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Reproductive Biology of *Evolvulus alsinoides* L. (Medicinal Herb)

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Abstract: Reproductive biology provides information on life forms, rate of flowering, type of breeding system, plant-pollinators interaction, fruit and seed output, overall fitness and survival of the species. The present study was carried out to reproductive biology of *Evolvulus alsinoides* Linn. (Convolvulaceae) is a medicinal herb of semi arid zone of Agra in North India. The study was carried out for a period of twenty four months to observe the reproductive parameters of the *E. alsinoides* in the different places of Agra. The flowers appear from February to June and attracted the vide variety of insects for the pollination. The fruiting nature indicates that maximum number of flowers set into fruit by xenogamy as compared to geitonogamy.

Key words: *Evolvulus alsinoides*, reproductive biology, pollen morphology, pollination biology and medicinal herb

INTRODUCTION

The reproductive biology of this plant species is essential for developing effective strategies for their sustainable utilization. This recognition has made reproductive biology a frontline area of research. Research in this area has shown profound changes from the traditional methods as a result of integration of the techniques of cell and tissue culture, cytochemistry, physiology, biochemistry and molecular biology. Studies of reproductive biology provide information about the nature of species, adaptation, speciation, hybridization and systematic (Anderson *et al*., 2002; Neal and Anderson, 2005).

The knowledge of reproductive biology is a prerequisite for attempting any breeding programme. Very little is known about the reproductive biology of this rare but important medicinal herb. Some workers paid attention to the biology of reproduction in medicinal plants, it is not adequate a little attention of biology of the reproduction in medicinal plants. Recently, Wani *et al*. (2006) studied the reproductive strategies of *Inula racemosa* (Asteraceae). Moza and Bhatnagar (2007) made the protocol for conservation of threatened and endangered medicinal plants via, reproductive biology. Raimundez-Urrutia *et al*. (2008) studied the reproductive biology of morning glory *Merremia macrocalyx*. Studies on this line were also conducted in a number of medicinal plants like, Safed musli (Geetha and Maithi, 2001), *Withania somnifera* (Kaul *et al*., 2005) and *Jatropha integerrima* Jacq (Gupta *et al*., 2006).

Evolvulus alsinoides L. (Convolvulaceae) known as shankhpushpi is a perennial herb. It occurs during rainy season in gardens, lawns, agricultural fields and on road sides. It is a common wild weed but it is also cultivated for medicinal purposes. The whole plant is used medicinally and possesses antidysentric and antiseptic properties. Shankhpushpi is also known as brain tonic, increasing memory, reducing nervous debility and so on (Chermexcil, 1992). Its paste is applied externally in skin diseases. The whole plant juice is traditionally used in various mental disorders. In epilepsy and hysteria, the fresh plant juice is recommended with kustha powder and honey. It promotes intelligence, enhances memory and retention especially of school going children. In spite of these uses in medicines little attention has been paid to its floral and fruit formation. These studies are essential to understand the evolution and survival of the species to develop effective conservation strategies and optimal utilization of the economic potential of the plant resources.

Keeping the above facts in view a detailed study of the reproductive biology of *Evolvulus alsinoides* plant growing at various places of semiarid regions of Agra has been undertaken.

MATERIALS AND METHODS

The present study was carried out during 2008-2009 in the semiarid zone of Agra in North India. Flowering phenology was observed at plant and inflorescence. For the latter 20 inflorescences, selected at random from

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different plants were tagged before the initiation of flowering. These selected plants were followed daily and the number of open flowers was recorded. The tagged inflorescences were followed until they ceased flowering. Twenty-five flowers were sampled to record floral morphology and pollen characteristics. The plant produces bisexual flowers and on this basis flower ratio was calculated from the daily anthesis records (Shivanna and Rangaswamy, 1992).

The morphology of anther and pistil was studied by scanning electron microscopy. Samples were fixed in 3% glutarledehyde in 0.1 M phosphate buffer at pH 6.8. The samples were dried with CO_2 at 1000lb per inch. The samples were mounted on stubs with the help of both side adhesive tapes and coated with gold 20 mm in a SCD 0.2 sputter coating unit (Polaron equipment Ltd., Walford, England). Observation and photographs were taken, using a Philips EM 501 SEM at All India Institute of Medical Sciences, New Delhi.

The number of pollen grains per flower was determined from 25 flowers following by Cruden (1977). The pollen size was measured with an ocular micrometer under light microscope, following the procedure of Mckone and Webb (1988). The number of pollen grains divided by the number of ovules per flower yielded the pollen ovule ratio. Pollen viability was checked by 1% terazolium chloride in 0.15 M tris HCL Buffer at 7.8 pH as per by Shivanna and Johri (1985). *In vivo* pollen germination was studied by aniline blue florescence microscopic method as described by Shivanna and Rangaswamy (1992). Breeding behavior by geitonogamy and xenogamy was tested through hand pollination studies. In order to observe the rate of natural fruit set, 25 inflorescences were tagged and flowed until fruit development. The foraging behavior of different insects was studied and the pollination efficiency was checked by observing the pollen load on different body parts of the insects under a microscope, according to the procedure given by Kearns and Inouye (1993). Fruit development was studied from the day of pollination until maturation and dehiscence type of fruit was also observed. The number of fruits formed on an inflorescence was estimated by randomly counting fruit formation per inflorescence. For estimation of fruit set, few inflorescences were selected at random, tagged and observed. The percentage of fruit set and seed set were calculated by following formula:

$$\text{Fruit set (\%)} = \frac{\text{No. of fruit per inflorescence}}{\text{No. of flower per inflorescence}} \times 100$$

$$\text{Seed set (\%)} = \frac{\text{No. of seeds per fruits}}{\text{No. of ovules per pistil}} \times 100$$

RESULTS

Evolvulus alsinoides L. is hairy perennial herb with spreading prostrate branches arising from the small woody root stock. The numerous branches are 20-30 cm. long and leaves are small entire elliptic to oblong, obtuse, apiculate base, acute and densely hairy with minute petiole or nearly absent. The flowers are produced in the month of February to June. However, maximum flowering was recorded in the month of March, while minimum flowering was recorded in the month of May. The flowers are funnel- shaped purple and white in colour and arranged in solitary or aggregated inflorescence (Fig. 1). The complete bisexual flowers consist of accessory as well as reproductive parts. The sepals are persistent in nature. However, petals are white and purple. The bicarpellary ovary has two ovule in each locule with a long style and a short bifurcated stigma. The size of flowers is not much varied but stamens are dimorphic (two types long as well as short) in nature. The anthers are small showing longitudinal slits dehiscence.

Flowers are 1.4 cm in size; consist of calyx (0.53 cm) and corolla (0.68). The dimorphic stamens are variable in size, the short stamens are 0.57 cm and long stamens are 0.78 cm. Stamens have 0.17 cm long anther with 0.54 cm long filament. They are five in number, polyandrous, epipetalous, staminal insertion near the base of the corolla tube. They become exerted, oppositisepalous with glabrous filaments. Anthers are tetrasporangiate,

Fig. 1: *Evolvulus alsinoides* plant with funnel shaped flower

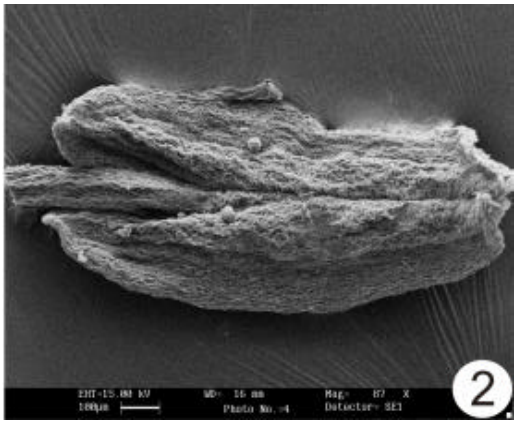

Fig. 2: SEM photograph of anther

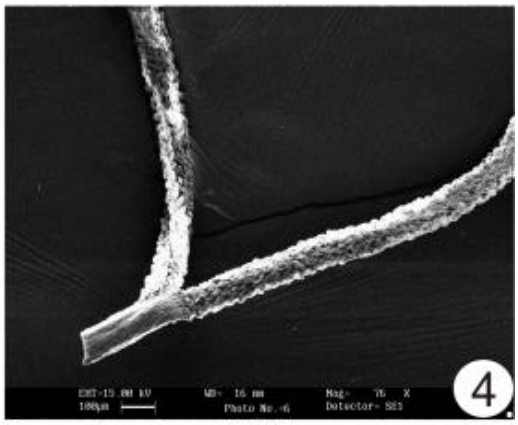

Fig. 4: SEM photograph of bifurcated stigma

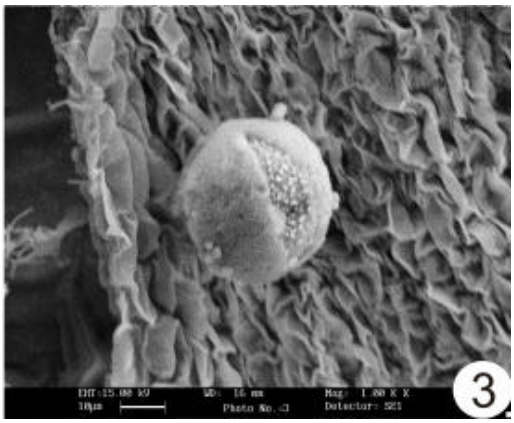

Fig. 3: SEM photograph of pollen grain

Fig. 5: Honey bees (pollinators)

dehiscing via longitudinal slits and pollen grains are tricolpate, rupate, psilate with long axis. The pistil is 0.66 cm long with 0.24 cm stigma and ovary 0.16 cm with a long style.

SEM studies reveal that pollen grains are 30×40 μm in size and spherical and oval in shape and tricolpate (Fig. 3) with reticulate exine sculpturing. Pistil has a long style with bifurcated dry type stigma (Fig. 4) and stigmatic surface consists of loosely arranged papillae. A very low amount of pollen grains was also observed on the stigmatic surface. Flowers open out in the early morning during 5:30-7:00 a.m. and the anthers dehisce by longitudinal slits (Fig. 2) at 6:30-7:00 a.m. Stigma becomes receptive after anther dehiscence at 6:00-8:00 a.m. At the time of receptivity stigmatic surface appears shiny and sticky.

The pollen viability as tested by 1% TTC and Alexander stain test, the pollen grains showed maximum viability in Alexander stain (85%) However, the minimum viability is recorded in 1%TTC test (80%). The number of pollen grains/anther is 250, the number of pollen grains/flower is 1000-1200. However, ovules per ovary in flower are 8 and the pollen ovule ratio is calculated as 312.5:1.

Flowers are self as well as cross-pollinated as confirmed by various hand pollination experiments. They were visited by different floral visitors (Table 1) like, honey bees (Fig. 5) i.e., (Apis *dorsata*, *Apis cerana indica*), small bees (*Melliopona sps.*) Bumble bees, Black ant (Componotus *compestris*), Beetle (*Coccinella punctata*), Butterflies (*Colias earytheme*), common wasps (*Polistes herbraeus*) (Fig. 6). They visit flowers between 7:00-8:00 a.m. It is interesting to note that all of them are not pollen- carriers. Honey bees, bumble bees and butter flies are pollen-carriers as evident from the presence of pollen on their body parts. On the basis of their visitation rate and pollen load on their body parts, the bees are found to be most efficient pollinators. Pollen-load on the

Table 1: Insect visitors of *Evolvulus alsinoides* L.

Vector order and family	Common name	Scientific name	Syndrome term	Nature
Hymenoptera (Apidae)	Honey bee	*Apis cerana indica*	Melitophily	PC
	Small bee	*Meliopona* sp.	Melitophily	PC
	Bumble bee	*Bombus lapidarus*	Melitophily	PC
	Honey bee	*Apis dorsata*	Melitophily	PC
Hymenoptera (Formicidae)	Black ant	*Componotus compestris*	Formicophily	PV
Cloeoptera (Carabidae)	Bettle	*Coccinella punctata*	Canatharophily	V
Lepidoptera (Piepridae)	Butterfly	*Colias earytheme*	Phycophily	PC
Isoptera (Kalotermitidae)	Termites	*Zootermopsis angusticollius*	Formicophily	V
Hymenoptera	Wasp	*Polistis herbraeus*	Melittophily	V

Fig. 6: Common wasp (floral pollinators)

bees is distinctly observed on the lower side of the abdomen of honey bees, a higher amount of pollen load than that of other insects. The fruit set percentage is calculated as 65%. The fruits are 6.00 mm in diameter; they are oval and spherical in shape. The type of fruit is capsule non-fleshy, non- hairy, dehiscent, a capsule long loculicidal. Fruits are usually 2-seeded. It is clear from the present observation that the average number of fruit/inflorescence is 6-8. Unripe fruits are yellow green blotched in color which becomes brownish yellow at the time of maturity. The total number of seeds/fruit is 2-4 and seed set percentage is 96.4%. Seeds are smooth, reniform, glabrous and black in color with 0.16 cm length. These seeds are hard under normal conditions. The weight of 100 seeds measured 1.2 g.

DISCUSSION

The observations indicate that *Evolvulus alsinoides* is an important medicinal perennial woody root stock herb and well-developed in semi-arid zone of North India. It produces white and purple coloured flowers from February to June. Flowers are white funnel shaped with twisted corolla. Similar observations made by Allard (1947) showed that morning glory is easy to spot in the field with their twining habit and generally large, white or brightly colored and funnel-shaped with twisted corolla and clockwise in bud and strongly plicate.

Anthesis took place around 6.00-8.00 a.m. with anthers already opened at the same time. Stigma becomes receptive at the time of anther dehiscence. But, it is receptive for a very short time. The results obtained are similar to those found by Maimoni-Rodella and Rodella (1992) and Maimoni-Rodella and Yanagizawa (2007) in *Ipomea* species (Covolvulaceae). Sarma *et al.* (2007) and Terada *et al.* (2005) have also supported the observations of the present authors. The present authors recorded higher pollen ovule ratio in *E. alsinoides* L. Such an investigation was also suggested by Sarma *et al.* (2007) in *Volvulopsis nummularium,* while, Cruden (1976) reported that higher pollen ovule ratio is important as out-breeding nature. A large number of insects visit the flowers of *E. alsinoides* L. and they play a significant role in the diffusion of pollination. The most efficient pollinators were observed honey bees and beetles. Austin (1997) has observed pollination in the family Convolvulaceae primarily by bees, although there are few instances of pollination by moth, birds and bats. Maimoni-Rodella and Rodella (1992) reported that various insects of the orders (Lepidoptera, Coleoptera and Hymenoptera) are the floral visitors in *Ipomoea accuminata* a member of the family Convolvulaceae. Ushimaru and Kikuzawa (1998) studied primary pollinators in *Calystegia* species (Convolvulaceae). According to them, several kinds of insects were observed but out of them, 50% were honey bees.

Sarma *et al.* (2007) have found that snaisl as well as honey bees were responsible for pollination during the rainy season weed *Volvulopsis nummularium*, a member of family convolvulaceae. However, snails are more active than honey bees for pollination. In most surveys, the group of bees that visited the largest number of plant species was considered to be the most important pollinators. The floral features are fairly homogeneous in Convolvulaceae and most of them show a melittophilous syndrome (Faegri and van Deer Pijl, 1979; Austin, 1998). Gottsberger *et al.* (1998) suggested that bees are potential pollinators. Pollination is a significant process and one of

the perquisites for ensuring fruit and seed set in all sexually reproductive seed plants (Richards, 1986; Kearns *et al*., 1998; Corlett, 2004). The 60-65% fruit set percentage was observed in *E.alsinoides*. It is interesting to note that seed set percentage is higher than the fruit set percentage. The higher fruit and seed set percentage were also recorded in *Calystegia* by Ushimaru and Kikuzawa (1998).

Thus, on the basis of the present observations and discussion it is clear that *Evolvulus alsinoides* is a cross as well as self-pollinated medicinal herb and exhibits xenogamy and geitonogamy. Therefore, the present study suggests that this herb shows various ways for reproduction in semi-arid zone of Agra. The population of this medicinal herb is slightly decreasing due to urbanization, expansion of road network, industrialization and low rainfall in the Agra region and requires cultivation in herbal gardens so as need to conserve *ex situ* and *in situ*.

REFERENCES

Allard, H.A., 1947. The direction of twist of the corolla in the bud and twining of the stems in Convolvulaceae and Dioscoreaceae. Castanea, 12: 88-94.

Anderson, G.J., S.D. Johonson, P.R. Neal and G. Bernardello, 2002. Reproductive biology and plant systematics: The growth of a symbiotic association. Taxon, 51: 637-653.

Austin, D.F., 1997. Dissoalution of Ipomoea species *Anisomerae* (Convolvulaceae). J. Torr. Bot. Soc., 124: 140-159.

Austin, D.F., 1998. Convolvulaceae. In: Flora of the Venezuelan Guayana, Caesalpinaceae- Ericaceae, Steyermark, J.A., P.E. Berry and B.K. Holst (Eds.). Vol. 4. Missouri Botanical Garden Press, St. Louis, Missouri, USA., pp: 377-424.

Chermexcil, 1992. Selected Medicinal Plants of India: A Monograph of Identity, Safety and Clinical Usage. Pharmaceutical and Cosmetics Export Promotion Council, Bombay, pp: 151-153.

Corlett, R.T., 2004. Flower visitors and pollination in Oriental (Indo-Malayan) region. Biol. Rev., 79: 497-532.

Cruden, R.W., 1976. Intra- specific variation in pollen-ovule ratio and nectar secretion- preliminary evidence of ecotypic adaptation. Ann. Missiour. Gard, 63: 277-289.

Cruden, R.W., 1977. Pollen-ovule ratios; A Conservative indicator of breeding systems in flowering plants. Evolution, 31: 32-46.

Faegri, K. and L. van Deer Pijl, 1979. The Principles of Pollination Ecology. Pergamon Prees, Oxford.

Geetha, K.A. and S. Maiti, 2001. Reproductive biology of *Chlorophytum borvilianum*. J. Trop. Med. Plants, 2: 287-290.

Gottsberger, G., J.M.F. Camargo and I. Silberbauer-Gottsberger, 1998. A bee pollinated tropical community. The beach dune vegetation of IIha de Sao Luis, Maranhao, Brazil. Botanische Jahrbucher fur Syst., 109: 469-500.

Gupta, G., C. Seema and S.V.S. Chauhan, 2006. Reproductive biology of *Jatropha integerrima* Jacq. Adv. Pollen Spores Res., 24: 93-100.

Kaul, M.K., A. Kumar and A. Sharma, 2005. Reproductive Biology of *Withania somnifera* L. Dunal. Curr. Sci., 88: 1375-1377.

Kearns, C.A. and D.W. Inouye and N.M. Waser, 1998. Endangered mutalism: The conservation of plant pollinator interaction. Annu. Rev. Ecol. Syst., 29: 83-112.

Kearns, C.A. and D.W. Inouye, 1993. Techniques for Pollination Biologist. University Press of Colorado Niwat, Colorodo.

Maimoni-Rodella, R.C.S. and R.A. Rodella, 1992. Biologia floral de *Ipomoea acuminate* Roem. Et Schult. (Convolvulaceae). Rev. Bras. Bot., 15: 129-133.

Maimoni-Rodella, R.C.S. and Y.A.N.P. Yanagizawa, 2007. Floral biology and breeding system of three Ipomoea weeds. Planta Daninha, Vol. 25.

Mckone, M.J. and C.J. Webb, 1988. A difference in pollen size between the male and hermaphrodite flower of two species of Apiaceae. Aust. J. Bot., 36: 331-337.

Moza, K.M. and A.K. Bhatnagar, 2007. Plant reproductive biology studies crucial for conservation. Curr. Sci., 92: 1207-1207.

Neal, P. and G.J. Anderson, 2005. Are mating system breeding system of inconsistent and confusing terminology in plant reproductive biology: Or is it the other way around. Plant Syst. Evol., 2501: 173-185.

Raimundez-Urrutia, E., L. Avendano and D. Velazquez, 2008. Reproductive biology of the morning glory *Merremia macrocalyx* (Ruiz and Pavon) O'donnell (Convolvulaceae). J. Torrey Botanical Soc., 135: 299-308.

Richards, A.J., 1986. Plant Breeding System. George Allen and Unwin, UK.

Sarma, K., R. Tandon, K.R. Shivanna and H.Y.M. Ram, 2007. Snail- pollination in *Volvulopsis nummularium*. Curr. Sci., 93: 826-831.

Shivanna, K.R. and B.M. Johri, 1985. The Angiosperm Pollen Structure and Function. Wiley Eastern Limited, New Dehli.

Shivanna, K.R. and N.S. Rangaswamy, 1992. Pollen Biology: A Laboratory Manual. Narosa Publishing House, New Dehli.

Terada, Y., A.P. Taniguchi, M.C.C. Ruvola, Takassusuki and V. de Alencer Arnaut De Toledo, 2005. Floral biology of four Ipomoea (Tubilfloral: Convolvulaceae) species. Acta Sci. Anim. Sci., 27: 137-143.

Ushimaru, A. and K. Kikuzawa, 1998. Variation of breeding system floral rewards and reproductive success in clonal Calystegia species (Convolvulaceae). Am. J. Bot., 82: 152-158.

Wani, P.A., K.A. Ganaie, I.A. Nawchoo and B.A. Wafai, 2006. Phenological episodes and reproductive strategies of *Inula racemosa* (Asteraceae)-a critically endangered medicinal herb of North West Himalaya. Int. J. Bot., 2: 388-394.