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Research Article

PHENOLOGY AND REPRODUCTIVE BIOLOGY OF *RHYNCHOSIA BEDDOMEI* BAKER, AN ENDEMIC MEDICINAL PLANT OF TIRUMALA HILLS

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ABSTRACT: Flowering phenology and reproductive biology of *Rhynchosia beddomei*, an endemic medicinal plant of Tirumala hills was studied in the natural habitat in the year 2010-11. Flowering was initiated in December 1st week and continued up to end of March. Peak flowering was observed in 4th week of December and another small peak was also observed in February 2nd week. The flowers are yellow in colour and are arranged in axillary or terminal recemes. Maximum pollen germination was observed in 12.5% sucrose solution + BBM at the time of anthesis (between12 noon to 12. 30PM) and in 37.5% concentration at 3.00PM. Maximum pollen viability was observed at 11.30 AM in TTC and FCR tests. Pollen tube growth was maximum in 12.5% sucrose with BBM at 11.30 AM. Pollen were lipid rich and starch poor. Predominance of outcrossing was observed and was brought about by insects, particularly bees.

Key words: Phenology, Reproductive biology, *Rhynchosia beddomei*,

INTRODUCTION

India has rich and varied heritage of biodiversity covering ten bio-geographical zones. The Indian subcontinent is blessed with a wide variety of aromatic and medicinal plants. India nurtures enormous plant diversity and as many as 140 genera out of 5285 angiosperm species are endemic to the country (Botanical Survey of India, 2001). This is largely because of favourable agro climatic conditions for this bioavailability. More than 7,500 species of medicinal plants grow in India which is considered as the botanical garden of the world. In the last 10 years, there has been a dramatic increase in export of medicinal plants and overwhelming interest in their products as well as in traditional health systems worldwide. However, most of these plants are wild and hundreds of species are at the verge of extinction because of over-harvesting, destructive collection techniques and conversion of habitats to crop-based agriculture. According to Singh and Khurana (2002), about 25% of higher plant species are expected to disappear in the next few decades. Cultivation of medicinal plants is a challenging task because less is known about their reproductive biology and seed biology. Improvement, protection and management of medicinal plants are impossible without a clear understanding of their reproductive biology. With the perception of reproductive processes in the plants, attempts are being made to improve medicinal plants by conventional breeding techniques as well as using biotechnological method.

Tirumala hills are the conglomerates of Eastern Ghats with hills rising from 800 – 3000 ft. MSL with diverse angiospermic and pteridophytic flora belonging to different taxanomic groups. *Rhynchosia beddomei* Baker is an endemic plant of southern part of Eastern Ghats (Pullaiah, 2006) including Tirumala hills. This plant belongs to tribe phaseoleae of legume family. As an endemic species, understanding the reproductive biology of this species is critical to formulate successful conservation strategies. Its present status is "Vulnerable" in the IUCN Red data book of Indian plants (Sudhakar Reddy *et al*., 2006) mainly because of its restricted distribution in Tirumala hills and small number of individuals left in the wild.

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The genus *Rhynchosia* consists of approximately 200 species and occurs in both the eastern and western hemisphere in warm temperate and tropical regions (Grear 1978). Bakshu and Raju (2009) reported that the leaves of *Rhynchosia beddomei* have abortifacient, antibacterial, antifungal, antidiabetic and hepatoprotective properties. The leaves are also used for healing wounds, cuts, boils and rheumatic pains by adivasi tribes. Reproductive biology of this important species has not been studied so far. Keeping this in view, the present investigation has been carried out to study the phenology and reproductive biology of this important medicinal plant for its better cultivation and protection.

MATERIALS AND METHODS

Ten plants each growing at different localities viz., Sandralamitta, Japaliteertham Gogarbham area and Patanjali vanam of Tirumala hills were marked for observations. Flowering phenology was studied periodically by counting flowers on marked plants throughout the flowering period. Floral morphology, floral biology, number of pollen grains/flower and number of ovules were studied by various methods given by Dafni, 1992. Pollen viability was checked by TTC and FCR (Fluro Chromatic Reaction) Tests. Pollen size was measured using an ocular stage micrometer under light microscope. Stigma receptivity was checked by localization of non-specific esterases after the method of Mattsson *et al*. (1974) using α-naphthyl acetate. *In- vitro* pollen germination was studied by using different concentrations of sucrose in BBM. *In vivo* pollen germination was studied by KOH and aniline blue method (Shivanna and Rangaswami, 1992). The mode of pollination was evaluated by emasculating the mature buds and hand pollination. Pollen morphology was studied by Acetolysis (Erdtman, 1963) and scanning electron microscopy (SEM) (Bozzala and Russel, 1998). For SEM studies, fresh anthers and pistils were fixed in 2.5% glutaraldehyde in 0.1 M Phsophate buffer (pH 7.2) and post fixed in 2% Osmium tetroxide. These were dehydrated in a graded series of alcohols, dried in CPD unit. The samples were mounted over the stubs and double coated with gold for 3 minutes using an automated sputter coater (model – JOEL JFC – 1600 and scanned under Scanning Electron Microscope (SEM model: JOEL – JSM 5600) at required magnifications as per the standard procedures at RUSKA Lab, College of Veterinary Science, SVVU, Hyderabad.

RESULTS AND DISCUSSION

Rhynchosia beddomei is commonly called as Adavi Kandi and Vendaku occurring in dry deciduous forests. It is endemic to Southern India, found in Andhra Pradesh and Karnataka states. In Andhra Pradesh it is distributed in parts of Kadapa, Chittoor and Anantapur districts. In Chittoor district, it is common in Talakona forest and Japaliteertham, Gogarbham area, Sandrala mitta, Patanjali vanam, and near deer park of Tirumala hills. Plants are erect under shrubs, and branchlets are tomentose. Leaves are trifoliate, reticulate, leaflets are white, silky, coriacious and lanceolate. Initiation of flowering was observed in the first week of December and maximum flowering occurred in the 4th week of December (1893.5 flowers) and a small second peak was also observed in the 2nd week of February (418.2) - Table 1 and Fig 1. Flowering continued up to the end of March. In plants, the phenomenon of flowering involves a transition from vegetative phase to reproductive phase. The floral transition is a major developmental event in the life cycle of flowering plants where by plants switch from a phase of vegetative growth to one of reproductive growth. The timing of this event is governed by many factors. Proper timing of seed production is critical for optimizing reproductive fitness in a given species (Mark Doyle et al, 2002). Onset and duration of flowering, relative maturation of male and female sex organs and the number and arrangement of flowers in a plant profoundly influence the pollinator visitation pattern in the taxa which has a direct bearing on the success of their life cycle (Siddique, 1991). Higher frequency of flowering by the endemic plants could be due to preference for localized pollinators and perhaps for a better perpetuation rate. Pollinator availability has been considered as probable reason for differential flowering time in tropical communities (Stiles, 1978; Bawa et al, 1985).

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Table 1: Number of flowers / plant in *R. beddomei* during the flowering period 2010-11

Week of observation	December	January	February	March
I week	11.9 ± 0.67	1240.7 ± 43.23	150.4 ± 9.15	73.8 ± 4.04
II week	334 ± 8.7	715.5 ± 24.01	418.2 ± 22.69	30.4 ± 0.96
III Week	817.8 ± 32.7	601.6 ±17.99	150.7 ± 5.43	13.7 ± 0.61
IV week	1893.5 ± 39.4	266.6 ± 14.74	127.2 ± 3.62	8.3 ± 0.52
Total no. of flowers	3056.7	2824.4	846.5	126.2

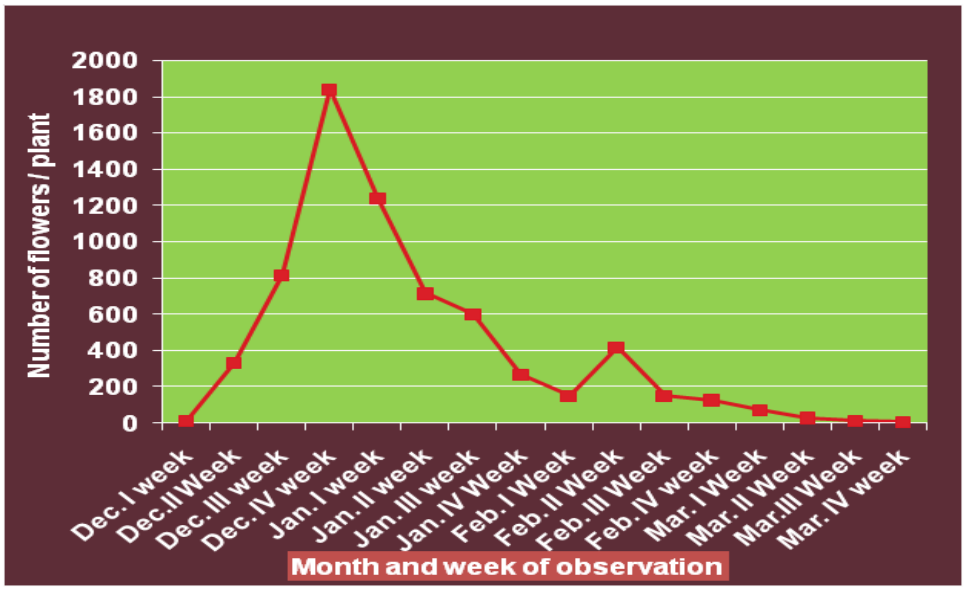

Fig 1: Flowering phenology in *Rhynchosia beddomei* in 2010-11

Flowers were yellow colored arising in axillary or terminal recemes. Calyx was oblong, obtuse, and longer than the corolla, upper two connate. Corolla was exerted, standard petal was obovate with inflexed auricles at the base, wings were narrow, and keel was incurved and hardly beaked. Stamens were diadelphous (9+1), anthers were uniform and vexillary stamen was free. Ovary was found to be sub-sessile, single ovuled, style was long, much incurved, pubescent below, and stigma was capitate. Two pairs of nectaries were present at the base of the ovary. Flower anthesis was observed between 12.00 noon and 12.30 PM and anthers showed dehiscence 24 hours before anthesis.

Pollen count was found to be maximum in the month of December (1044.2 pollen per anther) – Table 2 and Fig. 2. The maximum pollen size (Fig.4) was noticed in the month of December (49 X 47µm). Morphology of the pollen grains was found to be trizono colporate with reticulate exine surface (Fig. 3). Trizono colporate pollen in fabaceae were earlier described by Bera and Dixit (2010) in *Butea monosperma*, *Dalbergia sissoo* etc. A definite relationship is exhibited between pollen characters and pollen types especially regarding the mode of pollination. For instance, pollen grains of entomophilous taxa, are characterized by compound apertures i.e 3-6 colporate, large size, thick walled with granulate – reticulate- echinate sexine pattern, while pollen of anemophilous taxa are of simple apertures (Bera and Dixit, 2010). The reduction in aperture length tending towards pores with an associated increase in apocolpium and change to oblate shape is again regarded as specialization (Guinet and Ferguson 1989, Ferguson and Skvarla 1981, Ferguson 1984) and is **a** trend found in Phaseoleae and Desmodieae.

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Table 2: Month - wise pollen count in *R. beddomei* during the year 2010-2011

Month	Pollen count / anther
December	1044.2±14.5
January	949.2±10.9
February	1013.4±19.06
March	882.4±8.58

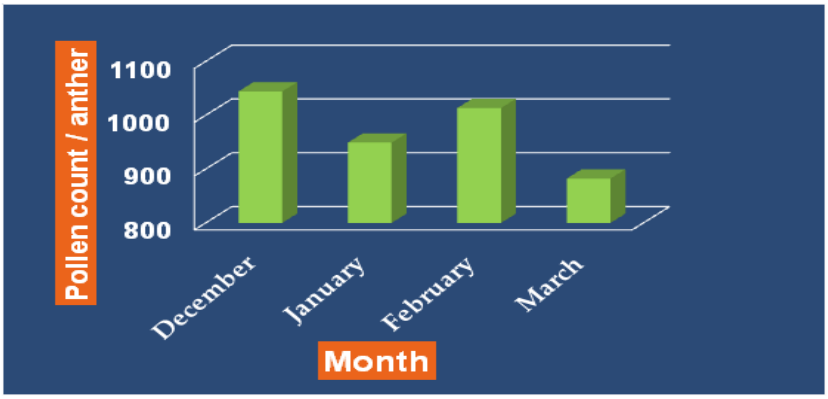
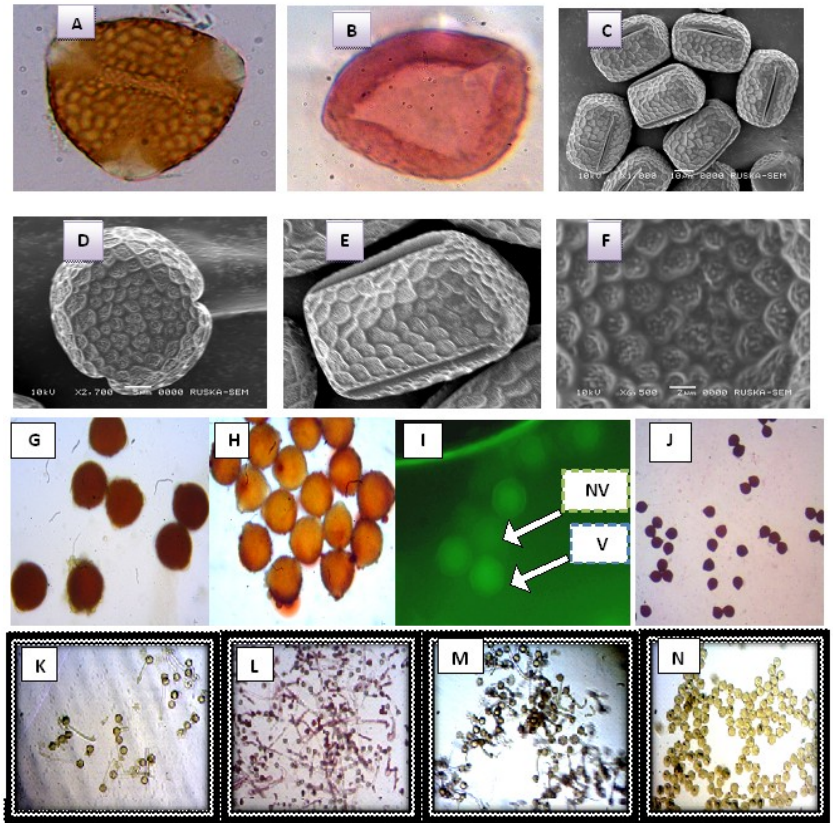


Fig 2: Month - wise pollen count / Anther in *R. beddomei* in the year 2010-11



A & B. Polar view & equatorial view of acetolysed pollen
C,D,E,F- SEM images of *R. beddomei*

C -View of pollen grains in different planes; D- Polar view of pollen grain showing tricolporate structure; E - Pollen grain in Equatorial view; F - Surface view of pollen grain (reticulate surface); G - Sudan III & Sudan IV test for lipids; H - IKI test for Starch; I - FCR test for viability – showing viable (V) & non viable (NV) pollen; J - TTC test for viability; K- Bursting of pollen in 2.5% sucrose + BBM; L - Pollen grains showing maximum pollen germination & tube growth in 12.5% sucrose + BBM; M - Pollen germination in 37.5% sucrose + BBM at 3.00 PM; N- Shrunken pollen in 47.5% sucrose + BBM.

Fig 3: Acetolysed & SEM images of *R. beddomei* pollen grains

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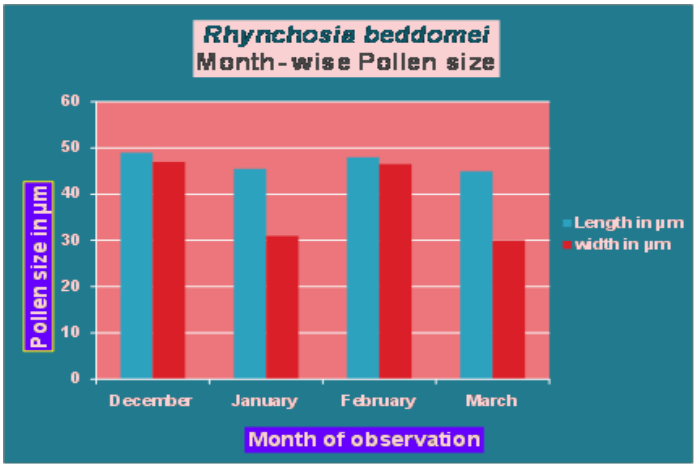
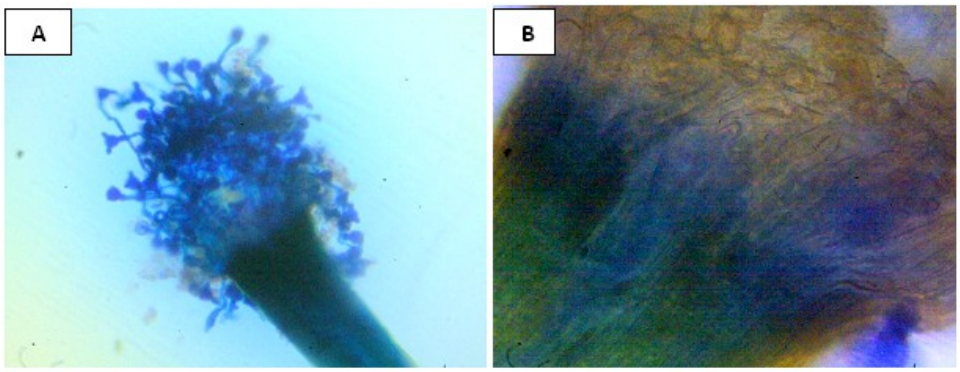


Fig 4: Month – wise pollen size during the year 2010-11



A and B showing germinating pollen on stigmatic surface in *R. beddomei* flower

Fig 5: *In vivo* pollen germination in *R. beddomei*

Pollen histochemical studies (Figure 3) revealed that pollen were rich in lipids and poor in starch. Baker and Baker (1983) have shown that starch poor pollen grains (which have a lot of lipids) are typical of bee pollination and also of fly pollination. Pollen germination / Pollen viability (Figure 3) was found to be maximum between 10.30 A.M to 4.30 P.M. The percentage of viability varied between FCR and TTC tests. Maximum germination was observed in 12.5% sucrose with BBM at 12.00 noon. But as the time progress pollen showed germination in higher concentrations (37.5%). Maximum pollen tube growth was noticed at 12.00 noon in 12.5% sucrose solution with BBM. *R. beddomei* is predominantly cross pollinated and was aided by insects, particularly bees**.** Pollen viability and stigma receptivity are critical for the effective initiation of pollen pistil interaction. Pollen viability refers to the ability of pollen to successfully complete post pollination events on a receptive, compatible pistil and to deliver functional male gamete to the embryo sac. Stigma receptivity tests (Fig. 5) revealed that stigma was receptive 24 hrs. before and after anthesis. High pollen load was observed on stigmatic surface. *In vivo* pollen germination was observed after 12 hours of anthesis.

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In leguminosae pollen are usually released while the flower is in the bud stage and in this stage pollen are viable (Asmussen, 1993; Rodriguez – Riano *et al*., 1999, 2001). Although, stigma is receptive in this phase, its receptivity is inoperative due to the presence of a stigmatic surface that blocks the germination of the pollen grain. Only the rupture of the surface by a pollinator will allow the pollen to germinate (Shivanna and Owens, 1989; Rodriguez – Riano *et al*., 1999).

Apart from people's awareness and participation, knowledge of reproductive biology is the key in achieving the required conservation (Moza and Bhatnagar 2007). Knowledge of reproduction is crucial to our understanding of the causes of rarity and for conservation of rare plant taxa (e.g., Drury, 1974, 1980; Harper, 1979; Ayensu, 1981; Kruckeberg and Rabinowitz, 1985). Knowledge on phenology and floral morphology are essential for conducting studies on breeding systems, particularly on pollination syndrome. Therefore, reproductive biology helps in developing strategies to preserve genetic potential of rare species which are crucial for restoration programmes.

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