Delayed autogamy during corolla abscission of *Barleria prionitis*: an efficient adaptation to overcome the pollinator crisis

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ABSTRACT

Barleria prionitis produces one-day flowers which are self-compatible and homogamous. In open flower state, pollen transfer from anther to stigma does not take place until and unless those are carried by a biotic vector. However, during the abscission of corolla, when it is dragged against the stigma, the receptive stigmatic surface brushes against the anthers of the shorter epipetalous stamens and also the secondarily presented pollens in the 'pollen pocket' constituted by the hairy base of the corolla tube. Such delayed autogamous pollination takes place in the flowers irrespective of pollen deposition on their stigmas before corolla abscission. The phenomenon acts as a failsafe mechanism to ensure pollination even in case of failure of pollinator activity.

Key-words: Pollen pocket, flowering strategy, flower longevity, pollen ovule ratio, *In vivo* pollen germination.

INTRODUCTION

Barleria prionitis Linn, belonging to the family Acanthaceae, is referred to as *Kuranti* and *Vajradanti* in Sanskrit and Hindi vernaculars respectively. The species is well known for its medicinal importance. Different plant parts are used in whooping cough, toothache, leucoderma, bronchitis, skin disease and inflammations (Kirtikar & Basu 1935). The leaves and stem contains iridoid glycosides like barlerin, barlerinoside, shanzhiside methyl ester, 6-O-trans-â-coumaroyl-8-O-acetylshanzhiside methyl ester, acetylbarlerin, 7-methoxydiderroside, lupulinoside and verbascoside (Ata, Kalhari & Samarasekera 2009; Chan et al. 1998). The plant is a native of Africa and tropical Asia including India. In West Bengal, the plant grows wild in southern districts. For medicinal purposes *B. prionitis* is solely used from the wild sources and at present the species has become almost threatened in the region. In order to formulate suitable strategies for conservation and successful cultivation of this medicinally significant species, studies in its reproductive ecology are being worked out.

In self-compatible species, self pollination may be carried out as an addition to cross pollination as a adaptive means to produce fruits and seeds even in absence of pollinators (Lloyd, 1979,1992; Lloyd & Schoen, 1992; Barrett 1988; Wyatt 1983) Lloyd and Schoen classified three phases of autogamy such as, prior, competing and delayed, according to the timing of self pollination relative to out breeding.
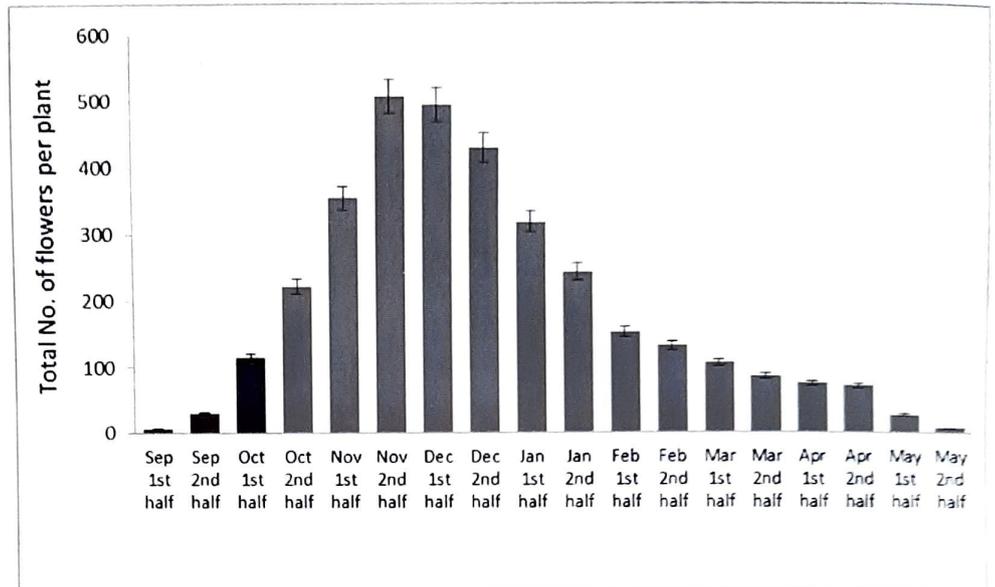
Several methods have been adapted by plants to achieve the delayed autonomy at the end of flower longevity such as - 1) Late curling of stylar branches and touching of the stigmatic surface with the anthers, as in *Hibiscus laevis* (Klips & Snow 1997.) and/or secondarily deposited pollen on nonstigmatic areas

as found in many *Campanula* species (Faegri & Van Der Pijl 1979). 2) Late bending of stamens resulting anthers to come in contact of the stigma, as in *Kalmia latifolia* (Rathcke & Real 1993). 3) Stigmas being brushed against the anthers of epipetalous stamens during corolla abscission, as in *Mimulus guttatus* (Dole 1990), while in *Incarvillea sinensis* var. *sinensis*, dragging of the abscising corolla with epipetalous stamens by breeze leads to anther stigma contact. (Qu et al. 2007.) 4) In *C. vernavia* the initial harkogamy, achieved by the early maturing anthers and the receptive stigma, changes due to the temporal and spatial overlap of the relative positions of that receptive stigma and the late maturing anthers resulting in delayed autogamy. (Kalisz et al.1999.)

Text-Figure 1. Flower production (Cumulative data per fortnight)

MATERIAL AND METHODS

The observations regarding the flower production, floral architecture, floral and flowering phenology together with floral events of the plant has been taken on the wild population of the species grown in Burdwan, Midnapur and Hooghly districts of West Bengal, India. Those observations were recorded daily throughout the flowering season. Each observation was based on at least ten plants from each of these regions. Floral organization was worked out in detail under Leica stereo-binocular microscope. Observations were based on hundred flowers from ten different plants. Details of flower opening, anther dehiscence and pollen presentation were studied regularly in every month of the flowering season. For the purpose, hundred flowers in ten different plants were tagged individually. Dynamic

changes of floral events has been observed and measured by digital scaling system after serial photography by WILD M3B Leica (Switzerland) sterio binocular microscope and Leitz Laborlux S (Germany) bright-field microscope. For macroscopic photography Nikon D5000 camera was used. For day to day field observation 15X hand lens was used.

OBSERVATIONS

Flowering strategy:

The plant flowers each year since late September through early May with peak flowering during late
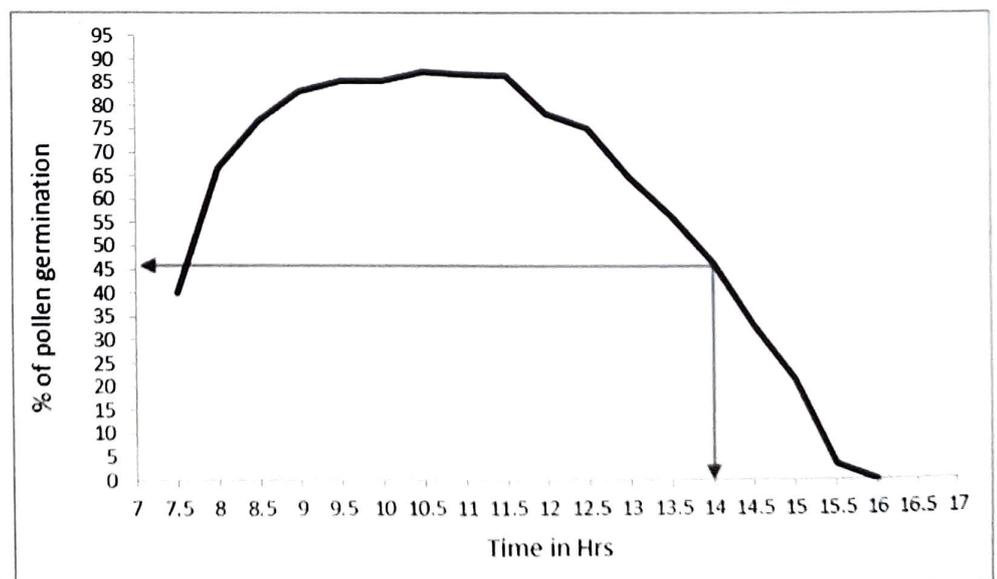
Text-Figure 2. Dynamic change of pollen germination throughout the flower longevity

November to early December. (Plate 1, Fig 1; Text-Figure 1).

Floral architecture:

The flower of *Barleria prionitis* comprises a gamosepalous calyx of four green sepals, a gamopetalous corolla of five bright yellow petals, four epipetalous stamens and a bicarpellary syncarpous gynoecium. A fully open flower exhibits the construction of typical gullet flowers. Among the four didynamously disposed stamens, anthers of the longer pair remain projected slightly beyond the corolla throat, while those of the shorter ones remain hidden (Plate 1, Figs 2, 3, 8). At the points of immergence of filaments of the stamens, the corolla tube is provided with closely spaced unicellular hairs which form an annular hairy zone inside the corolla tube (Plate 1, Fig 4). The stigma is bright pink in colour and two-lobed (Plate 1, Fig 11).

Dynamics of floral events:

The plant produces one-day flowers. Flower opening starts early in the morning from 5.30 AM to 9.45 AM varying with the months of the flowering season. Flower opening is gradual and its initiation is marked by the uncoiling of the posterior large corolla lobe together with the uncoiling of style inside. Simultaneous with the initiation of flower opening anther dehiscence is initiated by the appearance of a small slit. The stigma becomes receptive at the time of flower opening and receptivity lasts up to corolla abscission in the afternoon (Plate 1, Figs 5 – 11).

RESULTS

Pollination ecology of the species.

The flowers are visited regularly by two hymenopteran mambers, namely *Colletes sp* (Plate 1, Figs 12, 13) and *Chrysis sp* (Plate 1, Fig 14) and a lepidopteran one, *Papilio polytes* (Plate 1, Fig 15). As per the fulfillment of pollination postulates, pollination is mainly effected by the hymenopteran members. The role of the lepidopteran one is rather insignificant and it may be regarded as a nectar robber.

Polination during open flower phase: During open flower phase, pollen transfer from anther to stigma cannot take place until and unless those are carried by an insect vector (Plate 1, Fig 12). Though homogamous, exclusive pollinator dependence is due to herkogamous disposition owing to the special separation of the stigmatic head from the anthers of the didynamously disposed stamens.

A specific adaptation for delayed autogamy: The tuft of unicellular hairs inside the corolla tube functions as the 'pollen pocket' for secondarily presented pollen grains. During the abscission of corolla, when it is dragged against the stigma, the receptive stigmatic surface brushes against the anthers of shorter epipetalous stamens and also the secondarily presented pollens. Such delayed autogamous pollination takes place in the flowers irrespective of pollen deposition on their stigmas before corolla abscission (Plate 1, Figs 16, 17, 18).

Overall breeding system of the plant: Artificial pollination experiment reveals that the plant is self compatible. In open pollinated condition, the species is exclusively pollinator dependent and pollen transfer is carried out primarily by the hymenopteran. Moderately high pollen-ovule ratio (640.93 ± 15.71) indicates the predominence of out-breeding (Crudent 1977). Moreover, some degree of autogamous pollen transfer is also affected during corolla abscission at the end of flower longevity.

Relative efficiency of entomophylous pollination verses delayed autogamy.

In vivo germination experiment of pollen grains through flower longevity reveals that merely 40% pollen
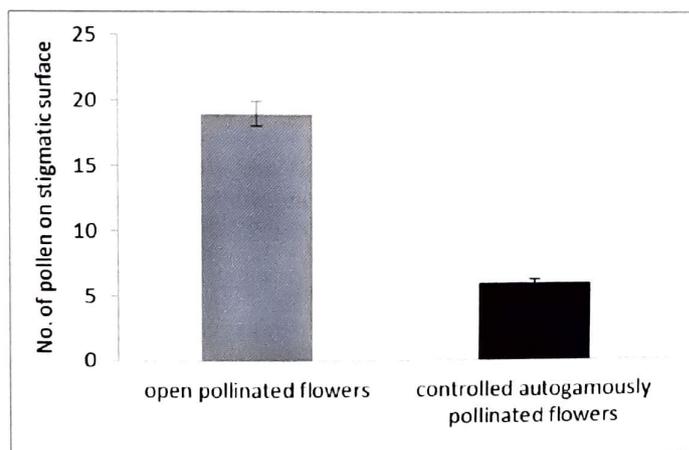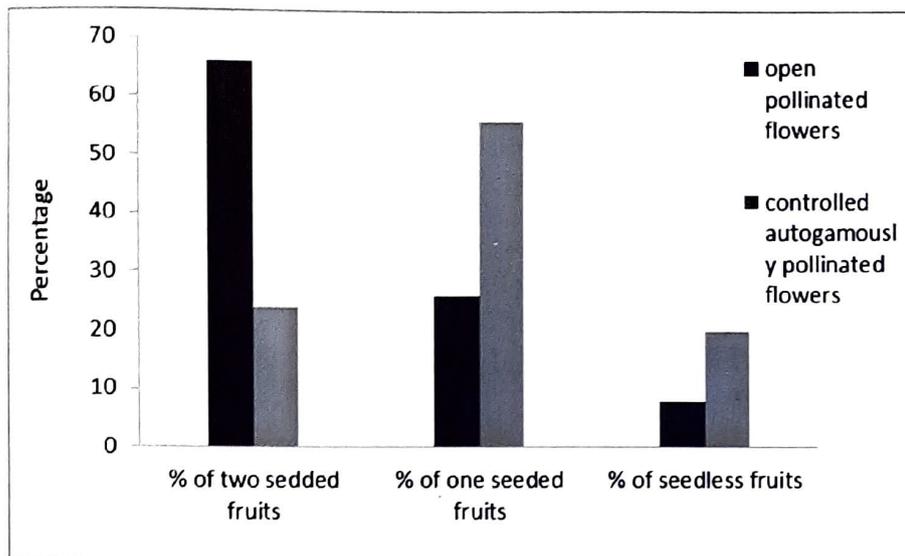

Text-Figure 3. Graphical representation of the number of pollen grains (pollen load) on the stigmatic surface of open pollinated flowers and controlled autogamously pollinated flowers.

Explanation of Plate 1:

Fig 1: Photograph showing a natural population of *Barleria prionitis*, Fig 2: Photograph showing an open *B. prionitis* flower, Fig 3: Photograph of a single amputated *B. prionitis* flower showing the calyx and corolla, Fig 4: Photograph of a vertically chopped corolla of *B. prionitis* showing the wrinkled inner surface of the corolla tube just above the hairy tuft, Figs 5-8: Photographs of different stages of flower opening, 5: Side view – commencement of corolla opening, 6: top view – separated corolla lobes in the next stage, 7: top view – diverged corolla lobes at an advanced stage, 8: side view – fully opened *B. prionitis* flower showing the disposition of posterior corolla lobe, stamens and stigma, Figs 9-10: Photographs of different stages of anther dehiscence, 9: slit of anther just appeared, 10: fully dehisced anther, Fig 11: Photograph of a receptive stigmatic surface in an open flower, Fig 12: Photograph showing the activity of *Colletes sp* (a hymenopteran pollinator) on the flower, Fig 13: Photograph of a single *Colletes sp* (a hymenopteran pollinator), Fig 14: Photograph of a single *Chrysis sp* (a hymenopteran pollinator), Fig 15: Photograph showing the activity of *Papilio polytes* (a lepidopteran pollinator) on the flower, Fig 16: Photograph showing the abscission of corolla together with epipetalous stamens, Figs 17-18: Demonstration of autogamy – during abscission the stigma travels through the corolla tube and touches the hairy tuft where pollens are deposited secondarily, Fig 19: Microphotograph of the stigmatic surface with germinating pollen grains.

Text-Figure 4. Graphical representation of the Fruit set percentage of open pollinated flowers and controlled autogamously pollinated flowers along with the number of seed(s) in each fruit.

germination is observed in case of pollen collected from freshly opened flowers. With the progress of flower longevity the frequency of pollen germination reaches 87.5% nearly at 3 hrs from flower opening (Fig 19). Subsequently pollen germination gradually diminishes and finally falls to 46.66% at the end of flower longevity. This low frequency of germination of pollen grains at the end of flower longevity limits the efficiency of autogamous pollination during corolla abscission (Text-Figure 2).

The stigmatic pollen load of an open pollinated flower at the end of flower longevity prior to corolla abscission is observed to be 13.14 (±3.57), while that of the bagged flower after autonomous pollen transfer during the corolla abscission is found to be 6.15 (±1.86) (Text-Figure 3).

The efficiency of entomophilous pollination verses mechanical autogamy was finally evaluated by the seed set percentage obtained from controlled pollination experiments. Open pollinated flowers that mature into fruit yield 66% of two seeded, 26% of one seeded and 8% of seedless fruits. On the other hand bagged flowers allowed to be pollinated exclusively by delayed autonomous pollination and matures in to fruit, give rise to 24% of two seeded, 56% of one seeded and 20% of seedless fruits. Thereby the delayed autogamy that takes place mechanically at the end of flower longevity is much less efficient than the entomophilly which takes

place during the open flower phase (Text-Figure 4).

CONCLUDING REMARKS

During open flower phase, 100% vector dependence for pollination may turn to be dangerous for the sustainence of the species if pollination crisis happens with respect to time or space. The phenomenon of delayed autogamy, however less efficient, is no doubt a means adopted by the plant as a failsafe mechanism to overcome any such incidence of pollinator crisis.

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