Short communication

Reproductive biology of *Piper longum* L.

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Abstract

Piper longum L. is an important medicinal species. Reproductive biology of *Piper longum* was studied in detail. Spikes were cylindrical and creamy white in female. In male and bisexual types, immature spikes were green, changing to dull yellow on maturity. Time taken for attaining full length of spike was 22 days in female, 43 days in male and 46 days in bisexual types. Anthesis and anther dehiscence were between 7.30 am and 4.30 pm with a peak between 10.30 am to 12.30 pm. Pollen fertility was maximum at 9.30 am (42.54 per cent). Complete opening of flowers in an inflorescence took seven days in male, female and bisexual types. Complete dehiscence of anthers also took one week in male and bisexual inflorescences. Fruits did not normally contain viable seeds. Viable seeds could be produced on artificial pollination.

Key words: *Piper longum,* Medicinal plant, Anthesis, Anther dehiscence, Stigma receptivity, Reproductive biology

Piper longum (Long pepper) is a very important and most extensively used medicinal plant in the ayurvedic system of medicine. It is used in over 320 classical medicinal formulations. Long pepper is indigenous to South Asian countries including India. It was well known in ancient India and was used as a spice in the early days. Dried female spike which consists of minute fruits embedded in a fleshy rachis is the officinal part in *Piper longum* which is being used in many ayurvedic preparations.

Earlier, *Piper longum* was reported as a dioecious species (Rahiman, 1981 and Ravindran, 1990). Sujatha and Nybe (2007) identified a bisexual type in *Piper longum* (Acc. P25). Even though *Piper longum* is a very important medicinal plant, the reproductive biology of the species has not been studied in detail. Information on reproductive biology is very important in crop improvement programmes. The species was reported to be apomictic (Ravindran 2000). However, seed propagation or seed viability were not reported in *Piper longum*. In the present study, reproductive biology of *Piper longum* utilising the three available sex forms has been analysed.

Reproductive biology of *Piper longum* L. was studied in detail utilising the three available sex forms. Observations were made on period of flowering and fruit set, number of spikes per plant, spike characters such as orientation, shape, colour, length, and type of hermaphroditism in the spike. Floral characters such as number of stamens per flower and number of stigmatic lobes were observed (in 100 flowers each). Time of anthesis, anther dehiscence and stigma receptivity were observed at hourly intervals. Pollen fertility was studied using one per cent acetocarmine. Viability of pollen was tested in eight concentrations of sucrose (2, 4, 6, 8, 10, 20, 40 and 50 per cent) and also in media reported by Ravindran (1979), Brewbaker and Kwack (1963), ME_3 medium (Leduc et al., 1990), Sunderland and Robert's (1977) and water.

Monthly variation in spike production and sex form in *Piper longum* L. was studied to find out the month

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in which maximum flowering was observed and to find out whether there is variability in the production of bisexual and male flowers in *Piper longum* in different months (Table 1). As can be seen from Table 1, the number of spikes per plant varied from zero (December) to 12.6 (June). Maximum flowering was observed from May to July which

Table1. Monthly variation in spike production and sex form in *Piper longum* L. (Acc. P25)

Month	Number of spikes per plant	Bisexual flowers per spike	Staminate flowers per spike
January	2.2	3.8	401.5
February	2.4	4.5	432
March	5.0	0	348
April	5.4	0.5	372
May	10.8	3.9	414.8
June	12.6	0.4	319.8
July	9.8	0	353.7
August	3.0	0	364.2
September	2.0	0	369.1
October	3.8	1.2	379
November	3.6	0	275.3
December	0	0	0
Mean	5.1	1.2	335.8
SD	3.9	1.8	113.6

was significantly higher in number compared to all other months. However, maximum number of bisexual flowers were produced during February (4.5) followed by May (3.9) and January (3.8). There was predominance of male flowers in the bisexual accession during March, July to September and November. The number of bisexual flowers recorded during these months was zero. Pistillate flower production was not noticed in the accession. Staminate flowers were produced in large number throughout the year (275.3 - 432). It was observed that there was variation in spike production and sex ratio in the bisexual type in different months of the year.

The spikes were cylindrical and erect in all the sex forms. In female type, the spikes were creamy white until fruit set, when and after that colour changed to green. In male and bisexual types, immature spikes were dark green which turned to light yellow and further to dull yellow on maturity. Ravindran (2000) also reported female spikes to be creamy white to yellowish white when young and male spikes yellow on maturity.

The number of spikes/ lateral branch varied from 1 to 3 in male and female types. In bisexual type, the number ranged from 1 to 4 (Table 2). As can be seen from the table, spike production / lateral branch was maximum in the bisexual type (Acc. P25) followed by male and female types.

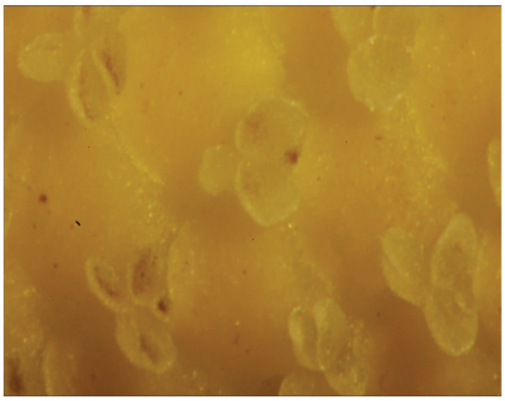
Table 2. Number of spikes per lateral branch in *Piper longum* L.

	Bisexual type	Male type	Female type
Range	1 to 4	1 to 3	1 to 3
Mean	2.7	1.9	1.7

(Average of 5 plants)

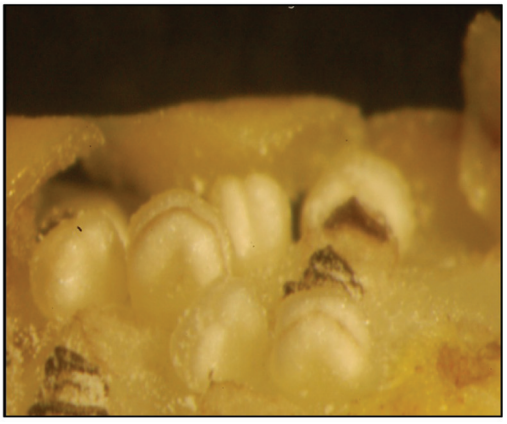
There was an increment in length of 0 to 3.9 mm/ day in the male, 0 to 2mm/day in bisexual types and 0.6 to 1.4 mm per day in the female type until they attained full length. The female spike attained full length (2.31 cm) in around 22 days, whereas male type attained full length in 43 days (7.76 cm) and bisexual type in 46 days (6.35 cm). As evident from Fig.1, growth of spike in all the sex forms showed linear pattern. Male and bisexual spikes were longer (6-7 cm) than female spikes (2-3 cm) in *Piper longum.* When compared to female spikes, long male spikes are seen in other dioecious species of *Piper* like *P. argyrophyllum, P. attenuatum, P. sugandhi, P. wightii and P. mullesua.* (Rahiman, 1981, Ravindran, 1990) Only in *Piper hymenophyllum,* female spikes are reported to be slightly longer than male ones (Ravindran,1990). It took only 22 days for attaining full length of spikes in the case of female types whereas more number of days were required (43 and 46) in male and bisexual types respectively. This shows that in *Piper longum,* more time was required for attaining full length in the longer spike of male and bisexual types compared to shorter spike of female type.

Female spike



Magnified view (x 40)

Male spike



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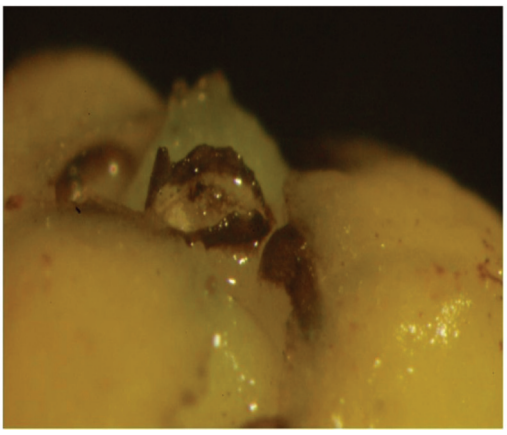
Bisexual spike



Magnified view(x 40)

Plate 1. Inflorescence and flowers of male, female and bisexual types

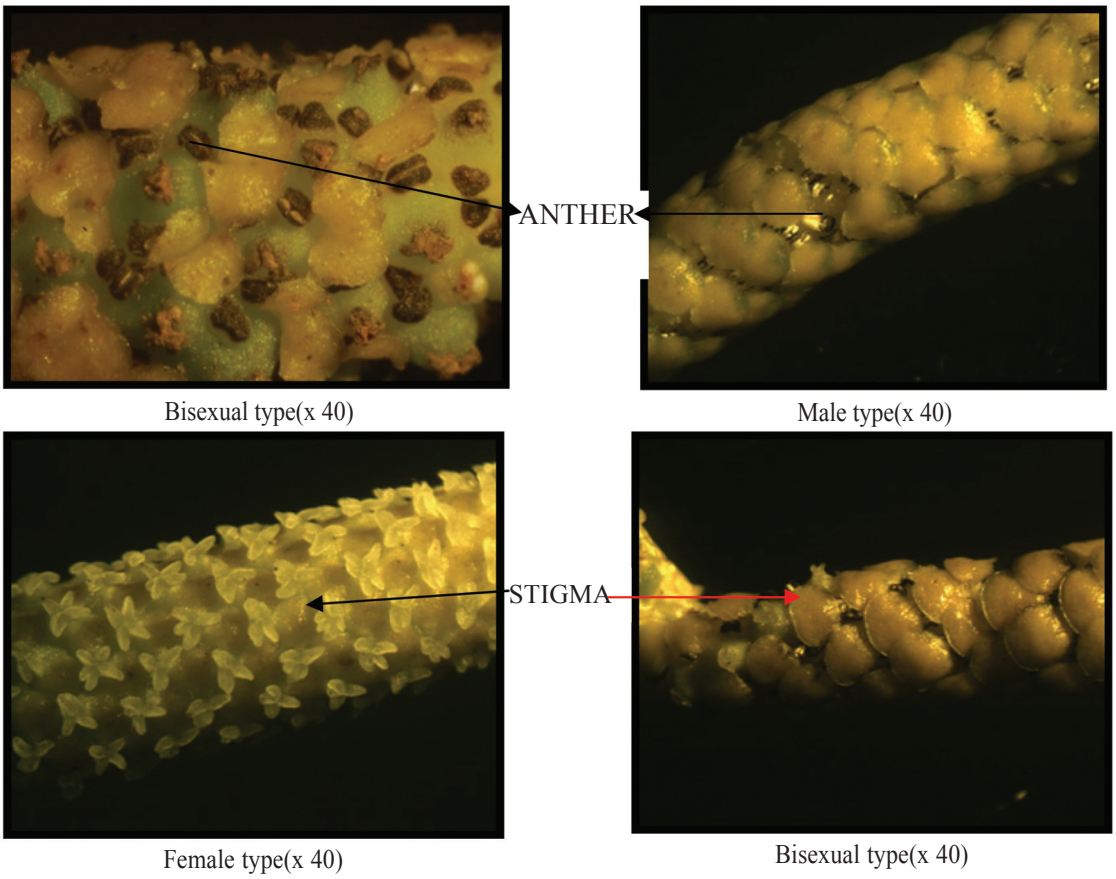


Plate 2. Anther and stigmatic lobes in male, female and bisexual types of *Piper longum* L.

Table 3 . Number of anthers in male and bisexual flowers of *Piper longum* L.

Type of flower	No. of anthers	Percentage in bisexual type	Percentage in male type
Bisexual	3	100	0
Male	2	0	100
Total		100	100

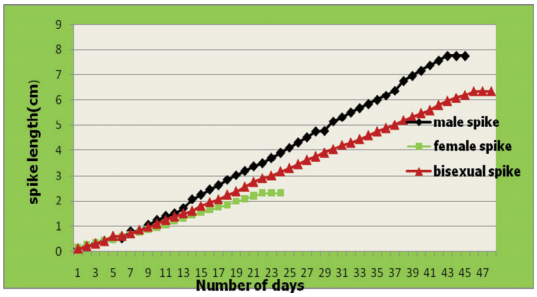


Figure 1. Growth pattern of spikes in three sex forms of *Piper longum* L.

The flowers were represented by ovary and stigmatic lobes subtended by bract in female. The male flowers were represented by anthers covered by peltate bract. Male, female and bisexual types did not possess perianth parts (Plate 1). Data furnished in table 3 shows that, the number of anthers in male and bisexual flowers were varying. Male flowers possessed only two anthers, whereas in bisexual flowers, three anthers could be seen surrounding the ovary (Plate 2, table 3). Stamens were reported to be 3 - 4 in *P. longum* (Ravindran, 1990). Rahiman (1981) reported two anther lobes in *Piper longum*.

Number of stigmatic lobes varied from 2 to 6 in bisexual flowers. In bisexual type, ninety per cent flowers showed bilobed stigma. Three and four lobed stigma were present in 5 per cent each of

Table 4. Number of stigmatic lobes in female and bisexual types of *Piper longum* L.

No. of stigmatic lobes	Percentage in female type	Percentage in bisexual type
2	0	90
3	20	5
4	74	5
5	2	0
6	4	0
Total	100	100

flowers. In the female type, predominant number of stigmatic lobes was four (74 per cent) (Plate 2). Twenty per cent of the flowers possessed three stigmatic lobes. Six (4 per cent) and five (2 per cent) stigmatic lobes were relatively less frequent. Two stigmatic lobes were not observed in the female type (Table 4). Data on time of anthesis in male, female and bisexual types are given in table 5. Anthesis started from 7.30 am and continued up to 4.30 pm, in all the sex forms. Peak time of anthesis was 8.30 am to 12.30 pm in male, female and bisexual types. Number of days taken for complete opening of

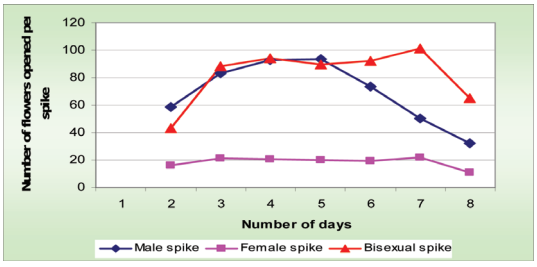


Figure 2. Number of days taken for complete opening of flowers in male, female and bisexual types of *Piper longum* L.

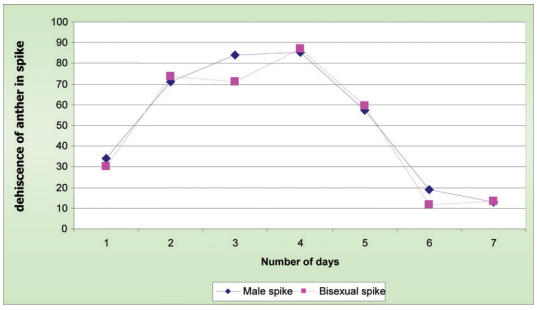


Figure 3. Number of days taken for complete dehiscence of anthers in male and bisexual types of *Piper longum*

flowers in a spike was seven days in male, female and bisexual types (Fig. 3). Maximum flower opening was on the 3rd and 4th day in the male type. In female and bisexual types, profuse opening of flowers was noticed from second to sixth day. Stigma remained white for two days from anthesis. The colour of stigmatic lobes changed to brown on the 3rd day around noon indicating end of receptivity. As in the case of anthesis, dehiscence of anthers also started from 7.30 am and continued upto 4.30

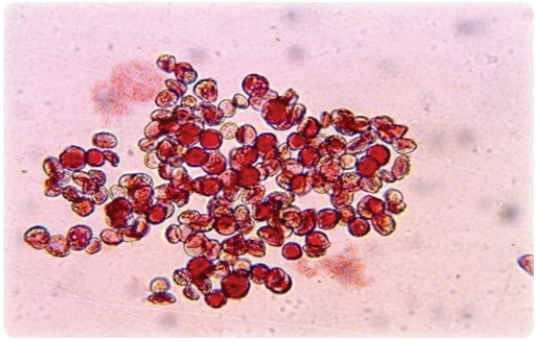


Plate 3. Pollen fertility in one percent acetocarmine (x 1600)

Table 5. Time of anthesis in male, female and bisexual types of *Piper longum* L.

Time	Male		Female		Bisexual	
	Mean/spike	SD	Mean/spike	SD	Mean/spike	SD
Before 7.30 am	0	0	0	0	0	0
7.30 am-8.30 am	73.83	11.02	0.6	1.89	16.67	3.08
8.30 am-10.30 am	120.7	18.55	39.7	7.10	123.83	10.38
10.30 am-12.30 pm	128.2	14.81	43	9.12	205.67	19.96
12.30 pm-2.30 pm	95.5	10.76	35.1	4.93	161.83	14.53
2.30 pm-4.30 pm	56.67	3.66	18.4	4.01	62.17	3.31
After 4.30 pm	0	0	0	0	0	0
Total	474.9	58.8	136.8	27.05	570.17	51.26

Table 6. Time of anther dehiscence in male and bisexual types of *Piper longum* L.

Time	Male		Bisexual	
	Mean/spikes	SD	Mean/spikes	SD
Before 7.30 am	0	0	0	0
7.30 am - 8.30 am	71.17	4.58	14.5	5.24
8.30 am - 10.30 am	97.16	4.36	63	8.15
10.30 am - 12.30 pm	100.33	5.92	98.1	12.92
12.30 pm - 2.30 pm	64.5	8.17	96.33	8.43
2.30 pm - 4.30 pm	35.33	5.24	73.5	10.60
After 4.30 pm	0	0	0	0
Total	368.49	28.27	345.43	45.34

pm. The peak time of anther dehiscence was from 8.30 am to 12.30 pm in male and 10.30 pm to 2.30 pm in bisexual types (Table 6). Anther dehiscence, as in the case of anthesis was between 7.30 am and 4.30 pm in male and bisexual type. Sujatha and Nybe (2007) reported anther dehiscence in the bisexual variant to be between 9.00 am to 11.00 am which extended further in cloudy weather. The report was a preliminary observation in the bisexual variant which coincides with the peak period of anther dehiscence in male and bisexual types in the current study. In *P. nigrum*, anther dehiscence was reported to take place around 4 pm (Ravindran, 2000).

Table 7. Percentage pollen fertility in *Piper longum* L. (average of 10 fields)

Time	Fertile pollen	Sterile pollen	Percentage fertility
8.30 am	3.5	17.4	16.75
9.30 am	4.5	6.3	41.67
10.30 am	3.1	8.4	26.96
11.00 am	2.8	6.1	31.46

The number of days taken for complete dehiscence of anthers was seven in male and bisexual types. Peak dehiscence of anthers was from second to fourth day in both male and bisexual types (Fig. 3) Pollen was collected from dehisced anthers at hourly intervals from 8.30 am to 11.0 am. The collected pollen was stained using one per cent acetocarmine and examined under research microscope. As can be seen from Table 7, the percentage pollen fertility ranged from 16.75 at 8.30 am to 41.67 at 9.30 am. Plate 3 shows pollen stained using one per cent acetocarmine.

Pollen viability of was studied in six different media and eight different concentrations of sucrose mentioned in material and methods. Fresh pollen collected was cultured in the media placed on a microscopic slide. Germination of pollen if any was examined under the microscope 24 hours after culturing. In none of the media, pollen germination could be achieved.

Pollen fertility in one per cent acetocarmine was observed as 42.5 per cent at 9.30 am. However, pollen viability could not be achieved in the six different media and eight concentrations of sucrose tested. Sujatha and Nybe (2007) also could not obtain pollen viability in the bisexual variant. There may be some plant factor, essential for pollen germination which is limiting in the different media tried. A detailed study on the effect of stigmatic fluids on pollen germination may throw some light into this.

No viable seeds were present in naturally set fruits. However viable seeds were obtained under artificial pollination.

Information on reproductive biology of a species is essential in any crop improvement programme. In the present study detailed observations were made on flowering, time taken for opening of flowers in an inflorescence, time of anthesis, anther

dehiscence, stigma receptivity, pollen fertility, pollen viability and fruit set in *Piper longum,* an important medicinal plant. The available information could be made use of in crop improvement programmes in *Piper longum.*

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