature, humidity, rainfall, *etc.* (Singh and Chauhan, 2018). The timing of various activities such as germination, bud break, flowering, fruit dehiscence and leaf drop is important for survival and reproductive success of many plant species (Kaur *et al.*, 2013).

Phenological observations of endangered and threatened plants can be helpful in finding a cause of rarity. *Meconopsis aculeata* Royle is an endangered, endemic perennial medicinal herb belonging to family Papaveraceae and distributed at an altitude of 3000–4700 m in Himalaya. It is famous as Queen of Himalaya and also known as Kalihari and Achatsarmum. There are total 49 species of the genus *Meconopsis* worldwide and about 38 species distributed in the west of China including Qinghai, Sichuan, Yunnan provinces and the Tibet region (Butola and Badola, 2008).

M. aculeata is rich source of phenols, flavonoids, cardiac glycosides, amino acids, carbohydrates, phytosterols, terpenoids, and steroids (Ahmad *et al.*, 2016). The entire plant is used as a tonic and shows cooling potency in spinal cord disorders (Aswal and Mehrotra, 1994). Traditionally the plants are used in the treatment of various ailments *i.e.*, fever, cough, and bone problems (Tsarong, 1994). Flowers of the species are used as antipyretic and analgesic and to treat asthma and cough (Bahukhandi *et al.*, 2018). The species is also used for ornamental and decorative purpose and has a great potential due to its showy flowers.

M. aculeata is monocarpic erect, prickly herb of about 40-60 cm tall. The plant bears a single, unbranched, erect, hard, prickly stem reaching up to height 50-65cm (0.5-0.6m) and deep tap roots of about 18-20 cm in length. Root is spongy and hollow type from inside at later stage. Leaves (approximately15-20cm length) are irregularly pinnately lobed and aculeate. The inflorescence is the cymose type, flower light blue-purple in colour, actinomorphic, hermaphrodite, hypogynous, complete and bisexual. Petals 4, obovate with obtuse or undulate margin. Sepals aculeate 2;

caducous. Bract or receptacle present along with pendicle and number of anthers were approximately 100. The androecium height measured that was 0.9±0.0cm. Flowering-mid July to August. Fruiting-mid August to September.

There was no detailed phenological coding available for *M.aculeata* thus present study was carried out to describe detailed phenological stages using the BBCH scale (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) at the experimental site.

Material and methods

Experimental site: Present study was carried out in Tungnath region of Garhwal Himalaya, India (30º29'23.50''N -79 º13'00.64''E) from altitude range of 3425-3600 msl. The area remains under snow cover for 5-6 months from November to April. The growing season of the plant is between May to October.

Maximum temperature ranges from 20 to 30 ºC and minimum temperature ranges between 3-15 ºC during summer season and annual rainfall ranges between 200-250 cm. Maxtech DT-9 Pen Type Thermometer, Maximum–Minimum Mercury Thermometer and Rain gauge were used to record temperature and rainfall in experimental site (Fig. 1). The soil type is black sandy loam.

Selection procedure and sampling: A total 50 individuals were randomly selected and tagged for phenological study and the observations were recorded from seed germination to fruiting and senescence at weekly interval. All the parameters were recorded through visual observation as well as measurement and photographs and date of observation were recorded as per BBCH scale (Meier, 2001). Two digit code was used to describe primary and secondary growth stages.

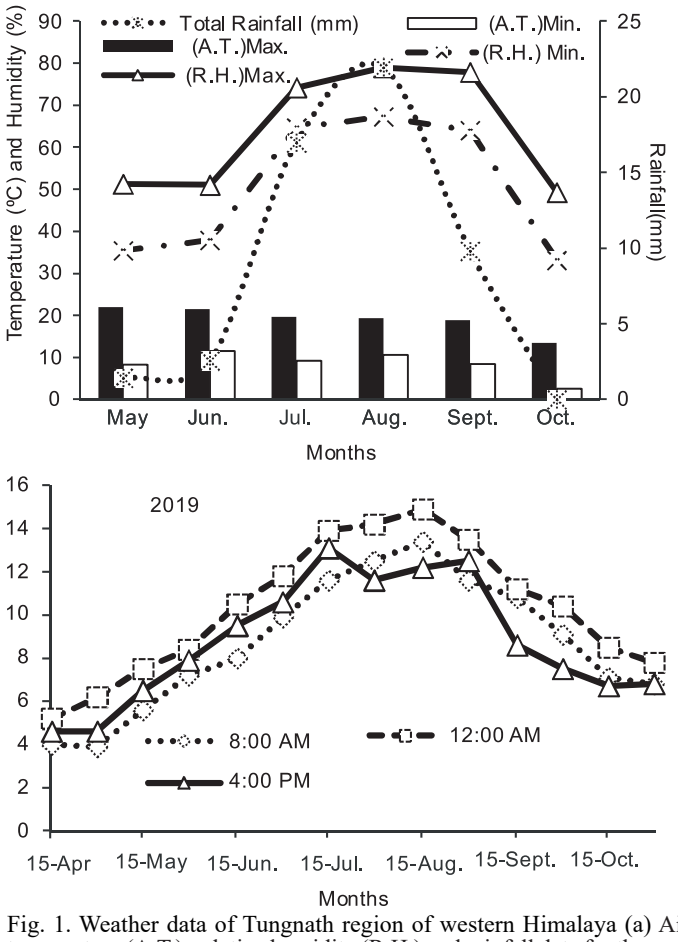

Fig. 1. Weather data of Tungnath region of western Himalaya (a) Air temperature (A.T.), relative humidity (R.H.) and rainfall data for the year of 2019, (b) Soil temperature data for the year of 2019

Results and discussion

The onset and completion of different phenological growth stages of *M.aculeata* were recorded in subalpine region of Indian Himalaya using BBCH scale with two digit numerical system. 7 Principal growth stages were recorded *i.e.*, stage 0 (germination phase), stage 1 (leaf development), stage 5 (inflorescence emergence), stage 6 (flowering), stage 7 (fruit development), stage 8 (fruit ripening) and stage 9 (senescence) and photographed to describe the growth out of total 10 stages (Table 1, Fig. 2, 3).

Stage 2 (formation of side shoots/tillering), stem elongation or rosette growth (stage 3), and development of harvestable vegetative parts (stage 4) has been omitted because *M. aculeata* does not produce any side shoot/tillers, rosette or stems and leaf are the major aerial part before flowering stage.

Principal growth stage 0: **Germination/sprouting/bud development**: Germination of seeds (first growth cycle) and sprouting (after second year onwards) are both considered as principal growth stage 0, as this species completes its life cycle in 3-4 years, however both the biological processes (germination and sprouting) are different from one another but phenologically analogous structures (Lancashire *et al.*, 1991).

Growth stage 0 consists of seed germination to cotyledon emergence (Fig. 2, a-d). After 5-6 months stratification period of seeds (in natural condition 5-6 months snow cover period from December to May) this stage takes 20-25 days or more for completion with temperature range around 8-20ºC. However, in second year (sprouting) this stage takes shorter duration as compared to first year similar to other perennial crops *e.g. Cynara cardunculus* L. (Archontoulis *et al.*, 2009).

After second year onwards re-growth starts from roots with vegetative bud on collar region and considered as stage 01 and this stage ends with appearance of green tips or leaf tips (stage 09). A single plant produces only one or rarely two vegetative buds and regenerates year to year for 3-4 years until fruiting. The plant regenerated from a long tap root having single vegetative bud which increases in size and mass after allocation of storage of food in root.

Principal growth stage 1: **Leaf development**: This stage illustrates the development of aerial part or leaves. The first leaf appeared after 25-30 days when the cotyledons were fully unfolded. During this stage leaves appeared similar to cotyledon having an elliptic or ovate shape (Stage 11) (Fig.2, e). After stage 12, leaves change their shape from ovate to slightly lobed margin. Stage 13 were recorded when 3 leaves were visible (Fig.2, f). This stage continues till the development of all leaves (up to 9) during the first growing cycle (Stage 19, Fig. 2, g,h).

Leaf development pattern in seed germinated plants and from rootstock is slightly different; a leaf from seed germination had elliptical or ovate shape with slightly lobed margin while those from sprouting have lobed margin and aculeate leaves.

Leaf development from first year onwards or sprouting begins when the first leaf appears during June (Stage 10). The second leaf appeared after 5-7 days of the first leaf (Stage 11). Stage 19

Table 1. Phenological growth stages of *M. aculeata* according to the BBCH scale

Code	Description	Description
		Principal growth stage 0
	Germination (First year only)	Sprouting, bud development (1st year onwards)
00	Dry seed	
01	Beginning of seed imbibition	Bud swelling
03	Completely imbibed seeds	End of bud swelling
05	Radicle emergence from seed	Beginning of bud breaking
06	Radicle Elongation, the formation of root hairs.	
07	Hypocotyl emerges from the seed	
09	The emergence of cotyledon	Bud showing green leaf tips
10	Cotyledon completely unfolded	First leaf separating.
		Principal growth stage 1: **leaf development**
11	First ovate or elliptic leaf visible	First leaf visible
12	Two ovate or elliptic leaves visible	Two leaves visible
13	Leaf changing shape from ovate or elliptic to lobed margin and three-leaves visible	Three leaves visible
1n	Stage continue till	Stage continue till
19	Plants attained final leaf number	9 or more leaves visible
		Principal growth stage 5: **inflorescence emergence and development (2nd year onwards)**
51		Inflorescence buds visible but still closed
52		Inflorescence development <20 %
53		First flower bud attains its 30 % size
54		Inflorescence elongates and reached to its 20 % of final size with 4-5 flowering buds
55		Inflorescence reached to its 50 % size, with 6-7 flowering bud and 1st flowering bud reached to its final size
57/60		Inflorescence reached to its 70 % size with 8-9 flowering buds and flowers still closed; sepals beginning to separate <30 %
58		Sepals beginning to separate <70 %
59		Inflorescence reached to its final size and final number of flowering buds
		Principal growth stage 6: **Flowering (2nd year onwards)**
60		First flowers open.
61		Beginning of flowering (about 10 % of flowers open)
63		Beginning of flowering: about 30 % of flowers open.
65		50 % of flowers open.
67:		Flower fading
69		All petals fall or dry: no flowers visible
		Principal growth stage 7: **Fruit development (2nd year onwards)**
71		Beginning of fruit growth; 20 % of final size
73		30 % fruiting and fruits reached 30 % of the final size
75		50 % fruiting appeared and 1st fruit reached its final size
76		60 % fruiting appeared and 30 % of the fruits reached their final size
77		70 % fruiting appeared and 50 % of the fruits reached its final size
78		90 % fruiting appeared and 70 % fruits reached its final size
79		Full fruiting appeared and all the fruits have reached its final size.
		Principal growth stage 8: **Fruit ripening (2nd year onwards)**
80		First fruit reached physiological maturity and change colour from green to blackish brown
81		20 % of fruits reached full maturity
83		30 % of fruits reached full maturity
85		More than 50 % of fruits reached full maturity and 1st fruit turns colour from black to brown. Advance ripening
87		70 % fruits reached the final maturity and 1st fruit had full seed dispersal
88		All fruits reached to full maturity and 50 % fruits had full seed dispersal
89		All fruits has full seed dispersal
		Principal growth stage 9: **Senescence**
	1st and 2nd year	3rd year
91	10 % yellowing of the plant	
93	30 % yellowing of the plant	
95	Leaves reached to 50 % yellowing and flowering stem turns yellow to brown	
99	Dormant phase	The whole plant complete senescence

was recorded when the plant attains 7-9 leaves (Fig. 2, h). Leaf development from sprouting is much faster compared to seedlings because the reserves food in root is more abundant to support growth (Archontoulis *et al.*, 2009).

Higher biomass allocation and growth were observed during July-August due to adequate moisture and temperature (rainy season, 15-25ºC). A fully developed basal leaf 20-25 cm long and 7-8 leaves per plant were recorded.

Principal growth stage 5: **Inflorescence emergence and development**: This stage describes the inflorescence growth and

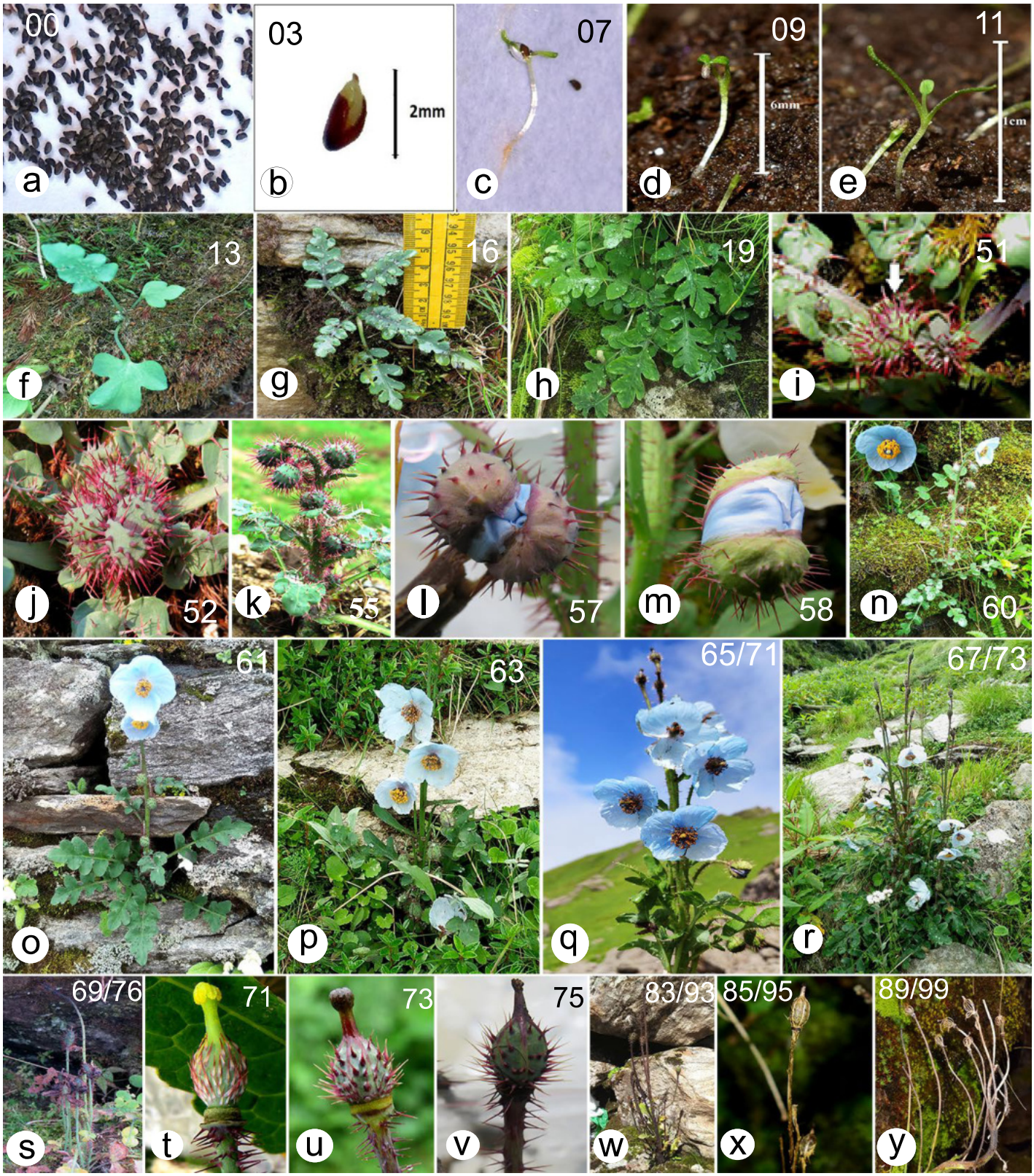

Fig. 2. BBCH growth stages of *Meconopsis aculeata*: stage 0: (a) dry seeds (b) redicle emergence (c) redicle elongation (d) cotyledon opening, stage 1: (e) cotyledon complete unfolded and first leaf visible (f) three leaf visible (g) six or more leaf visible (h) final number of leaf and full size , stage 5: (i) inflorescence bud visible (j) development of bud (k) inflorescence elongation; bud still closed (l, m) opening of sepal , stage 6: (n) first flower open (o,p,q) flowering (r) flower fading (s) all petal fall and 60 % fruiting appeared and 30 % of the fruits reached their final size (t) beginning of fruit growth (u) fruit reached to its 30 % of the final size (v) fruit reached to its final size (w) 30 % of fruits reached full maturity, 30 % yellowing (x) fruit reached to full maturity, 50 % yellowing (y) all fruit has full seed dispersal, plant senescence.

begins when the flower bud appeared (Stage 51) (Fig. 2, i) during the third year in late May or early June depending upon snow cover duration. These flowering buds grew and reached to their 20 % size and became easily visible during June 1st week (Stage 52) (Fig. 2, j). Along this cauline and radical leaves were also formed, two or three more buds were formed when earlier flowering bud attained 30 % of its final size during 2nd week of June (Stage 53). Along with this flowering shoot/inflorescence also elongated and

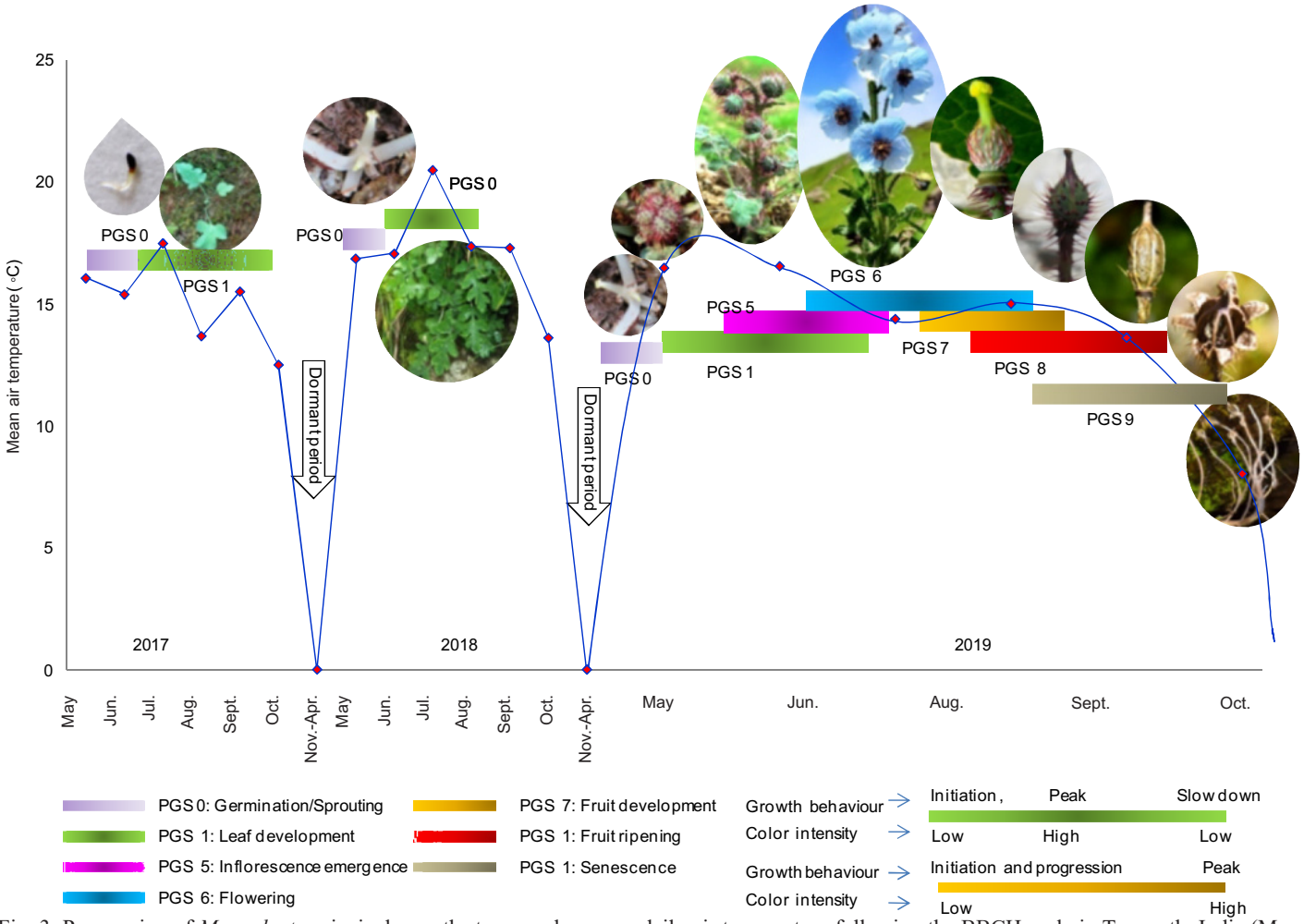

Fig. 3. Progression of *M. aculeata* principal growth stages and average daily air temperature following the BBCH scale in Tungnath, India (May-2017- Oct 2019). Length of color bar showing duration of PGS (Principal growth stage).

when it reached to 20 % of its final size with 4-5 flowering buds stage 54 were recorded during the third week of June. Flowering shoot reached to its 50 % size with 6-7 flowering buds and first flowering bud reached to its full size (Stage 55) (Fig. 2, k) during the fourth week of June.

During principal growth stage 5, stage 6 was also recorded when inflorescence elongated and reached to its 70 % size with 8-9 flowering buds at the same time 1st sepal was separated (Stage 57) (Fig. 2, l,m) during the first week of July. Along with this stem elongation continued till next stage (flower development) and reached to its final size with the final number of flowering buds (10-14 buds) during the second week of July and stage 59 was recorded. During stage 59, cauline leaves also attained the final number 6-10 and the stage ends. The main inflorescence is cyme or basipetal and the calyx is spiny abaxial and caduceus. Stage 5 takes 5-7 weeks.

Principal growth stage 6: **Flowering**: Principal growth stage 6 describes the flowering of *M. aculeata*, flowers appeared in basipetal manner (Stage 60) (Fig. 2, n). Stages 61-69 represent 10 %-90 % flowering (Fig.2, o-s). During 3rd week of July 50 % flowering or 6-7 flowers were open (Stage 65) (Fig.2, q) and the last flower opened during 3rd week of August (Stage 68) after that no flowers were visible (Stage 69) (Fig. 2, s). Stage 6 is overlapping with stage 7 and 8 due to indeterminate flowering growth. After completion of flowering vegetative parts seemed to grow and old leaves entered in senescence stage..

Principal growth stage 7: **Fruit development**: Principal stage 7 represents development of fruit and the increment of size. The fruit covered by minute erect pink spines, this stage concurrently runs with stage 6 and overlapping with stage 8. 10 % fruiting was observed and 1st fruit reached about its 20 % size (Stage 71) (Fig. 2,q,t) during the second week of July. 30 % fruiting appeared and fruits reached 30 % of final size during the 4th week of July (Stage 73) (Fig. 2, r, u). 50 % fruiting appeared and 1st fruit reached its final size (Stage 75) (Fig. 3, v) during 1st week of August. Stage 7 overlapped with stage 8 when 1st fruit had 10 % ripened and full fruiting appeared (stage 79/81) during 3rd week of August. Final size of fruit ranges from 1.6-1.9×0.6-1.3 in cm (Length×Width). At this stage cauline and radical leaves progressed towards senescence

Principal growth stage 8: **Fruit ripening**: First fruit reached physiological maturity and began to change colour from green to blackish brown (Stage 80) during 2nd week of August. 20 % of fruit reached their full maturity during 3rd week of August (Stage 81). When more than 50 % fruits reached to their full maturity and 1st fruit turned colour black to brown, stage 85 (Fig. 2, x) was recorded during 4th week of August. 70 % of fruits reached the final maturity during the 1st week of September (Stage 87) and 1st fruit ripened facilitating seed dispersal. After that, all fruits reached full maturity and complete seed dispersal took place in 50 % fruits during 3rd week of September (Stage 88). Finally, all fruits had completed seed dispersal (Stage 89) (Fig. 2, y) during the last week of September.

The seeds were black at full maturity with the weight of 100 seeds usually ranging from 8-10 mg. For the collection of seeds, fruit should be harvested during stage 85 when its colour turns black to brown to avoid further seed dispersal. Stage 85 onwards run concurrently with senescence stage (Stage 9).

Principal growth stage 9: **Senescence**: When flowering shoot and leaves turned green to brown, stage 91 was recorded (10 % yellowing of the plant) during late August. Leaves reached to 50 % yellowing and flowering stem turned yellow to brown (Stage 95) (Fig. 2, x) during last week of September. When whole plant attained full senescence reaching to dead stage 99 (Fig. 2, y) recorded during first week of October.

The phenological observation reading that *M.aculeata* takes 144 days to complete first year growth; leaf development stage is spread over 109 days and dormant period of 217 days, during second year growth leaf development takes 150 days and dormant period of 201 days, consequently during third year plants take 163 days to complete growth cycle. (Table 2)

Table 2. Major phenological events and complete life cycle of *M.aculeata* (2017-219)

Observed parameter	Period (Days)	Cumulative days
Seed germination	20	20
Cotyledon formation	9	29
Leaf formation	6	35
Leaf development	109	144
1st year total days	144	
Dormant period	217	361
Sprouting	12	373
Leaf development	150	523
2nd year total days	162	
Dormant period	201	724
Leaf development	92	816
Inflorescence	25	841
Bud break	4	845
Flower formation	2	847
Flower maturation	2	849
Fruit development	15	864
Fruit maturation	19	883
Seed dispersal	4	887
3rd year total days	163	

Phenological observations on *M. aculeata* have been performed for the first time over 3 years of life cycle using BBCH protocol. IUCN (1997) has categorized *M. aculeata* as endangered species and thus urgent measures need to be taken up for conservation of the species. Delineation of the phenophases over a temporal scale would help in devising conservation measures to ensure regeneration of the species. Detail information on different growth stages can be helpful for growers, breeders, conservationists and researchers. The plant completes its life cycle in 3 years entering flowering in third year leading to seed production. The reproductive phase is spread over 6 weeks wherein the dehiscence in the first fruit commences on nearly 75 % flowering and thus the seed dispersal continues till 2 weeks. Overlapping of phenophases, especially the reproductive stages, seems to be an adaptive strategy of the plant to ensure its regeneration in the unpredictable environment in its natural habitat.

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