Tropical Agricultural Research Vol. 7 1995, 124-131

Reproductive Biology of *Calliandra calothyrsus* in Relation to its Seed Production in Sri Lanka

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ABSTRACT. *The reproductive biology of seven provenances of Calliandra calothyrsus selected on growth performance and morphological characteristics, was studied. Calliandra flowers open in the evening and show receptivity to pollen grains from 5.30 pm to 6.00 am. The best time for pollination was from 7.00 pm to 9.00 pm as observed in the fixed samples under the microscope and also by allowing the pollinated flowers to be developed into pods. The major pollination vector is the bat belonging to the family Megachiroptera. The bats are attracted by the high sugar percentage of the nectar and not by the nectar volume. The provenance 10/91 has a high pollination efficiency in both Yala and Maha seasons.*

Crosses were made by keeping eight-polyad pollen grains on the stigma. Provenances 10/91 and 9/89 had a high response into the controlled crosses. Calliandra tolerates selfing upto 0.56%, which has been confirmed by additional observations in the two seasons.

INTRODUCTION

Calliandra calothyrsus Meisssner is a shrubby legume native to Central America and Mexico. Though seldom used in its native range, *Calliandra* has been cultivated extensively in Indonesia since 1936 (Duncan, 1993). More latterly, other wet tropical areas (*eg.* West Africa) have seen introductions of this acid-tolerant species. In Indonesia and elsewhere, however, concern exists over the genetic base that has been used to establishthe landraces. Consequently, the Oxford Forestry Institute began

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assembling a range-wide collection of germplasm in 1990 with a view of testing new material for a number of traits at a range of tropical sites. One of the first trials planted was under the University of Peradeniya-Oxford Forestry Institute Forestry Research Link. Following field planting in a woodlot design in October 1992, all seven provenances are flowering with some already setting fruit.

Research on *Calliandra* in Sri Lanka has been largely focussed on its use in tea plantations. Currently it is regarded as the best medium shade tree, and the demand for seed cannot be met by the Tea Research Institute. Germplasm presently used for planting has been introduced by various unknown sources from Indonesia. There is a need for local seed production in Sri Lanka to meet the increasing demand. Seed Orchards of genetically superior materials are required to avoid planting of inferior materials.

Little is known about the reproductive biology of *Calliandra.* Therefore, this study was undertaken to investigate the reproductive biology of *C. calothrysus* with the objectives of identifying the floral visitors which are effective pollinators, the pollination efficiency of putative pollinators, the variation in floral and fructal phenology within and between provenances, the success of intra and inter-provenance controlled crosses and the extent of selfing that occurs.

MATERIALS AND METHODS

The research was carried out in the provenance trial which was established in 1992 at the University Experimental Station, Dodangolla.

Seven provenances (namely 9/89, 10/91, 11/91, 12/91, 18/91, 9/91 and 20/91) were considered in the phenology (floral and fructal) study. The provenances 9/89, 10/91 and 20/91 which had superior growth and biomass production were chosen for detailed study in reproductive biology. Twelve trees from every selected provenance (four trees for every replication) were marked and labelled.

From every selected tree, required number (four) of panicles each were covered seperately by pollen proof bags, insect proof bags and bat proof cages to abtain measurements.

Floral visitors

Floral visitors were collected throughout the day to coincide with the timing of floral opening, stigma receptivity, male anthesis and nectar flow. Species of fruit bat which were caught in mist nets were identified, examined for presence of pollen on their muzzles and heads, sexed and marked.

Stigma receptivity was tested by applying H_2O_2 on the stigma surface and were confirmed by controlled crosses (Bawa,1983). Male anthesis was recorded relative to the complete opening of the staminal filaments. Nectar flow was measured at different times using microcapillary tubes and the sugar percentage was measured using a sugar refractometer.

Pollinator efficiency

Honey bees, wasps and bats (belonging to Megachiroptera) were observed. Early in the morning, flowers which had been visited (observed as broken or twisted filaments) were marked with labels. The percentage of broken styles were recorded along with the discernable number of the pollen grains. A week to ten days later, the presence or absence of developing fruits was recorded. Subsequent to the visit of the pollinators the flowers were covered with baggs to be unbagged at noon on the following day to prevent contamination.

Phenology

Phenological studies were done at the individual flower and panicle levels in all provenances. The following were recorded:

Number of flowers/panicle/tree
Number of pods/tree and the number of apices having pods
Duration between opening of the first and last flowers in one panicle
Duration between pollination and pod initiation
Duration between pod initiation and pod dehiscence
Number of seeds, aborted seeds/pod

Controlled crosses

Using pollen proof bags, the opened flowers on apices of chosen provenances 9/89 and 10/91 were bagged and the apices were removed in the late afternoon. Controlled crosses were carried out, later that day using freshly collected pollen. After pollination, the samples were fixed in FAA (70% alcohol, 5ml glacial acetic acid, 5ml formaldehyde) at 2, 4, 6, 8, 16, 24 hours post-pollination to ascertain the best time for pollination (Bawa, 1977). Pollination success was studied by taking samples of pollen from all parental plants and testing for viability using 10%, 20% and 30% sucrose solutions (Bawa, 1979). The percentage of crosses which set fruit, and the seed:ovule ratio in fruit set were recorded.

Selfing of *Calliandra*

Controlled crosses using pollen from the same plant with several replicates (depending on pollination success expected from out-crossed pollen) were carried out on four trees from each of the three provenances 9/89, 10/91 and 20/91.

The crosses were fixed at different time intervals after pollination and observed under a fluorescent microscope to determine pollination efficiency. Some pods were allowed to develop to observe any late-acting self-incompatibility (Bawa, 1977).

RESULTS AND DISCUSSION

Floral visitors and pollinator efficiency

Bats, honey bees and wasps were observed in the trial site. From March to July, wasps and honey bees were observed on *Calliandra* flowers from 5.30 pm to 7.00 pm. The bats belonging to the order Megachiroptera, known as the Sri Lankan Dogface Bat (*Rousettus seminudus)* and the Lankan Fruit Bat (kos atte vavula - *Cynopterus sphinx ceylonensis*) were observed striking the *Calliandra* flowers, after 7.00 pm in the field.

From October to January, wasps and honey bees were observed between 4.30 pm to 5.30 pm on the flowers, which had just unfolded. After 5.30 pm the entire field attracted the bats.

The receptive period of stigma was from 5.30 pm to 6.00 am. Provenances 9/89 and 12/91 showed receptivity from 6.30 pm to 6.30 am. This observation was confirmed by control crosses which were allowed to set fruits. The nectar flow and the sugar percentage of the two seasons given in Table 1 show that the sugar percentage in the nectar increased with the increasing volume of the nectar. Among the selected provenances (9/89, 10/91 and 20/91), the 10/91 had the highest sugar percentage in nectar.

Table 1. Nectar volume and the sugar percentage of Calliandra flowers during different season.

Provenance	April		September		October	
	volume (μl)	sugar %	volume (μl)	sugar	volume %	sugar (μl)%
9/89	70	18	55	15.0	45	17
10/91	50	20	50	16.0	45	18
20/91	50	17	35	13.0	30	12
12/91	-	-	30	12.0	30	13
18/91	-	-	25	13.0	30	15
11/91	-	-	35	10.5	35	12
9/91	-	-	35	14.0	30	13

The panicles which were bagged using either a pollen-proof or an insect-proof bag did not develop pods; but 98% of the panicles which were guarded with bat-proof cages showed pod development. Sixty five percent of the panicles which had broken styles and stamens on the day after flowering showed pod initiation. These results suggested that in Sri Lanka *Calliandra* is mainly pollinated by bats.

Since the provenance 10/91 contained the highest sugar percentage in nectar and there were other provenences which produced large volume of nectar, evidently the bats were attracted by the sugar % of the nectar and not by volume. This was confirmed by the results on pod formation (Table 2).

Table 2. Fructal phenology (*Calliandra* - March flowering).

Provenance	Total pods	Total No. seeds/100 pods	Aborted seeds/100 pods
10/91	737	617.4	103
12/91	257	453.0	114
18/91	108	530.0	65
9/89	532	566.0	112
11/91	174	486.0	142
9/91	369	586.0	133
20/01	176	496.0	50

Although 10/91 and 9/89 had an equal number of panicles more styles and stamens were broken in 10/91. The 10/91 provennce had a higher number of pods compared to 9 / 89 in Yala (March). In the Maha season (October to January) the same trend was observed. Provenance 10/91 had 622 in contrast to 59 pods in 9/89. The sugar content was higher in the nectar of 10/91 than in 9/89, though the volume of nectar of the latter was greater. Seed production of 11/91 was low (Table 2), although it had more flower panicles (Figure 1), and the least sugar percentage.

The best time of pollination was between 7.00 pm and 9.00 pm as observed in the fixed samples under the microscope and also by allowing the pollinated flowers to be developed in to pods.

Phenology

Provenance 10/91 had the highest rate of flowering throughout the year covering the two seasons, followed by 9/89 and 11/91 (Figure 1). The time from pollination to pod initiation was 10 -14 days and from pod initiation to pod dehiscence was 3 to 4 months. The time taken from the first flowering to last flowering (of a panicle) was 3 to 4 months in all seven provenances.

Controlled crosses

Table 3 gives the mean values of pod and seed setting obtained from

three replications of different pollination experiments. According to the results, the provenance 10/91 showed a comparatively better performance in selfing, within provenence crosses, between provenences and in open pollination.

Table 3. Fruit and seed - set and Number of seeds per fruit from different pollination experiments.

Crosses treated	No. of stigma	No. of pods	Seeds	No. of seeds/fruit
Selfing				
9/89 x 9/89	25	00	00	00
10/91 x10/91	25	02	02	01
20/91 x 20/91	25	00	00	00
Crossings within provenance				
9/89 x 9/89	50	15	132	8.8
10/91 x 10/91	50	24	224	9.3
20/91 x 20/91	50	11	77	7.0
Between provenances				
9/89 x 10/91	50	28	287	10.3
9/89 x 20/91	50	16	168	10.5
10/91 x 20/91	50	22	219	10.0
Open pollination				
9/89 x	25	4	34	8.2
10/91 x	25	6	54	9.0
20/91 x	25	3	23	7.8

CONCLUSIONS

Calliandra is pollinated by bats belonging to *megachiroptera.* Selfing occurs up to 0.56%. The peak flowering period is in October, and fruit setting takes place from January to March. Out of the seven provenences tested the provenance 10/91 proved to be the best seed producer.

The best time for pollination is between 7.00 pm and 9.00 pm, though the stigma shows receptivity from 5.30 pm till 6.00 am. The best cross was 10/91 x 9/89. In natural pollination, the effective pollinator bats are attracted to the flowers by the sugar content of the nectar but not by the volume of nectar.

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