3. PHENOLOGICAL VARIATIONS OF *S. obtusifolia* PROVENANCES

Phenological variations of plants help us understand the impact of different ecological conditions which may affect the traits of the species both in different vegetative and reproductive activities. Phenological patterns may provide useful information regarding the diversification and specification of species at intraspecific levels. Both biotic and abiotic factors contribute in favor of (positive covariance) or hinder (negative covariance) the phenological characters of a species. The reason of diversity at the intraspecific levels among the species of varied ecological environments may be expected due to the changes in the genotypic level resulting in the diversity of the phenotypes. Authors have studied phenological variations of various plants (Duraisamy and Subramaniam, 2010; Dinis et al., 2011; Ramos et al., 2014; Neji et al., 2015). In most of the cases, they have recorded variations in starting day (Nakar and Jadeja, 2015) and duration (Gienapp et al., 2005) of flowering and fruiting. The influence of climatic conditions on the phenology of plants have also been studied (Kushwaha et al., 2011; Cardoso et al., 2012; Pharswan and Mehta, 2013; Uppal and Singh, 2013; Biswas et al., 2017). Although, phenological variations of several non-medicinal and medicinal plants have been studied, there is no report on the study of phenological attributes of this species from different provenances. Though, recently the study of phenological variations of *S. obtusifolia* has been reported by Maiti and Nandi (2019).

S. obtusifolia, a widely distributed herb under Fabaceae, grows wild in many tropical countries including India (Sudi et al., 2011). The plant is useful in treating various ailments including clearing liver heat, lubrication of intestine, clearing vision (Mahwasane et al., 2013). Different parts of the plant containing various medicinally important secondary metabolites like glycosides, anthraquinones, and sennosides.

These chemicals possess cardioprotective, anti-cancerous, antiviral, and antitumor properties (Doughari et al., 2008). Therefore, the focus of the present work is to explore the phenological diversity, if any, amongst the provenances of *S. obtusifolia*, collected from different parts of India.

3.1 ACCESSIONS

Accession numbers and other details of the germplasms of *S. obtusifolia* are presented in Table 3.1. These accessions are referred across this thesis document including tables and figures.

Table 3.1 Accession number on location, altitude, temperature and rainfall

Accession Number	Provenance	District, State	Latitude, Longitude	Altitude (m)	Temperature* (Min, Max ºC)	Rainfall (mm)**
ASN	Asansol	Bardwan, West Bengal	23.6739° N, 86.9524° E	122	(26.3, 38.9)	1017
BDG	Bandhavgarh	Umaria, Madhya Pradesh	23.7225° N, 81.0242° E	440	(26.2, 41.4)	1066
BNP	Bishnupur	Bankura, West Bengal	23.0679° N, 87.3165° E	59	(26.2, 36.8)	878
BPR	Bolpur	Birbhum, West Bengal	23.6687° N, 87.6828° E	58	(25.5, 35.9)	933
DHD	Dahod	Dahod, Gujarat	22.8379° N, 74.2531° E	311	(26.1, 39.7)	680
DHN	Dehradun	Dehradun, Uttarakhand	30.3165° N, 78.0322° E	653	(21.6, 36.2)	1529
DNG	Devendra nagar	Devendranagar, Madhya Pradesh	24.6104° N, 80.3798° E	349	(26.8, 42)	845
GBP	Gobindapur	Hooghly, West Bengal	22.9151° N, 88.2011° E	18	(25.6, 35.2)	1138
HDR	Haridwar	Haridwar, Uttarakhand	29.9457° N, 78.1642° E	289	(23.4, 39.1)	1073
JBP	Jabalpur	Jabalpur, Madhya Pradesh	23.1815° N, 79.9864° E	412	(25.9, 41)	1075
JPR	Jaipur	Jaipur, Rajasthan	26.9124° N, 75.7873° E	431	(25.5, 40.5)	514
JRM	Jhargram	Jhargram, West Bengal	22.4550° N, 86.9974° E	81	(26.6, 38.5)	992
KLN	Kalyani	Nadia, West Bengal	22.9751° N, 88.4345° E	11	(25.5, 35.5)	998
KPR	Kharagpur	Paschim Medinipur, West Bengal	22.3460° N, 87.2320° E	53	(26.5, 37.6)	1095
LKP	Lakshmi kantapur	South 24-Pgs, West Bengal	22.1099° N, 88.3209° E	6	(26.3, 34.1)	1132
MGL	Mangalore	Mangalore, Karnataka	12.9141° N, 74.8560° E	107	(25.7, 32)	2650
NGP	Nagpur	Nagpur, Maharashtra	21.1458° N, 79.0882° E	310	(27.9, 42.7)	849
PRL	Purulia	Purulia, West Bengal	23.3321° N, 86.3652° E	248	(26.8, 39.9)	967
RPR	Raipur	Raipur, Chhattisgarh	21.2514° N, 81.6296° E	298	(28.7, 42.2)	1027
TNG	Tatanagar	Tatanagar, Jharkhand	22.8046° N, 86.2029° E	159	(26.8, 40)	1024

* Temperature of the month of May, ** Rainfall during May to October

3.2 MATERIALS AND METHODS

3.2.1 Collection of Germplasm

Fresh, mature and healthy seeds of *S. obtusifolia* were used as germplasms obtained from diverse phytogeographical regions of the various states of India and were used for different experiments. Naturally growing pods were collected from twenty different provenances and treated prior to sowing in the field for further studies. The collected *S. obtusifolia* specimens were identified at the CNH of the Botanical Survey of India, Shibpur, Howrah.

3.2.2 Seed Germination

As the seeds have a very hard seed coat and undergo dormancy, the scarification of the seeds was done by applying concentrated sulfuric acid for 5-7 mins. Then they were intensly washed for 15 mins under running water and transferred to sterile petri plates, kept covered with moist filter paper and left for germination. The moist seeds were then kept in the dark for 12 hours and then kept in a normal photoperiod for germination.

3.2.3 Raising of Plants

After two days, the healthy germinated seeds or plantlets were sown in the field in separate and distinct rows. Well-soaked seeds were sown in the field in separate rows for different provenances. A separate row was devoted for each provenance and marked accordingly. To minimize the position effect, an RBD was followed. The experimental plot for growing all species was selected at Panskura, West Bengal, India. The study was done based on the plants grown in this experimental plot. The whole process was repeated for three consecutive years 2015-'16, 2016-'17 and 2017-'18 in the experimental plot. Three-year average data were used for analyses. The span of growth of plants was from the germination day to the day when leaves turned yellow. The span

of flowering was from the day of emergence of buds to the appearance of the last flower. The fruiting was recorded from the day when miniature pod exposed from the flower and continued till all the flowers develop into fruits. For all the provenances, the plant growth, flower and fruit counts were recorded at an interval of 10, 5 and 5 days, respectively. The photographs of *S. obtusifolia* collected from different provenances are depicted in Figure (3.1 - 3.3). Germination of the plants in the experimental fields are shown in Figure 3.4, while the growth of the plants in the experimental field of different life cycle stages are shown in Figure (3.5 - 3.8).

Figure 3.1 *S. obtusifolia* growing in wild field

Figure 3.2 *S. obtusifolia* growing in wild field

Figure 3.3 *S. obtusifolia* growing in wild field

Figure 3.4 *S. obtusifolia* seedlings showing germination in the experimental field

Figure 3.5 *S. obtusifolia* from different provenances in the experimental field

Figure 3.6 *S. obtusifolia* young plants in the experimental field

Figure 3.7 *S. obtusifolia* in buds, flowers and pods grown in the experimental field

Figure 3.8 Mature *S. obtusifolia* plants in the experimental field

3.3 RESULTS AND DISCUSSIONS

In this section, growth of plants, flowering and fruiting patterns of *S. obtusifolia* from different provenances were studied. The relationships of the growth, flowering and fruiting patterns with that of altitude, temperature and rainfall were also apprehended. From the field experience, it has been observed that the plants can be grown in any season if adequate sunlight and water are available. Plants showed their maximum vigour for the first six to seven months and remained active of nearly a year. The plants can be grown in flowering pots as well. However, the vigour of the plants was witnessed to be quite less in the potted plants. The mature leaves dehisce first then from there secondary leaves grow in potted plants. Flushing of the leaves were also observed. The plants were not found in the proximity of about five km from the sea belt and above an elevation of approximately 1000 m.

3.3.1 Growth Patterns

Approximately, 93% - 98% of the treated seeds germinated after two to three days. After 5-6 days and 12-15 days, the first two leaves and leaflets emerged. The plants were established in the field after this. The growth curves for the twenty samples were drawn based on the collected data and are presented in Figure 3.9.

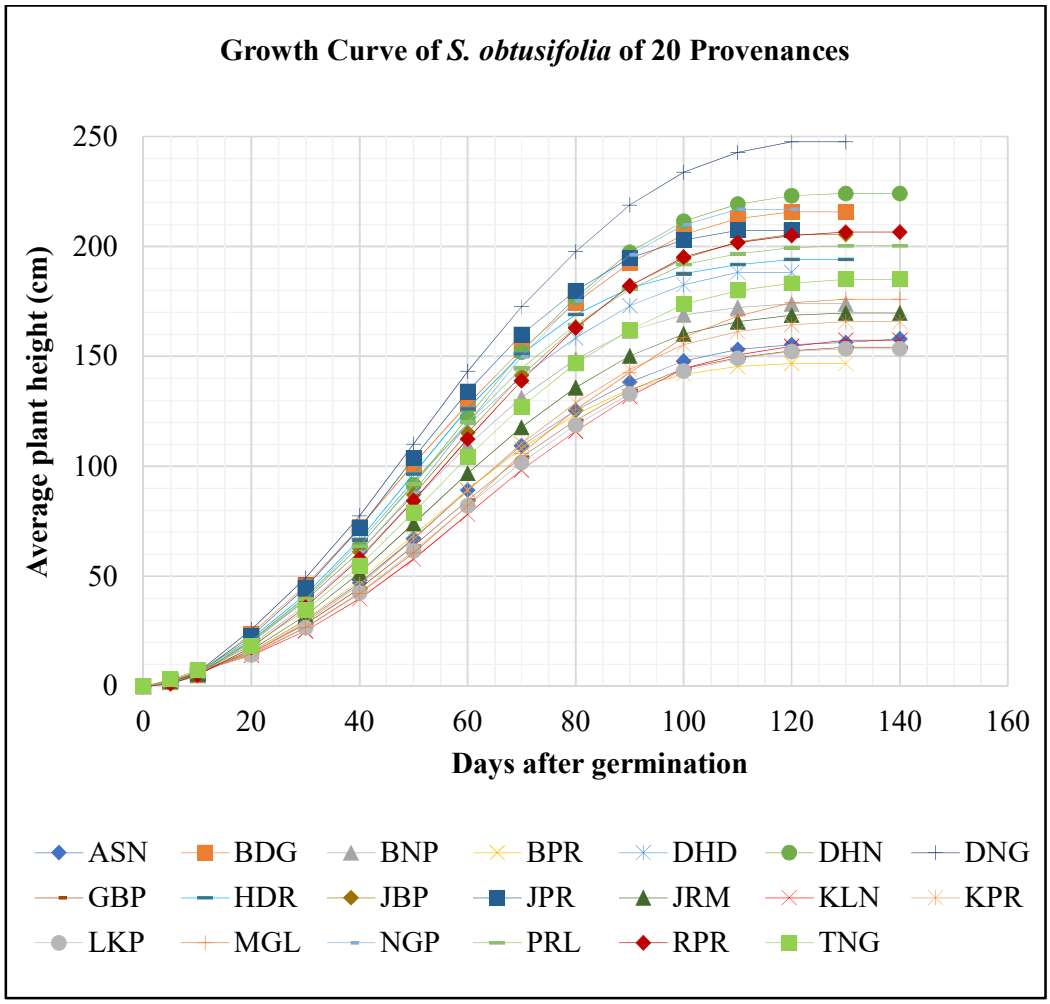

Figure 3.9 Growth patterns of *S. obtusifolia* of 20 accessions

From Figure 3.9, it was observed the plants from Devendranagar (DNG) achieved the maximum height with an average of 247.6 cm in 130 days. On the other hand, a similar growth pattern was exibited by plants from Dehradun (DHN), Nagpur (NGP), and Bandhavgarh (BDG). The average maximum height achieved by them are 224.1 cm, 216.9 cm, and 215.7 cm, in 140, 120, and 130 days, respectively. The plants from Jaipur (JPR), Raipur (RPR), Jabalpur (JBP), and Purulia (PRL) achieved an average maximum height of 207.3 cm, 206.5 cm, 205.5 cm, and 200.3 cm, in 120, 140, 130, and 140 days, respectively. The plants from Haridwar (HDR), Dahod (DHD) and Tatanagar (TNG) achieved an average maximum height of 194.1cm, 188.2 cm, and 185.0 cm in 130, 120,

and 140 days, respectively. The plants from Mangalore (MGL), Bishnupur (BNP), Jhargram (JRM), and Kharagpur (KPR) achieved an average maximum height of 176.0 cm, 173.9 cm, 169.7 cm, 165.8 cm in 140, 130, 140 and 140 days, respectively.

Finally, the shorter plants in the group are from Asansol (ASN), Kalyani (KLN), Lakhsmikantapur (LKP), Govindapur (GBP) and Bolpur (BPR) achieved an average maximum height of 158 cm, 157.4 cm, 153.4 cm, 154.2 cm, and 146.7 cm in 140, 140, 140, 140, and 130 days, respectively. Consequently, plants from different provenances took different days to attend their maximum heights. Out of twenty provenances, twelve took 140 days, five took 130 days, and three took 120 days to attent the maximum height. Correlation analysis was performed to find the relationships among average maximum height of plants and rainfall, altitude, maximum and minimum temperature of provenances. Similarly, relationship was obtained between the number of days the plants took to achieve their maximum height and the average maximum height. Results of correlation analysis is presented in Table 3.2.

Table 3.2 Correlation analysis for growths of *S. obtusifolia*

	Altitude	**Rainfall**	**Minimum Temperature**	**Maximum Temperature**
Maximum height	0.86	-0.12	-0.15	0.68
Number of days	-0.30	0.49	-0.06	-0.39

Table 3.2 purport that the average maximum plant height is strongly positive correlated with altitude and moderately positive correlated with the maximum temperature. Rainfall did not impact on the growth of the plants but did some weakly positive impact on the number of days in achieving the maximum height. This suggests that a minimum

amount of rainfall is enough for the plant growth. The number of days require to achieve the maximum height has shown a negative correlation with altitude and maximum temperature. However, the strength of relationship is not so significant.

3.3.2 Flowering Curves

The flowering of *S. obtusifolia* started between 45 and 55 days after germination. It has been observed that the flowering of the plants started at around 45 days in the provenances of Bolpur (BPR), Jaipur (JPR), Bandhavgarh (BDG), Bishnupur (BNP), Purulia (PRL) and Haridwar (HDR) in the beginning of the rainy season. For the provenances of Asansol (ASN), Dahod (DHD), Devendranagar (DNG), Govindapur (GBP), Jabalpur (JBP), Jhargram (JRM), Kalyani (KLN), Kharagpur (KPR), Lakshmikantapur (LKP), Nagpur (NGP), Raipur (RPR), and Tatanagar (TNG), the flowering started at around 50 days while for Mangalore (MGL), Raipur (RPR), and Nagpur (NGP) it started after 55 days. The flowering patterns are shown in Figure 3.10.

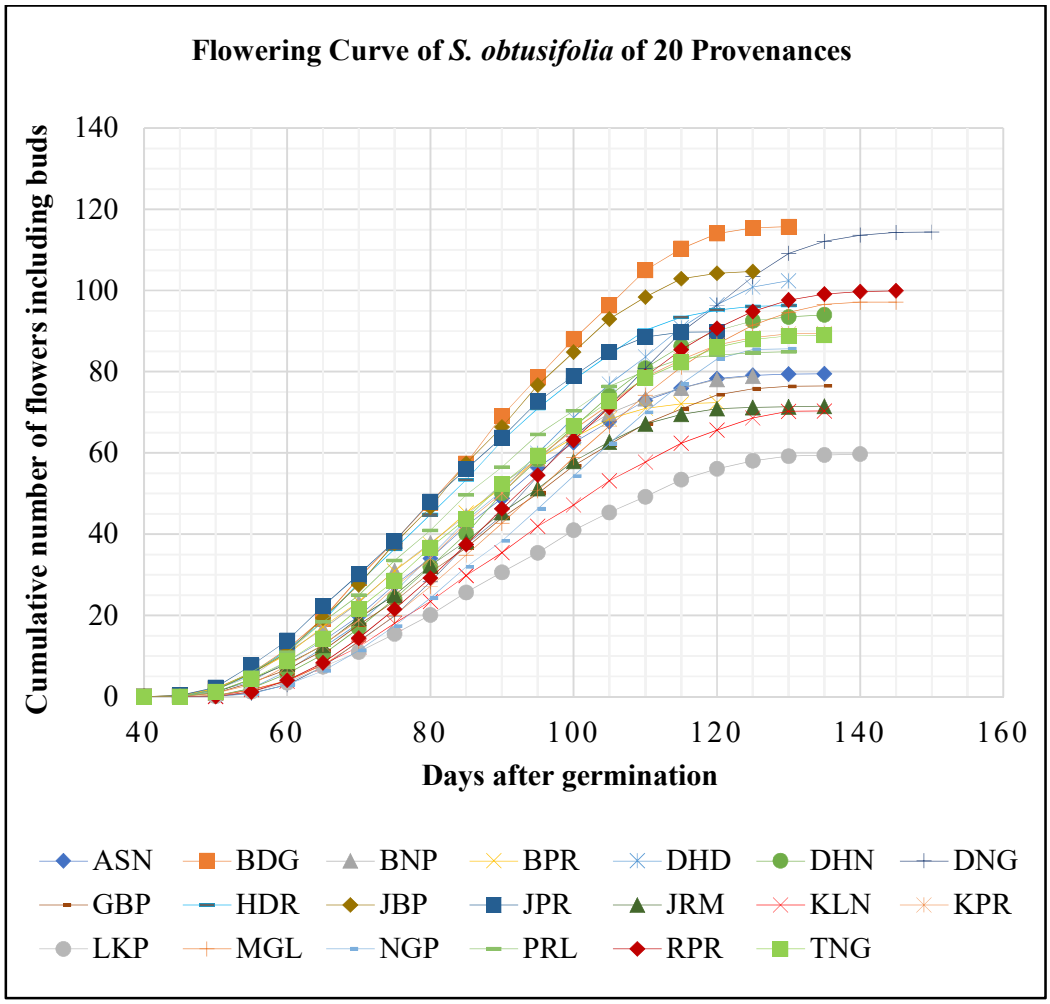

Figure 3.10 Flowering curve *of S. obtusifolia* of 20 accessions

From this study, it has been observed that the full bloom of the flowers occurred during 75-85 days in all the provenances and continued till about 115-120 days after the germination. Flowers started to appear after 120 days for the provenances of Bolpur and Jaipur with a cumulative flower count of 72 and 90, respectively. Maximum flowering occurred after 125 days for Bishnupur (BNP) and Jabalpur (JBP) with a cumulative flower count of 79 and 105, respectively and after 130 days for Purulia (PRL), Nagpur (NGP), Haridwar (HDR), Dahod (DHD), and Bandhabgarh (BDG) with a cumulative flower count of 85, 86, 96, 102, and 116, respectively. Flowers appeared maximum after 135 days of growth for Kalyani (KLN), Jhargram (JRM), Govindapur

(GBP), Asansol (ASN), Kharagpur (KPR), Tatanagar (TNG), and Dehradun (DHN) with a cumulative flower count of 70, 72, 77, 80, 89, 89, and 116, respectively. Flowers of maximum count were found in the provenances of Lakshmikantapur (LKP) in 140 days, Mangalore (MGL) and Raipur (RPR) in 145 days, and Devendranagar (DNG) in 150 days with a cumulative flower count of 60, 97, 100, and 114, respectively.

From the above results, we found that the flowers stopped emerging first at Bolpur (BPR) and Jaipur (JPR) after 120 days, and at last at Devendranagar (DNG) after 150 days. In terms of the number of flowers, Lakshmikantapur (LKP) had the lowest flower count of only 60 and Bandhabgarh (BDG) had the maximum flower count of 116. The relationships between the cumulative flower count and altitude, rainfall, minimum, and maximum temperature of provenances have been explored by finding the correlation coefficients. In a similar manner, relationships have been explored for the number of days after which the flowering ended. Table 3.3 presents the results of correlation analysis.

Table 3.3 Correlation analysis for flowering of *S. obtusifolia*

	Altitude	**Rainfall**	**Minimum Temperature**	**Maximum Temperature**
Flower count	0.71	0.08	0.00	0.56
Number of days	-0.06	0.46	0.22	-0.05

Table 3.3 shows that the cumulative flower count has a moderate positive correlation with both altitude and maximum temperature of the provenances. Rainfall and minimum temperature did not impact on the number of flowers. On the other hand, the number of days after which the flowers started to dehisce has a weak positive

correlation with both rainfall and minimum temperature. The other two factors have no impact on it.

3.3.3 Fruiting Performance

After 60-65 days of germination, the pod of *S. obtusifolia* arose. Fruiting patterns of plants from different provenances had shown a considerable variation. Fruits started to appear in around 60 days in Asansol (ASN), Bandhavgarh (BDG), Bishnupur (BNP), Bolpur (BPR), Jabalpur (JBP), Raipur (RPR), Purulia (PRL), and Tatanagar (TNG). For the other provenances except Mangalore (MGL), Nagpur (NGP), and Raipur (RPR), fruiting started in around 65 days. The fruiting patterns are shown in Figure 3.11.

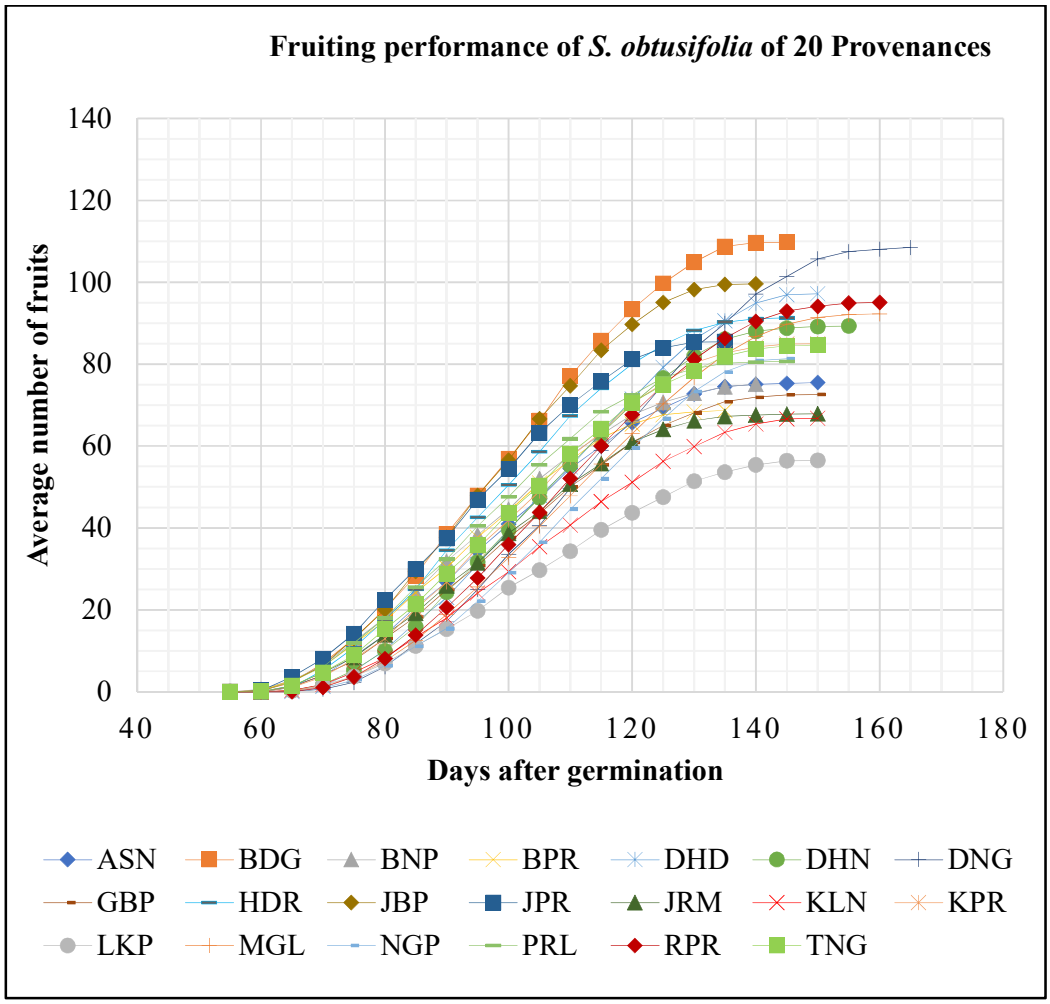

Figure 3.11 Fruiting performance of 20 accessions of *S. obtusifolia*

For the plants from Bolpur (BPR) and Jaipur (JPR), fruiting continued till 135 days from the germination date with an average number of 69 and 86 fruits, respectively. For the plants from Bishnupur (BNP) and Jabalpur (JBP), fruiting continued till 140 days with an average number of 75 and 100 fruits, respectively. For Purulia (PRL), Nagpur (NGP), Haridwar (HDR), and Bandhabgarh (BDG), fruiting continued till 145 days with the average number of 81, 81, 91, and 110 fruits, respectively. It was 150 days for the majority of provenances, which are Lakshmikantapur (LKP), Kalyani (KLN), Jhargram (JRM), Govindapur (GBP), Asansol (ASN), Kharagpur (KPR), Tatanagar (TNG), Raipur (RPR), and Dahod (DHD) with the average number of fruits 57, 67, 70,

73, 76, 85, 85, 95 and 97, respectively. Finally, for Dehradun (DHN), Mangalore (MGL), and Devendranagar (DNG) the fruiting continued till 155, 160, and 165 days, with the average number of fruits 89, 92, and 109, respectively.

From the above results, it was noted that the plants from Bolpur (BPR) and Jaipur (JPR) showed fruit emergence for the shortest phase during their growth for 135 days. The maximum duration of fruiting was found for the provenance Devendranagar (DNG) which lasted for 165 days. Lakshmikantapur (LKP) had the lowest fruit count of only 57 per plant and Bandhabgarh (BDG) had the maximum flower count of 110. The relationships between the fruit count and altitude, rainfall, minimum, and maximum temperature of provenances have been explored by finding the correlation coefficients. Similarly, relationships have been explored for the number of days till which the fruiting continued. Table 3.4 presents the results of correlation analysis

Table 3.4 Correlation analysis for fruiting of *S. obtusifolia*

	Altitude	**Rainfall**	**Minimum Temperature**	**Maximum Temperature**
Flower count	0.70	0.07	0.01	0.57
Number of days	0.02	0.49	-0.01	-0.16

Table 3.4 shows that the relationship strengths of fruiting with the considered factors are almost identical with that of the flowering (see Table 3.3). There is moderate positive correlation of fruiting with the altitude and maximum temperature and the other factors do not impact the same. However, only the number of days for which the fruiting continues shows a weak positive correlation with rainfall.

3.3.4 Major Findings

The germplasms of *S. obtusifolia* collected from different provenances of India vary in altitude, rainfall, temperature range and many other factors. All germplasms have been grown in the same field. This study showed variations amongst the provenances in growth of plants, flowering and fruiting patterns. These variations might have occurred due to their inherent differences in the genetic level. In a locally specialized ecotype through age and plasticity may help in the change in the genetic makeup of the species.

For understanding the phenological variation in intraspecific level, growth, flowering and fruiting patterns have been studied. A brief account of it is displayed in Table 3.5.

Table 3.5 Phenological characteristics of *S. obtusifolia*

	Maximum	**Minimum**	**Range**
Average plant height (cm)	247.6	146.7	100.9
Plant height achieves (days)	140	120	20
Flowering starts (days)	55	45	10
Flower counts	116	60	56
Flowers start to dehisce (days)	150	120	30
Fruiting starts (days)	65	60	5
Fruiting continues (days)	165	135	30
Fruit counts	110	57	53

Table 3.6 Relationship Strength

	Altitude	Maximum Temperature
Maximum height	SPR	MPR
Flower count	MPR	MPR
Fruit count	MPR	MPR

SPR: Strong Positive Relationship, MPR: Moderate Positive Relationship

Out of four climatic factors (Table 3.6), altitude and maximum temperature impacted growth, flowering and fruiting patterns at the maximum level. The other climatic factor rainfall had shown a weak positive relationship with the number of days after which the flowers started to dehisce as well as with the number of days till the fruiting continued. Interestingly, some negative impacts had also been observed. The altitude and maximum temperature had shown a weak negative impact on the number of days required to achieve the maximum height. These findings are at per with the literature (Cardoso et al., 2012) which reported that phenological variations in intraspecific level in tropical belts depends on climatic features.

The cluster analysis is employed to classify *S. obtusifolia* samples from twenty provenances. The dendrogram presented in Figure 3.12 shows the extenty of relatedness among 20 provenances of *S. obtusifolia* samples based on their phenological characteristics.

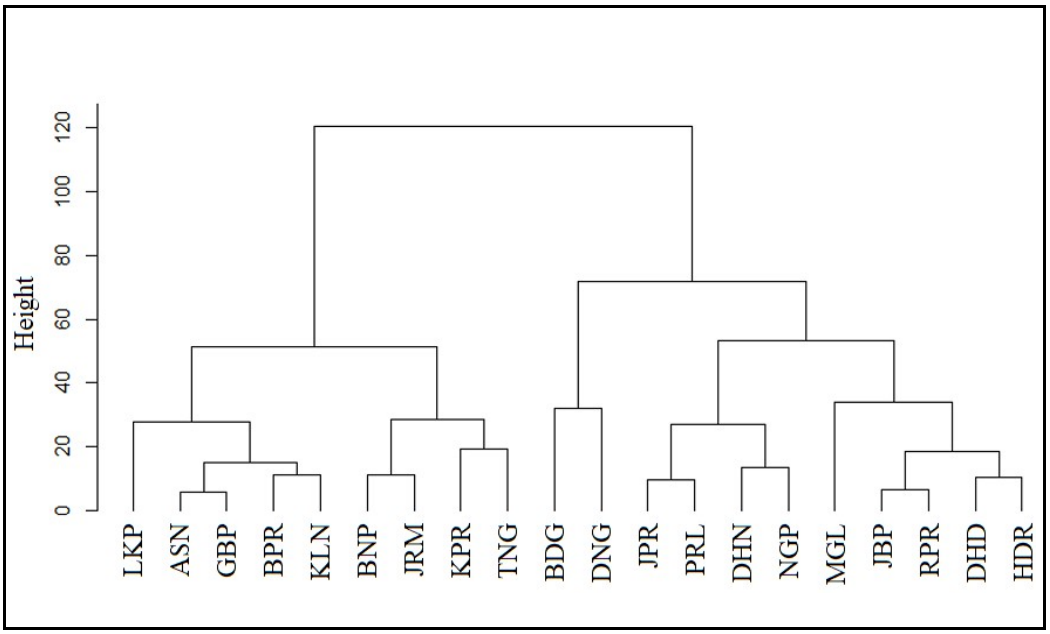

Figure 3.12 Clustering of *S. obtusifolia* accessions based on phenological characteristics

It is observed from Figure 3.12 that the twenty *S. obtusifolia* samples form five clusters. The samples from Lakshmikantapur (LKP), Asansol (ASN), Gobindapur (GBP), Bolpur (BPR) and Kalyani (KLN) belong to the first cluster. Out of these five samples, the phenological characteristics of Asansol (ASN) and Gobindapur (GBP) are similar. Phenological characteristics of Bolpur (BPR) and Kalyani (KLN) are also similar. The characteristics of these two pair are slightly different from the remaining member of the group. The samples from Bishnupur (BNP), Jhargram (JRM), Kharagpur (KPR) and Tatanagar (TNG) belong to the second cluster. Among these four samples, Bishnupur (BNP) and Jhargram (JRM), and Kharagpur (KPR) and Tatanagar (TNG) have similar phenological characteristics. The samples from Bandhavgarh (BDG) and Devendranagar (DNG) have similar phenological characteristics and belong to the third cluster. The samples from Jaipur (JPR), Purulia (PRL), Dehradun (DHN) and Nagpur (NGP) belong to the fourth cluster. In this cluster as well, the pair of samples from Jaipur (JPR), Purulia (PRL), and Dehradun (DHN), Nagpur (NGP) have similar

phenological characteristics. The remaining five samples from Mangalore (MGL), Jabalpur (JBP), Raipur (RPR), Dahod (DHD) and Haridwar (HDR) belong to the final cluster. Here also, the samples from Jabalpur (JBP) and Raipur (RPR), and Dahod (DHD), and Haridwar (HDR) have shown similar phenological characteristics and are marginally different from that of Mangalore (MGL).

3.3.5 Discussions

The samples of *S. obtusifolia* grown from the germplasms collected from various agroclimatic zones have shown a good length of variation in different phenological traits. Such difference is not merely evident in plants in their respective in situ conditions, but even while they are being grown from their seeds in a common place having same physical and chemical environment for growth. Flowering and fruiting phenophases among six medicinal plants of Nilgiri Biosphere Reserve, India, phenological behavior were observed by Duraisamy and Subramaniam (2010). The correlation analysis in the present study revealed that the average maximum plant height is strongly positive correlated with altitude and moderately positive correlated with the maximum temperature of the provenances. The flowering and fruiting count has moderate positive correlation with both altitude and maximum temperature of the provenances. In accordance with the present study Kushwaha et al., (2011) explored the relationships of different factors like temperature, rainfall and other climatic conditions on the phenological characteristics of the trees in dry tropics. Similarly, the effect of temperature and altitude in plant growth, development and seed yield of two oilseed crop *Lesquerella fendleri* and *Lesquerella pallida aff.* were studied elaborately by Dierig et al., (2006). Variations of various phenological attributes of chestnut trees regarding altitudes and different edaphoclimatic conditions were also studied by Dinis et al., (2011). Variation in seed morphological traits of *Balanites aegyptiaca* among

seven provenances, studied by Freigoun et al., (2019) are in accordandance provenance variation of phenological traits *S. obtusifolia* among twenty provenances. Thus, variation recorded in this study amongst such plants of *S. obtusifolia* indicates that the specificity of traits is of very much stable and inherent in nature. Apprehending a plausible genetic basis behind the expression of the concerned traits and for the existence of variations in them any selection made amongst the provenances, in search of best performance, is supposed to be most effective. Moreover, the documentation of these phenological events would serve as a very useful information for the best exploitation of the species.

The present study was directed to prospecting intraspecific phenological diversities of *S. obtusifolia*. For all the provenances, the average plant height, number of days required to attend maximum height, number of flowers, shedding off time of flowers, number of days required for fruit emergence from the day of germination, and fruit count per plant were 187.31 cm, 134.5 days, 88.65, 133.25 days, 148 days and 84.4, respectively. The most significant deviation was observed in case of plant height, flower counts, and fruit counts.

Registering these phenological details of different provenances would facilitate in handling them properly for propagation as well as harvesting. Such variations signify some basic differences inherent to them and may have implications at the level of secondary metabolite production.