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Floral biology of *Cajanus cajan* (L.) Millsp. [Leguminosae] in Tripura (India)

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Abstract

Cajanus cajan (L.) Millsp. (Leguminosae), the pigeon pea, is an ethnobotanical plant commonly used as food as well as medicinally by the ethnic people of Tripura. This species shows three floral morphs - complete yellow corolla, yellow corolla with red vexillum and yellow flowers but vexillum with red veins. The present paper deals with initiation of floral bud, floral morphology, anthesis, pollen viability, pollen germination and pollen production. Pollen : ovule ratio of the three morphs were also studied. The anthesis of pigeon pea flower occurs before the flower opening. Flowers were visited by many floral visitors during day time between 7 am to 5 pm in all three morphs whereas activity of thrips were recorded both in day and night. Floral visitors' activity was very frequent and highest in flowers with complete red vexillum, whereas lowest in yellow flowers. *Xylocopa* sp, *Bombus* sp. and *Apis* sp. were the common and frequent floral visitors.

Key words: *Cajanus cajan,* Floral biology, Floral morphs, Floral visitors.

INTRODUCTION

Over the centuries, the use of plants as medicine has become an important part of daily life despite the progress in modern medical and pharmaceutical research. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems (Joy *et al.* 1998; Mazid *et al*. 2012). In the context of increasing global interest in medicinal plants as potential sources of new bioactive molecules (Cordell 2000; Dahanukar *et al.* 2000), conservation of medicinal plants as well as the knowledge of their uses is vital for the future of human health care. For successful cultivation and conservation of plants a detailed knowledge of their reproductive biology is essential (Moza & Bhatnagar 2007).

Biological information such as reproductive ecology, life history strategies, distribution, habitat preference, and plant's responses to environmental perturbations are essential for conservation and sustainable use of ethnomedicinal plants, especially when they are collected in large volumes from wild populations. However, such information is lacking for most of the ethnomedicinal plants of Tripura.

Cajanus cajan (L.) Millsp., a short-lived perennial shrub, usually grown as an annual, is belonging to the legume family. Cotyledons of pigeon pea is one important pulse-crop in tropical and sub-tropical countries. In India it is known as *Rohor* in Assamese; *Orhar* in

Bengali; *Arhar* in Hindi; and *Tubarika* in Sanskrit. In Tripura, it is referred with different names by different communities: *Bethliang* by Darlong; *Khokhleng* by Jamatia and Halam; *Bethwi* by Hrangkhawl and *Muimasing* by Tripuri and Debbarma. It is increasingly grown in the semi-arid tropics, and India is the largest producer of pigeon pea (Wani *et al.* 2009; Taggar & Singh 2015).

Cajanus cajan is an important ethnobotanical plant used as medicine as well food by people of tropics and sub-tropics region of the world. It is a major source of protein to about 20 % of the world population (Thu *et al*. 2003) and is also an abundant source of minerals and vitamins (Saxena *et al*. 2002). According to Varshney *et al*. (2012), it is the sixth most important food legume of the world. It plays an important role in sustaining soil productivity by fixing atmospheric nitrogen.

The infusion of leaves are used to treat anaemia, hepatitis, diabetes, urinary infections, yellow fever, and genital and other skin irritations (especially in females) while floral decoctions are used for bronchitis, cough and cold, pneumonia, dysentery and menstrual disorders (Singh & Kaur 2012). In Tripura different communities uses leaves for treatment of dysentery, cough and cold, gastric troubles and jaundice. The main active constituent of *Cajanus cajan* are flavonoids, tannins, alkaloids, saponins, cyanogenic glycosides in the leaves and seeds (Aja *et al.* 2015). Young pods are used as vegetable and seeds are used as pulses by them.

METHODOLOGY

Six populations of *Cajanus cajan* (L.) Millsp. were selected for the present investigation in the natural conditions. The selected places were Suryamaninagar (23°45´51.55" N and 91°15'33.17" E), College Tilla (23°49´46.76" N and 91°18'2.74" E); Gajaria (23°48'59.45" N and 91°15'11.85" E); Malay Nagar (23°45´13.48´´ N and 91º18´43.80´´ E); Jirania (23º48´48.18´´ N and 91º25´50.27´´ E) and West Noabadi (23°51'43.19" N and 91°19'7.56" E).

During field survey it was observed that the species showed three types of floral morphs which could be distinguished by vexillum colour. The first morph type is with completely yellow vexillum; the second morph shows completely red vexillum; and the third morph is with yellow vexillum marked with red veins.

Five healthy plants were selected from each populationfor each morphs and observations were made on a day-to-day basis at the natural habitat for flower phenology. Observation includes habit of each morph, initiation of floral bud, time taken by a floral bud to bloom, and anthesis time. Floral morphology and floral structures were studied with the help of hand lens and dissection microscope. Floral visitors and their activities were observed and recorded. For pollen morphology, Acetolysed (Erdtman 1952), mounted in glycerine jelly (Wodehouse 1935) were measured under oil-immersion objective lens. For pollen production methods of Nair & Rastogi (1963) and Mandal & Chanda (1981) were followed. The pollen fertility was studied using 2 % acetocarmine (Radford *et al*. 1974). The pollen viability was assessed using 1 % TTC i.e. 2,3,5- triphenyl tetrazolium chloride (Norton 1966) solution and Fluorescein Diacetate (Heslop-Harrison & Heslop-Harrison 1970). *In vitro* pollen germination was carried out in different concentration of sucrose (2%, 5%, 10%, 15%, 20%, 25%, 30%, 35% and 40%) solution and 10% sucrose solution in combination with different concentration of Boric acid. Stigma receptivity was observed by fixing stigmas of varying ages at different time intervals in Carnoy's fixative for 3 – 4 hrs., then stained with aniline blue-lactophenol and observed under microscope (Hauser & Morrison 1964). The stigma having the germinating pollen grains were considered to be receptive. The pollen ovule ratio was measured following the method of Dafni (1992).

RESULTS AND DISCUSSION

Floral Phenology

Reproductive phenology or the timing of the gradual series of events for flowering and fruiting is a crucial determinant of reproductive success and offspring's genetic diversity in plant species (Rathcke & Lacey 1985; Fox 2003; Weis & Kossler 2004; Elzinga *et al*. 2007). Overlap in flowering time among individuals will determine the mating opportunities available and will constrain the genetic diversity within the progeny (Loveless & Hamrick 1984; Lyons & Mully 1992; White & Boshier 2000; Bawa *et al*. 2003; Kitamoto *et al*. 2006). Flowering phenology, coupled with plant density and spatial structure, will influence pollinator visitation, gene flow, and ultimately fruit production (Rathcke & Lacey 1985; Marquis 1988; Ollerton & Lack 1998; Bustamante & Búrquez 2008). Thus flowering phenology is an essential component in understanding the reproductive ecology of perennial plants.

The present observations were carried out during the months of July, 2014 to May, 2014 and_again July, 2015 to May, 2016 to observe all the phenophages of these plants. Plants take about 2 months to reach the flowering phase. Flowering of *Cajanus cajan* occurred during first week of September, whereas peak flowering continued from mid-November to mid-January and remain in flowering stage up to middle of April. Initiation of floral buds takes 6 to 8 days to develop from vegetative shoots whereas floral buds take 7 to 9 days to bloom. After pollination ovary takes 15 to 20 days to produce a mature fruit.

It was observed that anthesis starts before the flower opening i.e. in unopened bud and the process of anthesis was delayed by an hour during rainy days. The period of anthesis was 9.00 to 11.00 hrs and the flower opens during 11.00 to 13.00 hrs. Flower longevity was little over 2 days, i.e. 47 to 51 hr (mean ± SD = 49.08 ± 1.24).

Floral Morphology

Racemes axillary, rarely branching, peduncle erect with 1 – 3 additional nodes, usually slightly shorter than the leaves, mostly with 2-6 flowers at the tip; bracts about 5 mm long; pedicels to about 9 mm long; calyx campanulate, lobes triangular or lanceolate, tube glandular and pubescent, the upper lobe bifid, the lower lobe longest; vexillary petal basally inflexed biauriculate, mostly with yellow to reddish striate, bicallose in the target area, glabrous, with a claw, wings slightly obovate, with short auricle; keel apex obtuse, slightly inflexed. Stamens 9+1, 15 – 18 mm in length, tapering towards the top; anthers ellipsoid, about 1 mm long, dorsifixed, yellowish, slit longitudinal; pollen 3- colporate, ora-circular, exine areolate; ovary 1 chambered; ovary hairy; style long; stigma terminal, simple, Pods linear-oblong, compressed, bi-valved, depressed between the seeds,; upper suture swollen, the lower indistinct; beak down curved; seeds 1 - 5, compressed, subspherical about 6 x 4 x 1.5 mm, of various colours, hilum linear to oblong to somewhat elliptic, about 3 mm long

These three floral morpho-types also shows variation in pod colour. Morph one shows complete green pod with white and red marks, morph two shows completely green pods with white and red marked dark-red seeds and in morph three green pods with red marks and having light red seeds. Details of morphological parameters for these three floral morphs are given in the Table 1.

Floral Visitors

A total of 14 genera of floral visitors were recorded. The important floral visitors were *Xylocopa* sp., *Apis* sp. and *Bombus* sp . The activity of visitors were more from 09.30

Table 1. Comparison of different essential characters of three floral of morphs of *Cajanus cajan* (L.) Millsp.

Parameters	Morph 1	Morph 2	Morph 3
0Calyx size	0.7 cm x 0.6 cm	0.7 cm x 0.6 cm	0.7 cm x 0.6 cm
Corolla size	Vexillum 1.5 cm x 1.7cm Keel – 1.5cm x 0.7cm Wing -1.5cm x 0.6cm	Vexillum 1.5cm x1.7cm Keel – 1.5cm x1.7cm Wing -1.5cm x1.7cm	Vexillum 1.5cm x1.7cm Keel – 1.5cm x1.7cm Wing -1.5cm x1.7cm
Stamen size	1.4 cm	1.9cm	1.7 cm
Pollen size	PD 31.73 ± 2.25 µm; ED 26.36 ± 1.47 µm	PD 37.61 ± 4.21 µm; ED 30.23 ± 3.47 µm	PD 35.36 ± 3.47 µm; ED 29.77 ± 3.55 µm
Style length	1.5 cm	1.8 cm	1.6 cm
Ovule number	5 – 6	4 – 6	4 – 6
Fruit size	7.2 cm x 0.9 cm	6.8 cm x 0.85 cm	6.9 cm x 0.8 cm
Seed weight	0.145 ±0.002 gm	0.141 ±0.004 gm	0.120 ±0.004 gm

hrs. to 13.30 hrs. The floral visitors' activity were less in the afternoon, however, from 15.30 hrs onwards and towards the evening hours activity was again more. *Xylocopa* sp. visited the flowers regularly throughout the day time whereas Trips visited in day as well as at night. *Xylocopa* sp. visited one to ten flowers in a single spell. Duration of the visit was 5 to 20 seconds per flower. Bees visited one to seven flowers in a single spell and the duration was four to sixty seconds per flower. *Apis* sp. visited the flowers to collect pollen and nectar which was found to be most valuable process in the pollination. Their frequent visit from one flower to other may perhaps help transfer of pollen. The activity of floral visitors starts after the opening of the flowers. The insect activity diminishes in cloudy days. Floral visitors' activity were very frequent and highest in flowers with complete red vexillum i.e. Morph-2, whereas lowest in completely yellow flowers i.e. Morph-1.

Pollen Viability

According to Dafni & Firmage (2000), pollen viability is considered as an important parameter of Pollen quality. Pollen viability refers to the ability of complete the post pollination events on a compatible, receptive stigma and effective fertilization. The term 'pollen viability' should be used carefully and rather replaced by the more limited term 'pollen stainability', as it depends strictly on the staining assay (Bhowmik & Datta 2012). All the dyes used in this experiment showed good colour to differentiate between fertile and sterile pollens as well as viable and nonviable pollen grains. A number of authors have discussed the terms used to describe the viability of pollen grains and their ability to germinate and fertilize ovules, and have recommended different terms such as pollen sterility, stainability, viability, , and fertilization ability, pollen quality (Dafni & Firmage 2000; Klein 2000).

The pollens of *Cajanus cajan*, showed high viability and fertility rate which may ensures high chance of fertilization. In all the tests for fertility as well as viability, the pollens of *Morph 1* exhibited higher percentage than other floral morphs (Fig. 1).

Table 2. Floral visitors of Cajanus *cajan* (L.) Millsp.

Sl. no.	**Name of floral visitor**	**Family**	**Visiting time**
1	*Apis* sp	Apidae	Day (7am to 11 am and 3pm to 5 pm)
2	*Aulacophora nigripennis*	Chrysomelidae	Day (9 am to 12 pm)
3	*Bombus* sp	Apidae	Day (10 am to 3 pm)
4	*Elymnias malelas*	Nymphalidae	Day (10 am 4 pm)
5	*Eumenes* sp	Vespidae	Day (8am to 11 am and 2pm to 4 pm)
6	*Jamides celeno*	Lycaenidae	Day (9am to 4pm)
7	*Lampides boeticus L*	Lyeaenidae	Day (8 am to 11 am and 2 pm to 4 pm)
8	*Megacopta* sp	Plastuspididae	Day (7am to 5 pm)
9	*Musca sp*	Muscidae	Day (9 am to 11 am and 4 pm to 5 pm)
10	*Neptis hylas*	Nymphalidae	Day (10 am to 3 pm)
11	*Telamonia dimidiate simon*	Saticidae	Day (3pm to 5 pm)
12	*Thomisus* sp	Thomibicidae	Day (3pm to 5 pm)
13	Thrips	Thrpidae	Day and night Day
14	*Xylocopa* sp	Violacea	Day (7am to to 5 pm)

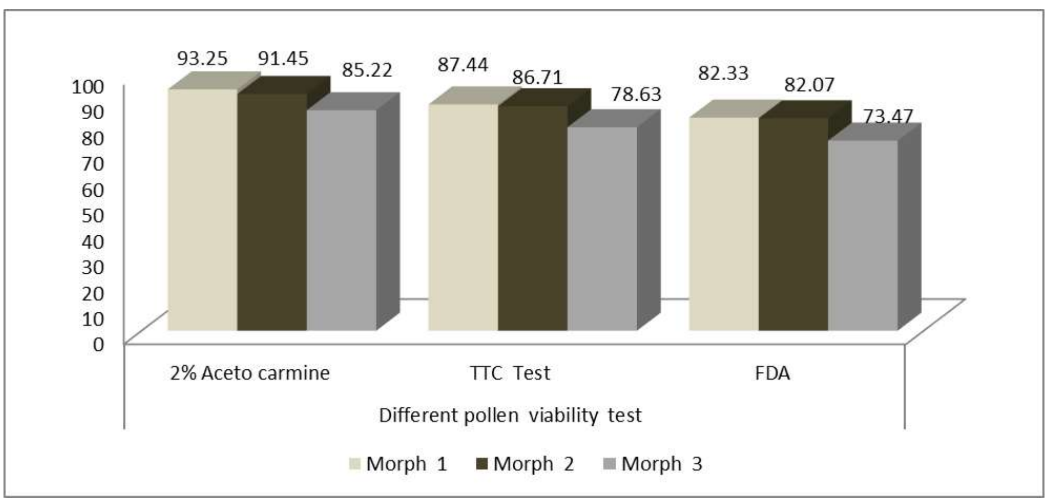

Fig. 1. Pollen viability of *Cajanus cajan* (L.) Millsp.

In vitro pollen germination method is rapid, reasonably simple and most commonly used for assessing pollen viability (Bhowmik & Datta 2012). According to Pfahler *et al*. (1997), *in vitro* pollen germination and pollen tube growth are very useful for explaining the lack of fertility. Pollen germination and the growth of pollen tubes are important and necessary for proper fertilization and seed formation in flowering plants.

Pollen Germination

In the present study, pollen grains showed the highest germination percentage (64.25±3.04, 66.24 ± 3.52 and 54.7±2.3 by Morphs 1, 2 and 3 respectively) and highest pollen tube development (139±2.38 µm, 98±1.54µm and 137.25 ±5.38 µm by Morphs 1, 2 and 3 respectively) in 10 % sucrose solution. In case of germination percentage, pollen grains of Morph 2 showed highest rate where as in case of pollen tube development the Morph 3 showed highest value (Figure 2).

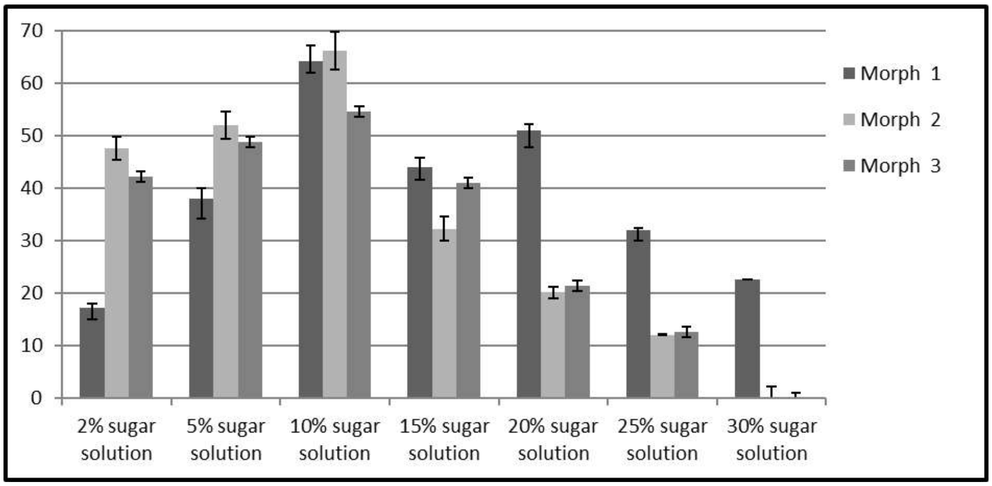

Fig. 2. Extent of pollen germination in three floral morphs of *Cajanus cajan* in different concentration of sucrose solution

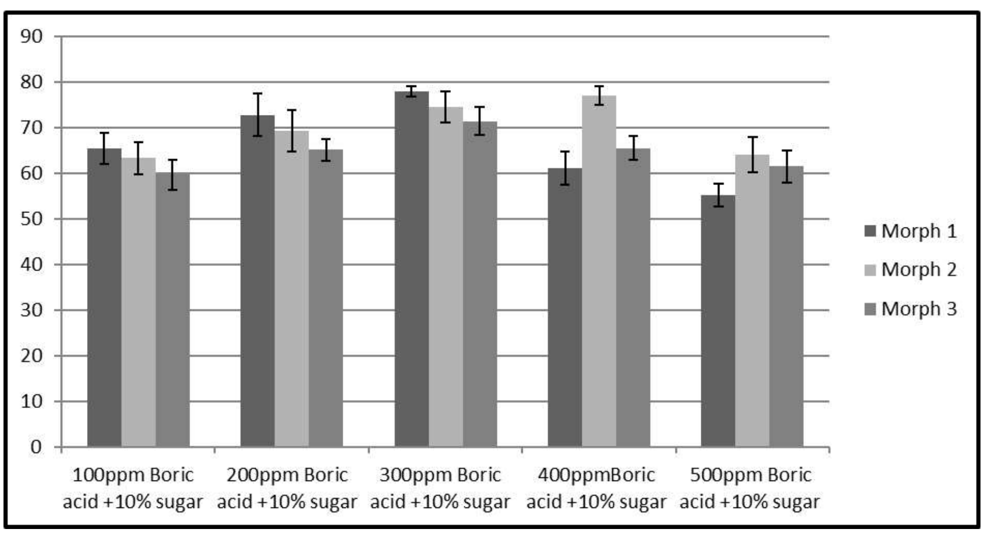

Fig. 3. Extent of pollen germination in three floral morphs of *Cajanus cajan* in different concentration of boric acid along with 10 % sucrose solution

Among different concentration of boric acid and 10 % sucrose solution, 300 ppm Boric acid +10 % sugar solution showed the highest pollen germination rate (78.0±1.16) by Morph-1 in and highest pollen tube development (148.±4.4µm) by Morph-3 in 400 ppm Boric acid +10% sugar solution (Figure 3).

This is attributed to the fact that sucrose is necessary for proper pollen nutrition, osmotic control and in combination with boric acid promotes pollen germination (Sidhu & Malik 1986). Boron combines with sugar and form a sugar-borate complex which is translocated with greater facility than sugar molecules without boric acid (Gauch & Duggar 1953). Boron may enhance the sucrose uptake and also stimulate germinating ability which has been confirmed by the study of other workers (Pal *et al.* 1989; Gupta *et al.* 1989; Mandal *et al.* 1982; Bhattacharya *et al.* 1997; Mohi-ud-din *et al.* 2007; Biswas *et al.* 2008).

Pollen : Ovule ratio

According to Shivanna and Johri (1985), rather than the total pollen per flower or per plant ratio, the pollen : ovule ratio is more accurate measures of reproductive success. During present investigation estimated pollen : ovule ratio was found to be 7212.0 ± 826.89 in Morph-1, 7783.91 ± 1048.29 in Morph-2 and 8254.55 ± 1105.5 in Morph-3 (Table 3). According to Cruden (1977) the pollen: ovule ratio ranges between 2108 and 19523 occurred in xenogamous flowers. The data of pollen : ovule ratio of *C. cajan*, ratified with the result reported by Cruden, indicates that the species to be xenogemous.

Table 3. Pollen production and pollen : ovule ration in three floral morphs of *Cajanus cajan*

Floral Attribute	Values		
	Morph 1	Morph 2	Morph 3
Number of Pollen per anther	3858.6 ± 86.92	3580.6 ± 93.22	3632.0 ± 98.91
Number of Pollen per flower	38586.1 ± 869.2	35806.0 ± 932.2	36320.0 ± 989.1
Number of ovule per flower	5.4 ± 0.55	4.6 ± 0.56	4.4 ± 0.55
Pollen / Ovule Ratio	7212.0 ± 826.89	7783.91 ± 1048.29	8254.55 ± 1105.5
Assessment	The high P/O ratio along with high pollen production attributes to its high seed set.		

Stigma Receptivity

The ability of the stigma to support pollen germination and tube growth of viable compatible pollen grain is referred as stigma receptivity (Shivanna 1998). According to Joshirao and Saoji (1989) for successful completion of the post-pollination events receptivity of the stigma is a critical factor. It is a critical stage during maturation of flower which highly influence the success of pollination in the life cycle of flowers (Barrett 2002).

In *C. cajan*, stigma becomes receptive at about 09.00 to 11.00 hrs before and flowers bloom during 18.00 to 19.00 hrs. On the 2nd day of flowering stigma remains receptive. During receptive state, stigma remain lustrous and yellowish and after losing the receptivity it becomes blackish red. The duration of stigma receptivity in *C. cajan* varies from a few hours to few days. The hour of the day and the presence or absence of stigmatic exudates may influence the stigma receptivity. The period of receptivity is influenced by environmental factors such as temperature and humidity. During the cloudy and rainy days normally receptive period of stigma is extends up to the third day of flowering. Stigma receptivity also shows

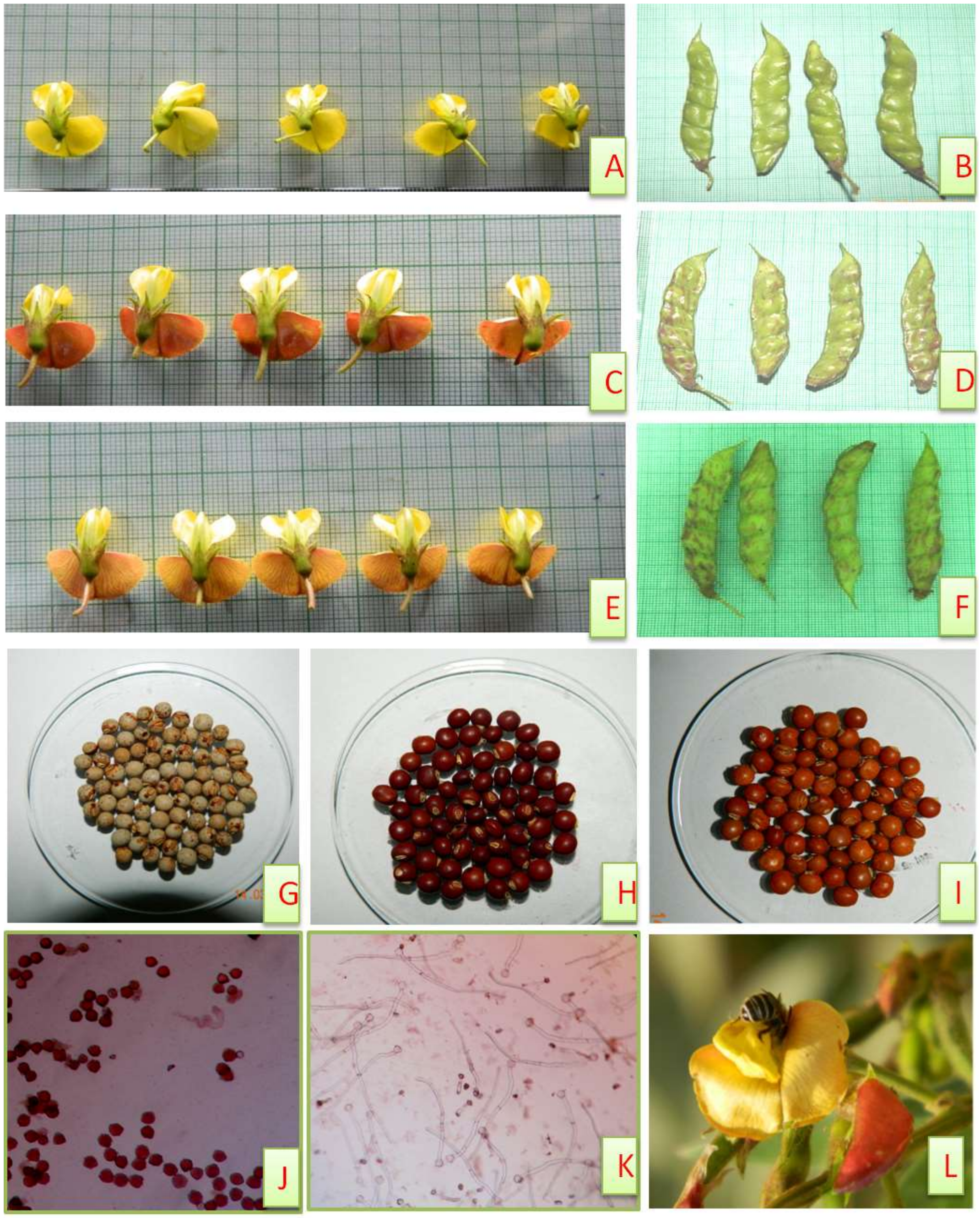

PLATE-I. (A) Flower and **(B)** Fruit of morph 1; **(C)** Flower and **(D)** Fruit of morph 2; **(E)** Flower and **(F)** Fruit of morph 3; **(G, H & I)** Seeds of morph 1,2 and 3 respectively; **(J)** Pollen viability in 1% TTC solution; **(K)** Pollen germination in 10% sucrose solution; and **(L)** *Apis* sp, a floral visitor of *Cajanus cajan*

relations with the change in flower colour. In present findings, stigmas of Morph-2 showing higher degree of receptivity than other two morphs.

Table 4. Absolute and Ecological Reproductive Potential

Floral attributes	**Morph - 1**	**Morph - 2**	**Morph – 3**
Inflorescences / plant	76. 6 ± 7.47	92.4 ± 5.13	71. 2 ± 3.03
Flowers / inflorescences	32.2.± 3.03	36.2 ± 3.11	33.6 ± 2.3
Fruit set / inflorescence	20.6 ± 2.41	16.2 .± 1.79	18.6 ± 2.41
Seeds per fruit	5.4 ± 0.55	4.6 ± 0.56	4.4 ± 0.55
Absolute / maximum reproductive potential (Rm)	13319.2 ± 1291.09	15386.4 ± 1369.67	10526.2± 1193.4
Ecological/realized reproductive potential (Re)	8620.98 ± 897.88 (64.73%)	6885.65 ± 751.79 (44.75%)	5827.01 ± 537.68 (55.36%)

Reproductive Success

Present study reveals that highest number of inflorescence per plant attained by Morph-2 ranged from 52 to 101 (mean ± SD = 92.4 ± 5.13, n = 50) and lowest in Morph-3 ranged from 38 to 92 (mean ± SD = 71. 2 ± 3.03, n = 50). Highest and lowest number of flowers / inflorescences showed by Morph-2 and 1 i.e. (mean ± SD = 36.2 ± 3.11, n = 50). and (mean ± SD = 32.2.± 3.03, n = 50) respectively, whereas fruit set / inflorescence showed highest and lowest values in Morph-1 (mean ± SD = 20.6 ± 2.41, n = 50) and Morph-2 (mean ± SD = 16.2 .± 1.79, n = 50). Each flower produce 2 to 6 seeds (n = 100) on an average, and the normal *C. cajan* plant indicates a potential to produce 13319.2 ± 1291.09, 15386.4 ± 1369.67 and 10526.2± 1193.4 seeds by Morphs-1,2 and 3 respectively, which is the absolute / maximum reproductive potential (Rm) (Table 4). The ecological/realized reproductive potential (Re) under conditions was, only 8620.98 ± 897.88 (64.73 %), 6885.65 ± 751.79 (44.75 %) and 5827.01 ± 537.68 (55.36 %) by Morphs-1,2 and 3 respectively (Table 4). This may be due to the presence of some strong ecological constraint(s). Factors responsible for low ecological reproductive potential is yet to be investigated.

CONCLUSION

From the present study on the reproductive biology of *Cajanus cajan*, three floral morphs were observed. During day time flowers are visited by different types of floral visitors. Pollinator activity was very frequent and highest in flowers with complete red vexillum on the other side lowest in complete yellow flowers. It also shows sucrose is necessary for proper pollen nutrition, osmotic control and in combination with 300 ppm concentration of boric acid, it promoted pollen germination. The high P/O ratio along with high pollen production attributes to its high seed set. The ecological/realized reproductive potential (Re) under natural conditions showed that complete yellow flowers showing highest yield as compared to other two morph which only 64.73 % of its absolute/maximum reproductive potential. This may be due to the presence of some strong ecological constraint(s).

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