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Floral Biology and Pollination Biology of *Cannabis sativa* L.

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ABSTRACT

Cannabis sativa L. (Cannabiaceae) commonly known as marijuana is dioecious. Maximum floral density was observed in the month of April–June in male plants and May–June in female plants. Male flowers are borne on loose panicle and yellowish green in colour, while female flower are greenish white and arranged in axillary crowded spikes. The anthesis of male flower occurs between the 1000–1100 h in February–March and anthers dehisce between 1100–1140h, while during the months between April–October, anthesis take place between 0800–0845 h and anthers dehisce between 0845– 0915 h. The anthesis of female flowers occurs between the 1030–1100 h during February– March and stigma becomes receptive between 1100–1230 h. In months of April–November anthesis takes place between 0830–0900 h followed by stigmatic receptivity between 0900–1000 h. Pollen viability was highest in the month of April. Unicellular trichomes were seen on the dorsal and ventral surface of tepals and ovarian surface. Pollen grains are 30µm in diameter and are triporate and suboblate. Number of pollen/flower 36,553 + 8.07 and number of pollen/anther is 7,256 + 9.86. There is one ovule per ovary and pollen ovule ratio is 36,553: 1. Pollination is anemophilous and fruit-set percentage is 16.6% and seed-set is 100%.

Keywords : *Cannabis sativa* L. floral biology, pollination biology.

INTRODUCTION

Cannabis sativa L. commonly known as marijuana, hemp, bhang, charas is a dioecious, aromatic annual flowering herb native of Central Asia and belongs to an extremely small family Cannabiaceae (Urticaceae) of the order urticales containing only two genera *Humulus* and *Cannabis* . The genus *Cannabis* is monotypic with one species i.e. *Cannabis sativa* L.

It is used as a remedy for asthma, bronchitis, headache, flu, epilepsy, cough and pains. In modern medicine the crude drug and some pure chemical derivatives are used for treating migraine, epilepsy, malaria, glaucoma, nausea from chemotherapy, for improving appetite in patients with cancer, AIDS, and anorexia nervosa and for suppressing muscular spasms in multiple sclerosis (Van Wyk 2000). The compounds which compress the active drug ingredients are Cannabinoids exist in form of carboxylic acids (Masoud & Doorenbos 1973, Small & Beckstead 1973, Turner *et al* . 1973). There are over 60 of these types of compounds present in the plant (Turner *et al* . 1980).

It grows wild In Northern India and Due to rapid urbanization and over exploitation for its use as a hallucinogenic agent, it is being ruthlessly cut and in years to come it may disappear from waste lands. In order to conserve this medicinally important plant the studies on its reproductive biology are essential (Moza & Bhatnagar 2007) and present paper is an attempt in this direction.

MATERIALS AND METHODS

Phenology and reproductive biology of *Cannabis sativa* L. was studied on plants growing in the fields at five sites in Agra. Plants were also raised in the Botanical gardens of School of Life Sciences, Agra. To evaluate the floral morphology and floral density, the 250 flowers of 25 marked inflorescence were counted during different seasons. Floral dimensions of 25 flowers on 10 plants at each site were measured. Flowering phenology was studied periodically by counting flowers on marked plants throughout the

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flowering period. Floral morphology, floral biology, number of pollen grains/flower and number of ovules were studied by various methods given by Kearns & Inouye (1993). Total number of pollen/ anther and pollen/flower was measured by a hemocytometer (Barret 1985). Pollen viability was checked by 0.2% TTC solution (2, 3, 5-triphenyltetrazolium chloride). The staining solution was prepared in 10% sucrose solution. The pH was adjusted to 5.8 using 0.15 M Tris HCl buffer (Hauser & & Morrison 1964); by Fluorochromatic reaction (FCR) after Heslop-Harrison & Heslop-Harrison (1970). *in vivo* pollen germination on the stigmatic surface was also carried out by aniline blue fluorescence microscopic method (Martin 1959) as described by Shivanna & Rangaswamy (1992).

Different pollinators, their population, types and visitation rates were recorded. The morphology of different floral parts was studied by scanning electron

Fig. 1A-F — *Canabis sativa* male and female plants and their floral parts. **a.** Male plants in full bloom growing in their natural habitat. **b.** Magnified view of a branch with male plant. **c.** Magnified view of male flowers. **d.** Flowering twig of female plants.

microscopy (SEM). The fresh anthers, pistils and other parts were fixed in 3% glutaraldehyde.

Mature fruits from plants were harvested and seeds were collected. Fruit set percentage was calculated by the following formula:

$$\text{Fruit set \%} = \frac{\text{Number of fruit per inflorescence}}{\text{Number of flowers per inflorescence}} \times 100$$

OBSERVATIONS & DISCUSSION

Cannabis sativa L. is a dioecious, evergreen annual herb attaining a height of 1–6 feet (male) (Figs.1a, b, c) and 1–5 feet (female) (Fig. 1d). The flowers are imperfect either staminate or pistilate. Flowering usually occurs when the light and dark periods are more or less equal. Sometimes it is day neutral also. The flowering cycle lasts for 5-10 weeks.

STAMINATE FLOWER — The male flowers are fig shaped, borne on loose panicle, incomplete, actinomorphic, and are yellowish green in colour (Fig. 1b). Perianth consists of five yellowish tepals, polyteplaous with sparse prostrate hairs and show imbricate aestivation. Stamens 5; opposite to tepals; and get caducous after anthesis (Fig. 1c). Filament short, erect, anther oblong, dorsifixed; dithecous dehiscing via longitudinal slit releasing pollen (Fig. 2a), introrse, 2-loculed. The anthesis of male flower occurs between the 1000–1100 h in February–March and anthers dehisce between 1100–1140 h. During the months of April–October, anthesis takes place between 0800–0845h and anthers dehisce between 0845– 0915 h by a longitudinal slit. The male flowers are small in size but produce large quantity small, light and dry pollen grains (36,553/ flower). Male plants shed pollen and die several weeks prior to seed ripening on female plants. Pollen grains are triporate, suboblate, sexine thickened at apertures with three germinal pores with 30 µm diameter (Fig.2b). The pores are circular or slightly elliptical. Their surface is smooth with a reticulate web (Fig. 2b). The margin of the pores usually appears as a rim between pores and annulus. Pores are circular or slightly elliptical (Fig.2b). During the month of April, pollen viability is highest as tested by Alexander stain (97.10%), 0.2% TTC solution (78.3%) and by FCR test (83.04%).

PISTILATE FLOWERS — Female flowers greenish white sessile, arranged in racemes and there are 5–7 flowers per inflorescence (Fig.1d). Ovary globose, superior, unicarpellary with one ovule. A single leaf like bract appeased to ovary. Placentation apical, pendulous. Stigma is V-shaped (Fig. 2c) with large number of dry papillae (Figs. 2d, 2f) to trap pollen from wind. The anthesis of female flowers occurs between the 1030–1100 h in the months of February–March and stigma becomes receptive between 1100–1230 h. In months of April–November anthesis takes place between 0830– 0900 h followed by stigma receptivity between 0900–1000 h. There are 7256 ± 9.86 pollen/anther and 36,552 ± 8.07/ flower. There is just one ovule per ovary. The ratio pollen to ovule member is 36553: 1. The ratio is an indication of anemophilous and xenogamous pollination (Cruden 1977). The stigmatic surface is covered with large number of unicellular papillae (Figs. 2d, 2f). A considerable amount of pollen grains are also observed on the stigmatic surface (Figs. 2d, f). There is one ovule in each locule of the ovary. A large number of trichomes with swollen base and several peltate scales are present on various floral and extra-floral parts. They are present on tepals, ovary and style (Fig.2 e). Dayanadan & Kaufman (1976) have observed stalked glands covering the tepals and massively stalked glands on the stamen filament.

In vivo pollen germination on stigmatic surface has revealed that a considerable number of pollen-grains are deposited on the stigma and they germinate readily showing 78-85% germination with considerably long pollen tubes to accomplish fertilization. Since *Cannabis sativa* is a dioecious plant therefore, pollination is xenogamous. The male plants are visited by various insects e.g. black ants, small bees, and housefly. It is interesting to note that these insects do not visit female plants. The pollination is anemophilous.

The number of fruits per inflorescence is 4-6 and the fruit-set percentage is 16.6%. The fruit is achene, oval in shape and unripe fruits are green, but turn brown on maturity. The fruit set percentage is low due to its dioecious nature and anemophilous its anemophilous mode of pollination. A dioecious species generally has a lower mating chance than both self- and self-incompatible hermaphroditic species (Tainaka & Itoh 1996). There is only one seed per fruit and the seeds are small, smooth, endospermic and brownish in colour with a diameter 3.87 ± 0.105 mm.

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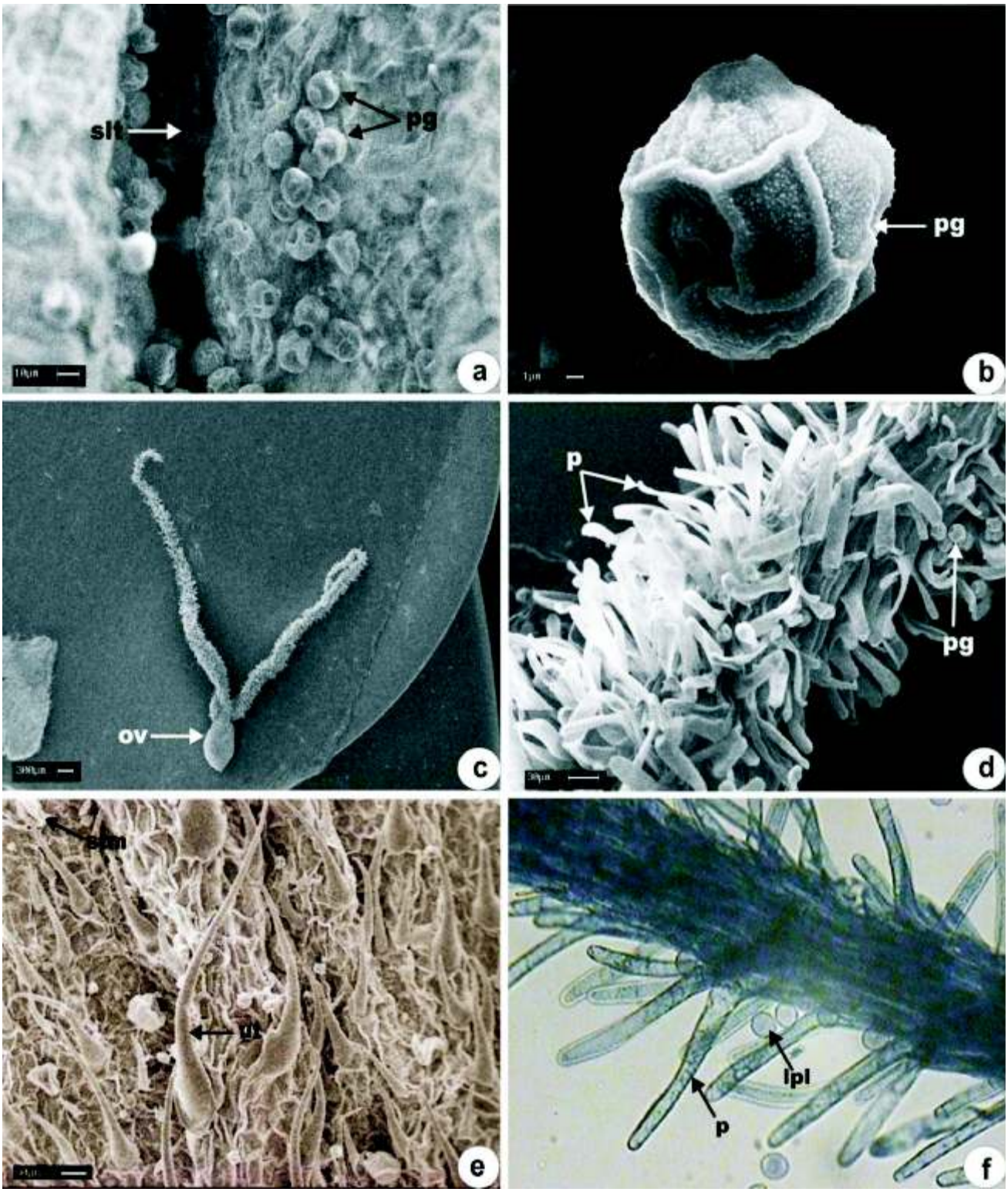

Fig. 2A-F — Scanning electron microsco-photographs of male and female reproductive parts. **a.** Dehisced anther with a slit (slt) releasing large number of pollen grains (pg). Bar=10 µm. **b.** Single triporate pollen with a distinct germ pore (arrow). Bar=1 ìm. **c.** Single pistil with 'v' shaped papillate stigma and small ovary (ov) at the base. Bar=300 µm. **d.** Papillate stigma with pollen grains (pg). The papillae (p) are unicellular and compactly arranged. Bar=30 µm. **e.** Dorsal surface of tepal showing unicellular trichomes (ut) with swollen base and peltate scales. Bar=30 µm. **f.** Light microscopic photograph of the papillate (p) stigma with pollen grains (lpl). 720X

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