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Reproductive Biology of *Pyrostegia venusta* (Ker-Gawl.) Miers (Bignoniaceae) with Special Reference to Seedlessness

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ABSTRACT

Pyrostegia venusta (Ker-Gawl.) Miers syn. *Bignonia venusta* (Bignoniaceae), commonly known as "orange trumpet creeper", "flame vine" or "golden shower" bears large number of orange tubular flowers arranged in dichasial cymes. There are four didynamous stamens and the gynoecium is bicarpillary, syncarpous with bilocular ovary, long style and bilabiate stigma. Various floral-polymorphic features e.g. increased number of stamens and stigmatic lobes and heterostyly are recorded in different flowers of the same individual. Flowers open in the morning between 6.00–7.30 am and remain open throughout the day and corolla abscises 12-14 h after anthesis. The flowers are protandrous as the stamens dehisce 1-2 h after their opening. A circular nectariferous tissue is present at the base of the ovary, secreting copious nectar throughout the life of flower. The stigma becomes receptive in the afternoon between 3.00 – 4.30 pm. However, the stigmatic lobes close down by various kinds of stimuli including self pollen, but reopens after some time. Only the honeybees, small bees and black ants visit the flowers, but are not effective pollinators as pollen grains on the stigmatic surface are either absent or only a few of them are present. Various hand pollination experiments conducted failed to produce fruits indicating that the plant is self-incompatible. The hand pollinated stigmatic surface shows only 2-3% pollen germination with small pollen tubes which fail to reach the ovules may be due to low temperature during the flowering period. Pollen-ovule ratio indicates that its facultative xenogamous nature.

Keywords : *Pyrostegia venusta*, floral biology, floral-polymorphism, pollination, self-incompatibility

INTRODUCTION

Reproduction is a natural means of increasing the number of individuals of the same species and is vital for its evolution and survival. The physiology of reproduction in most of the flowering plants is closely under the control of environmental factors.

For successful cultivation and conservation of plants a detailed knowledge of their reproductive biology is required (Moza & Bhatnagar 2007). Reproductive biology mainly focuses on flowering phenology, floral biology, pollen-pollinator interaction, pollen–pistil interaction, breeding systems and gene flow through pollen and seeds. Pollen-pistil interaction is unique to flowering plants and it covers all sequential events that take place in the pistil starting from pollination until pollen tube entry into the embryo sac (Shivanna 2003).

Flowers and flowering patterns of seed plants have evolved largely in response to selection for effective transfer of pollen grains. In a few plant species this transfer is accomplished by a single pollinator species and the flowers are highly specialized. General works on pollination often describes pollination 'syndromes' or group characteristics in plant species pollinated by different groups of pollinators.

Incompatibility is another significant aspect in the reproductive biological studies. It operates between species (intra-specific), as well as within the species (inter-specific).

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Pyrostegia venusta (family- Bignoniaceae) a neotropical vine grows in southern Brazil, Paraguay, Bolavia, and northeastern Argentina (Sandwith & Hunt 1974). According to Gobatto-Rodrigues & Stort (1992) the flowers of this beautiful climber are self-compatible and facultative out-breeding and reproduce solely by seeds in Brazil. On the other hand, in Indian sub-continent, this highly ornamental climber is grown in the gardens by vegetative propagation largely because in spite of profuse flowering, it remains seedless. Present investigation was undertaken to study of the reproductive biology in *Pyrostegia venusta* with special reference to seedlessness.

MATERIAL & METHODS

Observations were made on *Pyrostegia venusta* plants growing at 7 selected sites at Agra, namely, i. Church Road, Civil lines, ii. Lawyer's Colony, Bye Pass Road, iii. Circuit House, iv. Bhagirathi Devi Marg, v. Elora Enclave, Dayalbagh, vi. Professor's colony and vii. Fatehabad Road during 2005 and 2006. Flowering phenology was studied by recording flowering period with optimum flowering. Flowers were counted on marked stems periodically throughout the flowering period at each site. 200 inflorescences, selected at random from different individuals were tagged at the time of initiation of flowering. The inflorescence was followed daily and the number of open flowers was recorded. The open flowers from inflorescence were then removed to avoid recounting the next day. One hundred flowers/plant were sampled to record the floral morphology and pollination. Time of anthesis, anther dehiscence, number of pollen/anther/flower, pollen viability, number of ovules/flower and pollen-ovule ratio was recorded by various methods described by Kearns & Inouye (1993). Pollen viability was assessed by Fluorescence-FCR method, after Heslop-Harrison & Heslop-Harrison (1970). The number of pollen grains/ anther was calculated by hemacytometer method as described by Kearns & Inouye (1993).

Quantity of nectar was measured extracting with microcapillary tubes of 1-20μl volume from randomly selected 120 flowers attached to 10 plants. Nectar volume was computed by the equation given by Cruden & Hermann (1983). Sugar concentration was measured with a pocket refractometer.

The receptivity of stigma was studied by non-specific esterase test as described by Shivanna & Rangaswamy (1992). *In vivo* pollen germination was checked by aniline blue fluorescence microscopic method. Breeding behaviour was tested by controlled pollination studies in 200 emasculated and bagged flowers, of which 50 flowers received no further treatment, 50 flowers were pollinated with the pollen of another flower of the same plant, 50 flowers were pollinated with the pollen of another plant at the time when stigma flaps were open while another 50 emasculated flowers were left open for natural pollination. Time of stigma receptivity was determined by hand pollinating stigmas at various recognizable stages and revisiting to observe fruit set and maturation. The receptive surface of stigma was pollinated with different quantity of pollen grains (850±150, 1700±180, 2500±220 and 3500±250). The quantity of pollen was determined by cutting the anthers in two or four equal parts and each part was crushed in lactophenol-glycerine with aniline blue and making replicate counts using hemacytometer. The number pollen on stigmatic surface was by the method used by Bertin (1982). The virgin stigmas were exposed to a single visit by a visitor and the stigma was collected for counting the number of pollen grains deposited.

Foraging behavior of insects was recorded. Pollination activities were monitored by visual observations and the visitors were collected and identified. Patterns of visits number per minute were established by observing pollen load on their body parts under a microscope according to the procedure given by Kearns & Inouye (1993). Pollen load on the stigmatic surface was also checked under a microscope.

RESULTS AND DISCUSSION

Pyrostegia venusta (Ker-Gawl.) Miers Syn. *Bignonia venusta* is an ornamental perennial evergreen climber (Fig. 1). It flowers for about 14-16 weeks between December to March with optimum flowering between January to February in Agra. However, formation of fruits has not been observed. On the other hand, it blossoms in winter as well as in spring season with golden orange flowers and bear large number of elongated capsules in southern Brazil, northern Argentina and Paraguay.

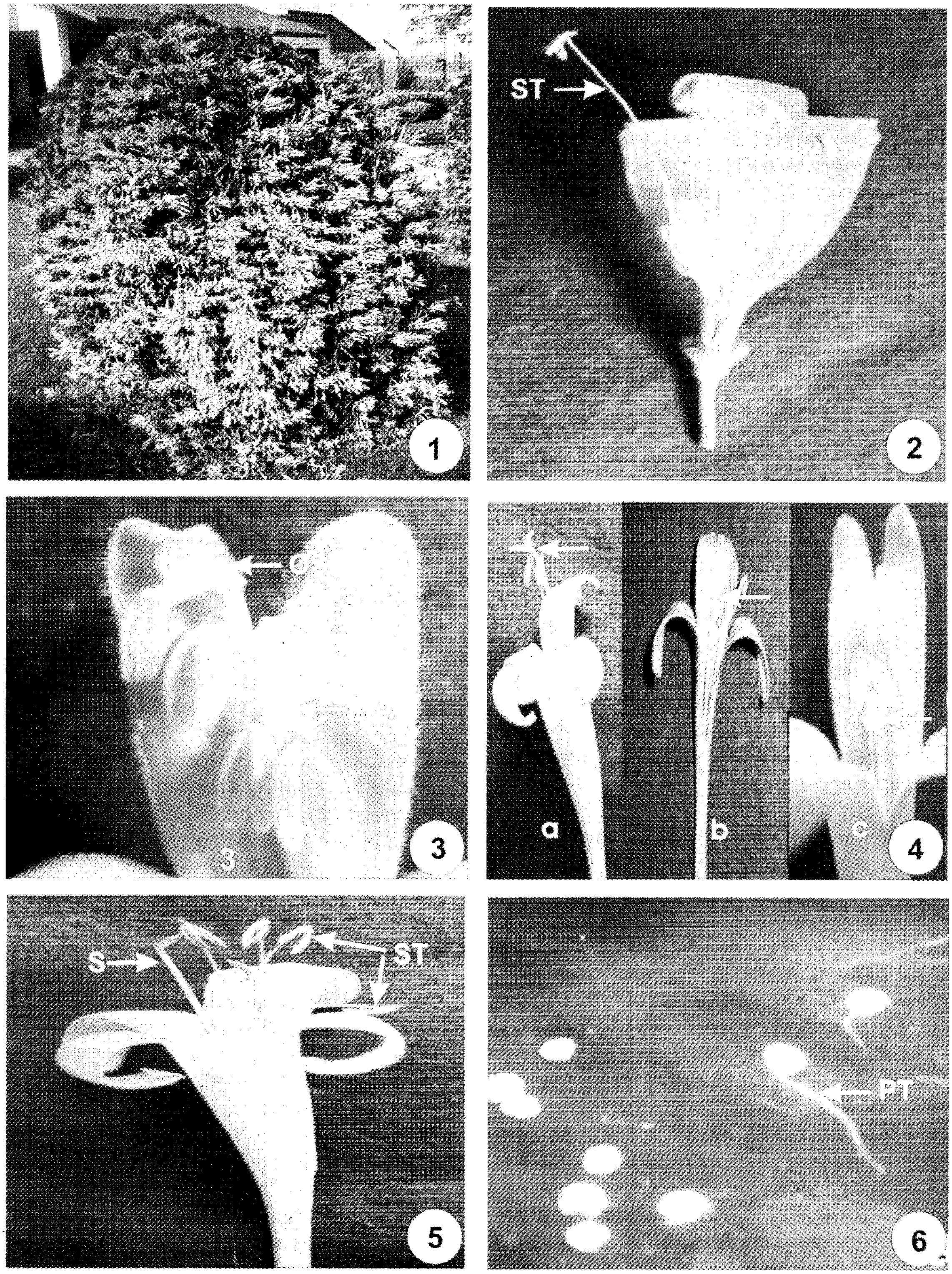
Each plant of *Pyrostegia venusta* bears several medium sized bright orange flowers arranged in dichasial cymes. In each inflorescence there are 8 to 1[illegible] open flowers and 10 to 20 buds. Number of flowers plant depends upon the size of the plant and at a time usually there are 8500-12800 flowers/plant (n=7). A large number of floral buds, corolla from open flowers with epipetalous stamens and unfertilized pistils abscise. Sampaio & Almeida (1995b) have also reported natural abortion of buds, flowers and fruits of *Pyrostegia venusta* in the urban region of Curitiba, Parana, Brazil. Corolla is orange and tubular with moderately curved 5 lobes; two corolla ridges are reduced, thick in texture, glabrous outside, odorless and tubular with an open mouth. The calyx is usually large, green, and thin and encloses the base of the corolla tube very loosely. Stamens are four exerted, epipetalous, didynamous, dorsifixed with a posterior staminode (Fig. 2). Gynoecium is bicarpellary, syncarpous with bilocular ovary with 100-120 ovules on axile placenta. A circular, light green, nectariferous tissue is present at the base of ovary. It starts secreting nectar since the time of anthesis and continues till corolla abscises. Each open flower produces 30-45 μl nectar in the morning, but gradually the quantity declines and lowest is seen in the afternoon (20-25 μl). The nectar predominantly consists of sucrose 53.3±6.4 mg/600μl nectar from 20 flowers in the morning hours, but concentration increases in the afternoon (65.5±2.5 mg/600μl nectar from 20 flowers). Characteristics of secretion of nectar in *Pyrostegia venusta* has been studied by Galetto *et al.* (1994). According to them removal of nectar throughout the flower lifetime increases the volume produced but reduces the concentration.

The long style terminates into a bilabiate stigma with more or less tetrahedral lobes, 0.3±0.01 cm in size. Stigmatic surface is covered by numerous medium sized unicellular papillae. According to Heslop-Harrison & Shivanna (1977) family Bignoniaceae is characterized by the presence of unicellular medium sized papillae and wet stigma. Flowering phenology in *Pyrostegia venusta* is similar to that reported by Bertin (1982) for *Campsis radicans* and is close to type 3 or 'cornucopia' pattern as described by Gentry (1974a). The main difference between the two species is that *Pyrostegia* produces flowers over a time span of several months instead of several weeks. The floral morphology of *P. venusta* is characterizes *Martinella*-type of Gentry (1974b) which corresponds to a hummingbird-pollination syndrome. Some authors have studied the floral morphology, pollinators and reproductive biology of *Pyrostegia venusta* in Brazil (Gobatto-Rodrigues and Stort 1992, Gusman & Gottsberger 1993, Sampaio & Almeida 1995a). Their description on floral morphology is more or less similar to present findings except that the number of flowers/plant is different and this species is pollinated by hummingbird and was found to produce large number of fruits/plant. They have earlier studied the effect of temperature and light on flowering in *Pyrostegia venusta* (Sampaio and Almeida 1994). According to them, both these climatic factors affect the production of floral buds, number of flowers/plant and anthesis.

Anthesis takes place in the morning after daybreak (6.30-7.30 h) and each flower lasts for 48 h only. The anther dehiscence occurs 1-2 h after anthesis. At this stage, the stigmatic lobes are folded and shorter than stamens hence fail to receive pollen from dehisced anthers. However, in a limited number of flowers stigma is present at the same level (Fig.5) or at higher level of stamens (Figs. 3, 4a). In the later case, the stigmatic lobes are wide spread and the anthers are non-dehiscent and interestingly are yellowish in colour indicating their sterile nature. Stigmatic lobes spread apart and become receptive 8-10 h after anther dehiscence showing protandrous nature (Fig.2, 3, 4a, c). The opening of stigmatic lobes and presence of hyaline exudates mark the receptivity of stigma. The stigma remains receptive up to 10-12 h after anthesis. Protandrous nature of flowers in the members of the family Bignoniaceae including *Pyrostegia venusta* is well known (Gentry 1974a, Bertin 1982, Gobattto-Rodrigues & Stort 1992, Galetto *et al.* 1994).

There are 6900-8138 and 27600-32552 pollen grains/anther and per flower respectively and only 27-55% of them are viable. There are 100-120 ovules/ovary and the ratio of pollen grains to ovule is 273.41:1 indicating facultative xenogamy (Cruden 1977). Facultative xenogamy has also been reported in this ornamental species by Gobatto-Rodrigues & Stort (1992) and Galetto *et al.* (1994). A large number of young floral buds, open flowers, and corolla with epipetalous stamens, and unfertilized pistils abscise.

It is interesting to note that stigmatic lobes close within 1-2 minutes when touched mechanically before pollination, but reopen after 4-5 hours. Similarly stigmatic lobes close within same time period (1-2 minutes) after self- or cross-pollination but reopen after 24 hours. According to Newcombe (1924), the sensitive stigmas occur only in the families of order Scrophulariales (Bignoniaceae, Lentibulariaceae, Martyniaceae and Scrophulariaceae). Among Bignoniaceae, sensitive stigmas are widespread. Hermaphrodite flowers, tubular corolla and bilobate stigma characterize these species (Fetscher & Kohn 1999). The permanent closure after cross-pollination has been recorded in several members of this family (Bertin 1982, Singh & Chauhan 1996, Richardson 2004). On the other hand, Shu-Xiang *et al.* (2004) has reported permanent closure of stigma even after self-pollination in *Campsis radicans*. In self-incompatible species with mass flowering and where pollinators promote high levels of geitonogamy, permanent stigma closer reduce fruit-set (Shu-Xiang *et al.* 2004). The factors affecting the permanent or temporary closer of stigma by mechanical touch or by pollination with pollen of different genera or species or even by self- or cross-pollination in the members of the family Bignoniaceae are not known. The members of various families of the order Scrophulariales including family Bignoniaceae, which show stigmatic movements, are also characterized by the presence of medium sized papillae on their stigmatic surface (Heslop-Harrison & Shivanna 1977).



Figs. 1-6 — **1.** Pyrostegia venusta plant in full bloom. **2.** L.S. of flower showing epipetalous stamens. Note the presence of one stamen with log filament (ST). **3.** Flowers showing open and receptive stigmatic lobes (OS) and un-dehisced sterile anthers (NA) of yellow colour. **4.** Flowers showing heterostyly a. stigma above the level of stamens with dehisced anthers. b. closed stigma lobes at the level of stamens and c. stigma below the level of stamens with dehisced anthers. **5.** Increase in the number of stamens (ST) with dehisced anthers at the level of closed stigma. **6.** Stigmatic surface showing and only two germinating pollen grains with short pollen tubes (PT) among large number of non-germinating pollen grains. 820 X

The flowers of *Pyrostegia venusta* under observation also exhibited following polymorphic features and these were found to be under direct control of temperature.

1. **HETEROSTYLY:** On the basis of stylar length, following three types of flowers were observed within an individual (Table 1).

(a) Stigma above the level of the stamens: The percentage of this type of flowers increases with the rise in temperature. At temperature ranging between 4.5- 24.4°C, 4.6- 28°C and 13.6-33.8°C, there were 52, 70, 80 and 90% flowers showing such condition respectively (Fig. 4a).

(b) Stigma up to the level of the stamens: The percentage of this type of flowers decrease with the rise in temperature and at 4.5–24.4°C 5–23°C, 4.6–28 °C and 13.6–33.8 °C, the percentage of type is 37, 25, 18, 7% respectively (Fig. 4 b).

(c) Stigma below the level of the stamens: The percentage of such types of flowers also decreases with the rise in temperature and at 4.5–24.4°C, 5–23°C, 4.6 – 28°C and 13.6 – 33.8°C, there were 11, 5, 2 and 3% flowers of this type respectively (Fig. 4c).

It was interesting to note that all these type of flowers were present on the same plant as well as on different plants as well.

2. **NUMBER OF STAMENS:** Usually a flower contains 4 stamens but in the months of December and January when temperature ranges between 2.8-24.4°C, the number of stamen increases from 4 to 5 (30%) or (25%) (Fig. 5).

3. **NUMBER OF STIGMATIC LOBES:** The stigma is usually bifid during December-January. However, in the month of March with temperature ranging between (13.4 – 33.8°C), 35% and 40% flowers (n=150) with tetrafid and trifid stigmas respectively.

Singh & Chauhan (1994) have recorded increase in the number of stamens, stigmatic lobes and heterostyly in *Tecoma stans* in different seasons. According to them, increase in the number of functional stamens enhances the chance of higher fruit-set in cross-pollinated flowers. Similarly, trifid and tetrafid stigmas also enhance the receptive surface for effective cross-pollination by insects.

The flowers are visited by honeybees (*Apis indica* sp), small bees (*Melliponia sp.*), and black ant (*Camponotus campessus*). They visit the flowers between 7:00 a.m. and 5.00 p.m. for nectar and/or pollen and they are not effective pollinators. Sunbirds (*Nectarinia asiatica*) have not seen to visit flowers. Sunbirds forage *Campsis grandiflora* flowers largely for nectar and penetrate their beak into the base of the corolla tube to collect nectar from the base of ovary without coming into contact of pollen grain. On the other hand, some authors have regularly recorded hummingbirds visiting the flowers of this Neotropical vine in Brazil (Gusman & Gottsberger 1993, Mendonca & dos Anjos 2005) and in northern eastern Argentina (Galetto *et al.* 1994). However, this bird is not found in India and the sunbirds have not seen to visit this plant even as a nectar robber.

The results of open and hand pollination experiments show that there is no fruit–set. There is no pollen germination on the stigmatic surface of open pollinated flowers. However, only 2–3% pollen germinates with very small pollen tubes (Fig. 6) are seen on the stigmatic surface pollinated with different quantity of pollen grains (850±150, 1700±180, 2500±220 and 3500±250). These pollen tubes are too small and fail to enter in the stylar tissue and ovules remained unfertilized. Bertin (1982) have succeeded in producing fruits in *Campsis radicans* by hand pollination of the receptive stigmatic surface with higher quantity of pollen. The low temperature during entire flowering period at Agra may be held responsible for reduction in pollen germination and inhibition of pollen tube growth on the stigmatic surface.

This is supported by the fact that *in vivo* pollen germination and tube growth is highly sensitive to climatic factors particularly, to the temperature and relative humidity. The compatibility of pollen appears on the stigmatic surface and probably it plays the most important role in self-incompatibility (Shivanna 2003).

Table 1 — Percentage of heterostyly during different months

Month and year	Temperature (°C)		Types of Heterostyly %
Jan (1-15) 2005	18.4–24.4	4.5–6.0	52 of condition (a), 37 of condition (b), 11 of condition (c)
Jan (16-31) 2005	17.2–23	5–10	70 of condition (a), 25 of condition (b), 5 of condition (c)
Feb (1-28) 2005	19.5–28	4.6–13.4	80 of condition (a), 18 of condition (b), 2 of condition (c)
March (1-15) 2005	30.0–33.8	13.4–16.8	90 of condition (a), 7 of condition (b), 3 of condition (c)

Homomorphic self–incompatibility is well known in Bignoniaceae (Shivanna 2003).

Thus, from the present observations it is clearly that flowers of this beautiful ornamental climber remains fruitless because of the absence of main pollinator-the hummingbird from India and sunbirds found in India do not visit the plant. The pollen-ovule ratio indicates facultative xenogamy. Inhibition of *in vivo* pollen germination on stigmatic surface by various hand pollination experiments in this species indicate self-incompatibility which may be due to low temperature prevailing during the entire flowering period in this otherwise self-compatible and facultative xenogamous species (Gobatto-Rodrigues & Stort 1992).

It seems that the cultivated populations of *P. venusta* at Agra were originated from a reduced genetic pool or even by vegetative reproduction. Thus, fruitlessness can be explained by a combination of environmental, biological and genetic particularities.

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