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Phenology of *Piper mullesua* Buchanan-Hamilton ex D. Don (Piperaceae) - a medicinally important dioecious plant

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Abstract

Phenological events in details of dioecious *Piper mullesua* Buchanan- Hamilton ex D. Don (Piperaceae) found in Arunachal Pradesh were observed in nursery condition during 2013 – 2014. Different vegetative and reproductive phenophases like leaf initiation, leaf opening, spike formation, initiation of flowers, peak flowering period, number of flowers, maturation of spikes, fruit formation and fruit maturity were studied. It is found that the production of leaves and spikes are simultaneous and mostly throughout the year. Peak flowering time was found in the month of March for both the sexes and maximum fruiting was observed during June – July. A longer flowering period was noted in male plants in comparison to the females. However, flowering frequency was low in male plants.

Key words: *Piper mullesua*, Phenology, Dioecious, Spikes, Fruiting

INTRODUCTION

The pantropic genus *Piper* Linnaeus (Piperaceae) is comprising of nearly 2000 species (Quijano-Abril *et al*. 2006). A recent phylogenetic analysis suggests three major clades for this genus (Jaramillo & Manos 2001), representing three large geographical regions: America (1300 spp.), Asia (600 spp.) and the South Pacific (100 spp.) (Quijano-Abril *et al*. 2006). Although the new world *Piper* species are bisexual and are trees or bushy shrubs, the old world *Pipers* are mostly dioecious and climbers. In India, so far, over 100 species of *Piper* have been reported, concentrating their distribution in Western Ghats and Eastern Himalaya with about 65 species in north eastern states (Gajurel *et al*. 2008). *Piper nigrum* Linnaeus, *P. betle* Linnaeus, *P. longum* Linnaeus and *P. methysticum* G. Froster are the well-known economically important species, besides many species of medicinal and ethnobotanical uses (Jaramillo & Manos 2001; Quijano-Abril *et al*. 2006; Gajurel *et al*. 2008). During the past 15 years the two senior authors have extensively undertaken studies on *Pipers* of north eastern region of India and tried to understand the taxonomic diversity, ethnobotanical uses, ecology etc. (Gajurel & Rethy 1998; Gajurel *et al*. 2001; Gajurel 2002).

The genus *Piper* contributes many economically and ecologically valuable many species those are of ethnobotanical interest and have been commercially used as spices and medicines and because of this the species are preferred in management and conservation programs in tropical areas. *Piper longum* is used in about hundreds of Ayurvedic preparations. Many wild species of *Piper* (*P. mullesua* Buchanan- Hamilton ex D. Don, *P. pedicellatum* C.

DC., *P. beteloides* C. D. C, *P. wallichii* (Miquel) Handel-Mazzetti, etc.) have been used as medicines for the treatment of common diseases or as preservative (Gajurel & Rethy 1998). The root of *P. sylvaticum* is used as medicinal as antidotes against snake poison in the indigenous India (Kirtikar & Basu 1933). Phytochemical screening of *Piper* revealed the presence of a variety of natural products such mevalonic acid (monoterpenes and sesquisterpene), cinanamoyl amides and alkyl amides, aristolactams, flavone, dihydroflavone, dihydrochalcone and O-methylflavonoids (Parmar *et al*. 1997) that may accounted for its medicinal value.

Phenology is the study of timing of recurring biological events; the causes of their timing with regard to biotic and abiotic forces, and interrelation between phases of the same or different species (Leith 1974) which provide useful information. For plants, recurrent biological events include vegetative processes such as leaf flushing and shedding as well as reproductive events such as bud formation, flowering and production of fruits (Francis *et al*. 2007). Plant phenological study has great significance because it not only provides knowledge about plant growth pattern but it also provides the idea on the effects of environment and selective pressure on flowering and fruiting behaviour (Zhang *et al*. 2006). The phenological studies considering both the sexes of dioecious *Piper* species have not been attempted so far in India where numerous economically important species of the genus are found. The present paper deals with the phenological events of both male and female plants of *Piper mullesua* occurring in the East Himalayan region.

MATERIALS AND METHODOLOGY

Piper mullesua Buchanan- Hamilton ex D. Don (Piperaceae) is an erect shrub of about 1 m high with few stolons and runners (Fig. 1). Leaves alternate, petiolate; stipules 5 – 10 mm, adnate to the petiole. Prophylls 6 – 9 mm, dark green, stout and finely pubescent; lamina ovate to lanceolate. Plants dioeceous. Male spikes erect, 5 – 8 cm long, flexuous; female spikes globose, 4 – 6 x 3 – 4 mm; flowers densely arranged, rachis pubescent; bracts peltate-orbicular. Males with 2 – 3 stamens supported by orbicular bract, anthers exerted beyond the bracts. Females 1 mm long, ovary ovate, stigmas 3, sessile. Infructescense spikes almost globose, 10 – 15 x 8 – 13 mm, fruits densely aggregated.

The present study was carried out during the years 2013 and 2014 at the NERIST campus in the state of Arunachal Pradesh that is located between 27°07' N latitude, and 93°22' E longitude and situated 126 m a.m.s.l.. Both the male and female plants of *P. mullesua* were collected from different parts of Arunachal Pradesh and were raised in the nursery of the Department of Forestry, NERIST and were studied for the phenological events. To observe different phenophases, 25 individual plants each for male and female were tagged for the study. For flowering phenological data 30 spikes each from both the sexes were randomly selected and marked. Continuous observations on leafing, flowering and fruiting behaviour were made periodically for one full calendar year for recording phenological data. The different phenological events were observed in regular intervals for each phenological event following standard methods (Marquis 1988; Kasarkar & Kulkarni 2011; Nanda *et al* 2014). Leafing phenophases include initiation, opening, peak period of leaf formation, time taken for maturity, size of mature lamina, number of new leaves developed, senescence and fall. Flowering phenophases include spikes formation, flower initiation, maturity of spikes and flowers, peak flowering period, maturity of a flowers, length of the mature spike, number of flower in a spike, completion of flowering period in a spike, duration of flowering period in a spike and number of flowers in a plant. For fruiting phenology all the events from fruit initiation to fruit shed including the number and size of fruiting spikes, fruits and seeds were recorded. All the recorded data were then analysed and presented.

RESULTS

Vegetative phenophase

Detail observation on leaf initiation and development is presented in Table 1. It was observed that leaf initiation in the species occurred throughout the year with peak in the month of February. Number of average new leaves developed per plant is of about 69.92 in males and 36.88 in females. An average of 8 to 10 days period was taken for opening of leaf from the day of its initiation. An open leaf took 3 months to reach its full mature size. About 11 months time was recorded as the life span after which senescence initiate and leaves start falling. Falling of the matured leaves was observed almost for more than 8 months where September to April and June to April was recorded in male and female plants respectively. Size of the mature leaf lamina is about 13.47 x 7.23 cm and 10.84 x 5.63 cm in male and female plants respectively.

Table 1. Vegetative phenophases of male and female plants of *Piper mullesua*

Sl. No.	Vegetative phenophases	Male	Female
1.	Leaf initiation	Throughout the year	Throughout the year
2.	Number of new leaf developed in the fertile shoot	69.92 ± 25.92	36.88 ± 11.61
3.	Peak time of leaf initiation	February	February
4.	Time taken for leaf opening after initiation	8.07 ± 2.37 days	9.53 ± 2.90 days
5.	Time taken for leaf maturity	3 months	3 months
6.	Time taken to initiate senescence leaf after emergence	11 month	11 month
7.	Main leaf fall period	September to April	June to April

Flowering phenophase

It was observed that both, male and female plants of *P. mullesua* produce spikes almost throughout the year. It was noticed that all the individuals bear spikes in the months of March and April in male plants and in March in female plants (Fig. 2). In male plants number of budding spikes were found highest in February with mean value (± s.d.) of 21.56 ± 13.97 and highest number of flowering spikes in March 17.44 ± 14.38 (Figs. 3 & 4). As in male, in female plants also budding spikes were found highest in February (10.28 ± 4.24) and flowering spikes in March (11.24 ± 4.82) (Figs. 5 & 6). A mature male plant produces 33.8 ± 6.16 spikes while female plants produce19.52 ± 6.35. However, the number of spike ranges from 30 to 40 and 15 to 20 in male and female plants respectively. The development of flowers in the spike took considerably long period in male plants as the flowers start immersing till the end of the elongation of the spikes. In a developing male spike the basal portion of spike bear matured flowers while the apical part consists of very young flower-buds. The total time

Figure 1. ***Piper mullesua:*** **A.** Male; **B.** Female; **C.** Male spike; **D.** Female spike; **E.** Infructescence

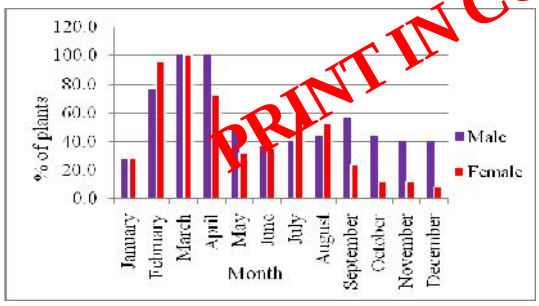

Figure 2. % of plants bearing flowering spikes in different months

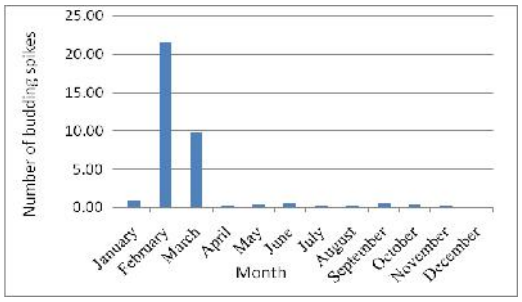

Figure 3. Number (mean value) of male budding spikes

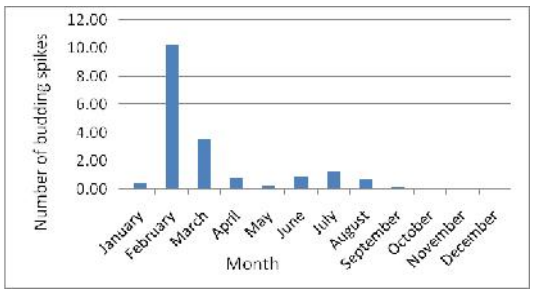

Figure 4. Number (mean value) of female budding spikes

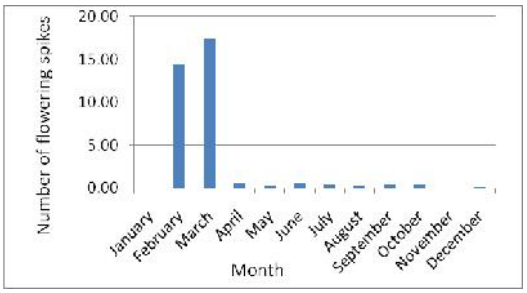

Figure 5. Number (mean value) of male flowering spikes

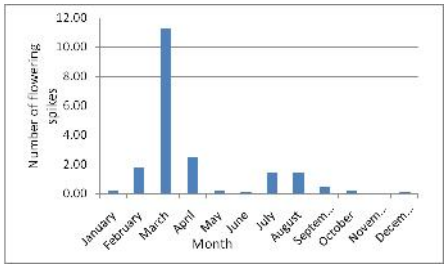

Figure 6. Number (mean value) of female flowering spikes

taken for spike elongation is 57 days in male plants and 28.2 days in female plants. In male plant flowers were observed after 15 days of spike initiation and anther dehisces after 5 days of flower initiation i.e., maturity of a flower took around 19.73 days and flowering complete in 42.00 days from the first day of initiation of flower. Spike fall after 9 days of maturity of spike having a total flowering duration of 66 days. Length of a mature spike varies from 4 to 12.5 cm with an average length of 7 cm comprising 664 flowers. The length of female spikes (0.49 cm) is much shorter than the male.

In female plant flower-formation initiated after 19 days from the day of initiation of spike; gets mature after 6 days of flowering initiation and flowering complete after 9.33 days from the day of flower initiation in a spike. So the overall duration of flower in female plant is 28.2 days. Average length of the spike is 0.5 cm with 82 flowers and the length of the spike varies from 0.4 to 0.7 cm (Table 2). In female plants flowering spike does not fall and it remain on the plants to develop into an infructescence.

Table 2. Flowering phenophases of male and female plants of *Piper mullesua*

Sl. No.	Flowering phenophases	Male	Female
1.	Spikes formation period	Throughout the year	Throughout the year
2.	Pick period of flowering	March	March
3.	Time taken for flower initiation after initiation of spike	15.2 ± 7.23 days	18.9 ± 5.90 days
4.	Time taken for maturity of a flowers	4.53 ± 3.07 days	5.73 ± 1.39 days
5.	Completion of flowering period in a spike from the 1st day of initiation of flower in a spike	42.00 ± 17.7 days	9.33 ± 2.85 days
6.	Time taken for spike elongation	56.87 ± 26.82 days	28.2 ± 7.1 days
7.	Time taken for falling of a mature spike	8.93 ± 4.48 days	NA
8.	Total life span of a flowering spike	65.8 ± 26.59 days	28.20 ± 7.1 days
9.	Length of the mature spike	6.99 ± 2.82 cm	0.49 ± 0.50 cm
10.	Total number of flower / inflorescence	664.2 ± 302.9	81.7 ± 12.28
11.	Number of spikes per plant	33.8 ± 6.16	19.52 ± 6.35

Fruiting phenophase

The female spikes turn into infructescence and the fruiting spikes were seen almost throughout the year. It was observed that 100 % of the female plants bear infructescences during the period March to October (Fig. 7). First initiation of fruiting was observed in the month of December and continues till May with peak fruiting initiation period in March having 11.00 ± 4.39 spikes in each plant (Fig. 8). Fruiting duration was around 8 months (239 days). Fruiting completed after 8 days from the first day of initiation of fruiting and it took 204 days i.e.,

Table 3. Fruiting phenophases of *Piper mullesua*

Sl. No.	Fruiting Phenophases	
1.	Fruiting initiation period	December - May
2.	Peak fruiting initiation period	March
3.	Time taken for completion of fruiting in a spike after initiation of fruiting	8 ± 2 days
4.	Time taken for ripening of fruit after its initiation	204 ± 26.05 days
5.	Time taken for dropping of mature infructescences	52.80 ± 34.09 days
6.	Season of dropping of matured infructescences	September to February
7.	Total fruiting duration	239 ± 70.18 days
8.	Fruiting season	December to January
9.	Number of infructescence per plant	16.2 ± 5.38
10.	Length of infructescences	0.91 ± 0.13 cm
11.	Total number of fruit per inflorescence	55.90 ± 16.84

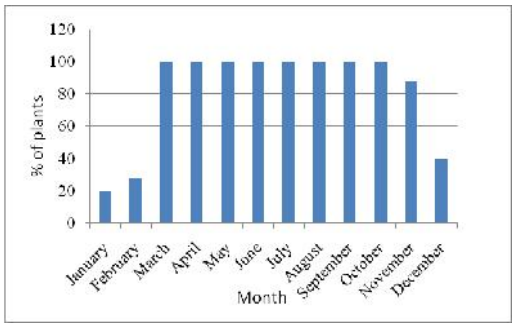

Figure 7. % of plants under study bearing infructescences

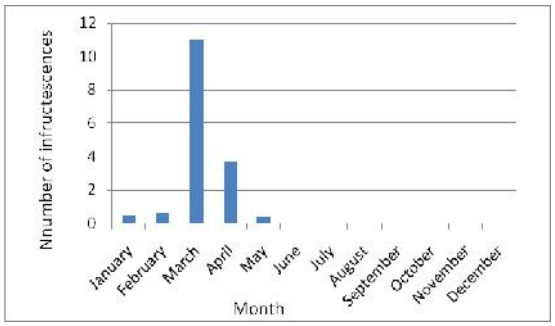

Figure 8. Number of newly developed infructescences in plants under study

around 7 month to get matured. In mature plants number of infructescences varies from 15 to 20. However, total number of infructescence produce by a plant during the study period was 16.2. Fruit fall was observed after 52.80 days of maturity. Length of the infructescences varies from 0.6 to 1 cm. Average length of a fruiting spike is 0.91 cm with 55.90 numbers of fruit.

DISCUSSION

The present study on the phenology of both the sexes of dioecious *P. mullesua* revealed that both of its vegetative and reproductive phenophases remain active almost round the year. The result on leaf flush occurring throughout the year in the studied species was found similar with other bisexual *Piper* species of the new world (Opler *et al.* 1980; Marquis 1988; Thies & Kalko 2004; Valentin–Silva & Vieira 2015). Leaf flush is almost synchronous with spike production, and such kind of synchronous phenological events are marked as characteristic in seasonal tropical forests (Thies & Kalko 2004). Production of more flowers in males than the female plants in dioecious species is a near universal pattern (Gracia & Antor 1995; Guitian 1995). In *P. mullesus* also the same pattern has been observed. In some dioecious plants male flowering period start earlier than the female flowering period (Bawa 1983). Spike developments and flower formation were observed throughout the year in both

the sexes with one peak period and similar phenomenon has also been observed in other new world *Pipers* like *P. arieianum* (Marquis 1988), *P. cernuum* (Mariot *et al*. 2003) and *P. vicosanum* (Valentin-Silva & Vieira 2015). Most of the individuals produce spike twice a year in male plants. Like *Piper,* the dioeceous *Withania aristata* also flowers simultaneously on both sexes year round (Anderson *et al.* 2006). Occurrence of more than one flowering episode have been observed in many other species like *Trichilia pallida* (Morellato 2004), Ficus sp. (Jia *et al.* 2007), etc.

It has been found that in female plants of *P. mullesua,* the flowering spikes develop during June to September mostly fail to set fruit, whereas those spikes initiated from October onwards successfully produces fruits. It is also seen that *Piper* species prefers to set their fruits during the winter – when there is comparatively low humidity, temperature and light intensity. The spikes develop during June to September are commonly subjected to high rainfall, temperature and light intensity. Hence, the environmental factors play the important role in their fruit setting. It has also been noticed that many of the dioecious *Piper* can produce fruits without the occurrence of male plant in the population (Gajurel 2002). Development of apomixes fruits in *Piper* species have already been reported (Ravindran *et al*. 2000). The mature male individuals produce about 30 to 40 spikes while the young plants produce only about 10 spikes. So, the relative number of spike formation is proportional to the age of the plant. However the health of a plant may also become a determinative factor in flowering and fruiting. Valentin-Silva & Vieira (2015) in their study on *Piper* suggested that one should select plants that scare of spikes from previous reproductive episodes that located on the nodes opposite to the leaf insertion site. Infructescences in *P. mullesua* were observed round the year. The infructescences developed during October to January were comparatively less in number from those developed in February to April. Marquis (1988) also encountered more than one infructescences per flowering bout, resulting from February and September to October blooming flowers, which disperse in different times i.e. October to January and April to June respectively. Snow (1965) and Thies & Kalko (2004) observed sequential fruiting periods in *Piper* species that relates to the year-long fruit production. In case of *Ficus* fig crops bore several times throughout the year (Zhang *et al.* 2006; Kuaraksa *et al.* 2012).

It is clear that the phenological events of leaf development and spikes formation take place throughout the year but with some particular peak periods. Spike production is directly correlated with leaf production. Male flowering spikes take more time to get maturity and have long flowering as compared to female flowering spikes which may supportive to provide pollen during the flowering period of the female.

CONCLUSION

This study has helped to understand the timing of different phenophases of the dioecious *Piper* species. The real time of availability of fruits in the plants has been understood which may be useful for selection of timing for the collection of fruits. The fruits are used medicinally in local, pharmaceutical and in different ayurvedic formulations. The different phenophases recorded in the present study would be very useful for management and cultivation of this plant. Moreover, the present study has significantly contributed to understand the flowering pattern and phenophases of the dioecious Indian *Pipers*.

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