Eco. Env. & Cons. 29 (May Suppl. Issue) : 2023; pp. (S438-S444)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i03s.076

Coding the Phenology of endangered *Lilium mackliniae* Sealy using extended BBCH scale

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(Received 19 December, 2022; Accepted 22 February, 2023)

ABSTRACT

Lilium mackliniae Sealy is an endemic lily of North-East India. The changing climate conditions, human activities, and natural resource exploitation have pushed the lily population to the brink of extinction. It has been well documented that the need to conserve *L. mackliniae* is in its critical stage. Plant phenology is closely correlated with environmental and human-caused factors. Therefore, a well-defined study of phenological traits can aid conservation aims. This paper utilized the BBCH scale to study its phenological characteristic, described by Phenological Growth Stages (PGS) in response to environmental conditions. Our findings can help the researchers as a means of conservation strategies.

Key words : *BBCH scale, Endemic lily, Phenological growth stages, Lilium mackliniae Sealy*

Introduction

Phenology (from the Greek *phainein*, to show or appear) is the study of a species' temporal behaviour, and it refers to the study of a plant's seasonal appearance and timing of life cycle events (Forrest and Miller-Rushing, 2010). Budburst, leaf-expansion, abscission, blooming, fertilisation, seed set, fruiting, seed distribution, and germination are all seasonal events in plants. Some species have developed great adaptations to the long-term climate pattern, while others are vulnerable to slight changes. Data from phenological research have shown that global climate change has caused dramatic changes in the phenology of numerous species during the last few decades (Primack, Higuchi and Miller-Rushing, 2009). Temperatures in the air and soil govern different phenophases (Diekmann, 1996), and they vary with latitude, altitude, community type, and plant growth, thus influencing species dispersion. Understanding the relationship between climate change and plant phenology (Suttle, Thomsen and Power, 2007; Yang and Rudolf, 2010) became a vital role in the conservation of plant species. It becomes more pertinent as the rare, endemic, and endangered species are already undergoing reproductive stress and habitat loss. As a result, data on various phenophases of these species in extant natural habitats realize the significance of environmental factors in conservation and domestication and assess the threat of global warming to their survival.

L. mackliniae is an herbaceous perennial plant. The lily was first reported as rare, endangered, and endemic to Shirui hills in Ukhrul district, Manipur, in the "Red Data Book" of "Indian Plants: Volume I" (Kataki, 1987). On March 21, 1989, it was designated as Manipur's state flower. *L. mackliniae* is a species of Lilium. Frank Kingdon Ward discovered *L. mackliniae* during a botanical study excursion in the Shirui Hills near the Ukhrul district of Manipur, India, and named it after his wife, Jean Macklin. The lily in its natural habitat grows from mid of March to August, with an annual temperature between 0^0C to 23^0 C. Changing climate conditions, human activities, and natural resource exploitation have pushed the lily population towards extinction. The invasion of dwarf bamboo species that grows abundantly in the Shirui hills threatens Shirui Lily's habitat (Meitei, 2011). As a result, the plant's growth and replication might be hampered, which is a matter of concern. Few attempts to investigate the leaf anatomy and pollen structure of *L. mackliniae* in the Shirui hills have been made (Devi *et al.,* 2020; Mao and Gogoi, 2013). There was a limited study related to the phenology of the *L. mackliniae*. As a result, the phenology of the *L. mackliniae*, an endemic and rare plant species, remains muddled. So to understand this, phenological studies were conducted to assess BBCH for *L. mackliniae* and demonstrate its key events related to environmental changes.

The application of the (BBCH) Biologische Bundesanstalt Bundessortenamt and Chemische Industrie (Bleiholder, Van Den Boom, LangelÜ Ddeke and Stauss, 1989; Hack *et al.,* 1992) helps in defining the phenological events and critical points of agriculturally important plant's life cycle. Based on the coding system, the BBCH scale designed a plant's growth into a principal or general stage (0–9) and a secondary or specific stage (0–9) (Zadoks *et al.,* 1974). To date, using this coding system, many plant's life events have been well defined, such as cereals (Uwe Meier *et al.,* 2009), vegetables (Archontoulis *et al.,* 2010), ornamentals (Meier *et al.,* 2009), stone fruit (U. Meier *et al.,* 1994), and medicinal plants (Martinelli and Sulas, 2015). The merit of the BBCH scale lies in its simplicity and ease of use while describing both vegetative and reproductive stages of plant growth, whether annual or biennial, or perennial. The use of BBCH scale is not only limited to defining the phenology of plants but is also approved as a standard for species protection by the European and Mediterranean Plant Protection Organization (EPPOC), the Global Phenological Monitoring Network, and the European Phenology Network. The BBCH scale has hitherto been restricted to agricultural use. However, it is currently being used to trace the phenology of endemic plants effectively (Jacinto *et al.,* 2022; Singh *et al.,* 2022).

Materials and Methods

Study area

The study area (Shirui hill) is in Ukhrul district, Manipur, India (Fig. 1). The physical boundary is surrounded by Myanmar in the East, Imphal East district and Senapati district in the West and Nagaland state in the North. Its location globally is 24^0 27′-$25^0$41' North latitude and $94^0$35′-$94^0$57' East longitudes. Vegetation is of temperate evergreen forest type (Mao and Gogoi, 2013), plant species such as *Rhododendron* spp., *Quercus* spp., *Lithocarpus pachyphylla, Magnolia* sp., *Daphne* spp., *Michelia* sp., etc. can be seen in the surrounding forest. The common species that grow along with *L. mackliniae* are dwarf bamboo (*Sinarundinaria* sp.), *Iris* sp., *Aconitum* sp., *Satyrium nepalensis*, etc.

Research method

The study was conducted adopting adaptive cluster sampling, which has an upper edge in sampling rare clustered species compared with other conventional methods (Thompson, 1990). Sampling was carried out by simple random sampling using 5ft x 5ft plot. Phenological stages of *L. mackliniae* was studied according to BBCH scale. Shoot growth was tracked weekly, whereas flowering, fruit development, and fruit maturation were tracked every three alternate days. The study lasted from January 2021 to December 2022 i.e., 48 months. The photographs of *L. mackliniae* with unique phenological stages were selected and arranged to describe the life events occurred.

For recording a comprehensive growth stage encompassing phenological growth stages, standard BBCH coding methods (Meier, 2001) were adopted. Decimal coding schemes were used to observe the whole development cycle of *L. mackliniae* (Meier, 2001).

Results and Discussion

The phenological stages of *L. mackliniae* have been described using the data collected through observation and recording and demonstrated how the plant progressed during its life cycle (Fig. 2). The two-digit extended BBCH scale developed for *L. mackliniae* in this work intends to bridge knowledge

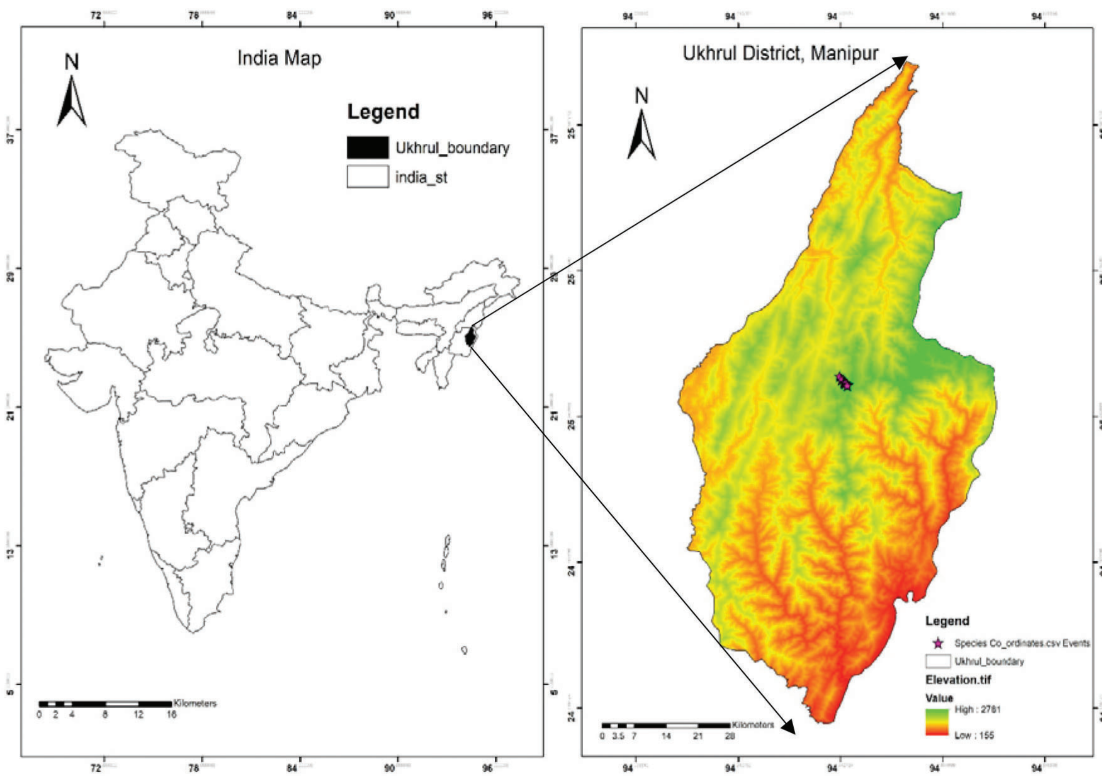

Fig. 1. Study area map showing the distribution of *L.mackliniae* (star shape points)

gaps about this endemic lily. The coding of the phenology of *L. mackliniae* has been done based on only the external morphological traits that can be seen with the naked eye (Table 1). The graphical representation of overall phenological growth stages of *L. mackliniae* is presented (Fig. 3).

The Lilium genus, which belongs to the Liliaceae family, contains approximately 110–115 taxa that are found predominantly in the northern hemisphere's cold and temperate zones (Du *et al.*, 2014). In Manipur, Lilium genus has 19 species belonging to 11 genera (Gogoi, 2010). The growth and development of lilium can be greatly dependent on the climatic condition especially temperature of the habitat (Xu *et al.*, 2021). The ideal temperature for the growth of genus Lilium is between 18 and 22 ^{0}C. (Xin *et al.*, 2010; Yin *et al.*, 2008).

Phenology of *L. mackliniae*

The seeds of *L. mackliniae* dispersal happen after the dehiscence of the dry capsule. The seeds remain dormant until mid-March, and as soon as there is enough moisture in the soil followed by a warm season, the seeds undergo imbibition in a short period. The presence of moisture and ambient conditions favours growth, emergence of radicle, and lateral root hairs formation, followed by cotyledons formation. The bulb produced by lily was labelled ovoid, scales elliptic, and fleshy. It has sessile leaves with irregularly spiral and hairy joints. The flowers are solitary, terminal, and campanulate, with a single three-lobed stigma and six stamens (Mao and Gogoi, 2013). The flowering window lasts between 20 to 25 days. The fruit of this lily is a dehiscent capsule that contains six chambers.

In the first PGS (00) (Table 1), sub-stages 01,03,05 and 07 could not be observed, but sub-stage 08 was observed for epigeal germination in which elongation of hypocotyl occurs, and a new sub stage (09) is followed, which we can be seen emerging from the ground (4-4.5 cm) (Fig. 2). PGS 01 begins with scaly leaf development. *L. mackliniae* leaves have a linear elliptical form and are born in opposite positions. Substages 12 and 19 (Fig. 2) are observed in PGS 01 stage. The leaves are sessile and spiral in an uneven pattern. The first pair of photosynthetic leaves appear at stage 12, and the stalks flourish to complete the crop canopy. After a certain period, most of the

Table 1. Application of extended BBCH scale for phenological studies in *Lilium mackliniae*

Code	Principal growth stage	Subcode	Secondary growth stage	Date of the life event
0	Germination/Sprouting			
		00	Dry seed/seed dispersed	
		01	Beginning of seed imbibition	
		03	Seed imbibition complete	
		05	Radicle (root) emerged from the seed	
		06	Elongation of the radicle, formation of root hairs and lateral roots	
		08	Elongation of hypocotyl and bringing up cotyledons towards soil surface	
		09	Emergence of cotyledons through the soil surface	March 25
1	Leaf development			
		10	Cotyledons fully unfolded	
		12	Second true leaves visible	
		13	Third true leaves visible …. stages continue	
		19	Nine leaves visible	April 15
3	Stem elongation: shoot development (main shoot)	31	Stem visibility 10% of maximum height	
		32	Stem visibility20% of maximum height	
		34	Stem visibility40% of maximum height	
			Stem visibility50% of maximum height…. goes on till	
		39	90% of maximum stem height	April 30
4	Development of harvestable vegetative plant parts or vegetativelypropagated organs / booting (main shoot)			
		41	10% of maximum biomass reached	
		45	50% of maximum biomass reached	
		49	90% of maximum biomass reached	May 10
5	Inflorescence emergence (main shoot) / heading	51	Inflorescence or flower bud visible	
		55	First individual flowers are visible (still closed).	
		59	First flower petals visible (in petalled forms);	May 25
6	Flowering (main shoot)			
		60	First flowers open (solitary)	
		61	Beginning of flowering: 10% of flowers open	
		62	20% of flowers open	
		63	30% of flowers open	
		64	40% of flowers open	
		65	50% of flowers open	
		67	Flowering about to finish	
		68	Majority of petals fallen or dry	
		69	End of flowering, fruit set visible	June 25
7	Development of fruit			
		71	10% of fruits have reached final size or fruit has reached 10 % of final size	
		72	20% of fruits have reached final size or fruit has reached 20% of final size	
		75	50% of fruits have reached final size	
		79	Nearly all fruits have reached final size	July 25
8	Ripening or maturity of fruit and seed	81	Beginning of ripening or fruit colouration	
		85	Fruits on main stem at 50%DM	

Table 1. *Continued ...*

Code	Principal growth stage	Subcode	Secondary growth stage	Date of the life event
		89	Fruits on main stem are dry (>80%)	August 18
9	Senescence, beginning of dormancy	93	Beginning of leaf fall	
		94	50 % of the leaf fall	
		95	All plant leaves are dead; stem colour turns into brown	
		97	End of leaf fall	
		99	Main stem, ramification	September 27

leaves are visible and distinguishable from the main stem. Linear elliptical leaves of the particular species can be seen in April. The transition from the vegetative to reproductive flower growth stages is a magnificent and exciting process (Poethig, 2003). A complex genetic system, an array of signal transduction pathways that regulate the developmental stage of the plants, and environmental signals such as light (day length, photoperiod) and temperature influence the transition from vegetative to reproductive phase (Amasino, 2010; Simpson *et al.*, 1999; Srikanth and Schmid, 2011).

PGS 03 illustrates stem elongation where 32 and 34 stages depict 20% and 40% of stem height attained. The booting of the main shoot in which about 50% of the biomass is observed is depicted in stage no 45 of PGS 04. It can attain a height of 1–3 feet (0.30–0.91 m). There is a rapid growth observed in *L. mackliniae* after this stage, and it onsets the PGS 05 stage where 55 stage (Fig. 2) is observed. As the flower unfolds, the edges of the petals wrinkle, implying that differential growth promotes the deployment of these laminar shell-like structures. The calyx is tubular, and corolla tube is long and widened at the mouth. The flowering stage is observed in PGS 06 shown in stages 61 and 67 (Fig. 2). Each plant produces one to seven flowers. It has delicate bluish-pink flowers. Fruit development is represented by PGS 07 where in stage 79 (Fig. 2), fruits have reached their maximum size and are ready to enter the stage PGS 08 for maturity. Stage 89 (Fig. 2) of PGS 08 shows the dried fruit followed by PGS 09; (99; Fig. 2) stage in which the plant enters the senescence/dormant phase.

The present study strongly hinted on the possible threats of fire (human activities) and dwarf bamboo (acting invasive species) as the two biggest risks to this endemic plant. Further human activities involve tourism, infrastructure, and recreational activities. *L.*

Fig. 2. Phenological Growth Stages (PGS) of *L. mackliniae* according to extended BBCH scale. Selected images of phenological stages.

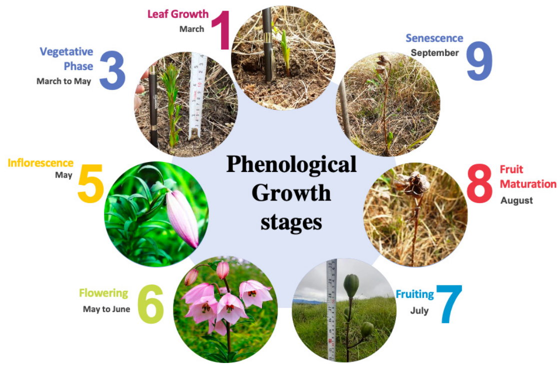

Fig. 3. Graphical representation of Phenological Growth stages of *L.mackliniae*

mackliniae habitat has reportedly deteriorated due to periodic forest fires, trace passage, tourists, visitors, and climate change. Climate change and other stressors are causing the plant to disappear from its natural environment. The effect of fire-invasive plant species on endemic plants has been well documented, and even it can prevent the recovery potential of native plant species (Whisenant, 1990). During the investigation, it was observed that these factors could collectively alter the phenology and population of *L. mackliniae*. The most serious problems posed by invading species are those that directly impact ecosystems and species by acting as rivals or predators (IUCN, 2001) (code 8.1.2; IUCN, 2012). They can also indirectly impact native plants and ecosystems by modifying soil stability, encouraging erosion, colonizing open substrates, impacting litter and other soil resources build-up, and stimulating or suppressing fire (Richardson *et al.*, 2000; Vitousek, 1996). If significant progress in the conservation of target species is to be made, it is evident that a coordinated effort will be required to address these concerns. As a result, it is critical for the general public and government officials to recognize the current scenario, which is jeopardizing the State flower's existence, and to adopt conservative steps as soon as possible. Otherwise, the *L. mackliniae* will lose its natural habitat, and it might lead to its extinction.

Conclusion

For the first time, the phenological stages of *L. mackliniae* have been described using the BBCH scale, which clearly distinguishes each life event stages. The paper's description will serve as a tool for developing a consensus-based unified method for standardising phenological stages as well as implementing conservation strategies such as accurate timing for dwarf bamboo and control fire management. Furthermore, phenological stage identification is important for characterisation of germplasm and endemic plant enhancement programmes. Since the phenology can detect the climate change (Zhao *et al.*, 2013), the basic information of phenology obtained from this study can help in assessing the impact of climate variables on germination, vegetative growth, reproductive growth, fruit development, and senescence of *L. mackliniae.*

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