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Pollination ecology of safflower (*Carthamus tinctorius* linn)

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Abstract
Safflower is basically self-pollinated but bees or other insects are generally necessary for optimum fertilization and maximum yield. If anther dehiscence occurs before the style elongates, the stigma pushes through the mass of pollen, becomes coated with pollen and gets self-fertilized. If anther dehiscence occurs after style elongates, the stigma passes through the anther tube without becoming pollen coated, then cross pollination is needed. Absence of pollinators results in self-pollination. Anthesis of disc florets takes place between 0530 - 0800 h. The number of pollen grains produced per anther/per flower is 236±74/ 5906±372 respectively. The stigma remains receptive up to 32-56 hours after anthesis. There is about 85.99% seed set achieved in open pollinated heads followed by 38.15% in muslin cloth and 35.54% in butter paper bagged flowers.

Keywords: *Carthamus tinctorius* - pollination mechanism - pollen-pistil interaction.

Introduction

Safflower is a minor oilseed crop, often neglected and very much underutilized. The primary centers of origin of safflower are mountainous regions of Ethiopia, Afghanistan and India (Vavilov, 1951; Chavan, 1961; Weiss, 1971). India is largest producer of safflower in the world (Hegde, 2004). Safflower producing states in India are: Madhya Pradesh, Maharashtra, Karnataka, Jharkhand, Andhra Pradesh, Orissa and Bihar.

The genus *Carthamus* Linn. (tribe Cardueae, family Asteraceae) includes about 17 species (Bremer, 1994). In India, the genus is represented by three species of which *Carthamus tinctorius* (safflower) is grown as an important oilseed crop. The seed oil is used for edible purposes due to the high percentage (70%) of linoleic acid (Baydar, 2002).

Seed production in safflower is directly related with success of pollination because the plants show self-pollination in absence of pollinators (Knowles, 1969). Classen (1950) reported zero to 100 percent cross-pollination. In most of the plants, cross-pollination ranged from 5-40%. Pollinators contribute to various degrees of pollination of the flower (Kadam and Patankar, 1942; Levin and Butler, 1966; Butler et al., 1966; Levin et al., 1967). Safflower is usually considered to be a self pollinated crop. Insects, particularly bees, are the major agents of pollination (Boch, 1961; Eckert, 1962; Rubis et al., 1966). Temperature and humidity affect seed setting of bagged flowers (Patil and Chavan, 1958).

The family Asteraceae, to which safflower belongs is said to be homogenous in features of the pollination syndrome and generalist with respect to pollinators (Lane, 1996). Members of many insect groups visit the capitula, apparently indiscriminately. The role of different species of insect and their relative contribution in pollination of the crop is still not well studied. The present study attempts to describe floral biology, pollination mechanism, and self incompatibility in safflower.

Material and Methods

The present study was conducted on crops growing in the Department of Botany, T. M. Bhagalpur University, Bihar (India). Observations were made on three successive crops in the years (2005-2007). Morphological characteristics were noted by field and microscopic observations. Insects visiting safflower crops were collected by an insect net. Bees, moths and butterflies were killed by crushing on their thorax by the thumb and forefinger and preserved in butter paper bags. The insect packets were preserved inside a cardboard box with naphthalene

balls inside it. These insects were identified by the Entomology Laboratory, University Department of Zoology, T M Bhagalpur University, Bhagalpur. Visits of important pollinating insects to the crops were studied throughout the flowering span of the crop. Observations were recorded between 0530 - 1730 h.

Detailed studies of various parts of male and female reproductive structures (stamens and pistil) were made using a dissecting microscope. Details of the pollen and stigma surface were studied using light as well as scanning electron microscopes. For the study of anthesis and anther dehiscence, 24 plants were randomly selected in the field taking two plants from each plot. The flowers in these plants were carefully observed with the help of a hand lens throughout the course of anthesis and anther dehiscence. Flowering behaviour of an individual floret and also that of a head was carefully watched.

Separate experiments were conducted for stigma receptivity test. Entire heads were bagged with butter paper bag one or two days before the disc florets started unfurling and the mouth of the bag was clipped with a paper clip. When disc florets start to unfurl, the bag was removed for a short while and all the disc florets were removed with a forceps and the head was rebagged. At different time intervals (4 hours before to 72 hours after anthesis) the heads were hand pollinated by rubbing the dehisced anthers on the stigma or picking the pollens with a needle and dusting on the stigmatic surface. The head was left in bagged condition for at least 3 days after pollination and then the bag was removed. The head was properly tagged and left for seed development.

Self-incompatibility behaviour of safflower was determined on seed setting pattern of this crop in open pollinated and bagged conditions. Butter paper bags and muslin cloth bags were used to exclude cross-pollinating insects from visiting the flowers. The data presented in the results is an average of 30 heads for both bagged and open inflorescence of each collection.

Results

Pollination

During the flowering period, several insect species visited the safflower crop from early morning (0530h-1700h). The pollinators were ascertained on the basis of pollen adherence on their body parts, and also from relevant literature confirming their role as pollinator of the members of family Asteraceae. The identified insects fall into 5 different orders of Class Insecta (Table 1). There are a number of characteristics found associated with pollination (Table 2).

Table 1. Insect visitors of safflower crop

Order	Species
Hymenoptera:	*Apis indica, A.mellifera, A.dorsata, Solenopsis geminate, Dalius cucaris, Pyntomis* sp., *Xylocopa* sp., *Spex* sp., *Camponitus compressus*
Lepidoptera:	*Pieris brassicae, Danus plexipus, Euplea core, Pieris rapae, Pthima* sp
Diptera:	*Syphus latifaciatus, Musca domestica, Sarcophaga* sp., *Calophora* sp., *Musca domestica, Dacus cucurbitae*
Coleoptera:	*Epilachna sparsa, Chilomenes sexmaculata*
Odonata:	*Ischnura* sp., *Libula* sp., *Crocothemis sirvilia servilia*

Table 2. Some important characteristics of safflower associated with display of flower for effective pollination

	Mean	Range
Height of plant (cm)	90 cm	30-150
Number of branches/plant	9	5-15
Number of flowers/branches	5	1-9
Number of flowers/plant	15	5-45
Number of disc florets/head	56	45-71
Diameter of a head	3.25mm	2.5-4cm
Size of the disc floret (Length)	29mm	28-30mm
Size of the disc floret (Breadth)	1.5mm	1-2 mm
Length of the corolla tube	18 mm	15-20mm

Foraging behaviour of some of the important and most frequent pollinators is presented in Table 3. *Apis* species are most abundant in the middle of the flowering periods when maximum number of flowers are at bloom. *Apis dorsata* visits the head in the early afternoon whereas, *Apis indica* remains active only in the morning and evening hours. The flies (Dipterans) become active early in the morning and remain in the field for the whole day. They are most abundant and active towards the end of the flowering season when heads start drying. Butterflies

(Lepidopterans) are regular visitors in the middle of the flowering season. They visit heads only in the early morning (0630-1100h). Out of which *Danus plexipus* and *Eupoloea core* are noted as casual pollinators (Table 3).

Table 3. Foraging behavior of some important pollinators of safflower.

Name of insect	Duration of insect visit (h)	Average time spent by the insect in one bout (min.)	Average time spent by the insect in one flower (min.)	Number of flower visited in one bout (min.)
Apis indica	05:30-7:00& 4:15-5:30	10-25	3.12	6-15
Apis dorsata	09:30-12:00	12-15	0.30-1.30	7-11
Apis mellifera	10:30-16:00	15	0.25-2	5-8
Solenopsis geminate	10:00-14:30	4 –7	1.28-2	3-5
Dacus cucurbitae	10:00-14:00	10	2:00-3:00	5-6
Surphis latifaciatus	11:30-16:00	8	0.20-0.45	7
Danus plexipus	06:30-10:30	2	0.15-0.28	4
Pieris brassicae	07:00-11:00	3-4	0.25-0.36	5-7
Calophora spp.	06:00-12:00	5	0.20-0.30	8
Musca domestica	07:30-16:00	11	0.3-0.15	6-10

If anther dehiscence occurs before the style elongates, the stigma pushes through the mass of pollen, becomes coated with pollen and gets self fertilized (Figure 3A). If anther dehiscence occurs after the style elongates, the stigma passes through the anther tube without becoming pollen coated (Figure 3B). In the latter case cross pollination is needed.

Anthesis and Anther Dehiscence

Ray florets are absent in safflower. The disc florets are hermaphrodite. When the florets fully emerge from the corolla tube, size of the anther is about 3.5-4.5 mm. Anthesis of disc florets takes place between 0530-0800 h depending upon time of sunrise. Before flowering, the stigma is enclosed by five fused anthers, which are attached by very short filaments to the tip of the corolla tube. Usually all florets that open during a given day have begun to elongate by the sun rise times. It takes 3-4 days to open all the florets in an inflorescence (Figure 1).

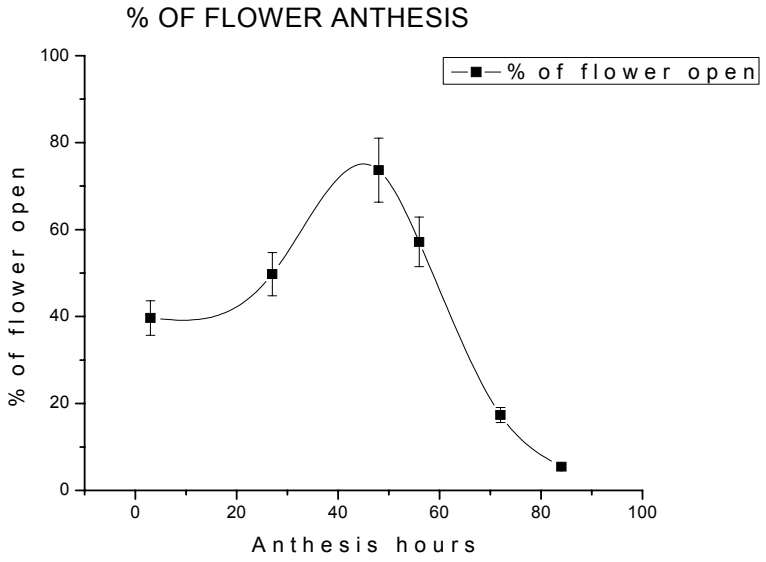
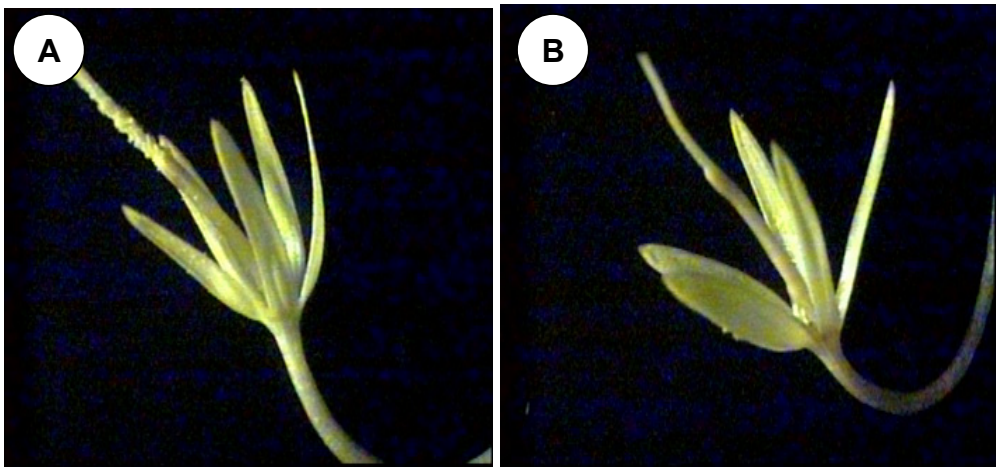


Figure 1. Graph showing percentage of opening of florets in an inflorescence.

Anther dehiscence, which normally occurs soon after sunrise, takes place at the top of the anther column as the stigma emerges from within the anther tube. The combined elongation of the style through the corolla tube pushes the brush-like stigma through the anther until all the stigmatic surface of the pistil has grown well beyond the tip of the anthers. By the time this process of elongation is completed, the stigma is usually well-covered with the floret's own pollen (Figure 3 C, D).



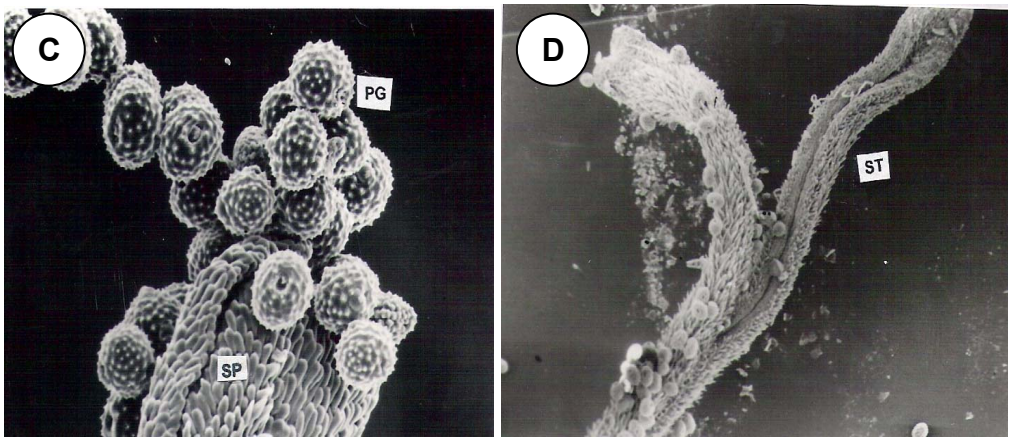


Figure 3. Safflower pollen at the time of anthesis. (A) safflower pistil coated with pollen at the time of anthesis; (B) safflower pistil without pollen at the time of anthesis; (C) Pollen grains lodged on the surface of stigma papillae at the time of anthesis; (D) Bifid stigma with Stigmatic papillae after anthesis well-covered with the floret's own pollen (pg-pollen grains; sp-stigma papillae; pt-pollen tube).

The style is solid. A core of transmitting tissue traverses the whole length of the style. The transmitting tissue is made up of elongated parenchymatous cells, which in trans-section; appear circular with conspicuous intercellular spaces. There are about 236±74 pollens in an anther and about 5906±372 pollens in a flower.

Stigma Receptivity

The unvisited stigmas remain receptive for a longer time. When aging stigmas twist toward the centre of the head, they pick uncollected pollen from neighbouring styles. Receptivity of the stigma was tested in a series of controlled self and cross-pollinations at various stages of pistil development. Before anthesis the stigmatic papillae are short and closely appressed to one another. The papillae are covered with uniformly thickened cuticle overlaid with a layer of pellicle. No surface secretion is present on the stigma (dry type). The stigma remains receptive 14 hrs before anthesis and after 72 hrs the receptivity is completely lost. The seed setting is maximum 20.58% in the cross pollinated florets from different plant. The percentage of seed setting declines in the florets pollinated after 2, 6, 24, 32, 48 and 56 hrs after anthesis. There is no seed setting in the florets pollinated after 72 hrs after anthesis (Figure 2).

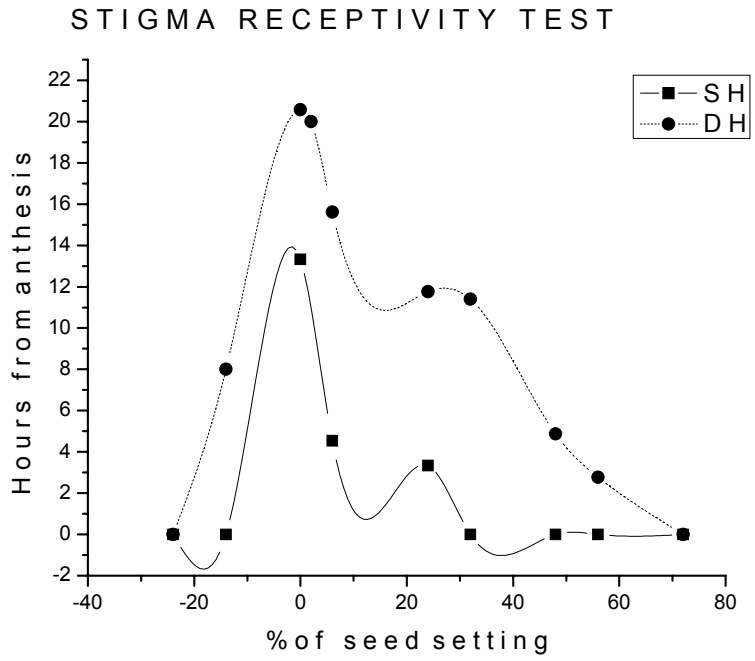


Figure 2. Graph showing percentage of seed setting in controlled pollination.
SH = Cross pollination from same plant different head
DH = Cross pollination from different plant head

Self-Incompatibility

Safflower is a short day plant. A single plant when put in isolation does not set seeds and thus the plant is basically self-fertile as well as self-sterile. In an attempt to understand the incompatibility behaviour, seed setting pattern in open pollinated and bagged conditions was made. At 50% flowering stage, flowering branches of different plants with young unopened heads are bagged with butter paper and muslin cloth bags. When seed setting is completed and heads start drying, seeds are counted from both bagged and opened heads. There is about 85.99% seed setting in the open pollinated heads followed by 38.15% (in muslin cloth) and 35.54% (in butter paper) bagged condition (Table 4).

Table 4. Self incompatibility test in different experimental conditions

Types of Pollination	Total number of flowers	Number of filled seeds	Number of unfilled seeds	Percentage of seeds setting
Naturally pollinated heads	54.75±7.70	47.08±13.78	7.67±6.07	85.99
Butter paper bagged head	54.76±5.50	19.46±8.88	35.3±3.38	35.54
Muslin cloth Bagged heads	51.53±5.35	19.66±4.67	31.87±0.68	38.15

Discussion

Safflower is basically self-pollinated but bees or other insects are generally necessary for optimum fertilization and maximum yield. Absence of pollinators results in self-pollination. Each branch in the moderately branched stem is terminated by a head which gives rather complex synflorescences, the basic unit of which is the dichasm. The cymes are arranged in nearly

corymbose fashion. In this behaviour safflower differs from other Asteraceae members which are mostly self-incompatible (Kumari and Pandey, 2005). Amongst pollinating insects, Hymenoptera (bees), Lepidoptera (butterflies) and Diptera (flies) play a major role in pollinating the safflower crop. Foraging behaviour of bees observed in present study is basically similar to sunflower (Linsley, 1978; Parrish and Bazzaz, 1978; Prasad and Rao, 1984) in dahlia (Heslop Harrison and Shivanna, 1977) and niger (Panda et al., 1995; Pyke, 1984; Reddy, 1976; Dhakal and Pandey, 2003; Pandey and Dhakal, 2004; Neff and Simpson, 1990) and in safflower (Kumari and Pandey, 2005).

The flowering lasts for 40-50 days and this period are remarkably constant but a single plant can flower only for three to four weeks. Ants act as a nectar eater and continue up to mid day. Within a single head the insects move centrifugally visiting the newly opened flower and then moving to the older flowers. Safflower, like *Senecio* and *Guizotia* are generalist with respect to pollination and thus can be an excellent genus for comparison of pollen dispersal movements by pollinators with different energy requirements foraging on the same plant population (Lack, 1982a). Unvisited stigmas remain receptive for a longer period than visited ones in *Pyrrhopappus* (Lack, 1982a), *Helianthus annuus* (Choudhary, 1993) and *Guizotia abyssinica* (Dhakal and Pandey, 1999) and *Carthamus tinctorius* (present study).

In Asteraceae the florets are protandrous when hermaphrodite, and among the species in which the level and type of self-incompatibility has been measured, the vast majority are sporophytically self-incompatible (Nettancourt, 1977; Patil et al., 1979; Stuessy et al., 1986). Consequently vector mediated crossing is extremely important. Free (1970) reported that in *Helianthus,* 63% of achenes set resulted from cross between florets in different heads on different plants (outcrossing), 35% of achenes set resulted from crosses between florets within a capitulum (selfing), and 1% of achenes set resulted from selfing within a single floret. Chavan (1961) reported 79.57 % seed setting in self pollinated safflower plant. During present study bagging experiments reveal that there was about 85.99% seed set in open heads while in bagged condition seed setting varied. It was 38.15% in muslin cloth and 35.54% in butter paper. Landgridge and Goodman (1980) reported about 10% natural crossing only. According to Hegde (2004) extensive self sterility occurs in safflower. It shows that temperature and humidity influence compatibility behaviour in safflower.

In safflower (present study), highest seed set (%) was obtained with stigma pollinated 24h after anthesis a feature also observed in *Guizotia abyssinica*(Sujatha, 1993; Dhakal and Pandey, 1999; Pandey and Dhakal, 2004). Self-compatibility as well as self-incompatibility in safflower have been reported earlier by some workers (Li and Mundel, 1996; Knox, 1984). In niger, Vandana (1990) observed that pistils at all stages of development failed to promote seed set after self-pollination. Controlled self- and cross-pollinations indicated that the natural population of niger is self-incompatible and there is absence of pre-anthesis self-pollination in this crop. Stigma receptivity in terms of seed set (%) confirmed the non-receptivity in buds.

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