RESEARCH ARTICLE

OVULE ABORTION AND ITS POSSIBLE IMPLICATIONS IN *FUMARIA INDICA* (HAUSKN.) PUGSLEY – AN ANNUAL WEED

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Abstract: Our studies on the breeding systems and pollination biology of number of annual weed species of Jammu region of J&K, India has revealed most of them to be annuals, sexually reproducing and selfers. Majority of them are prolific fruit and seed producers. Predominant / obligate selfing enables these plants to set seeds even in uncertain pollinator conditions, helping both in their survival and rapid spread. Two plant species of this group namely *Trifolium dubium* Sibth and *Fumaria indica* (Hauskn.) Pugsley however defy these generalisation. Plants of these species like their other counterparts are selfers with high fruit set. Seed set in these two species however averages below 50 %. This dip in seed set is because they invariably abort a proportion of their ovules. The abortion is mostly prefertilization and fruits are invariably one seeded. The details of the studies in different populations of *Fumaria indica* point towards this mechanism to be universal, although the pattern differs. Investigated abortion seems predetermined and is uniform indicating it to be genetically determined.

Keywords: Cleistogamous, *Fumaria indica*, *In situ* pollen germination, Ovule abortion

INTRODUCTION

Weeds are unique in being opportunistic, highly competitive, and in their capability to thrive well in diverse types of habitats including the disturbed ones like agricultural fields, waste lands and lawns. They exhibit diverse life forms; majority are however annuals. In a large proportion of these annual weed species, vegetative propagation is absent; these reproduce sexually and thus get a single chance to produce seeds in their life; their survival and spread being threatened if they miss this opportunity [1] [2] [3]. An important strategy required in this group of plants is thus a form of reproductive assurance which enables them to set seeds even in uncertain pollinator environments. Selfing has evolved as the predominant system in these weeds. Prolific seed production by resorting to autogamy is an important adaptation in many of these weed species. It helps in their invasion and rapid spread both, once they enter a new area [4] [5] [6] [7] [8] [3] [9]. Annual weeds are thus mostly selfers and high seed setters.

Our work on a group of annual weeds forming overlapping populations in Jammu province of J&K state India has revealed some interesting results. Most of the investigated weeds follow the general rule in being selfers and prolific fruit and seed producers. These include *Mazus japonicas* Thunb., *Medicago polymorpha* L., *Stellaria media* (L.) Vill., *Trifolium fragiferum* L. and *Vicia hirsuta* Gray [1] [2] [10]. Two of the common annual weeds of the area however defy this trend. These are *Fumaria indica* and *Trifolium dubium* both lack vegetative propagation and are selfers with high fruit set (averaging above 80 %). Seed set is however below 50 % in them. A proportion of flowers in these species display *in situ* pollen germination and a sizable number of ovules in both of them show prefertilization abortion. Patterns of abortion and some more details of fruit and seed set in *Trifolium dubium* has been published by us [11] [12]. Present communication describes the details of reproductive events in *Fumaria indica* which has been studied in detail recently.

MATERIAL AND METHOD

This study is based on three populations of *Fumaria indica* all sprawling in Jammu district of J&K state, India.

Morphology

In order to study the vegetative and floral morphology, the plants and flowers were tagged in the field itself at three distinct sites, named as JP1, JP2 and JP3. All these populations were separated by a minimum distance of 1000 m from each other. Data was collected on various morphometric aspects of the plants. Reproductive features including length of the pistil, length of stamens, length of anthers, length of flower, length of outer and inner petal pair and length of sepals was studied in laboratory. All these measurements were obtained using a scale and/or stage micrometer.

Anthesis and anther dehiscence

Observations on anthesis and anther dehiscence were made in the field at regular intervals of time. Inflorescences and flowers were regularly monitored throughout the day and over the blooming period to record the time of peak flowering and for the time taken by the individual inflorescence or flower to bloom. Flowers of different sizes were collected before and after anthesis to check anther dehiscence.

Stigma receptivity

It was checked from stigmas of different ages fixed in a mixture of three parts of absolute alcohol and

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one part of acetic acid for 6 -8 hours. Fixed stigmas were washed in distilled water and stained in a mixture consisting of lactic acid, distilled water, glycerine and phenol in ratio of 1:1:1:1 [10]. These were then mounted in lactophenol. The stigmas with germinating pollen grains attached to their surface or showing copious exudations were considered receptive.

Ovule abortion

Pistils were dissected out from the flowers, fixed in Carnoy's fixative for 24 hours and transferred to 70% alcohol. After 24 hours, the carpels were thoroughly washed in distilled water and cleared in 4N NaOH in an oven maintained at 60°C. These pistils were washed and stained with mixture as specified for stigma receptivity and mounted in a drop of lactophenol on a clean glass slide. The prepared slides were observed for the position and number of ovule developing/aborting within a carpel under compound microscope. Ovules appearing flaccid and shrunk and reduced in size were treated as aborted. All these readings were then clubbed to determine the ovule abortion rate and patterns [12].

Fruit and seed set

In order to estimate the reproductive potential, the inflorescences were initially monitored for number of flowers per inflorescence and then for number of fruits per inflorescence. Solitary flowers were also monitored individually for fruit set. Fruits were later collected and studied for number of seeds. Percentage fruit and seed set, was calculated as per [1].

RESULT

Plant and floral morphology

Plants of *Fumaria indica* (Fumariaceae) are annual herbs, suberect and diffusely branched. Flowering in them occurs during winter months of December to April [13], when the temperarture in the area of study fluctuates between 4.3 °C to 29.5 °C. Plants bear small flowers aggregated into racemes, each of which is terminal and axillary consisting of 8- 21 flowers (Figure 1a). Flowers are zygomorphic, hermaphrodite, 4- 6.5 mm long, pale pink with purplish tips. Each flower comprises of two scale like ovate sepals with medianly placed fimbriated margins. Petals four in number are present in two whorls erect and united at the base. One of the outer petal is spurred at the base. Inner two petals are joined at tips and enclose the reproductive apparatus completely. The stamens are six in number and diadelphous, three on each side of the carpel (3+3). The middle anther of each set is dithecous while two laterals are monothecous. One of these bundles is produced into a basal nectariferous gland enclosed inside the petal spur. Each stamen has got a winged base and anthers are small, averaging only 315.2 ± 1.9 µm in size. Pistil 3.8± 0.1 mm to 4.1± 0.1 mm long consists of ovary, thread like style and bilobed, wet stigma. It remains surrounded by anthers (Figure 1b). The style is curved so as to bring the stigma close to anthers. The ovary is unilocular with 1- 4 ovules (averages x = 2.2) (Figure 1e). Stigma is non-papillate and wet (Figure 1c). Pollen output per flower averages 217.05 ± 23.23. Pollen ovule ratio thus has an average value of 104.43: 1

Pollination mechanism

Dehiscence of anthers in the flower usually occurs before the opening of lower lip of outer petal. Only lower lip of the outer petal is seen opened in mature flowers. The flowers can thus be termed cleistogamous. Stigma receptivity initiates along with anther dehiscence, it is retained for 1 – 2 days. The period of anther dehiscence and stigma receptivity thus overlaps to a great extent. Floral architecture, placement of anthers and stigma close to each other, and the curvature of style at top allows stigma to be clogged by self pollen as soon as the anther dehiscence occurs. Pollen germination on self stigma surface is high and averages 92.87±1.65 %.

During the observation period, (2014-2016), plants of *Fumaria indica* were never observed to be visited by any insect.

Of a sample of 150 flowers of the species studied at different timing of the flowering period, 34.66% showed the unique phenomenon of *in situ* pollen germination (Figure 1d). In these flowers, pollen grains germinate inside the anthers and receptive stigma gets covered with numerous germinated pollen grains. Pollen grains may come out individually or the pollen tubes formed by the grains *in situ* reach to the nearby stigma traverse the style and ovary and are available to fertilize the ovules within the unilocular ovary.

Fruit and seed set

The percentage fruit set is very high on open pollination and averages 94.61 %. Each fruit is invariably one seeded. Fruit set averages 89.47% in flowers bagged to allow autoselfing. As in open pollination fruits developed in bagged inflorescence also contain only one seed. Since the number of ovules per ovary averages 2.2 (1-4). The percentage seed set is low and averages 45.89% on open pollination and 43.42% on unassisted selfing.

Ovule abortion

Three flowers/ buds sizes were selected to see the pattern of seed set:

1. Small buds (2- 2.5 mm), unpollinated
2. Medium sized (3-4 mm), pollinated
3. Large sized > (4mm), pollinated and fertilized

Number of ovules observed in each of these bud sizes in the three different populations is tabulated (Table 2). As is evident from Table 2 majority of small buds in population JP 2 and JP3 carry 4 ovules each, others carry 2 or 3. In JP 1, however the number of buds carrying 4 ovules is less (10 %), majority of these buds have 2 ovules. As the buds grow in size, the number of ovules decreases and in a mature fertilized bud the number is invariably 1 in JP

1, 1 or 2 in JP 3 and 4, 3 or 2 in JP 2. Mature fruit is however invariably one seeded in all these plants.
Scanning of styles in mature buds reveals the presence of large number of pollen tubes in the style in all these populations, out of which only one pollen tube is seen to reach the ovary in all the pistils in JP 1 and JP 3 population (Figure 2b, 2c, 2d). In JP 2 in approximately 25 % of the ovaries multiple pollen tubes also invade the ovary (Figure 2a). Fruits however are one seeded here too.

DISCUSSION

Floral structure, events of floral biology and lack of insect visitation reveal *Fumaria indica* to be an obligated self sreproductive efficiency in this sexually reproducing species as high fruit set is assured. As such *Fumaria indica* falls in league of a number of annual selfers studied till date [14] [15] [16] [2] [10] [3].
What makes this annual, interesting is the high rate of ovule abortion occurring in the species. Ovule abortion leading to a dip in seed set is not a new observation and has been reported in number of taxa [17] [18] [19] [20] [1] [12]. A number of reasons have been cited for the same. What makes it intriguing in the present case is its association with a self pollinated species without any pollen limitation.
Observations on ovule abortion reveal that in majority of the pistils, phenomenon is pre fertilisation. However in one population some indications of post fertilization abortion are also there. Whether this abortion is a sieving mechanism or a dispersal tactic, needs to be authenticated.

Table 1. Data on reproductive parameters

S.NO	Character	*Fumaria indica*
1	Percentage fruit set/inflorescence (Open pollination)	94.61 (n= 45)
2	Percentage fruit set/inflorescence (Unassisted selfing)	89.47 (n= 45)
3	Percentage seed set/ fruit (Open pollination)	45.89 (n=150)
4	Percentage seed set/ fruit (Unassisted selfing)	43.42 (n=150)

*n= sample size

Table 2. Percentage frequency of flowers with different number of ovules

Population	Flower size(mm)	Number of developing ovules per ovary	Percentage frequency (%)
JP 1 (n=50)			
	2- 2.5	4 ; 2 ; 1	10 ; 80 ; 10
	3	2 ; 1	63.63 ; 36.36
	3.5	2 ; 1	20 ; 80
	4	1	100
	4.5	1	100
JP 2 (n=50)	2 - 2.5	4 ; 3	75 ; 25
	3	4 ; 3 ; 2	25 ; 62.5 ; 12.5
	3.5	4 ; 3 ; 2	33.3 ; 33.3 ; 33.3
	4	4 ; 3	50 ; 50
	4.5	4 ; 3 ; 2	42.8 ; 42.8 ; 14.2
JP 3 (n=50)	2- 2.5	4 ; 2	50 ; 50
	3	4 ; 2 ; 1	20 ; 60 ; 20
	3.5	3 ; 2 ; 1	16.6 ; 66.6 ; 16.6
	4	2 ; 1	25 ; 75
	4.5	2 ; 1	57.14 ; 42.85

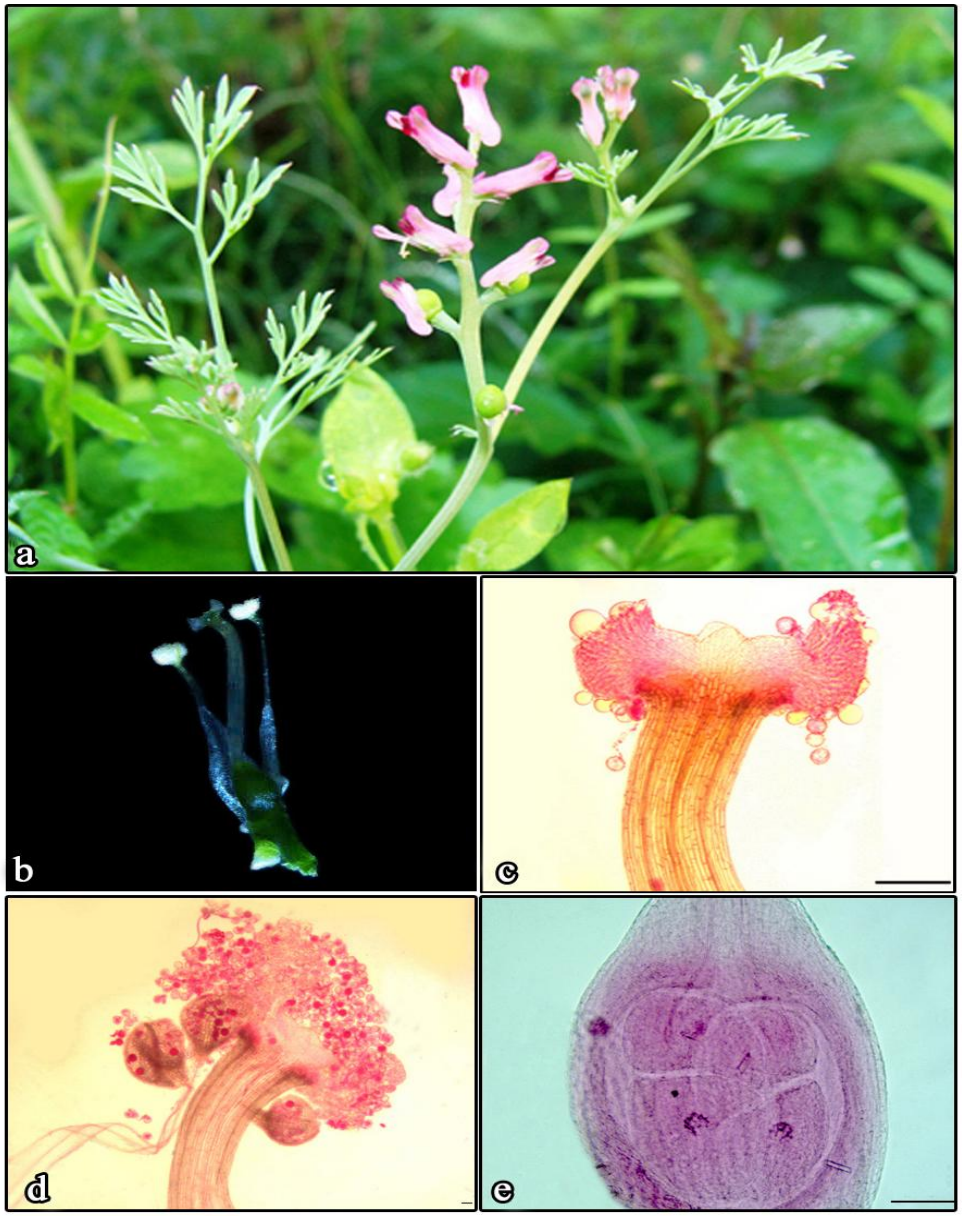

Figure 1.a. A flowering twig of *Fumaria indica* bearing an inflorescence(x1.83)
b. Reproductive apparatus of *Fumaria indica* (x0.86).
c. Enlarged view of receptive stigma of *Fumaria indica* with copious yellow exudates (Scale bar =10µm) .
d. Stigmas and anthers excised from different flowers showing *in situ* pollen germination and pollen tube invasions (Scale bar =10µm)
e. Ovary of *Fumaria indica* bearing different number of ovules. (Scale bar =10µm)

CONCLUSION

To conclude *Fumaria indica* comes out to be a obligate self pollinated species with high fruit set associated with high rate of ovule abortion also. The ovule abortion may be a pre or post fertilization event and may possibly be serving as a sieving mechanism to ensure healthy future populations.

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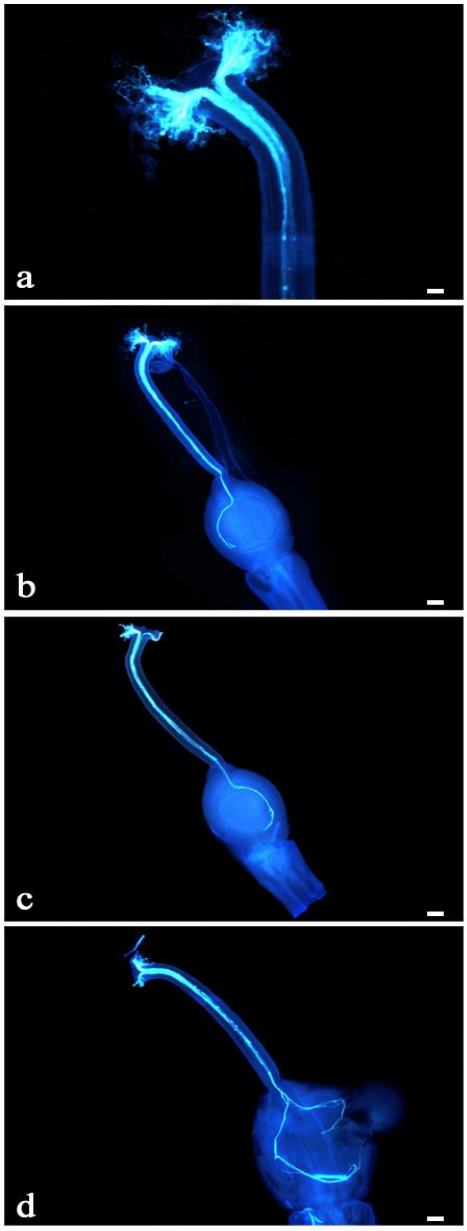

Figure 2. Fluorescence micrographs showing pollen tube growth through the style (a) and in the ovary (b,c,d) (Scale Bar= 10 µm).

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