

Reproductive Biology and Phenology of *Croton scabiosus* Bedd. - An Endemic Tree Species of Southern Eastern Ghats of Kadapa District, Andhra Pradesh

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Abstract: *Croton scabiosus* Bedd. is an endemic tree species of Southern Eastern Ghats of Kadapa District, Andhra Pradesh. In the present study observations were made on reproductive biology and phenology of *Croton scabiosus* at Idupulapaya reserve forest, Vempalli, Kadapa. The observations on phenological events i.e. leaf initiation, flowering, fruiting and leaf fall were recorded between December 2013 and November 2014. Observations revealed a pattern of two episodes of flowering in the months of January to June and September to October. The peak flowering was observed in the month of March and flower anthesis occurs during morning to evening (0600h to 1800 h). Inflorescence is terminal simple raceme. Male flowers are on the upper portion and female on the lower portion of the inflorescence. Flowers are minute, monochlamydeous and actinomorphic. Floral morphology, breeding systems - geitonogamy and xenogamy, stigma receptivity, pollen output per anther/flower, pollen ovule ratio and fruit set percentage were recorded.

Keywords: *Croton scabiosus*, endemic tree, reproductive biology, phenology.

1. Introduction

Croton scabiosus Bedd. (Euphorbiaceae) is a small tree of about 6m high, endemic to Southern Eastern Ghats of Andhra Pradesh (Ahmedullah and Nayar, 1986; Babu, 1997). The Species is represented by small populations with scattered distribution in dry deciduous forests of Southern Andhra Pradesh (Balakrishnan and Chakrabarty, 2007). It is found in Anantapuram, Kadapa and Nellore Districts of Andhra Pradesh, India. It is assessed as vulnerable according to IUCN Red list status (Salamma and Rao, 2014). This plant is locally known as *Puruguchekka*, *Verrichilla* or *Yerrichilla*. It is medicinally important as the seeds of this species are used as antidote for snake bite and scorpion sting in the local markets (Devi *et al.*, 2011). It has a very attractive silvery to golden foliage and can be recommended as an ornamental garden plant (Neginhal, 2004). Population of this species is declining in the native habitat. As this is an endemic and economically important tree species, and populations are small, the conservation of this species is an important aspect. For the same thorough knowledge of reproductive biology, breeding systems and phenology is required. Hence the present study.

2. Materials and Methods

The present study was undertaken in the dry deciduous forest of Idupulapaya. Total of 25 individual trees were selected in the natural populations in Idupulapaya reserve forest, Vempalli, Kadapa district. The study site lies N-14°19.418 Latitude, E-78°31.468 Longitudes and the elevation of 275m, above the sea level. Phenological observations were made for the permanently marked trees. Observations on flowering initiation, duration of flowering season, anthesis, anther dehiscence, stigma receptivity,

breeding systems, fruit set, and seed dispersal were studied as described by Shivanna and Rangaswamy (1992). The number of inflorescences per tree and number of male and female flowers per inflorescence were counted. From each tree ten inflorescences were maintained for counting the opened flowers. The other observations include the structure of male flowers, number of stamens, anther dehiscence, number of pollen in each anther and structure of female flowers and gynoecium, number of carpels and number of ovules per carpel.

Flowering phenology was recorded based on flowering initiation, anthesis and anther dehiscence during the flowering season of January to June. The anthesis was recorded on daily basis on the mature floral buds of 10 inflorescences each from ten marked trees. Fifteen mature buds were observed for recording the time taken to reach various developmental stages from anthesis to anther dehiscence in successive days. Breeding behavior (geitonogamy and xenogamy) was tested by controlled pollination in bagged flowers. Pollination experiments were performed by dusting pollen obtained from freshly dehisced anthers on the receptive stigmas of the female flower of same tree (geitonogamy) to test for self compatibility and also on the female flower of different trees (xenogamy) to test for out crossing ability. The pollinated flowers were bagged and observed periodically for fruit formation (Shivanna and Rangaswamy 1992).

2.1. Pollen size

Pollen size and pollen tube length was measured with an ocular micrometer under light microscope following the procedure of Mekone and Webb (1988).

2.2. Pollen viability and germination

Pollen viability was assessed by 2, 3, 5 - Triphenyl tetrazolium chloride (TTC) test. To study the pollen germination *in vitro*, pollen grains were incubated in Brewbaker's medium with different concentrations (5, 10 and 15%) of sucrose (Brewbaker and Kwack, 1963). After 4 hours the percentage of pollen germination and tube elongation was noted.

2.3. Pollen output/ anther/flower

Pollen production was recorded on 10 individual trees during flowering and the pollen count was made by selecting anthers. In each tree, five mature buds were selected and in total 10 trees were observed for pollen output per anther and flower following the method of Kearns and Inouye (1993).

2.4. Pollen ovule ratio

The number of ovules per flower was observed by taking the cross section of ovary to determine pollen ovule ratio. Pollen ovule ratio was calculated following the method of Cruden (1977).

2.5. Stigma receptivity

During anthesis 10 flowers each from 5 inflorescences were selected to test stigma receptivity. Experiments were conducted with Hydrogen Peroxide; a drop was added to stigma of flower during anthesis. Observations were recorded if stigma showed pure effervescence producing bubbles resulting from catalase enzyme activity (peroxidase) method (Dafni, 1992). The time of stigma receptivity was checked on 1st day, 2nd day and 3rd day of anthesis of flowers.

3. Results

3.1. Phenology

In *Croton scabiosus* leaves appear orange-red colour in November. Leaf fall occurs in November-December and leaf flush in January to March (Fig-1A-C). Flowering occurs in two episodes per year. In the first episode it starts from the first week of January and extends up to last week of June. The peak flowering was observed in the March month. It flowers again from September to October. The flower buds take 10 days from initiation to anthesis. The average life span of each flower is 2-4 days. It takes at least one hour for complete unfolding of floral parts. Day long anthesis was observed, i.e. anthesis occurs from morning to evening between 0600h to 1800, though it is low between 1000h to 1600h. Anthers dehisce after flower anthesis. Flower bud initiation to termination of the flowering at inflorescence level takes 40 days time. The time taken from initiation to dehiscence of fruit is 28 days.

3.2. Floral Biology

Croton scabiosus is a monoecious tree with separate male and female flowers borne together on terminal simple racemes. Female flowers are borne on lower and male on upper portion of the inflorescence (Fig-1D). Each inflorescence is about 5.5cm to 13.2cm length and bears 5-12 female flowers and 20-52 male flowers. Female and male flowers ratio is approximately 1:4. The male flowers are small dichlamydeous, actinomorphic and pentamerous. The size of the flower is 0.6-0.7mm x 0.5 – 0.6 mm. The sepals are five in number, polysepalous, valvate and pale green in colour. The corolla contain five petals, polypetalous, valvate and pale green in colour. Stamens are 12 -20 in each flower, cream coloured and 1mm in size. Anthers are dithecous and dehisce by longitudinal slits (Table -1). Anther dehiscence occurs after flower anthesis. Pollen grains are light yellow in colour, wet and monads. Pollen size is $44.82 \pm 4.06\mu\text{m}$. Pollen output per anther is 219.4 ± 24.76 and per flower is 2637.18 ± 53.23 . Female flowers minute, pedicellate, monochlamydeous, pentamerous, actinomorphic and hypogynous. It is oval shaped, light green in colour. The size of the flower is $6.8 \pm 0.6\text{mm} \times 5.4 \pm 0.4\text{mm}$. Perianth lobes are five, light green, free and valvate. Gynoecium light green in colour, tricarpeal, syncarpous, ovary superior and trilobular. Each locule contains a single ovule on axile placentation. Styles are three, stigmas are forked, wet and shiny. Flower opening is acropetal at inflorescence level. Fruits are schizocarpic capsules, globose to sub globose and 3- valved (Fig. 1 F). Each fruit contain 3 seeds, very rarely four seeds occur (Table 1, 4). Seeds are small and black with caruncle.

3.3. Pollen output/ anther/flower

Pollen output per anther is 219.4 ± 24.76 and pollen output per flower $2,637.18 \pm 53.23$

3.4. Pollen viability and In vitro pollen germination

In *Croton scabiosus* the pollen grains are round, monads and wet. The size is $44.82 \pm 4.06\mu\text{m}$. On the first day of anthesis the viability percentage is 48.25 and they were viable for 14 hours and on the second day pollen grains are not viable (Fig.2B). Effect of sucrose on *in vitro* pollen germination studies revealed that 38.7% pollen germinated with tube length of $373.2 \pm 6.45\mu\text{m}$ in 5% sucrose, 44.97% pollen germinated with tube length of $428.8 \pm 5.15\mu\text{m}$ in 10% sucrose and germination is 36.52% and tube length is $286.6 \pm 4.74\mu\text{m}$ in 20% sucrose i.e. the maximum number of pollen germinated is 10% sucrose (Table -2, Fig 2-A, B).

3.5. Stigma receptivity

The stigma receptivity was checked on first, second and third day of anthesis of flowers. Stigma receptivity occurs only on second day anthesis of flower. Stigma was not receptive on first and third day of anthesis of flower.

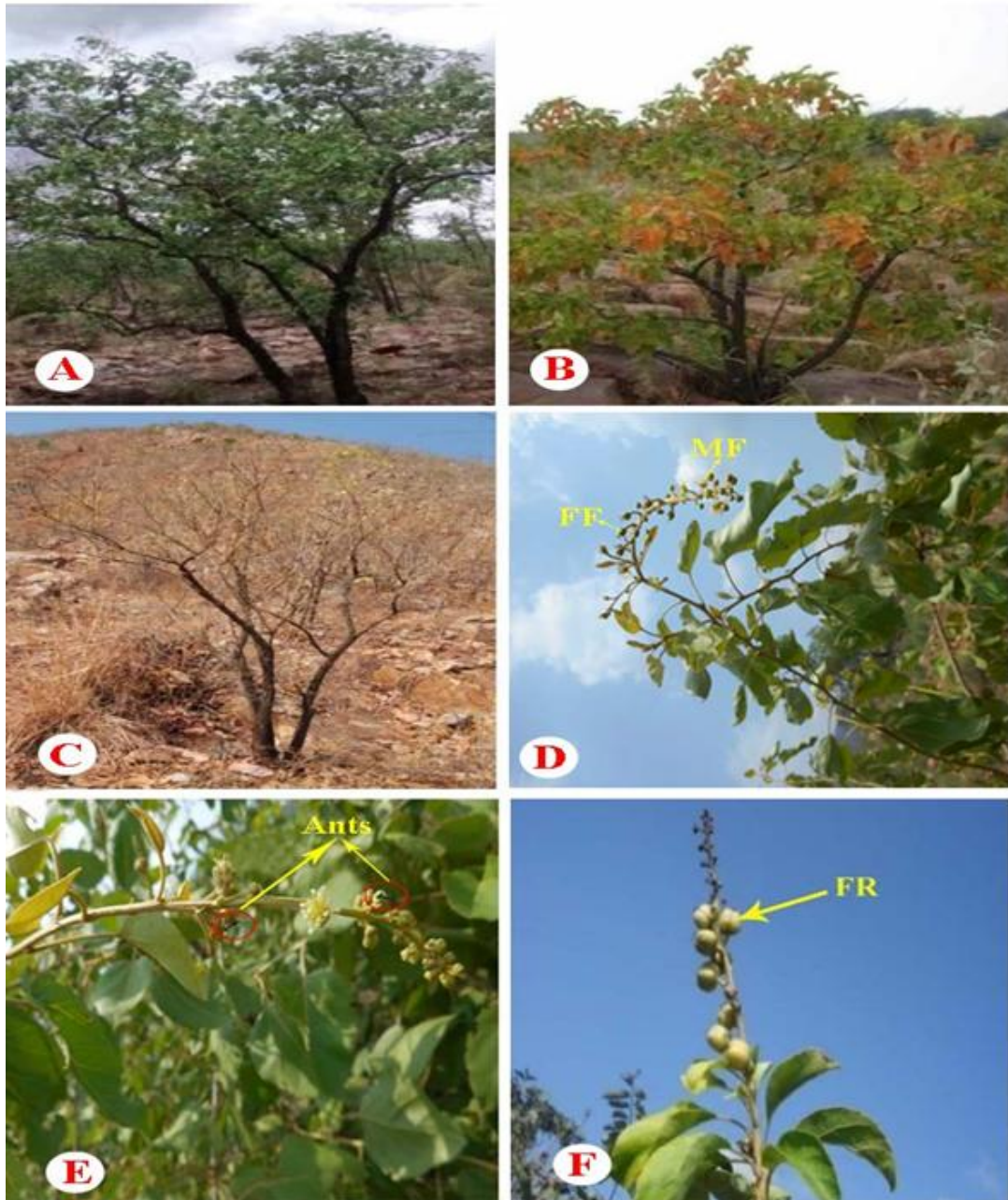


Figure 1A: *Croton scabiosus* Habit B: Tree with orange – red coloured leaves C: Tree with total leaf fall D: Inflorescence showing male and female flowers E: Inflorescence with ants F: Branch which fruits.(MF:Male flowers, FF:Female flower,FR: Fruits

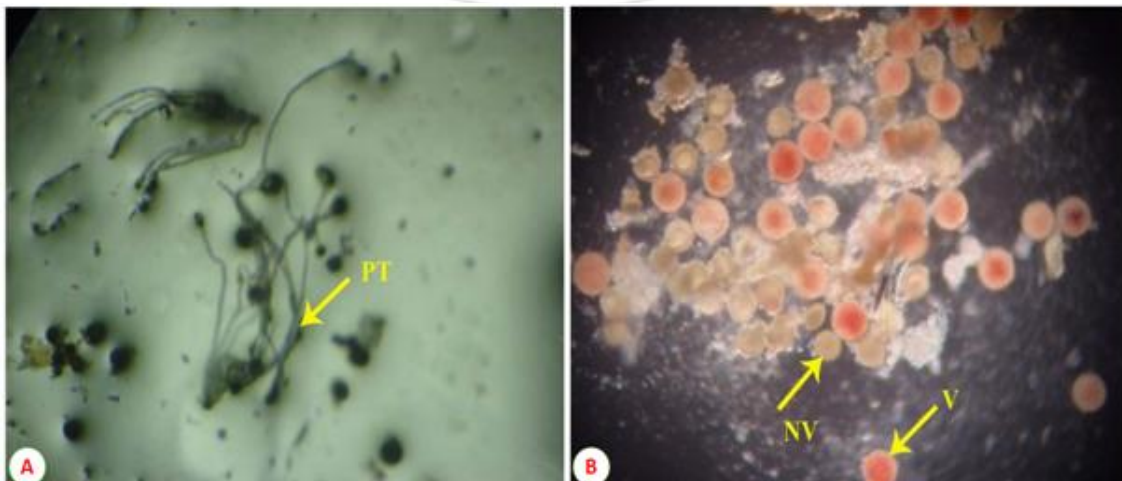


Figure 2A: *In vitro* pollon germination PT- pollen tube **B.**Pollen viability cheaked by TTC NV: Non viable pollen V.Viable pollen

3.6. Pollination and Breeding behavior

In *Croton scabiosus* the flowers are minute and unisexual, male and female flowers are borne on simple racemes inflorescence. Flowers have nectar in traces. The flowers were visited by good number of ants and thrips throughout the flowering season (Fig. 1E). They were found during the day carrying and transfer considerable amount the pollen grain to the female flower. The other insects like honey bees and butterflies are not observed on *Croton scabiosus*.

In natural conditions fruit set was 46%. The percentage of fruit set through xenogamy was 17.65% and through geitonogamy was 23%. The results show the species is self-compatible. The time required from initiation fruit to dehiscence is 28 days. The seeds obtained by natural and artificial germinated up to 10% only.

Table 1: Floral biology in *Croton scabiosus*

Male flower:	Observations
Shape	Cup
Flower colour	Creamy white colour
Number of male flowers/Inflorescence	20 - 52
Male flower length	0.6 ± 0.7mm
Male flower width	0.5 ± 0.6mm
Calyx	5 Sepals, free, valvate
Corolla	5 Petals, pale green in colour, free, valvate
Stamens colour	Pale green
Stamen size	0.47 ± 0.04 mm
Anther colour	Creamy colour
Anther size	1mm
Filament colour	Creamy colour
Filament size	0.36 ± 0.04mm
Pollen grain colour	Light yellow
Pollen grain nature and shape	Wet pollen grains and monads
Pollen size	44.82 ± 4.06µm
Pollen output per anther	219.4 ± 24.76
Pollen output per flower	2,637.18 ± 53.23
Pollen ovule ratio	265492.33
Female Flower:	
Shape	Oval
Flower colour	Light green in colour
Number of female flowers / Inflorescence	5 - 10
Female flower length	6.8 ± 0.6 mm
Female flower width	5.4 ± 0.4 mm
Perianth	5 lobes, light green, free, valvate
Gynoecium	Tricarpellary
Style and stigma colour	Light yellow
Ovary position	Superior, trilocular, with single ovule in each locule on axile placentation.
Ovary colour	Light green
Styles and stigmas	Styles 3, each stigma is bilobed
Number of locules per flower	Three locular
Number of ovules per flower	Three ovules
Nectar	Nectar is produced in traces
Pollination	Entomophilous –ants and thrips observed as pollinators.
Fruit	Schizocarpic capsule, globose to subglobose, 3-valved
Fruit length	8.1 ± 0.4mm
Fruit width	9.4 ± 0.6mm
Seed	Small black with caruncle.

Table 2: Pollen germination and pollen tube growth

Media (Sucrose)	No of pollen grains	Germination of pollen grains	Pollen germination percentage	Pollen tube length(µm)
5%	165	64	38.7%	373.2 ± 64.5
10%	229	103	44.97%	428.8 ± 51.5
20%	115	42	36.52%	282.6 ± 47.4

Table 3: Pollen Viability

Total no of pollen	No. of viable pollen present	% of viability
143	69	48.25%

Table 4: Frequency of seed number per fruit level in *Croton scabiosus*

Number of fruits observed	Frequency of seed number per fruit level					
	1	2	3	4	5	6
330	0	3	306	20	1	0

4. Discussion

The observations on phenology and floral biology of *Croton scabiosus* revealed that flowering occurs in two spans i.e., from January to June and September to October in the dry deciduous forests of Southern Eastern Ghats of Kadapa district. Similarly two flowering seasons are reported in other Euphorbiaceae species like *Jatropha curcas* (Sukarin et al. 1987) and *Jatropha gossypifolia* (Bebawi et al. 2005). Flower anthesis occurs throughout the day i.e., morning 0600 to 1800 hrs. But in *Croton bonplandianum* the flowers open in evening (17.30-19.00 hrs) but continued up to next day morning (9.30hrs) and sporadic flower opening occur throughout the day depend upon the climate condition (Biswas 2012). In the present study it was observed that the flowers are visited by only ants and thrips. They help in pollination by carrying a considerable amount of pollen through their body surface. As the flowers are unisexual both geitonogamy and xenogamy operate. The fruit set in high in natural condition (46%) when compared to artificial pollination. Fruit set by geitonogamy (23%) is high compared to xenogamy (17.67%) indicating that plants are self compatible. Though average fruit set is observed poor, germination of seeds with 10% is recorded. This is due to seed infestation and predation. Many seedlings were found congregated near the mother plant indicating vegetative propagation. Nutrient poor habitat, frequent fires, rocky habitat and habitat degradation are the factors of threat to this species. The information on floral biology, pollination and breeding systems is a prerequisite for knowing the life history of *Croton scabiosus* which is an endemic tree and shows restricted distribution. It is monoecious tree and self compatible. Information on flowering period, floral anthesis, pollen dehiscence, pollen viability and floral characters will be helpful for further studies on breeding systems.

5. Acknowledgements

The authors are grateful to University Grants Commission New Delhi, India for the financial support to the project and Forest Department, Andhra Pradesh for giving permission to work in the forests of Kadapa

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