Pak. J. Bot., 54(3): 1073-1076, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-3(38)

THE REPRODUCTIVE BIOLOGY OF *FAGONIA INDICA* BURM. f. (ZYGOPHYLLACEAE) FROM PAKISTAN WITH SPECIAL EMPHASIS TO MODE ON BREEDING SYSTEM

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Abstract

The reproductive biology of *Fagonia indica* Burm. f. (Zygophyllaceae) reveals that it is a facultative autogamous taxon i.e. predominantly self-fertilized but also gets benefit by crossing as normal seed set was observed in both selfing and crossing. Values of pollen ovule ratio did not show the positive correlation with breeding system. Opening and closing timings of flower govern by temperature and light intensity. An initiated bud required about 19-21 days to convert into a mature fruit. The flower simultaneously serve as primary as well as secondary attractant as it offers nectar, floral odor, waxes and glowing colour, due to which a variety of insects regularly visited the flower.

Key words: Breeding, *Fagonia indica*, Pollen, Zygophyllaceae.

Introduction

The genus *Fagonia* belongs to the family Zygophyllaceae with about 30 species distributed in Africa, America, Mediterranean region and Mid-East to East (Mabberley, 2008). *Fagonia indica* Burm. f. is evenly distributed in all over Pakistan (Ghafoor, 1974). Reproductive biology has major role in genetic diversity, evolution as well as crop production as lack of proper pollination mechanism results in less reproductive rate and the reason of becoming endangered therefore, pollination biology found useful to solve various problems for many plants (Richards 2001; Xiao-Ying *et al.*, 2011; Boreux *et al.*, 2013; Rech *et al.*, 2016; Yao *et al.*, 2019). Moreover, a remarkable work on pollination biology for the different genera of the family Zygophyllaceae reported by various workers such as Ruiz-Zupata & Kalting-Arroyo (1979) observed self-compatible breeding system within *Guaiacum sanctum*. The species of genus *Larrea* (Zygophyllaceae) was studied to characterize and compare the floral structure and development (Rossi *et al.*, 1999). Moreover, mode of pollination i.e. self or cross pollination was studied for *Tribulus terrestris* and results showed both phenomena within the species (Reddi *et al.*, 1981; Ganaie, 2011). Similarly, Yankova-Tsetkova *et al.*, (2011) studied reproductive biology of *Tribulus terrestris* and estimated high pollen and embryo viability. Later on, Naghiloo *et al.*, (2019) studied the effect of flower structure and shape of *Reopera* spp. (Zygophyllaceae) on foraging activity of *Apis mellifera*. They concluded that position of reproductive organs affects the pollination accuracy and efficiency. Similarly, Naghiloo & Siahkolaee (2019) studied the effect of breeding system on pollen morphology within Zygophylloideae (zygophyllaceae). They found that large sized pollen was produced in self pollinating species and small sized pollen was associated with cross pollination. Further, Sandoval-Molina *et al.*, (2020) recorded the *Apis mellifera* and *Exomalopsis zexmeniae* as pollinators of *Kallstroemia pubescens* (Zygophyllaceae). Inspite of the presence of the above reports there is no detailed report available on reproductive biology of *Fagonia indica*; therefore the present study was carried out for determining the mode of reproduction and insect's behavior on *Fagonia indica* from Pakistan.

Materials and Methods

Study sites: Present studies were conducted within the vicinity of Karachi i.e., Karachi University employees cooperative housing society, Gulzar Hijri Karachi, Department of Chemistry (University of Karachi), Department of Applied Physics (University of Karachi), Botanical Garden (University of Karachi) and Near Silver Jubilee Gate University of Karachi.

Floral phenology: To determine the phenological changes from initiation of bud up to fruit formation, 5-10 young floral buds/population were tagged.

Insects (visitors): Flower visiting insects were observed for their foraging behavior. Insects were collected by hand net and chloroformed and observed microscopically for the pollen load. The insects carrying pollen were evaluated as pollinators.

Breeding studies

Pollen–ovule ratio: The flower buds were collected prior to anthesis and pollen ovule ratio was determined by dividing the total number of pollen grains/flower by the total number of ovules/flower (Cruden, 1977) and following counts were made (i) Total number of anthers/flower (ii) Total number of pollen grains/anther (iii)Total number of pollen grains/flower (iv) Number of ovaries/flower (iv) Number of ovules/ovary (v) Number of ovules/flower.

Bagging experiments: Following pollination treatments were given in flowering bud stage (8-12 for each treatment).

Control (Open pollination): Buds were tagged and left to study the normal seed set.

Direct autogamy: Buds were bagged without manipulation to determine the self-pollination.

Indirect autogamy: Buds were hand pollinated and bagged to test the self-pollination.

Apomixis: Buds were emasculated and bagged to test the apomixis.

Geitonogamy: Buds were pollinated by hand with the pollen grains of the other flowers of the same plant and bagged to test the geitonogamy.

Xenogamy: Buds were cross pollinated by hand with the pollen grains of different plants of the same species and bagged to test the xenogomy.

Observations and Results

Floral phenology: The initiated bud of *Fagonia indica* took about 19-21 days to complete its floral phenology. Initially, bud was directed upwards and covered by green colored calyx; this condition continued for about 3-4 days, bud increased its size during 4 days. After 4 days pedicel and white petals arised, within 5-6 days bud fully matured and pinkish-purple petals exposed. Flowers opened early morning at about 06:00-07:00 am, and then closed nearly 11:00-12:00 pm, the petals also change colour from pinkish to white. Opening and closing time of flower showed that it was light intensity and temperature dependent and flower survived for about 24 hours, after that floral parts (corolla and stamens) shed and formation of fruit was started. After 6-7 days, mature fruit was formed which was light brown in color and directed downwards. Floral nectaries were present beneath the ovary.

Pollen-ovule ratio (P/O): The mean pollen ovule ratio was found 2107 and according to Cruden's classification (1977) observed value indicated it was a xenogamous taxon (Table 1).

Bagging experiments: Fruits were normally formed in all pollination treatments except the apomixis. Although control, direct and indirect autogamy showed highest seed set compared to the geitonogamy and xenogamy (Table 2; Fig. 2).

Insect's behavior: Different varieties of insects mainly including *Apis mellifera, Sarcophaga* sp. and *Cupido comyntas* regularly visited the flowers of *Fagonia indica*. Mainly insects visit the flower in search of food (pollen or nectar), whereas some insects were also attracted due to the presence of glowing color and fragrance of the flower (Fig. 1).

Apis mellifera: *Apis mellifera* (Honey bee) was found to be the most common and effective pollinator of *Fagonia indica.* Bee visited the flower just prior to the opening of flower. Alighted on the top of the flower for about 60 seconds, grabbed the anthers with the help of legs and entered its proboscis towards the base of ovary (Fig. 1 A-D). Bee revolved and twisted around anther in this manner pollen grains adhered to abdomen, thorax and legs, after that bee moved to another flower within same population which favors the chances of crossing.

***Sarcophaga* sp.:** *Sarcophaga* sp. (Flesh fly) was also observed as the pollinator of *Fagonia indica*. Flies visited the flower in the same manner as honey bee but the duration of visiting the flower seems to be shorter than bee, observed about 30-35 seconds. After visiting 4-5 flowers of a population, flies moved towards another population (Fig. 1 E-H).

***Chilades parrhasius*:** *Chilades parrhasius* (Moth) seemed to be a visitor of *Fagonia indica*. Moth started its visitation around 09:30-10:00 am, alighted on petals without touching reproductive organs in search of nectar. Visit duration was observed about 10-15 seconds (Fig. 1 I).

Table 1. Pollen ovule ratio of *Fagonia indica*.

Populations	Total no. of anthers/flower	Average no. of pollen/anther	Average no. of pollen/flower	Average no. of ovaries/flower	Average no. of ovules/ovary	Pollen-ovule ratio	Mean pollen-ovule ratio
1	10	2080 ± 23.30	20800 ± 233.0	1	10	2080	
2	10	2100 ± 26.07	21000 ± 260.7	1	10	2100	2107
3	10	2349 ± 35.53	23490 ± 355.3	1	10	2349	
4	10	1900 ± 11.36	19000 ± 113.6	1	10	1900	

Table 2. Effect of various pollination treatments on fruit set in *Fagonia indica*.

Population	No. of buds	Fruit sets					
		Control	Direct autogamy	Indirect autogamy	Apomixis	Geitonogamy	Xenogamy
01	10	10	10	06	00	04	00
02	12	12	10	08	00	02	02
03	10	10	08	04	00	00	00
04	08	05	08	06	00	02	04

Fig. 1. Insects visiting flowers in search of food and collecting nectar. A-D, *Apis mellifera*; E-H, *Sarcophaga sp*; I, *Chilades parrhasius*

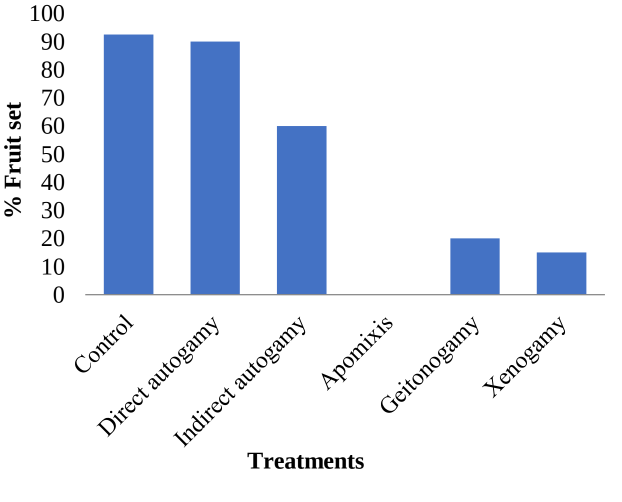

Fig. 2. Percentage of fruit set among different pollination treatments.

Discussion

The floral phenology indicates that the flowers of *Fagonia indica* persist for about 24 hours and requires 19-21 days to become a mature fruit from an initiated bud. Flowering period remains throughout year and flower opening and closing depend on light and temperature. Frequent insects visits were observed during high temperature and high light intensity compared to shady areas that might be due to high production of nectar, as it was previously suggested that environmental factors like light intensity, humidity or temperature could affect the nectar production (Petanidou, 2007; Jones & Koptur, 2015). Similarly, the fragrance and pinkish-purple flower colour might act as the signal to the insects (Proctor & Yeo, 1973) as the osmophores were frequently detected on petals and all the floral parts. Therefore, both the secondary attractants may advertise the presence of nectar that oozes out from the nectaries present beneath the ovary. *Apis mellifera, Sarcophaga* sp. and *Chilades parrhasius* were frequent pollinators and they visited the flower for sucking the nectar and visitation started just after the opening of flowers and continued till about 2 hours. Whereas, *Chilades parrhasius* also visited the flower for getting the nectar but it did not touch the pollen grains. So, it is assumed as visitor instead of pollinator. Bagging experiments proved that the species is predominantly self-fertilized with slight mode of crossing. Previously it was suggested that pollen ovule ratio is a general indicator of breeding system (Cruden, 1977; Jurgens *et al*., 2002; Jurgens & Witt, 2014; *Abid et al.*, 2010). However, present finding showed that there was no positive correlation between pollen-ovule ratio and breeding systems as the values of pollen-ovule ratio were quite higher (2107) in *Fagonia indica*. Higher values of pollen-ovule ratio normally represents the out crossing (Cruden, 1977). While our findings may be well supported by the findings of Lozada-gobilard *et al*., (2019) where they also found the negative correlation of pollen-ovule ratio and breeding system in the family Balsaminaceae. Thus based on bagging experiments *Fagonia indica* seems to be a facultative autogamous taxon that gets benefit by both selfing and crossing.

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(Received for publication 22 November 2019)