



Research article

Chrono-spatial reproductive phenology of *Swertia bimaculata* Hook.f. & Thomson ex C.B. Clarke reveals suitability as climate-changing bio-indicator along the Sikkim Himalayan altitude

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ABSTRACT

Genus *Swertia* under the family Gentianaceae is morphologically and taxonomically distinct with about 14 species from Sikkim Himalayan region. A Chrono-spatial floral phenology study was conducted on *Swertia bimaculata* from 1580 to 2400 m altitude in West Sikkim Himalayan region. The floral phenology was studied in a field nursery at 1200 m altitude and spatial phenology was studied over a 1580–2400 m altitudinal range in the West Sikkim Himalayan region. Detailed study on flowering pattern and seed formation in *S. bimaculata* revealed late flowering at 1200 m compared to 2400 m. Plants at the lower altitude showed prolonged and variable flowering from September to November and seed formation from November to December. During September to mid-October, pentamerous and bisexual flowers were observed, followed by 9–16 % tetramerous until November; and up to 24 % trimerous and 10 % bimerous until the end of flowering season in December. By the end of December, 43 % of flowers become stamen-less (monoclinous). Less than 2 % seed formation was observed in stamen-less flowers. The temporal phenological behavior reveals a successful reproductive adaptive strategy, depicting pentamerous flowers during favorable conditions, reducing to tetramerous, followed by tri- & bimerous with female (monoclinous) flowers until the end of flowering with stressful conditions, suggesting its suitability as a stress indicator to the changing climatic conditions. Along the altitudinal range floral anomalies from the usually observed pentamerous (and very rare hexamerous) flowers in favorable conditions change to tetramerous and/or trimerous, as the environmental conditions become more stressful, suggesting that the changes in this species could be used as an indicator of stress in changing climatic conditions.

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1. Introduction

The world is facing climate change as the greatest challenge where temperature increase causes cascading effects on biotic and abiotic ecosystem components. Climatic conditions are projected to shrink by 66 % over polar and mountainous regions [1]. Climate warming consequences are higher in the Himalayas than in the rest of the world [2]. The unsustainable anthropogenic activities aggravate the Himalaya's susceptibility to climate change, posing serious threats to various life forms for which timely assessment of the changing climate and taking of precautionary steps is preferable to having to mitigate consequences. Climate change is a slow and steady process, wherein most flowering plants are affected. Most flowering plant species are coping through adaptive mechanisms and changing phenology [3,4]. Flowering phenology of olive trees is an indicator of climate change affected by altitude and crown-flowering branches exposure [5].

Observation of adaptive mechanisms and changing phenology is a new venture of understanding the changing climatic scenario. Iversen et al. [6] mentioned consideration of plant growth form along with topography as predictors for assessing the role of phenology in an ecosystem. Phenotypic variations have been reported in *Morus alba* along an altitudinal gradient [7]. Himalayas being most prone to climatic vagaries, offer the most fitting predictive opportunity for assessing the climatic changes through floral phenological variation along altitudinal ranges. Sikkim Himalaya, being narrowest and thus highest too, offers the most suited place among Himalayas for studying patterns of responses to climate change. Sikkim is biogeographically important in the entire Himalayan chain as a part of the Himalayan biodiversity hotspot designated by Conservation International [8]. Geographic location and climate make Sikkim Himalaya a junction for Indo-Chinese tropical lowland, Indo-Malaysian, the Western Himalayan and the Sino-Himalayan east Asiatic vegetation, comprising approximately 9000 species with high endemism [9]. Sikkim Himalaya suitably offers opportunities for studying climate changing patterns through selection of suitable floral species having ample area of occupancy and phenological variations. Through field observation *Swertia bimaculata* Hook. f. & Thomson ex C.B. Clarke (Family: Gentianaceae) is an appropriate sentinel species because of its area of occupancy and altitudinal phenological variation.

The genus *Swertia* comprises worldwide about 150 species [10], with 40 in India and 15 in the Eastern Himalaya [11–13]. The genus *Swertia* is recognized as a group of essential medicinal plants in the Indian System of Medicine (ISM) and Traditional Chinese Medicine (TCM), used as a bitter tonic to treat various ailments including diabetes, malaria fever, bone fracture, liver diseases, pneumonia, asthma, inflammation, and leucorrhoea [14–18]. In the changing climatic scenario, we studied *S. bimaculata* for the temporal and spatial floral phenological observations. *S. bimaculata* is spread across the Sikkim Himalayas [19] and also found in Southeast & East Asian countries like China, Japan, Bhutan, and Republic of Korea, besides India. *S. bimaculata* is taxonomically identified through ovate leaves having conspicuous three nerves, flowers in common morphology are pentamerous, show two green distinct orbicular nectaries at the middle and black spots at the tip of each corolla lobe [19,20] (Fig. 1) and considered endangered by area of occupancy in India [21]. Usually in the congenial growing conditions pentamerous flowers are observed with very rare

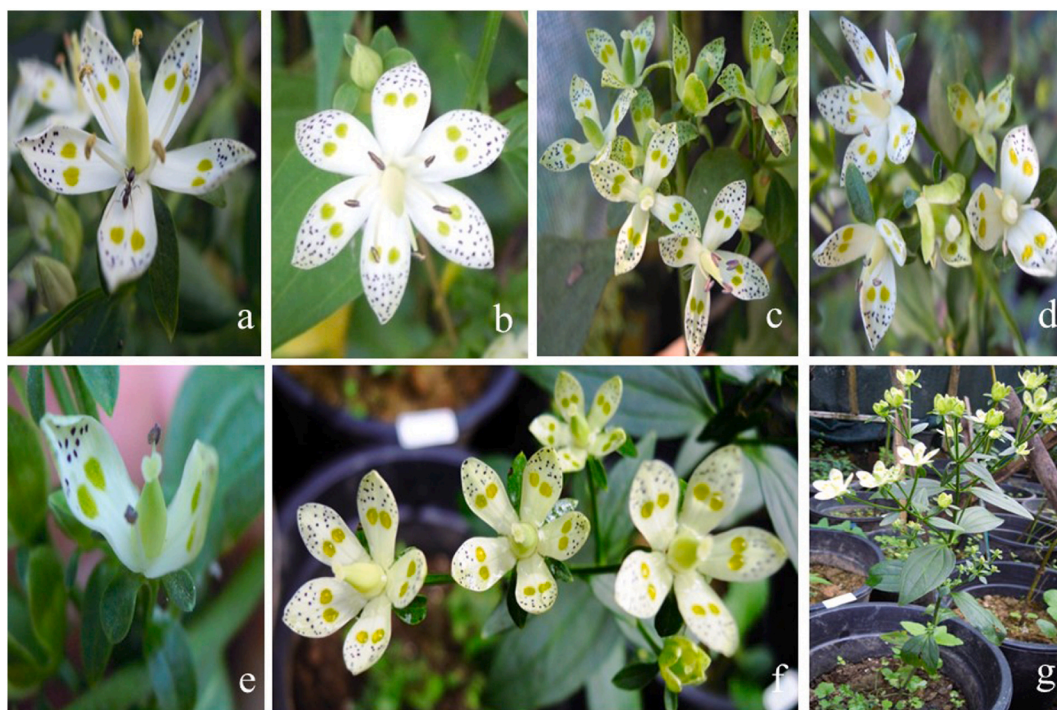


Fig. 1. Floral morphological pattern showing pentamerous (a), hexamerous (b), tetramerous (c), trimerous (d), bimerous (e), monoclinous (f) and plant bearing monoclinous flower (g) of *Swertia bimaculata*.

hexamerous flowers in very good growing conditions (when the abundance of pentamerous flowers is more). Tetramerous, trimerous, bimerous and/or monoclinal flower are observed as the flowering season ceases or the environmental conditions become stressful. No previous study has described climate change impacts on the phenology of *S. bimaculata*. Boral and Moktan [22] predicted decrease in *S. bimaculata* habitat range in the Darjeeling-Sikkim region of Eastern Himalaya. Phenotypic plasticity with varying floral traits across altitudinal gradients is reported in *Swertia thomsonii* [23]. Gallagher et al. [24], using herbarium records, reported an Australian alpine species as a climate change indicator, mentioning conspicuous flowering patterns and accessible population making the species suitable for monitoring responses toward future climate, which is also suitably depicted in *S. bimaculata*. While studying the tropical ecosystem Numata et al. [25] emphasized on improved mechanistic understanding of environmental drivers of reproductive phenology in diverse species to decipher complex phenological responses. Numata et al. [25] advocated early biotic change detection and prediction of magnitude and direction of changes in plant reproductive phenology to facilitate a sustainable future of the diverse ecosystem on earth. Detailed study of phenology and breeding system can provide valuable clues about the effect of projected climate change [26].

The varied floral phenological temporal behavior with changing growing conditions from floral bud formation to fruit formation and seed shedding prompted spatial study along the altitudinal range. Porter and Semenov [27] advocated spatiotemporal climatic variation studies during the flowering phase for better modelling of future global warming effects. The floral phenology depicts a range of periodic phenophases, useful in understanding the changing climatic pattern across time in a single location and along an altitudinal gradient with changing locations. Looking at the range of floral phenophases, its usefulness in understanding the changing climatic pattern across time in a single location and along an altitudinal gradient is hypothesized. *S. bimaculata* floral phenology, in addition to its noticeable morphological variations over an altitudinal range in Sikkim Himalaya, influenced by varying climatic factors, makes it ideal for examining phenological responses to climate change. The study was conducted with the objective of deciphering the range of periodic phenophases temporally and spatially with its suitability as a climate change indicator. The study evaluate the influence of altitudinal gradient and other flower types on the pod formation.

2. Material and methods

2.1. Experiment site

With the objective of deciphering the range of periodic phenophases temporally and spatially with its suitability as a climate change indicator the phenological observations for *S. bimaculata* flowering were recorded using phenophases defined in international BBCH scale [28,29]. Both the temporal and spatial floral phenology observed from flower bud stage (BBCH code 55) till the fruit ripe (BBCH code 89) following Meier [28] and Bleiholder et al. [29] for weed species. The floral phenology was studied in the planted plants at the institutional experimental field nursery. The experimental field nursery area for the temporal floral phenological study is at 1200 ± 10 m from msl under the temperate to sub-tropical hills of Sikkim Himalaya, India. The maximum annual temperature in the nursery reached up to 33.5°C during May and minimum 7.37°C in January. The maximum and minimum average relative humidity varied from 89.3 % (June) to 39.2 % (March). In addition, spatial floral phenology was studied along the altitude range from 1580 to 2400 m above mean sea level (msl) in the West Sikkim part of Sikkim Himalayan region. The diurnal temperature during observation period ranged at lower altitude (1580m) from 13°C to 25°C ; whereas, at higher altitude (2300m–2400m) from 3°C to 12°C . The relative humidity ranged at lower altitude (1580m) from 38 % to 92 % and at higher altitude (2300m–2400m) from 34 % to 82 % [30].

2.1.1. Temporal range: For the temporal range, *S. bimaculata* seeds from the mature plants were harvested during mid-January of 2012 and sowed in pots during May 2012 under nursery shade house conditions with 70 % sunlight cut off, akin to the natural field conditions. The seeds germinated about one month after sowing. Routine irrigation and manual weeding were performed in growing seedlings as and when needed. Seedling thinning was practiced to avoid internal competition. The germinated seedlings remained in rosette form till June–July of 2014 and thereafter rose to the erect stage with flowering commencing from September and lasting until the end of November. In the observation process, data were recorded for pentamerous and tetramerous flowers, followed by various flowering patterns. Flowering occurred from mid-September to November, whereas seed formation was observed from November to December. The observations are pooled data of two seasons, taken in three replicates with one plant in each replicate. All the flowers from commencing until the end were observed for each plant.

2.1.2. Spatial range: For the spatial range the naturally growing *S. bimaculata* populations at altitudes ranging from 1580 msl to 2400 msl (at 1580, 1800–1900, 1900–2000, 2000–2100, 2100–2200, 2200–2300 and 2300–2400 msl) were observed for number of buds, flower & their types and pod formation of individual plant in a 20 m transect along the area of occupancy. An aerial camera view of the individual studied plant was captured for record and observation. The observations were taken in three replicates with minimum of two and maximum of three plants in each replicate within each altitudinal range.

2.2. Statistical analysis

The generated data were statistically analyzed as per Gomez and Gomez [31]. The critical difference (CD) value was calculated to compare significant and highly significant means of flowers and their types and pods at different altitudes through generated single factor ANOVA table by using the formula “ $CD = t \times \text{Square root of } (2 \times \text{MSSerror}/n)$ ”. Where, ‘t’ is the value of the Student’s t-distribution at a given confidence level at a particular degree of freedom, and ‘MSSerror’ is the mean sum square of error and ‘n’ is the number of replicates. Statistical analysis was further conducted by applying linear regression to evaluate the influence of altitudinal gradient and other flower types on the pod formation.

3. Results

S. bimaculata showed various flowering patterns with flowering commencing from September to November, whereas seed formation was observed from November to December.

3.1. Temporal range

The total number of flowers ranged from 66 in September to mid-October to 21 in November to December. From September to mid-October maximum pentamerous and bisexual flowers ranging from 41 to 21 flowers per plants were observed, declining to about 5 flowers up to December (Fig. 1a). Tetramerous flowers (Fig. 1c) were observed with 3 per plant in September to 9 per plant in November; whereas the trimerous flowers at 4 per plant from mid-October to 5 per plant till December (Fig. 1d). The rare bimerous flower with 2 per plant was observed only during November–December (Fig. 1e). The monoclinal (i.e., male sterile usually without stamen or rudimentary & infertile stamens) flowers (Fig. 1f) were observed from mid-October to December ranging from 16 to 9 per plant (Fig. 1f and g). Therefore, in temporal sequence the floral morphology changed from usually observed pentamerous flower to tetramerous, trimerous followed by bimerous to monoclinal flowers (Fig. 2). The pod formation ranged from 3 per plant in September to 33 per plant up to December. The average seed in bisexually produced pods was 126 per pod; whereas, in monoclinal flowers there were only 2 seeds per pod. The unopened flower buds (likely to open later) throughout the floral phenological phase ranged from 23 to 2 commencing from September to December (Table 1).

3.2. Spatial range

Variable flower types were observed along the altitudinal range from 1580 to 2400 msl. The plant population along each 100 m altitude, ranged from 3 plants at 1580–1800 msl to 9 plants at 2300–2400 msl. The number of flowers per plant along the altitudinal gradient ranged from 15 to 49 but did not differ significantly. The number of pentamerous flowers (Fig. 1a) per plant ranged from 12 at 1580 and 2100–2200 msl to 40 at 2300–2400 msl, but differences were not significant. The hexamerous flowers observed between 1900 and 2100 msl ranging from 1 to 0.67 flowers per plant (Fig. 1b). The tetramerous flowers (Fig. 1c) ranged from nil at 1580 msl to 9 at 1900–2000 msl. No trimerous or bimerous flowers were observed throughout the altitudes studied. More unopened flowers were observed at lower altitude compared to higher altitude, ranging from 2 at 2100–2200 msl to 16 at 1580 msl. No pod formation was observed at 1580 msl; however, 5 pods at 1800–1900 msl and a maximum of 47 pods were observed at 2200–2300 msl. The data were highly significant for the number of tetramerous, and hexamerous flowers, and significant for unopened flowers and pods and non-significant for pentamerous and total number of flowers (Table 2). Statistical analysis conducted by applying linear regression analysis was used to evaluate the influence of the altitudinal gradient. In regression analysis the number of pods formed is affected by two dependent variables, i.e., altitude and the number of tetramerous flowers along the altitudinal gradient. The 92 % variation in the

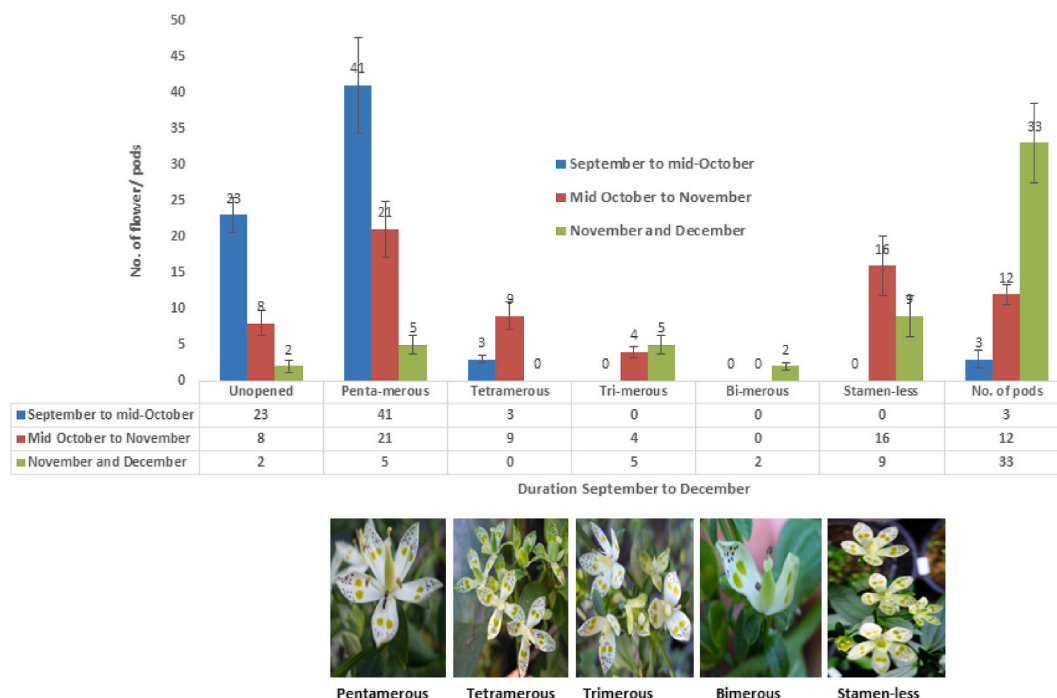


Fig. 2. Temporal adaptive reproductive phenological behaviour of *Swertia bimaculata*.

Table 1
Temporal adaptive reproductive phenological behaviour of *Swertia bimaculata*.

S. No.	Duration	No. of flower						No. of pods	No. of seed formation		
		Total	Unopened	Pentamerous	Tetramerous	Trimerous	Bimerous		Stamen-less	Bisexual flower	Stamen-less flower
1	September to mid-October	66 ± 5.5	23 ± 2.49	41 ± 6.6	3 ± 0.5	–	–	–	3 ± 1.25	–	–
2	Mid October to November	58 ± 11.8	8 ± 1.7	21 ± 3.86	9 ± 1.89	4 ± 0.82	–	–	16 ± 4.11	12 ± 1.41	126 ± 23.13
3	November and December	21 ± 0.81	2 ± 0.82	5 ± 1.25	–	5 ± 1.25	2 ± 0.5	9 ± 2.87	33 ± 5.56	–	2 ± 1.36

number of pods depends on the altitude and number of tetramerous flowers along the altitudinal gradient (Table S1). Along the altitudinal gradient pentamerous and total number of flower are not significantly affected showing the suitability of habitat along the altitudinal gradient. However, the large number of unopened flowers and lower pod formation at lower altitude compared to higher altitude shows the phenophase shifting in synchronization with the location-specific climatic conditions.

4. Discussion

Phenotypic plasticity is the genotype ability to regulate development in response to environmental fluctuations [32]. Phenotypic plasticity is an important means of coping with the changing climatic conditions. Predicting the changing climatic pattern through study of plants' adaptive plasticity is a new application of plant phenomics. Knowing the plants' functional traits through which plasticity can respond to the changing climatic conditions is important [33]. Phenotypic plasticity influenced by changing environmental conditions/tolerance, creates plastic responses that help the inhabiting species in coping throughout the prevailing environmental range [34]. The greater the phenotypic plasticity the greater the variability and adaptability of species in response to the environment. The morphological variability within the *Swertia* species is high, which is based on the small number of fixed floral characters. The seed morphology, nectaries shape and their appendages are features of the genus, which are used in identification keys [35–37]. In *S. bimaculata*, along the temporal range, during the favorable growing season (mid-September to mid-October), pentamerous and bisexual flowers were observed, which is described as the usual flowering pattern [38]. However, from September to mid-October 9 % and mid-October to November 16 % tetramerous flowers were observed. Similarly, mid-October to November 7 % and November to December 24 % trimerous flowers were observed. Also from November to December about 10 % of the flowers turned bimerous. The data showed that, as the number of flowers declines the floral anomalies increase. To add to it further, during mid-October to November 28 % and November to December 43 % flowers become stamen-less.

The varied floral phenological behavior has been reported by Wang et al. [39], who observed monoclinal and female (i.e., male sterile usually without stamen or rudimentary & infertile stamens) flowers in *S. bimaculata*. Less than 2 % seed formation in stamen-less flowers at the end of the flowering season suggests the successful reproductive adaptive strategy during the stressful environmental conditions. van Ginkel and Flippi [40] envisaged that stress-induced male sterility assists and/or enables outcrossing in selfing plants. Wang et al. [39] mentioned protandry (i.e., stamens developing, or pollen releasing, prior to carpel maturity or stigma receptivity) and herkogamy (i.e., space separation of stigma & anthers in hermaphroditic plants as a self-fertilization reducing strategy) as successful mating strategy in *S. bimaculata*; helping the species to produce highly outcrossed and quantitatively high seed-set rate. The stress-induced male sterility mechanism in certain species significantly impacts the survival in changing conditions mentioned as "Diversity Assurance", which is an adaptive mechanism under stress, supported by male reproductive system function loss [40]. Wani et al. [23] mentions xenogamy followed by geitonogamy and autogamy, envisaging adaptive features through phenotypic plasticity along an altitudinal gradient in *S. thomsonii*. Knowing that these plasticity-oriented functional traits help to cope with the changing conditions, helps in confirming changing climatic conditions.

In the present spatial floral phenological observation the flower morphology changes from the usual pentamerous to tetramerous at

Table 2
Spatial adaptive reproductive phenological behaviour of *Swertia bimaculata*.

S. No.	Altitude (m)	No. of flower					No. of pods
		Total	Pentamerous	Hexamerous	Tetramerous	Unopened	
1	1580	28 ± 3.6	12 ± 3.06	–	–	16 ± 2.08	–
2	1800–1900	30 ± 10	16 ± 9.52	–	1 ± 0.51	13 ± 0.95	5 ± 1.41
3	1900–2000	34 ± 11.5	20 ± 6.82	1.00 ± 0.58	9 ± 3.75	13 ± 2.47	44 ± 32.38
4	2000–2100	48 ± 34.6	28 ± 6.10	0.67 ± 0.00	2 ± 0.29	18 ± 9.09	35 ± 13.53
5	2100–2200	15 ± 3.3	12 ± 4.0	–	4 ± 1.41	2 ± 0.71	41 ± 23.03
6	2200–2300	37 ± 2.4	28 ± 5.68	–	3 ± 0.35	7 ± 1.04	47 ± 13.00
7	2300–2400	49 ± 25.0	40 ± 3.73	–	2 ± 0.88	9 ± 4.62	44 ± 6.26
	CD	NS	NS	0.53**	5.045**	7.74*	30.72*

CD= Critical difference; ** = Significant at 1 % probability; * = Significant at 5 % probability level; NS = Non-significant.

the end of the flowering season. The statistically significant variations in unopened flowers and highly significant variation in hexamerous and tetramerous flowers along altitudinal gradient as the diurnal temperature (13°C – 25°C) declines to (3°C – 12°C) and relative humidity (38 %–92 %) declines to (34 %–82 %) from 1580m to 2400m. The floral morphological anomaly observed temporally coincides with the spatial observation along altitudinal gradient. In regression analysis the 92 % variation in the number of pods depends on the altitude and number of tetramerous flowers along the altitudinal gradient. Plants have survived and adapted to climate change-induced challenges through similar maneuvering during their evolution [40]. Many plant species show transitional, mixed-mating capabilities to self and outcross [41–43]. Plant sexual behavior under temperature stress adjusts during changing climatic scenarios through gametophytic selection and phenotypic plasticity [44]. Temperature or other stresses can lead to selfing or outcrossing as favored, and phenotypic plasticity enables mixed-mating systems. When insects or pollinators are scanty, hindering outcrossing, reproductive assurance prevails through selfing. Whereas, under limited selfing, as in heat stress, cross-pollination is facilitated through pollinators achieving reproductive assurance through outcrossing [45]. The receptive stigma of stress-induced male-sterile plants remain viable to receive male-fertile pollen from stress-tolerant plants thus effectively outcrossing and acts as an assisting factor in reproductive success. Hedhly et al. [44] speculated there could be an increase in such reproductive adaptations in further climate change. In the present study under changing climatic scenarios the varied floral phenological temporal & spatial behavior of monoclinal flowering seems adaptive toward stress-induced male sterility and is the new information reported herein for *S. bimaclata*.

The present study revealed the non-significant distribution of the flower number per plant, ranging from 15 to 49 along the altitude range suggesting that there is no altitudinal effect on the number of flowers, proving the entire environment can be suitable for the plant growth and survival. As altitude did not significantly affect the total flower numbers, this makes the plant ideal for selection in climatological studies along its altitudinal distribution and for seasonal climatological effects at a single location. In the future climate scenario, with possible shrinkage of habitat and upward shifting of species, Boral and Moktan [22] predicted *S. bimaclata* vulnerability towards extinction in the near future. In usual congenial conditions, *S. bimaclata* flowers are pentamerous [38]. The number of pentamerous flowers increases with the suitability of growing conditions with observance of rare Hexamerous flowers in very good growing conditions. The non-significant distribution of the number of pentamerous flowers ranging from 12 at 2100–2200 msl to 39 at 2300–2400 msl, depicts the entire altitudinal range congeniality for the optimal growth of the plant. The anomaly in the flower morphology from pentamerous (and very rare hexamerous flowers) to tetramerous or trimerous or to other form as bimerous and/or monoclinal is observed as the flowering season ends or the environmental conditions become unsuitable.

The tetramerous flowers were absent at 1580 msl and at other altitudes ranged from 1 at 1800–1900 msl to 9 flowers per plant at 1900–2000 msl. No trimerous and bimerous flowers were observed along the altitudinal range. The unopened flowers ranged from 1.5 at 2100–2200 msl to 15.67 at 1580 msl. The more unopened flowers at lower altitude compared to higher altitude shows the congeniality of wide range altitude for the growth of *S. bimaclata* with the shifting of phenophase in synchronization with the prevailing climatic conditions. No fruit formation was observed at 1580 msl; however, 4.5 fruits at 1800–1900 msl and maximum 46.72 fruits were observed at 2200–2300 msl. The trend of more unopened flowers and no fruit formation at low altitude and fewer unopened flowers and maximum fruit formation at higher altitude shows shifting reproductive phenophases along the altitudinal gradient. Based on similar observations, Boral and Moktan [22] predicted *S. bimaclata* spatial range declination by 2050 in future climatic scenario, with further declining by 2070 [46]. In our study, the highly significant tetramerous and hexamerous flower numbers and the significant unopened flowers & fruits and non-significant pentamerous & total number of flower numbers, suggests that over-all congeniality of growing conditions for the plants and shifting trend of reproductive phenophases along the altitude, make *S. bimaclata* ideal for studying the climatic trend. The temporal phenological behavior of successful reproductive adaptive strategy depicting stress-induced male sterility enabling and/or enhancing outcrossing along with protandrous and herkogamous as successful mating strategy makes *S. bimaclata*, an ideal indicator of changing climatic parameters.

5. Conclusion

Plants adapt myriad strategies to combat the changing climatic conditions as vividly depicted by *S. bimaclata*. The temporal phenological behavior of successful reproductive adaptive strategy depicting pentamerous flowers during congenial conditions, reducing to tetramerous, followed by tri- & bimerous flowers with monoclinal (i.e., male sterile) flowers during ceasing & stressful flowering conditions, suggest its suitability as a stress indicator to the changing climatic conditions. Similarly, along the spatial altitudinal range floral morphology anomaly from the usually observed pentamerous (and very rare hexamerous flowers) in congenial conditions to tetramerous and/or trimerous, as the environmental conditions become uncongenial suggests its suitability as a stress indicator to the changing climatic conditions. The stress-induced male sterility enabling and/or enhancing outcrossing along with protandrous and herkogamous as a successful mating strategy makes *S. bimaclata* an ideal indicator of changing climatic parameters.

Data availability statement

The authors confirm that the data supporting the paper are available within the article beside as supplementary table and will be made available on request from the corresponding author.

CRedit authorship contribution statement

Jitendra Kumar Shukla: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources,

Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kishor Basor:** Writing – review & editing, Visualization. **Preeti Dhakal:** Writing – review & editing, Visualization. **Sandhya Thapa:** Writing – review & editing, Visualization. **Evanylla Kharlyngdoh:** Writing – review & editing. **Sunil S. Thorat:** Writing – review & editing. **Pardeep Bhardwaj:** Writing – review & editing. **Sushil K. Chaudhary:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Pulok K. Mukherjee:** Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e35275>.

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