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Floral biology, Pollination and Breeding system in *Alcea rosea* (L.) syn. *Althaea chinensis* Wall. (Malvaceae)

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ABSTRACT

Present communication deals with the floral biology, pollination and breeding system in *Alcea rosea* (L.) syn. *Althaea chinensis* Wall. (Malvaceae). It is annual, biennial, or perennial plant usually erect, unbranched and about 1-3 m tall. Inflorescence primordia develop in the first week of January and flower buds start opening during the first week of February. The full bloom occurred in the second week of March and lasted until the end of April. Inflorescence is a spike-like raceme and axillary solitary flowers are also produced from the axils of the upper leaves. Flowers are pentamerous with a columnar structure in the center with numerous stamens which coalesce with filaments, constituting a staminal tube which encloses the gynoecium with thread-like stigmas at the tip. Stamens are monadelphous, anthers reniform, and monothecous. Gynoecium consists of numerous fused carpels with a disk-like ovary and stigma in equal number to the carpels. Anthesis occurred 28±4 days after initiation of bud at 1200 h. The flowers are protandrous and the anthers dehisced by longitudinal slit a day before anthesis. The number of pollen grains in each anther is 94.2±2.7. Pollen viability is 88.2±12.5%. Pollen grains are echinate and dimorphic. The number of ovules/flower are 30.8±5.5 (n=25) and the pollen ovule ratio is 952:1. The flowers are often visited by various pollinating insects. The most frequent visitors are carpenter bee (*Xylocopa* spp.) and honeybees (*Apis cerena indica*). They visit to extract nectar and bring about pollination also. Small black ants (*Formica* spp.), red cotton bug (*Dysdercus koengii*) and butter flies [*Graphium doson* (C. & R. Felder) and *Graphium chironides* (Honrath)] also visit flowers, but they are nectar robbers only. The stigma becomes receptive after 45±5 h of anthesis and remains receptive for 24±2 h. Fruit set percentage in open pollinated flowers is 93±5; by self-pollination (unemasculated and bagged flowers) it is 25.3±5.2% with 0.44 self incompatibility index. Fruit set percentage was 43.6±8.2 and 56.4±14.2 by getinogamy and xenogamy respectively. Thus, *Alcea rosea* is predominantly out-crossing but in the absence or scarcity of pollinators it produces fruits by selfing also.

Keywords: *Alcea rosea* (Linn.) syn. *Atlthea chinensis*, Phenology, Pollination, breeding system.

INTRODUCTION

The Malvaceae is a family containing over 200 genera with close to 2,300 species (Tang *et al*. 2012). Earlier, Wroblewska (2009) have reported only 70 genera and over 100 species. These occur both in the semi-desert tropics or subtropics as well as in temperate climatic zones. *Alcea* commonly known as hollyhocks is

a genus of about 60 species of the family Malvaceae and are native to Asia and Europe (Tang *et al*. 2012). *Alcea rosea* syn. *Althaea rosea* is a very popular ornamental plant grown for its showy flowers of various colours. Apart from having ornamental value, it has several medicinal uses. Roots are considered as an astringent and used to cure dysentery and fever, jaundice, stomach, urinary ulcers and in liver disorders (Steward 1977). The flowers are diuretic, demulcent and used as tonic as well as for soothing skin, and in chest complaints (Scott 2007). It is also used in the treatment of irritable forms of spermatorrhea and chronic sensitiveness of the prostrate. It is given in rheumatism also. Its seeds are also demulcent, diuretic and febrifuge. The present study covers the structural and temporal details of flowers, pollination and breeding system in *Alcea rosea* syn. *Althaea chinensis*.

MATERIALS & METHODS

Present study was undertaken during two vegetation seasons in the years 2006 and 2007 at Kota, Rajasthan, India (75º37' to 77 º 26' East longitudes and 24º25' to 25º51' North latitude).

Floral biology — Observations were recorded every day between 0600-1000 h during the entire flowering period from 20 marked plants growing in the Garden of C. B., Office of Chhatra Bilas Garden, Kota, beautifully landscaped garden. The average number of flowers borne on an inflorescence was recorded from a set of randomly tagged flowering branches (n=25) following the procedure after Dafni (1992). Floral development was recorded in marked floral buds (10/plant). The dimensions of 100 flowers and their parts from the marked plants were measured with a vernier caliper. Subsequent events such as fruit maturation and fruit dispersal were recorded once a week.

Flowering phenology — Date of initiation of flowering, optimum flowering and end of flowering, fruiting period were recorded. The data on sequential development of buds, into flowers, floral morphology and flowering phenology, fruits and seeds were recorded from the buds tagged (n=250) on marked plants. Number of open flowers/plant on each day and flower longevity was determined by the method after (Gill *et al.* 1998). Anther dehiscence and stigma receptivity were recorded two days before and two days after anthesis. Stigma receptivity was determined by recording fruit formation in hand pollinated floral buds (n=100) at different stages of development.

Pollen production & viability — Mature anthers were crushed in lactophenol-glycerine with aniline blue. A known dilution was placed on the grid and 10 replicate counts were made using a hemocytometer (Barret 1985). The pollen viability of plant was checked by by 0.2% TTC (2, 3, 5 triphenyl tetrazolium chloride) solution at 5.8 pH using 0.15M Tris-HCl buffer (Hauser & Morrison 1964).

In vivo pollen germination — Pollen germination in stigmatic and stylar tissue was verified by staining technique after Alexander (1987).

Number of ovules/ovary — Stain-clearing technique after Stelly *et al*. (1984) was used to count the number of ovules/ovary. Ovaries fixed in FAA were stained in Mayer's hemalum and cleared in methyl salicylate and xylene mixture. Number of ovules/ovary were counted from the cleared ovaries (n=20). Pollen-ovule ratio was calculated following Cruden's (1977) method.

Nectar — Nectaries on different floral parts were located by the method after Meeuse (1982). Small pieces of floral parts were dipped in neutral red solution (1:10,000) in distilled water for 2-3 h and examined under microscope. Red spots appeared on the floral parts indicating the presence of nectaries.

Breeding system — Hand pollination experiments were conducted. Marked floral buds (n=25) were left for open pollination. Emasculated flowers were pollinated with pollen collected from fresh dehisced anthers from flowers of the same plant (geitinogamy) or from flowers of different plants (xenogamy). For emasculation, the method described by Chandrasekharan *et al*. (1960) was followed. The sepals and petals were removed from the selected floral buds (n=25). The staminal tubes were covered with the polythene straw (used for drinking soft drinks) leaving the stigmas exposed. The straws were cut slightly larger in the size of staminal tubes, inserted on the tube and sealed on the top to cover the young un-dehisced anthers. In order to find out self-pollination, calyx and corolla were removed from the floral buds and the staminal tubes (with both stamens and stigmas) thus exposed were covered with butter paper bags (n=25). Percentage of fruit formation was recorded in open pollinated and hand pollinated flowers.

Pollination biology — Observations on the floral visitors and their foraging behavior were carefully made during entire flowering period at regular intervals throughout the day following the procedure after Faegri & van der Pijl (1980).

Statistical analysis — Results obtained were statistically analyzed by calculating standard deviation.

RESULTS & DISCUSSION

Habit — *Alcea rosea* syn. *Althaea chinensis* are annual, biennial, or perennial plants usually erect, unbranched or sparingly branched and about 1-3 m tall (Fig. 1). The stems usually have a coating of star-shaped hairs. The blades of the alternate leaves are up to 15-20 cm long and 10-15 cm across and are palmately lobed (with 3-7 blunt lobes each) and crenate along their margins. The upper surface of leaf blade is moderately green, slightly pubescent to hairless, and wrinkled from fine veins; the lower surface is light green and pubescent. The petioles of the leaves are as long as or a little longer than their blades; they are light green and hairy.

Phenology — Plants of *A. rosea* marked for phenological observations (n=20) showed not much variation with respect to flowering, fruiting and leaf fall. Leaves attained their maximum size by December. The inception of inflorescence primordia occurred in the first week of January. The flower buds are green, hairy, grooved and commenced opening during the first week of February (Fig. 1). The full bloom occurred in the second week of March and lasted until the end of April and a plant produces 150±50 flowers (Fig. 1). The length of blooming of a single flower is 3±1 days. Wroblewska (2009) observed that *Alcea rosea* in Lublin, Poland flowers during the months of June-September and a plant on an average produces 659 flowers in a vegetation season.

Floral biology — The central stem terminates in a raceme of flowers; axillary solitary flowers are produced from the axils of the upper leaves as well. The average number of flowers born on raceme is 14±1.38. The youngest visible bud was 0.15±0.05 cm in size, grooved, hairy and light green in colour (Fig. 1). Fully open flowers are large and about 7.5±0.5 cm wide and 8.28±1.5 cm long. Flowers are pentamerous, hermaphrodite, hypogynous, and infundibuliform. Each flower has 6-9 sepal-like bracts, 5 sepals, 5 petals, and a columnar structure in the center with the reproductive organs (stamens on the central part and thread-like stigmas at the tip). The epicalyx of each flower are located underneath the sepals; they are light green, hairy, ovate, and joined together at the base. The sepals are light green, ovate, and much smaller than the petals (2.14 ± 0.05 cm × 0.86 ± 0.02 cm). The overlapping petals provide the flower with a funnel form shape; they are white, pink, or purplish red (5.3 ± 0.06 cm. × 5.56 ± 0.16 cm) (Figs. 3 & 4). The protandrous flowers are characterized by the presence of numerous stamens which coalesce with filaments, constituting a staminal tube which encloses the gynoecium (Fig. 2a; b). Staminal column is hollow with an apical pore, glabrous, 1.48 ± 0.25 cm long. Stamens are monadelphous, anthers reniform, monothecous and there are 259±51 stamens/flower (Fig.2b). Anthers are clustered at apex, creamy yellow (Fig. 2c). Gynoecium consists of numerous fused carpels with a disk-like ovary (2.81 ± 0.07 cm × 0.77 ± 0.02 cm at maturity). Number of stigma is equal to the number of carpels and stigma are cream coloured, hairy at one margin facing towards center and soon after the release of pollen, the stigmas overgrow the staminal tube (Fig. 2d). In the absence of pollinators, the stigma curl down and come in contact with the dehisced anthers (Fig. 2e). Shome *et al.* (1991) have made a comparative study to distinguish morphological features of *Aclea officinalis* and *Alcea rosea* syn. *Althaea rosea*. Mehrotra *et al.* (1999) have made detailed pharmacognositic studies on the flower of *Alcea rosea* syn. *Althaea rosea*. Nectaries are present in five pits, each of which occurred by the side of the base of each petal (Fig. 5) and protected by hairs on the lower margin of petals. The structural organization of flower of *A. rosea* is comparable with that of other members of Malvaceae, indicating that these features are conserved (Kumar *et al.* 2011). Their nectariferous, large flowers with gaudy corollas attract insects. Protandrous flowers in several members of family Malvaceae have earlier been recorded (Faegri & Piji 1980, Duwar *et al.* 1994, Abid *et al.* 2010 and Kumar *et al.* 2011.

Flowering phenology — Anthesis occurred 28±4 days after initiation of bud at 1200 h. The flowers are protandrous and the anthers dehisced by longitudinal slit at the loose bud stage, just prior to opening of petal a day before anthesis (Fig. 2c) and release large quantity of pale yellow pollen on the cup like corolla. The release of pollen starts by the stamens located at the upper part of the androecium. Maturation and anther dehiscence proceeded towards the base of the staminal tube. The number of pollen grains in each anther is 94.2±2.7. Pollen viability is 88.2±12.5%. Pollen grains are echinate and dimorphic. Larger pollen grains are 136 ± 2.35 µm and smaller 97±1.25 µm in diameter. The number of ovules/flower are 30.8±5.5 (n=25) and the pollen ovule ratio is 952:1. Initiation of pollen germination (Fig. 7) on stigma is seen after 54 h of anthesis.

Pollination — The flowers are often visited by various pollinating insects. The most frequent visitors are bumblebees or carpenter bees (*Xylocopa* spp.) and honeybees (*Apis cerena indica*) and which are interested in nectar harvest (Figs. 3 & 4). Their visiting rates increase in the afternoon. Freshly opened flower attract visitors. Nectariferous large flowers with gaudy corolla help to attract these bees. Nectar is excreted in five pits, one of which occurred by the side of the base of each petal (Figs. 4 & 5). *Xylocopa* spp. (Fig. 3) and *Apis cerena indica* (Fig. 4) land on the corolla, move inside

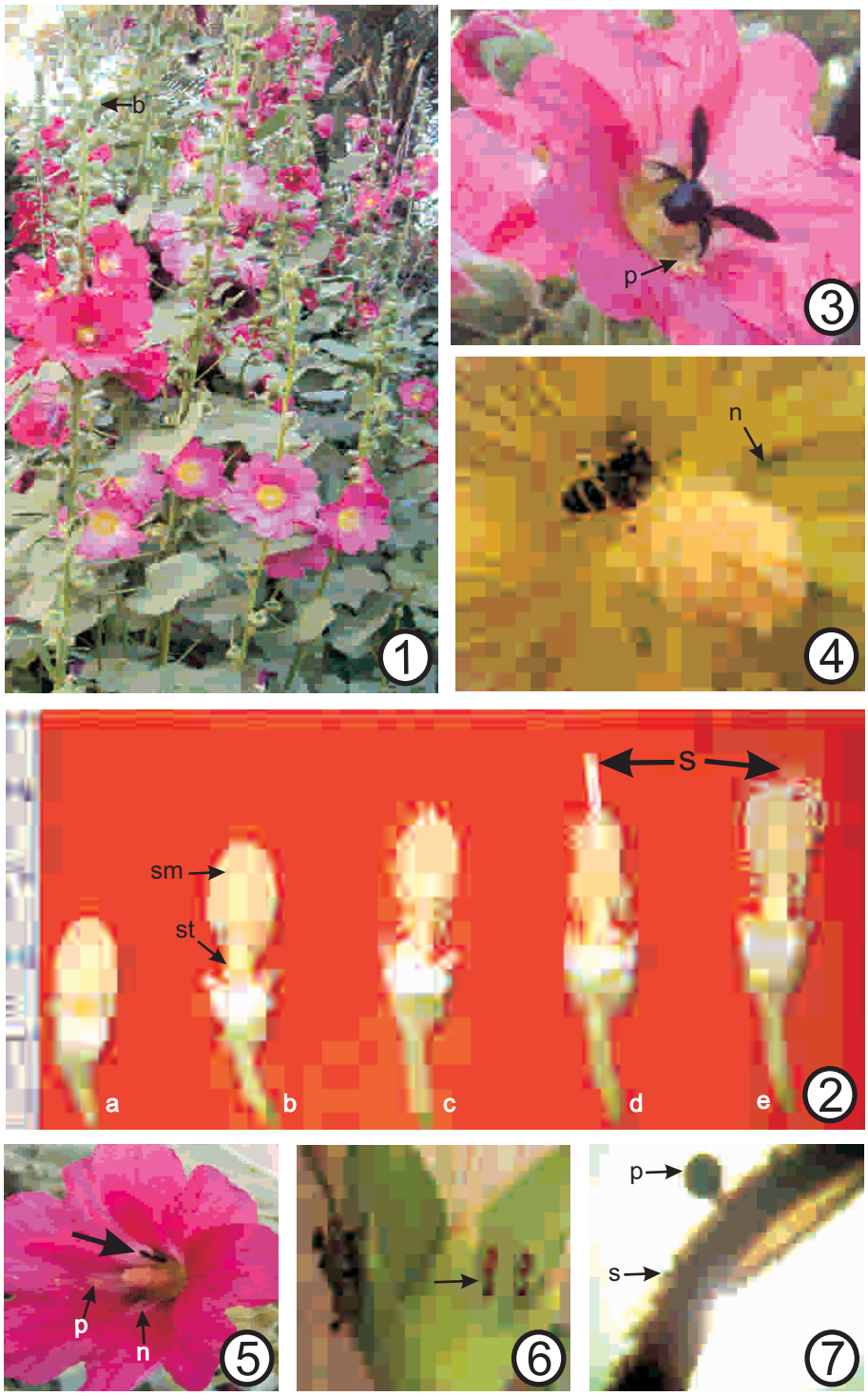

Fig. 1. *Alcea rosea* plants with pink flowers. **Fig. 2.** Reproductive structures at different stages of development (a: stamens in young floral bud; b: dehisced anthers of stamens on staminal tube a day before anthesis; c: dehisced stamens two days after anthesis; d: stigma elongating above the stamina tube and e. stigma bending down to touch the dehisced anthers. **Fig. 3.** Carpenter bee feeding inside the flower. **Fig. 4.** *Apis cerena indica* laden with pollen feeding on the flower showing nectaries. **Fig. 5.** A large black ant feeding on the nectar. Note the presence of pollen (p) on the corolla surface and nectaries at the base of petals. **Fig.6.** A group of ants on the surface of sepals (arrow). **Fig. 7.** Pollen (p) germinating on the stigma surface. (b: buds; p: pollen; n: nectar; s: stigma; sm: stamen; st: stigma)

towards the basal pits in search of nectar. While landing and searching for nectar, the dust of pollen covers their whole body parts (Fig. 4). The bees laden with pollen grains transfer these pollen grains to the stigma of other flower while landing, foraging or harvesting nectar. Bees are seen to visit the flowers in both morning and afternoon and they visit the flowers of the same plant and flowers of different plants and their foraging rates were very fast (2-5 seconds/flower). They extract nectar from the base of the reproductive column and come in contact of the dehisced anthers and stigmas.

Large & small black ants (*Formica* spp.) (Fig. 5 & 6), red cotton bug (*Dysdercus koengii*) and butter flies [*Graphium doson* (C. & R. Felder) and *Graphium chironides* (Honrath)] also visit flowers, but they are only nectar robbers. They crawl on the petals to feed nectar for several hours during the blooming period, but do not come in contact with stigma. *Dysdercus koengii* are seen on sepals and pedicels and they also do not come in contact with the reproductive parts of the flower.

Cross-pollination is favoured by protandry. In the absence or scarcity of pollinators, self-pollination is accomplished by stigmas coming in contact with anthers via style curvature (Fig. 2 e). After pollination petals shed off, sepals come close and twist to protect the young fruit. After the maturation of fruit twisted sepals get separated to disperse loose mericarps.

Pollination biology in several other members of Malvaceae has been recorded by various investigators (Vaidya 2000, Hajimi 2004, Abid 2006, Abid *et al.* 2010, Kumar *et al.* 2011). They have also found that *Apis* spp. is the effective pollinator. Interestingly, Franceschinelli (2005) has found that the species of Helicteres (Malvaceae) are pollinated mainly by hummingbirds and bats. In *H. brevispira* there is an unusual mechanism of depositing pollen grains as the hummingbirds deposit pollen under their tail or on the abdomen. Pleasants & Wendle (2010) have studied several aspects of reproductive biology, including potential pollinators, floral biology, and diurnal and seasonal flowering phenology in the endemic Hawaiian cotton, *Gossypium tomentosum* (Malvaceae). Primary visitors were introduced species, honeybees and carpenter bees, both of which were pollinating the flowers.

Breeding system — Fruit set percentage in open pollinated flowers is 93±5; by self-pollination (unemasculated and bagged flowers) it is 25.3±5.2% with 0.44 self incompatibility index Fruit set percentage was 43.6±8.2 and 56.4±14.2 by getinogamy and xenogamy respectively.

Ramirez & Navarro (2010) observed that monomorphic species of Melochia (Malvaceae) were self-compatible, while distylous species were self-incompatible. Studies made by Li *et al*. (2012) on floral morphology and mating system of *Alcea rosea* indicated that when stigmas were in contact with anthers via style curvature, stigma receptivity and pollen viability was 89.7 and 42.33%, respectively, indicating the potential of delayed selfing. It was concluded by Li *et al*. (2102) that *Alcea rosea* is dominantly out-crossing species with potential delayed selfing when pollinators are absent or scarce.

Fruits & seeds — Fruiting commences from mid February continues up to end of April It is technically, a schizocarp and carcerulus, disc shaped with persistent calyx which turn brown on ripening. Fruits mature in 15-20 days. There are fruits per raceme is 9.8±0.66. The fruit is divided into 15-20 sections the mericarps each containing a black seed. The seeds are kidney shaped, flattened, and notched on one side. The mericarps are released from the cup like structure formed by calyx. They disperse in air due to the movement of plant with air currents but do not travel much distance from the plant. The mericarps release seeds after decaying.

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