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Research Article

Breeding system study in sunnhemp (*Crotalaria juncea* L.) A stepping stone in crop improvement

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Abstract

Sunnhemp (*Crotalaria juncea* L.) belonging to the family Fabaceae is having wide range of industrial utilization as fibre crop, fodder crop and green manure. Pollination is the key mutualism between two kingdoms of organisms. The ambiguity of whether sunnhemp is self-pollinated or cross-pollinated crop paved the way for our study. The simplest and most efficient approach to investigate the breeding system of *C. juncea* would be the combination of controlled hand pollination followed by pod set analysis. Floral biology study revealed the protandrous characteristics of sunnhemp flowers. Flowers are zygomorphic and complete with dimorphic anthers. Breeding system study confirmed the prevalence of cross pollination in sunnhemp by higher per cent pod set in cross-pollination in comparison with self-pollination.

Keywords

Crotalaria juncea, Pollination mechanism, Cross-pollination.

INTRODUCTION

Sunnhemp (*Crotalaria juncea* L.) is grown extensively for fibre, green manure and fodder purpose (Mondin *et al.*, 2007). It belongs to the Kingdom: Plantae; Family: Fabaceae; Genus: *Crotalaria*; Species: *C. juncea* L. Sunnhemp is commercially cultivated for its soft, slightly lignified stem fibres. These fibres have high cellulose, low lignin and negligible ash content (Tripathi *et al.*, 2013), which makes it suitable for manufacturing cigarette paper, high quality tissue paper, currency paper and also for medicinal purposes (Rajesh *et al.*, 2014, Chopra *et al.*, 1956). It is used as fodder and culinary purpose are only to a limited extent. The nutritive value of sunnhemp is found to be on par with other leguminous fodders. In addition to these industrial values, sunnhemp can be grown on fallow or freshly reclaimed soils, where its major role would be renovator or soil builder and as deterrent to nematode. Sunnhemp as green manure, it adds about 50-60kg N ha⁻¹ through biomass in addition to 50-60kg N ha⁻¹ through biological nitrogen fixation by root nodules. Sunnhemp has gained the status of substitute for N fertilizer application as it is capable of fixing 60-80kg of N ha⁻¹. The leguminous crop sunnhemp is cultivated in many parts of the world such as India, Brazil, Pakistan, China, Korea, Bangladesh, etc., China is the highest producer of sunnhemp fibre in the world. India

contributes about 23% of world's production with 27% of total area under cultivation. Despite all known economic and ecological information about the sunnhemp, only a little information is available about the breeding system and its pollination mechanism. Moreover, all the available information is found to be contradictory. One school of thought prescribed, sunnhemp as a self-pollinated crop (Fryxell, 1957) while another school of thoughts prescribed it as a cross pollinated crop (Howard *et al.*, 1919, Mitra, 1970, Patel and Kamat, 1950). Some others prescribed, sunnhemp possess self-incompatibility and its takes the advantage of pollinators to set seeds (Kundu, 1964, Purseglove, 1972). Such a scientific dilemma not only describes the knowledge gap but, also highlighted the need of master maneuvering breeding works comprising the prospects of both plant genetic resource management and its utilization in genetic improvement of the species.

Hence, the simplest and most efficient approach to investigate the breeding system of *C. juncea* would be the combination of controlled hand pollination and pod set analysis. So, the present study was carried out to understand floral biology and pollination mechanism in *C. juncea* L.

MATERIALS AND METHODS

The current investigation was carried out at the Department Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore during *Kharif* 2019–2020 and *Rabi* 2019–2020. The experimental material comprised of five germplasm accessions collected from different parts of Tamil Nadu and also from CRIJAF (ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore). Each accession was raised in 4m x 2m sized plot with 60cm spacing between rows. The above location was situated at an altitude of 27 meters above MSL, 11°N latitude and 77°E longitude.

Flowers in *C. juncea* are found to be zygomorphic and complete as that of Fabaceae flowers. Flowers consist of corolla with polypetalous petals (Standard petal, Wing petals and Keel petals), androecium with dimorphic anthers and gynoecium with hairy stigma (Fig. 1). For the floral biological study, four growth stages of flowers were identified (Fig. 2) viz., stage 1 (S1- unopened bud), stage 2 (S2- petals start to break out of the calyx), stage 3 (S3- petals expanded) and stage 4 (S4- open flowers) as described by Chandrashekar *et al.* (2013).

Observations were taken from 20 randomly selected plants per plot. The relative growth of floral parts and time of anther dehiscence were recorded on ten random flower buds at different stages. To determine stigma receptivity in relation to pollen viability ten flowers bagged at each flowering stages (S1, S2, S3 and S4) were randomly collected during 6:00–18:00 hours. Stigma receptivity was investigated by immersing stigmas in 3% hydrogen peroxide solution, stigmas that produced the bubbles within 2–3 minutes were considered receptive (Zeisler, 1938). Pollen viability was tested by following acetocarmine staining method (Dafni *et al.*, 2005) and *in vitro* pollen germination method using 10 % sucrose + 100 ppm boric acid solution with aniline blue dye (Rathod *et al.*, 2018). The samples were viewed under a fluorescent microscope (Nikon Eclipse Ni-U, Japan) using Nikon filter (330–380nm excitation filter, 410nm barrier filter). Images were recorded with the help of a Nikon DS-Fi3 camera using NIS-Elements F v.4.60.00 image processing platform. To ensure the comparative fitness of dimorphic anthers in effecting fertilization, 20 randomly selected flower buds were emasculated and hand pollinated with pollen from both types of anthers, separately. The number of pods with seed set were recorded upon maturity.

To study the breeding system, flower buds from more than 200 individual plants of *C. juncea* was randomly selected and tagged. Plants were evenly and randomly subjected to different pollination treatments as follows; (i) selfing (hand pollination with pollen of the same flower), (ii) cross-pollination (pollen from flowers of different accessions)

and (iii) control (flower buds were covered and allowed natural selfing if any to take place). Upon pollination treatments, each flower bud was immediately covered with a butter paper bag. Mature pods were harvested and per cent pod set was calculated. To examine statistical differences in pod setting between hand self-pollination and hand cross-pollination t-test was performed using SPSS software version 16.0 (Released, 2007). The experiment was repeated in the next crop season to identify seasonal variations, if any. Index of self-incompatibility (ISI) is used to assess the breeding behaviour of hermaphrodite plants. ISI is a ratio between the percentage of pod set from hand self-pollination to hand cross-pollination and calculated for all the cultivars.

$$\text{Index of self-incompatibility (ISI)} = \frac{\text{Percentage of pod set in self-pollination}}{\text{Percentage of pod set in cross-pollination}}$$

Species with ISI value < 0.25 are considered self-incompatible and those with value > 0.25 as self-compatible (Bawa, 1974, Bullock, 1985, Nayak and Davidar, 2010).

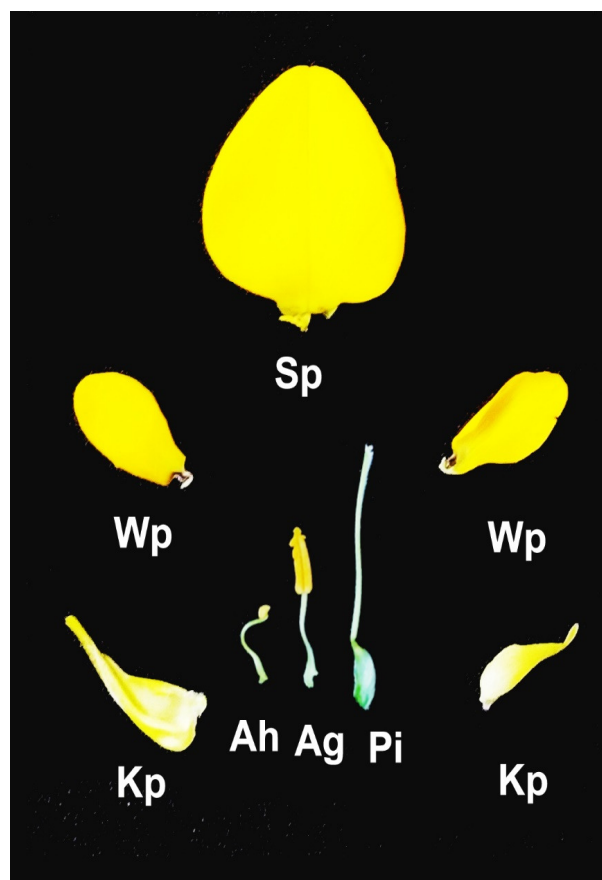


Fig. 1. Sunnhemp (*C. juncea*) floral parts. Sp- Standard petal, Wp- Wing petal, Kp- Keel petal, Ah- Heart shaped anther, Ag- Globose anther, Pi- Pistil



Fig. 2. Sunnhemp (*C. juncea*) flower showing four anthesis stages, a) complete flower, b) flower showing dimorphic anthers at different stages, c) Size of dimorphic anthers in relation to pistil at different stages. S1 - unopened bud, S2 - petals start to break out of the calyx, S3 - petals expanded and S4 - open flowers

RESULTS AND DISCUSSION

Flowers in *C. juncea* are found to be zygomorphic and complete as that of Fabaceae flowers. Of the four floral components, the calyx exhibited only minor changes across the anthesis stages, while standard petal showed rapid expansion from stage 1 (16.3mm) to stage 4 (18.9mm) (Table 1). The species is characterized with anther dimorphism condition, where it exhibited dimorphism in shape and size as one having bigger heart shape while the other having small globular shape (Fig. 2b). The filaments of globose anthers remained almost at the same length while the filaments of heart shaped anthers elongated from stage 2 onwards. Filaments of heart shaped anthers exhibited rapid

expansion from stage 2 onwards to reach the stigma (Fig. 2c). Results established that anther filament growth of globose anthers remained stagnated throughout anthesis and released pollen from anthers at stage S2. On the other hand, filaments of heart shaped anthers started growing from stage S2 onwards and carried the pollen mass towards stigma. Filaments of heart shaped anthers reached a height of $2/3^{\text{rd}}$ of stylar length by stage S3. The heart shaped anthers release pollen at this stage, enhancing probability of self-pollen landing on the stigmatic surface naturally. However, pistil length remained unchanged.

Table 1. Relative growth of floral parts across different anthesis stages in *C. Juncea*

Stage	Calyx		Standard Petal		Style	Filament Length		Anther Length	
	Length (mm)	Width (mm)	Length (mm)	Width (mm)		Globose Anthers (mm)	Heart shaped Anthers (mm)	Globose Anthers (mm)	Heart shaped Anthers (mm)
S1	16.8	13.8	16.3	15.2	16.1	6.7	6.2	4.0	0.7
S2	17.2	14.2	17.7	15.8	16.4	7.1	8.6	4.4	0.9
S3	17.5	14.3	18.4	16.9	16.7	7.1	11.5	4.2	1.0
S4	17.8	14.5	18.9	17.6	17.0	7.2	12.2	4.2	1.0
SE \pm	0.21	0.15	0.56	0.54	0.19	0.11	1.49	0.08	0.07
CV (%)	2.42	2.02	6.38	6.67	2.39	3.37	28.51	3.30	14.48

Both types of anthers were investigated for their dehiscence at 2 hours interval from 06:00–18:00 hours across the anthesis stages. The globose anthers start dehiscence at stage S2 (around 08:15 hours), remained inflated up to stage S3 and deflated at stage S4. However, heart shaped anthers began to dehiscence from stage S3 onwards (around 10:15 hours) and remained inflated up to stage S4. Despite the differential growth phenomena pollen of both globose and heart shaped anthers found insignificantly deviating for pollen fertility and viability. Acetocarmine staining results showed 90.1% and 88.6% (t-test, $p = 0.424$) of pollen fertility for globose and heart

shaped anthers, respectively (Fig. 3). Results of *invitro* pollen viability test also witnessed that there is no significant deviation for pollen viability of both globose and heart shaped anthers (Fig. 4). A similar result was found for pollen viability, both globose and heart shaped anthers produced 81.2% and 82.4% pod (t-test, $p = 0.889$), respectively in emasculated flowers. At stage S1, pollen exhibited viability since 09:00 hours while stigma becomes receptive during 12:00 hours onwards and maintained till stage S4 of anthesis as indicated by the presence of effervescence after immersion of stigmas of each stage in a drop of 3% hydrogen peroxide solution. Pollen viability

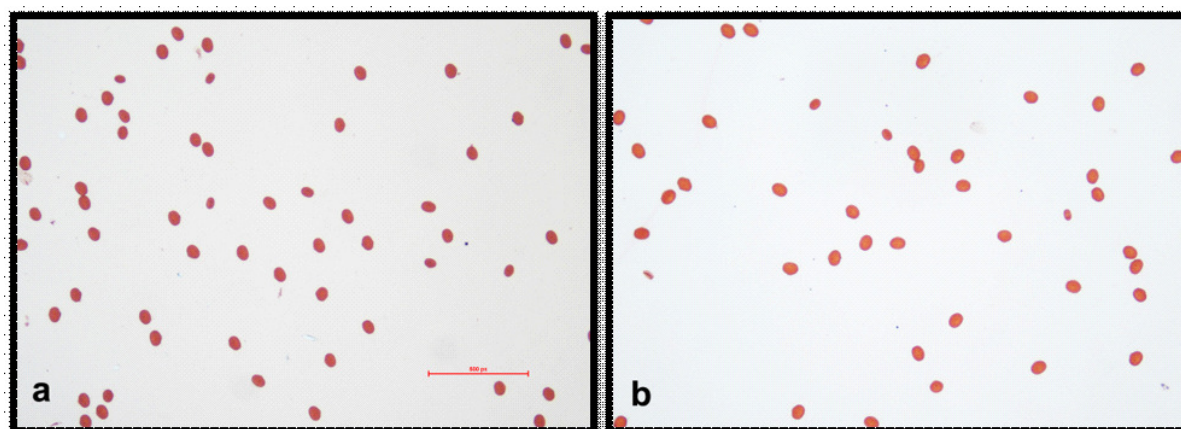


Fig. 3. Sunnhemp (*C. juncea*) pollen fertility a) Pollens of globose anther b) Pollens of heart shaped anthers

and stigma receptivity study found that *C. juncea* is a protandrous plant species. In addition, the study also revealed that both type of anthers are viable and equally competitive in effecting fertilization. Hydrogen peroxide based assay established that stigma of the flower remained receptive throughout the anthesis. Taking into account of all these outcomes, natural self-pollination in *C. juncea* appears obvious at a later stage of anthesis and the phenomenon called delayed autonomous self-

pollination. Similar selfing mechanism was reported in some wild *Crotalaria* species like *C. micans* (Etcheverry et al., 2003) and *C. stipularia* (Etcheverry, 2000) that support our hypothesis. Floral biology study ruled out probability of herkogamy as a causal factor for the preferential success of cross-pollination, as pollen from dimorphic anthers could reach stigma of pistils belonging to the same flower but failed to set the seed.

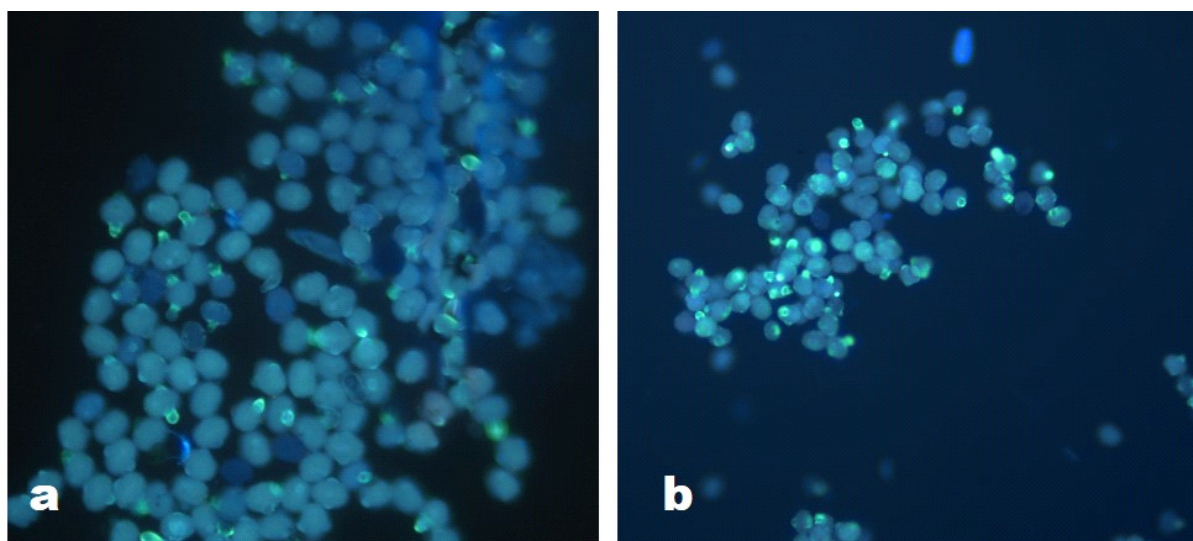


Fig. 4. Sunnhemp (*C. juncea*) pollen viability a) Pollens of globose anther b) Pollens of heart shaped anthers

There was no significant difference in per cent pod set among hand self-pollinated (2.64%) and paper bagged (control) flowers (2.75 %) (T-test, $p = 0.648$). The per cent pod set in cross pollination was found to be 53.12 %. It shows a significant difference for percent pod set in comparison with self-pollination (t-test, $p = 0.000$). In selfed pods average of 3–4 shrivelled and none viable seeds were obtained per pod whereas in crossed pods, average of 10–11 bold and viable seeds were developed per pod. However, the results of the experiment indicated the prevalence of cross-pollination in *C. juncea*. (Table 2). The results of hand pollination revealed that

C. juncea is preferably a self-incompatible species that usually reproduce through cross-pollination as there was a significant difference in pod setting between self and cross-pollinated flowers. Self-pollination yielded significantly very less pods. Index of self-incompatibility (ISI) is an indirect measure to determine the breeding behaviour of hermaphrodite plants, where lower value of ISI (< 0.25) indicate the presence of self-incompatibility (Bawa, 1974, Bianchi and Gibbs, 2000, Bullock, 1985, Nayak and Davidar, 2010). Low value (< 0.25) of ISI in the present study convincingly established the self-incompatible breeding behaviour of the species.

Table 2. Per cent pod setting from different methods of pollinations in *C. Juncea*

Treatments	TNCJ-11	TNCJ-37	TNCJ-97	SN-17	SN23	Mean \pm SE
Control	2.7	2.57	2.9	3.2	2.4	2.754 \pm 0.14
Selfing	2.3	2.87	2.1	3.1	2.84	2.642 \pm 0.19
Crossing	48.6	54.3	52.7	53.1	56.9	53.12 \pm 1.35
ISI value	0.04	0.05	0.04	0.05	0.05	0.04

It can be elucidated that *C. juncea* is a cross pollinated crop with the presence of self-incompatibility. In order to identify the mechanism involved in recognition of self pollen, an elaborated histological, biochemical and transcriptional studies is needed further.

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