



STUDIES ON FLOWER BIOLOGY AND POLLINATION IN MUSTARD (*BRASSICA JUNCEA* L.)

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ABSTRACT

Studies were carried out on flower biology and pollination of mustard (*Brassica juncea* L.). Observations were recorded on stages of flower bud development, time and duration of flowering, mode and rate of anthesis, number of pollen grains, pollen viability, stigma receptivity and effect of different modes of pollination on seed set. The flower bud took 10 to 15 days to pass from first stage of flower formation to full bloom. A single flower may remain open for approximately 3-7 days. The flowering period was of 38 ± 2 days (15 December and continued till 21 January). The average length of sepal was 0.53 cm and that of petal 0.74 cm. The average length of stamens was 0.7 cm and 5 in number/flower. The anthesis starts between 08.00 hrs and continue till 18.00hrs, was maximum between 10.00 to 12.00hrs. Pollen viability was 91.34 %. Stigma receptivity was maximum on the day of anthesis. Highest seed set was obtained by open pollination which gave 77.24 percent seed set followed by hand pollination (52.50 %). The minimum seed set (25.53%) was observed in case of controlled pollination by bagging.

KEYWORDS: phenology, pollination, mustard and fruit set.

INTRODUCTION

Brassica juncea (L.) belongs to the Cruciferae (Brassicaceae) plant family, commonly known as the mustard family. The inflorescence of *Brassica juncea* is an elongated corymbose raceme, borne terminally on the main stem and branches, carrying bright yellow flower (Langer and Hill, 1991 and Pua and Dogulas, 2004). *B. juncea* have an annual growth habit. Flowers of mustard are a prime source of pollen and nectar, making them a desirable site for pollinating insects. Mustard comes in to flowering in January, anthesis with in one two days. A few flower buds started to open in the evening a day before anthesis, but the majority of flowers opened in the morning, mainly between 06.00hrs and 09.00hrs. Opening of new flowers was observed to be completed by 10.00h. Stigma was receptive for three days. A single flower may remain open for approximately 2-7 days depending upon climatic condition Goswami and Khan (2014). *Brassica* is an excellent research crop to study patterns of variation in pollinator behaviour because it is mostly pollinated by insects and has a rapid life cycle, does not self pollinate and require insects for cross pollination (Stewart, 2002). The structure of *Brassica* flowers is well adapted to generalist insect pollinators; it has colourful petals, large amounts of pollen, scent production and nectar production during the whole flowering period, which attracts insects to feed (Free,1970). Indian mustard (*B. juncea*) is a naturally autogamous species, yet in the crop frequent out- crossing occurs which varies from 5 to 30 per cent depending upon the environmental conditions and random variation of pollinating insects (Kumar *et al.*, 2013). The pollinating

efficiency of anthophilous insects is intimately related to the floral biology of the many species and to their foraging behaviour. Plant phenology is an important tool for measuring global change in ecology because it is one of the easiest way to research species behaviour and is significant indicator of climate change (Menzel, 2002). Flowering is the most sensitive stage for temperature stress, probably due to vulnerability for pollen development, anthesis and fertilization (Hall 1992). Pollination plays extremely important role in obtaining high and sustained yields of mustard seeds.

Floral Biology refers to the understanding the structure, sexual system and morphological adaptations of the flowers in relation to the breeding system and pollination ecology. Therefore, the present studies were undertaken on floral biology of mustard and effect of modes of pollination to obtain the relevant information on the various aspects of flowering.

MATERIALS & METHODS

The investigations were carried out at Entomology farm of Dr. Y. S. Parmar University of Horticulture and Forestry Nauni, Solan during 2015-2016 with the mustard cultivar Raya RCC-4. Before ploughing, the field was incorporated with farm yard manure in order to enhance the soil fertility. The land was ploughed in the month of September and beds of 2.5 x 2.5 m size were prepared for sowing of mustard crop. The seeds of mustard were sown in lines (30 cm apart) by hand, which had been made by using a hoe. Observations on floral biology of mustard crop were recorded. Fifty plants were tagged randomly to record the observation on formation of buds. Hundred

freshly formed buds were tagged on these plants at the time of bud initiation to record the day of opening and sequence of opening on the inflorescence. Such observations were recorded on daily basis till the opening of buds. Observations on onset of bloom, full bloom and end of bloom were recorded to observe time and duration of flowering for the mustard crop. To study the time of anthesis, flower on all the sides of plant were marked and the opened flowers were counted at 09.00hrs. The mode of opening of flowers was recorded at two hours intervals from 09.00 to 1800 hrs. The opened flowers were recorded and pinched off at each interval in order to avoid recounting of the flowers. Percentage of opened flowers was calculated.

Flowers for pollen studies were tagged at pre-anthesis stage to prevent contamination with other pollen grains.

$$\text{Pollen Viability \%} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

Stigma receptivity was studied by visual observation of stigmatic surface. The different mode of pollination (open and natural, selfing by bagging, hand pollination and artificial cross pollination) and their effect on seed set was studied.

Studies on the modes of pollination on fruit set of mustard

Open pollination

Observations were recorded in mustard flower exposed to all insect pollinators under open condition. Fruit set was recorded on the flower basis. Flowers was counted (20-30) at balloon stage for recording data on fruit set. The observations on fruit set were recorded 3 weeks after petal fall. Per cent fruit set is calculated from the number of flowers counted.

Hand pollination

Hundred flowers at balloon stage were enclosed in the selfing bags. At the time of anthesis selfing bags was removed and pollen was applied on the stigma by means of camel brush. The stigma receptivity was examined by magnifying hand lens and stigma showing greenish yellow to yellowish colour with droplets of secreted fluid was considered to be receptive. After application of pollen, flowers were bagged to avoid any contamination after

Five completely matured anthers just before dehiscence were transferred to a cavity slide with a drop of water and crushed well with a spatula to get all the pollen grains in to the water. The contents were transferred on to a glass vial and the volume was made up to 10 ml using 70% ethyl alcohol. From this a known amount about 1 ml was transferred to a cavity slide and the pollen grains were counted using a haemocytometer (Belavadi and Ganeshiah, 2013). Pollen viability was observed in one percent acetocarmine solution. One to two drops of acetocarmine solution were taken on a slide and then the pollen grains were dusted, covered with a cover slip and examined under microscope. The deeply stained, normal looking grains were counted as viable, whereas weakly stained as non viable.

pollination. The observations on fruit set were recorded as described above.

Controlled pollination

Hundred flowers at balloon stage were enclosed in selfing bags. The observations on per cent fruit set for different modes of pollination were recorded as below:

$$\text{Fruit Set (\%)} = \frac{\text{Number of fruits}}{\text{Total numbers of flower}} \times 100$$

RESULTS & DISCUSSION

Brassica juncea is an annual crop. Flowers of brown mustard, as in other crucifers, are perfect and have four sepals, four petals, an inner whorl of four long stamens and an outer whorl of two shorter stamens. The ovary is superior and surmounted by a style with a two-lobed stigma. The flower bears four nectaries located at the base of the stamen filaments.

i) Flower bud development: After initiation the flower buds passed through several morphological changes in reaching the anthesis stage. The whole development was divided into the following stages.

Stage 1: The buds were slightly conical in shape, compact in nature and yellowish in colour.

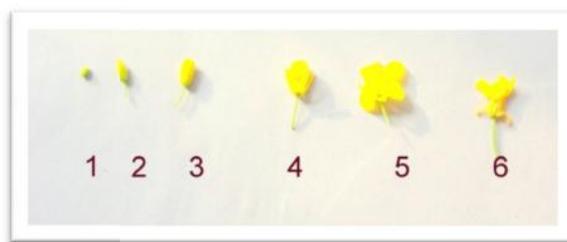


FIGURE 1: showing stages from bud to opening of flower/ bloom

Stage 2: The first stage was pushed into second in 2-4 days. There was no change in colour.

Stage 3: The calyx tip got separated and the yellow corolla tip appeared.

Stage 4: Corolla was pushed more upward due to developing pressure and the calyx spread more outward. The petals were loosely jointed

Stage 5: The buds entered into full bloom stage/opening of flower.

Stage 6: The corolla start drying and shed out from flower.

The time taken by mustard from first stage (bud) to full bloom was 10 to 15 days. A single flower may remain open for approximately 3-7 days. The flowers were bright yellow with 4 small sepals, 4 petals arranged in a cross formation pattern. There were 6 stamens in a flower, 4 long and 2 short, respectively and 1 slender pistil. The average length of sepal and petal were 0.53 and 0.74 cm, the average length of stamens was 0.7 cm.

ii) Time and duration of flowering: The mustard sown on 6 October started flowering on 15 December and continued till 21 January with 38 ± 2 days of flowering duration. The crop was at full bloom stage during 29 December to 11 January for 14 days.

iii) Mode of anthesis: The mode of flower opening was observed when the petals started unfolding till the flowers

were fully opened. Firstly the calyx segmented separated out slowly due to the pressure exerted by developing corolla. Finally the petals spread out to a horizontal position, exposing the stamen open. The process of anthesis was completed between 2-4 hours from the stage 4 (Fig 1).

iv) Rate of anthesis: The observation on rate on anthesis was recorded from 08.00 to 18.00 hrs at an interval of 2 hours. It is evident from the Fig. 2 that anthesis started between 08.00 to 09.00 hrs and continued till 18.00 hrs. The maximum percentage of anthesis took place between 10.00 to 12.00 hrs thereafter it decreased. The anthesis is the flower's opening period and the beginning of the stamen's and pistil's senescence (Faegri and Pijl, 1979).

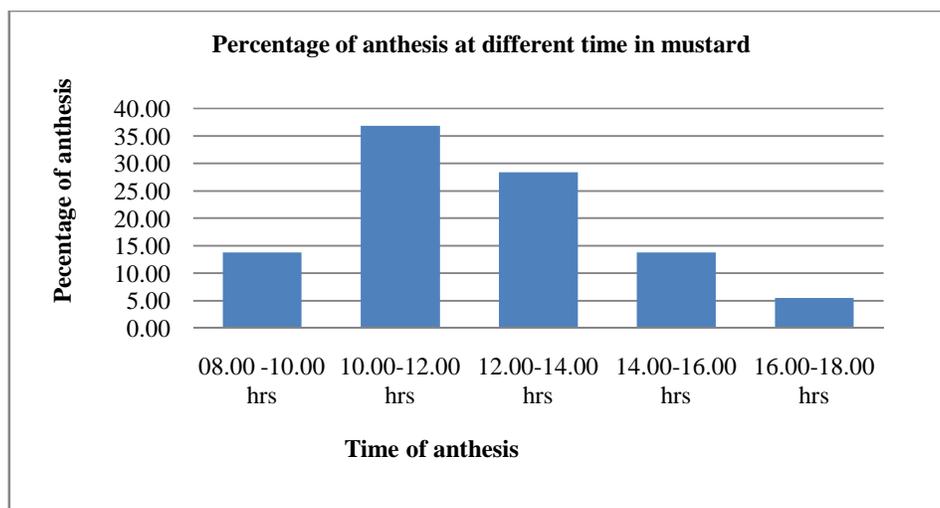


FIGURE 2: Percentage of anthesis at different time in mustard

Goswami and Khan (2014) observed that few flower buds of mustard (*Brassica juncea* L.: Cruciferae) at Pantnagar started to open in the evening a day before anthesis and the majority of flowers opened in the morning, mainly between 06.00hrs and 09.00hrs. Opening of new flowers was usually completed by 10.00h.

v) Pollen studies: The pollen was collected during peak flowering and slide of pollen were made and grains were found slightly oval to roundish (fig. 3) in shape under the microscope.

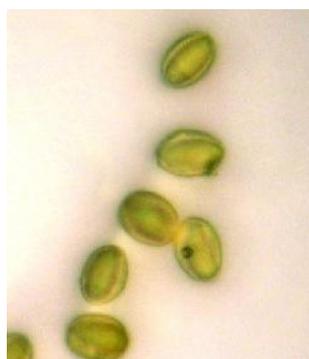


FIGURE 3: Pollen of mustard

The number of pollen grains counted using a haemocytometer was 1473.43 / 5 anther in 1ml of volume of aliquat. The viability of pollen grain was studied in 2 % acetocarmine solution. The deeply stained and normal looking pollens were considered as viable. In this crop 91.34 % of pollen grains were viable.

vi) Stigma receptivity: The receptivity of stigma by visual observation in mustard was 9 to 45.87 % three days prior to anthesis (Table 1). The receptivity on the day of anthesis was 100 %. The quick decline of stigma receptivity was observed after anthesis may be due to aging and their drying by environmental factors. The

stigma presents itself receptive in the second and third stages of anthesis, the period in which there is some secretion on the stigmatic surface. When it ceases to be receptive, this secretion dries and causes the colour to change from yellow to dark beige (Rosa *et al.*, 2010). A single flower may remain open for approximately 2-7 days depending upon climatic condition. Duration of flowering

is month in mustard crop (Goswami and Khan, 2014). Mankar *et al.*, (2007) observed that stigma in *Brassica* may remain receptive 6–8 days after anthesis though, the degree of receptivity reduced drastically (60 %) after 3 days. Rai (1991) reported that stigma in *Brassica* remains receptive for 6 days.

TABLE 1. Stigma receptivity by visual methods

Age of stigma	% age receptivity By visual method
3 days before anthesis	9
2 days before anthesis	21.43
1 day before anthesis	45.78
On the days of anthesis	100
1day after anthesis	71.87
2 day after anthesis	21.4
3 day after anthesis	4.77

Effect of modes of pollination

Three pollination treatments were applied on mustard flower viz. hand pollination, controlled pollination and open pollination and their impact/effect on seed set was recorded. Mustard crop in open pollination recorded significantly highest seed set 77.24 percent followed by in

hand pollination (52.50 %). The minimum seed set (25.53%) was observed when flowers were bagged at bud stage (controlled pollination) (Fig 4). This study suggests that insect pollinators are playing an important role in seed set of mustard crop.

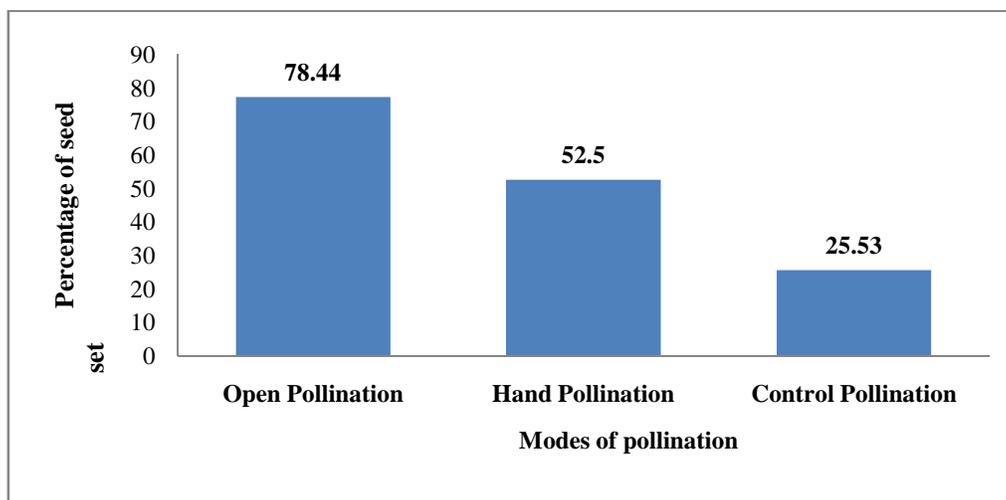


FIGURE 4: Effect of different modes of pollination on seed set

These results are in conformity with the earlier recorded observations of Tara and Sharma (2010) on *Brassica campestris* var. *Sarson*, which revealed that seed set was less (79.96%) in controlled experiment as compared to open pollinated flowers (88.05%). Goswami and Khan (2014) also studied the impact of different modes of pollination in Mustard (*Brassica juncea* L.: Cruciferae and reported that highest percent pod set was in open pollinated (83.42%) plots followed by bee pollinated (75.41%) and caged pollinated (62.80%) and recorded an increase of 8.09% pod set in open pollinated flowers as compared to controlled ones. Similar observations were also reported by Singh (1997) on *Brassica juncea* and Singh *et al.*, (2004) on var. *toria*.

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