Pollination Biology of *Merremia vitifolia* Hallier f. (Convolvulaceae)

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Abstract

Background: The purpose of present investigation is to unravel the mechanism of pollination in the plant *Merremia vitifolia* ,commonly known as Grape Glory which is common in our area. **Methods:** Flowering phenology and floer morphology was studied by simple observation. Pollen production was calculated by Cruden method. Pollen histochemistry was studied by using IKI solution, drop of Sudan Black B and Coomassie Brilliant Blue solution. Pollen-ovule ratio was also calculated. Pollen morphology studied accurately by acetolysis. Pollen fertility was studied by acetocarmine-glycerin technique and pollen viability was assessed by Tetrazolium. *In-vivo*ans*in-vitro* pollen germination also studied.Biochemical analysis of stigma also studied by IKI solution, Sudan black and Coomassie Brilliant Blue solution. Role of wind in pollination was studied by hanging Vaseline coated slides around the flower. Pollinators and their behaviour were observed through observation from the time of anthesis to flower closing. Breeding system and seed germination rate also studied. **Findings:**Flowers are produced in terminal cymose inflorescence and flower takes 10-12 days from initiation to bloom. Pollengrains are roughly spherical in equatorial view. Pollen cotain protein as it intensely stained with Coomassie Brilliant Blue and is estimated 240mg per ml in 0.03g anther sample. Pollen viability was peak at 1 pm. Stigma stained with IKI, Sudan Black and Coomassie Brilliant Blue which indicates the presence of starch,lipid and protein. Stigma is receptive at the time of anthesis. Wind has no role in pollination. Cross pollination lead to fruit set and seed set.

Keywords: *Merremia vitifolia,* Breeding behaviour, Pollination biology, Viability, Histochemistry, Flowering phenology.

1.Introduction:

Flowers have served as an excellent offering to God, an invaluable aid for personal make up, and a source of inspiration to poets. For plants it is the organ of sexual reproduction. Flowers exhibit a great variation in size, color, shape and insertion of floral whorls. Floral diversification is the outstanding characteristic of angiosperm radiation. In a broad sense, abundant evidencesnow confirms that pollinators play a major role in this diversification[1].

Convolvulaceae, commonly known as the Bindweed or Morning glory family, is a family of about 60 genera and more than 1650 species of mostly herbaceous vines, but also trees, shrubs, and herbs, and also including the sweet potato and some other tubers. The Convolvulaceae have a cosmopolitan distribution but 90% of the species found in the tropics. The genus *Convolvulus* is present around the Mediterranean basin. *Calystegia* is very diverse on the West Coast USA with some species growing in Europe. *Ipomoea* and *Merremia* are most diverse in Tropical America but they are also common in Africa and Asia.*Merremia* is a genus of Convolvulaceae. *Merremia vitifolia* Hallier f. in Engl. is a climber or twining shrub which is global in distribution. They are commonly known as Grape Glory or Wood roses. They mainly include climbing or creeping shrub with cord like stem rooting at the node. The plant generally found in river banks, roadsides, grass lands, degraded forests and also in the plain. The flower is medium sized, lemon yellow and found in clusters. The floral traits suggests exclusively insect mediated pollination syndrome (entomophily). Little is known about the phenology, floral biology, anthesis, stigma receptivity and pollination biology of this particular species. Present study has been undertaken to study the pollination biology of *Merremia vitifolia.*

2.Materials and methods:

Present study was undertaken on *Merremia vitifolia* plant growing at back side of the campus, KAHM UWC Manjeri.

2.1 Flowering phenology: The initiation, peak and end of flowering was recorded properly. Time of anthesis, anther dehiscence, pollen viablity, stigma receptivity was recorded. Anthesis and anther

dehiscence observed in field using hand lens by the method of Reddi and JanakiBai(1981)[2], Mathur and Mohan Ram (1986)[3] and Ramasubbu et al., (2009) [4].

2.2 Pollen production per anther/ flower: pollen production was calculated by the method described by Cruden(1977)[5]. Pollen morphology was identified by acetolysis method described by Erdtman[6].

2.3 Pollen histochemistry: presence of starch, lipid and protein were diagnosed with IKI solution, Sudan Black and Coomassie Brilliant Blue. Protein estimated by using Folin-Ciocalteau reagent to the method of Lowry *etal.,* (1951)[7].

2.4 Pollen viability: Pollen viability was checked by using 0.2% Tetrazolium solution.

2.5 Stigma receptivity:stigma receptivity was studied by cytochemical localization of esterases on surface of stigma by the method described by Shivanna and Rangaswami (1992)[8].

2.6 Stigma histochemistry: The presence of starch, lipid, and protein was checked by using IKI solution, Sudan Black and Coomassie Brilliant Blue.

2.7Pollinators and behaviour: Pollinators, non-pollinating visitors were observed continuously. Their number, visiting time, foraging nature was recorded by using stop watch.

3. Results:

Merremia vitifolia Hallier f. is a climber or twining shrub which is global in distribution. In India it is found in Assam, Andaman and Nicobar Islands, Gujarath, Meghalaya, Odisha, Maharashtra, Karnataka, Kerala and all districts of Tamil Nadu. The plant generally found in river banks, roadsides, grass lands, degraded forests and also in the plain. The flower is medium sized, lemon yellow and found in clusters. The floral traits suggests exclusively insect mediated pollination syndrome (entomophily).

3.1 TAXONOMIC TREATMENT OF *M. vitifolia*

M. vitifolia is a perennial climber. Stem brownish black or purplish, hispid, terete. Leaves simple, alternate, 5-18 × 4-15cm long, base cordate, palmately 5-lobed, lobes broadly triangular or ovate-lanceolate, sparsely fulvous hairy on both sides, basally 7- ribbed, Margin coarsely serrate or sub-entire, apex acuminate to obtuse. Inflorescence 1-3 or several flowered, peduncle 2-5cm long, bracts subulate, pedicel 1-1.6cm long, thicker distally. Sepals oblong or ovate-oblong, 1.4-1.8cm long, enlarged

in fruit, leathery, shiny, pitted adaxially, pellucid glandular, apex obtuse or acute, 1.7×0.9 cm long. Corolla lemon yellow, petals fused forming infundibuliformis corolla, 3.5-5cm long. Stamens 5, differently sized (3 long and 2 short), epipetalous. Anthers creamy white, 5mm long, filament white, slender, terete, basifixed, dithecous, extrose, split longituidinally. Ovary superior, tetracarpellarytetralocular, syncarpous, placentation axile, ovules 4. Style terete, white, 1.7cm long, glabrous, narrowing towards tip.Stigma bicapitate, wet type, 1mm long and 3mm wide, creamy white. Fruits dry dehiscent capsule, straw colored, 1-1.2cm across, globose. Seeds black brown, trigonous-ovoid, glabrous, 7mm long.

3.2 Flowering phenology

Under normal climatic conditions of the Unity campus *M. Vitifolia* started flowering from second week of December 2018 and reached its peak during January. The flowers are produced in terminal cymoseinflorascence. Approximately 6-10 flowers are produced in each inflorescence, but maximum three flowers opens at a time.

Flowering declined towards the end of February. The flower takes 10-12 days from initiation to full bloom. Flower opening starts from 8.20 am in the morning and lasts up to 9 am. The life span of individual flowers is 7-9 hours. Anther dehiscence commenced after 30 minutes to 1 hour of anthesis. On the second day of flower closing the corolla tube along with stamens will fall off and the calyx cup along with gynoecium persist for about a week and gradually develops to fruit. Fruit development takes approximately 10-12 days.

3.3 Flower morphology

The flowers are born in axillary cymes with divaricating branches. Each inflorescence with 2-3 flowers and 5-8 buds of different stages. Flowers are pedicellate, lemon yellow, 5.8cm long and 4.7cm wide. The flowers are infundibuliformis, actinomorphic, bisexual, and without having any particular odour. The stamens are epipetalous, creamy white and free. There are 3 long (1.4cm long) and 2 short (5mm long). The filaments slightly bend inward. The anthers are 5 mm long, basifixed, dithecous and extrose. The ovary is superior and tetralocular, syncarpous, containing single ovule in each carpel on axile placentation. The style tipped with creamy white bicapitate stigma, 1mm long and 3 mm wide when fully grown.

3.4 POLLEN GRAINS

Pollen grains are roughly spherical in equatorial view. Pollen grains are non-porate and hexacolpate type (SuraponSaensouk and PiyapornSaensouk, 2018)[9]. Rarely pentacolpatepollen grains also observed. The average diameter of pollen grains was 120 µm. The total pollen production in a flower is 3968. The pollen ovule ratio is 992: 1.

3.4.1 Pollen histochemistry

Pollen grains not stained with IKI solution indicating that it does not contain carbohydrate. Pollen grains stained intensely with Coomassie Brilliant Blue indicating the presence of protein. Pollen grains intensely stained with Sudan Black B indicating the presence of lipids.

3.4.2 Estimation of total protein content of anthers

0.03 g anther sample contain 240mg protein per ml.

3.4.3 Pollen fertility

A small proportion of pollen grains were found to be fertile during the first hours of anthesis as tested through acetocarmine-glycerine technique. From the third hour of anthesis the pollen fertility was found to be higher. After the fourth hour 100% pollen were found to be fertile. After that pollen viability found to decrease.

3.4.4 Pollen viability

The pollen viability at the time of flower opening was too low. It was found to be increasing during each hour and get maximum during the peak sunny time, 1pm. Then it gets gradually decreased in successive hours.

3.4.5 Effect of organic and inorganic nutrients in *in-vitro* pollen germination

An *in-vitro* pollen germination study showed that the pollen of *Merremia vitifolia* is special and was not giving pollen tube in the given media.

3.4.6 *In-vivo* pollen germination

The test using cotton blue and Lactophenol does not show any pollen germination under microscope.

3.5 PISTIL

The pistil is differentiated into ovary, style and stigma. The ovary is superior and is divided into four equal halves by the formation of septum. It is tetracarpellary, tetralocular and syncarpous. The ovules are arranged on axile placenta. Ovules 4. Style is creamy white, glabrous and cylindrical. Stigma is bicapitate and creamy white with many ridges and furrows.

3.5.1 Stigma receptivity

The stigma treated with Hydrogen peroxide instantly releases oxygen bubbles, showing that the stigma is highly receptive at the time of anthesis.

3.5.2 Biochemical analysis of stigma

Stigma stained with IKI solution indicates the presence of starch in stigma. Stigma stained with Coomassie Brilliant Blue R indicates the presence of protein in the stigma. Stigma stained with Sudan Black B indicates the presence of lipid in stigma.

3.6 Pollination biology

3.6.1 Role of wind in pollination

The possibility of wind pollination was studied by hanging Vaseline coated slides at various heights around the flower. Microscopic examinations of the slides indicate that pollination of *M. vitifolia*does not take place through wind (no anemophily).

3.6.2 Pollinators and their behavior

The study on *Ipomoea habeliana* found that ants are most frequent floral visitors[10].foraging patterns of floral visitors affect the relationship between physical distance and genetic variability[11]. Flowers of *Merremia vitifolia* were regularly visiter by insects. *Halictus* bees were found to be prolific forager during the first hours of anthesis. They are the most frequent pollinators. Other frequent visitors include carpenter bee (*Xylocopa sp*.), several species of ants like Monomorium, black carpenter beetle, and winged ants. The flower is not visited by any butterflies and moths.

Non-pollinating visitors includes white fly(*Hemiptera*), crickets(*Orthoptera*), spiders(LynX spider), leaf hoppers, white flies, grass hoppers and some species of ants.

Breeding behavior

M. vitifolia does not give successful fruit setting on self pollination. Fruit setting is observed in flowers which are cross pollinated from other flowers of the same plant and also by the pollen from other flower of other plant.

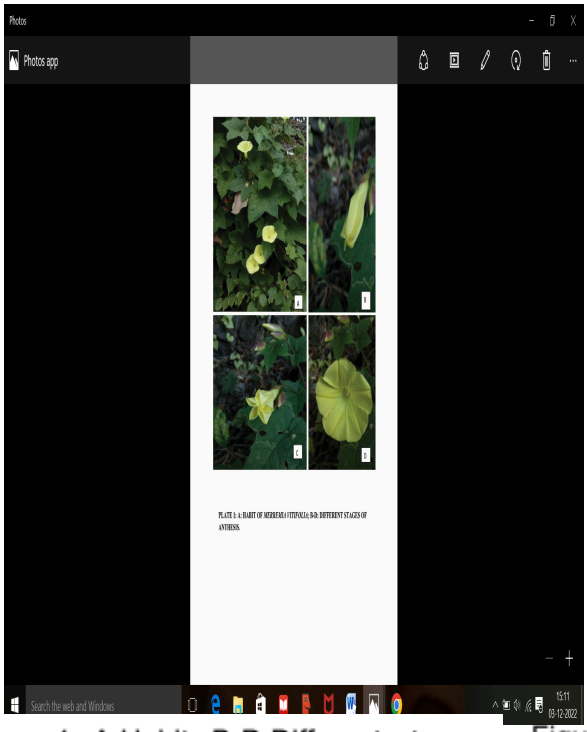

Figure.1: A:Habit; B-D:Different stages of anthesis.

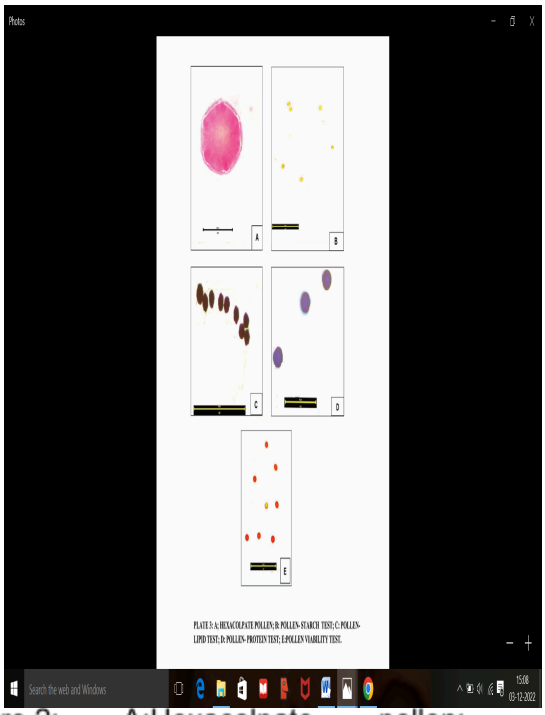

Figure.2: A:Hexacolpate pollen; B:Pollen starch test; C:Pollen lipid test; D:Pollen protein test; E:Pollen viability test.

Figure.3: A:Stigma receptivity; B:Stigma protein test; C:Stigma lipid test; D:Stigma starch test.

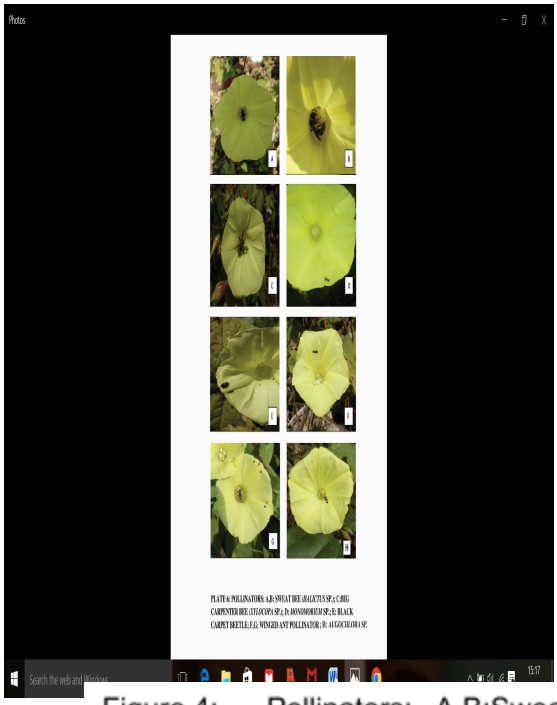

Figure.4: Pollinators; A,B:Sweat bee (*Halictus* sp.); C:Big carpenter bee (*Xylocopa* sp.); D:*Monomorium* sp.; E:Black carpet beetle; F,G:Winged ant pollinator; H:*Augochlora* sp.

Figure.5:Non-pollinating visitors; A:Cricket (*Orthoptera* sp.); B:White fly (*Hemiptera*sp.); C:Lynx spider; D:Leaf hopper(*Hemiptera*); E:Nymph of a katydid (*Orthoptera*); F:Short horned grass hopper (*Orthoptera*).

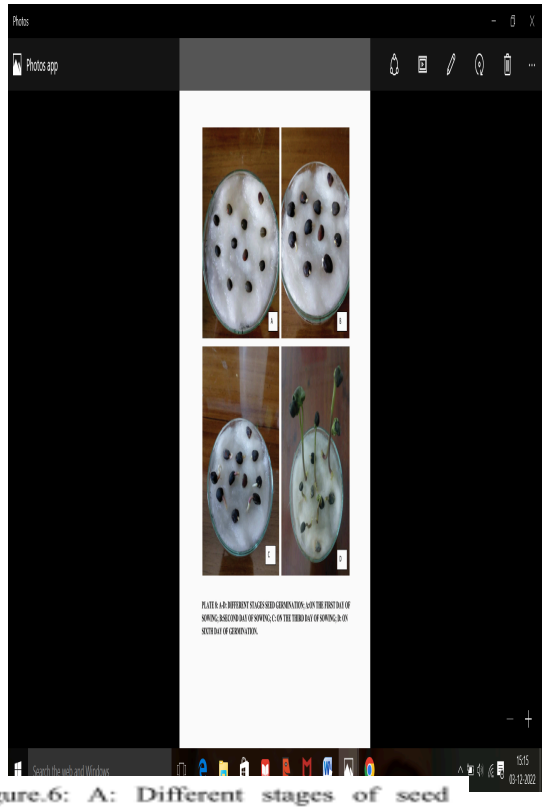

Figure.6: A: Different stages of seed germination; A:first day of sowing; B:Second day of sowing; C:Third day of sowing; D:Sixth day of germination.

3.7 Seed germination

The seeds of *M. vitifolia*are extremely hard with mechanically resistant coats that protect embryo from damage. The seeds sown in petridishes with moist cotton do not gave germination. Seeds that are scarified with conc. H_2SO_4 for 30 minutes also do not germinated. Seeds that are soaked in conc. H_2SO_4 for overnight also resulted the same. Then the seed coat at the hilum part is removed mechanically by using sterilized razor. The seeds treated in this way gave germination just next day of sowing in moist cotton. Seeds of this type also sowed in the soil. The seeds in the soil also gave germination, but not as much growth in the seeds of moist cotton. They show repressed growth. Seeds of moist cotton produced leaves after seven days of germination. The germination rate is 80%.

4. Discussion:

The present work is the first comprehensive study on the pollination biology of *Merremia vitifolia.* The basic knowledge on reproductive biology is not only essential for evolutionary and systematic studies(Anderson, 1995) [12] but also important for effective conservation strategies(Holsinger, 1991; Bernardello *et al.,* 1999) [13][14]for endangered species.

Under favourable climatic conditions of Korambayil Ahmmed Haji Memorial Unity Womens College, *M. vitifolia* started flowering on second week of December, 2018 and reached its peak during January. Flowering declined towards the end of February. It is essential to have a basic knowledge about the floral morphology for the studies of pollination biology and breeding systems. The flowers of *M. vitifolia* were born in terminal cymose inflorescence. Flowers are companulateor infundibuliformis, 5.8cm long and 4.7cm wide. It is lemon yellow in color and without having any particular odour. Each inflorescence has 2 or three open flowers at a time and the flowers are bisexual, actinomorphic and pedicellate. Flower opening starts at 8.20am in the morning and were completely closed by approximately 4pm in the evening. Anther dehiscence takes place sometime after anthesis. In pollinated flowers, the calyx cup was persistent and the fruit emerges out. Pollen grains are non-porate and hexacolpate type. The average dimeter of pollen grains was 120µm. The average number of pollen grains per flower was calculated as 3968, the number of ovule as 4 and the pollen-ovule ratio as 992:1.

Acetocarmine-glycerine technique indicates that pollen fertility at the time of anthesis was low and it is maximum at 1pm. Then the viability decreases. *In vitro* and *in vivo* pollen germination tests did not give any result.

Pollen grains stained deeply with Coomassie Brilliant Blue indicate the presence of protein, pollen also stained with Sudan Black B indicate the presence of lipids. Pollen grains do not stain with IKI solution which indicates the total absence of starch in them.

Pistil has a long style and bicapitate stigma. Stigma was receptive at the time of anthesis itself. The stigmatic surface is with many ridges and grooves. The style is erect. The stigma is the recipient of pollen grains.

There is a great variation in the morphology of stigma. Angiosperm species that produces trinucleate pollen typically have dry stigmas, whereas binucleate pollens often interact with wet type stigmas (Shivanna.,Heslop Harrison, 1977) [15].

The present study showed that wind has no role in pollination and the pollination occurs exclusively by insects. The major pollinator is found to be *Halictus* bees. Many other insects visit the flower but they not contributing anything to pollination.

Fruit setting occur only on cross pollination. Seeds are with very tough seed coat and germination rate is 90% on moist cotton.

5.Conclusion:

Merremia vitifolia Hallier f. is a perennial climber belongs to the family Convolvulaceae. Lemon yellow flowers are born on cymose inflorescence. No previous studies on pollination biology of *Merremia vitifolia* have been conducted. The present study was carried out for a period of four months (December-March 2018-19), to observe the reproductive parameters of *M. vitifolia* in the back yard of Korambayil Ahmed Haji Memorial Unity Women's College. The flowers appeared from December 2018 to February 2019 and attracted many bees and insects. Flower opening starts at 8.20am in the morning and lasts about 9 am. The life span of individual flower is approximately 8 hours. Stamens fall off in the closed flowers whereas gynoecium persists for about two or three days and gradually develops into fruit. Anther dehiscence commenced after anthesis. Pollen grains at the time of flower anthesis have low viability but attain maximum viability at peak sunny noon. Acetocarmine-Glycerine technique shows that a high proportion of pollen grains are fertile at that time.Receptivity of stigma is the critical factor for the successful completion of the post-pollination events. The test by using hydrogen peroxide shows that the stigma is receptive at the time of anthesis itself. Wind has no role in pollination of this species. The plant promotes cross pollination and fruit development in self pollinated flower is poor or completely absent. 90% fruits contain four fully viable seeds; some are with one or more non-viable seeds. The seeds have a very tough and hard seed coat, and thus it takes many months to germinate in natural soil conditions. However, 90% of treated seeds were germinated in moist cotton.

6. Acknowledgment:

I express my profound gratitude to Dr. C. Saidalavi**,**Principal, KorambayilAhammed Haji Memorial Unity Women's College Manjeri, Malappuram, Kerala, India for providing necessary laboratory facilities for the smooth progression of our work.

7. Reference

1.Kay KM, Voelckel C, Yang JY, Hufford KM, Kaska DD, Hodges SA. Floral characters and species diversification. *Ecology and Evolution of Flowers,* ed. LD Harder, SCH Barrett, New York: Oxford University Press. 2006;311.

2.Reddi CS, Janki B. Floral Biology of *Mimusops elengi* L.J. Bombay Nat. Hist. Society.1981;77: 471-475.

3.Mathur G, Mohan Ram H.*Phytomorphology.*1986;36: 79-100.

4.Ramasubbu R, PanduranganAG, Sreelekha AK.Reproductive biology of three endemic endangered Balsams (*Impatianscoleotropis*Fischer, *Impatiansphoenaicea*Beeld and *Impatiansplatyaldena,* Fisaher.) Ph.D thesis, University of Kerala. 2009.

5.Cruden RW. Pollen-Ovule Ratios: A Conservative Indicator of Breeding Systems in Flowering Plants. *Evolution.*1977; 31:32-46.

6.Erdtman G. The Acetolysis Method. *SvenskBotaniskTidskrift.* 1960*;*54:561-564.

7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.Protein measurement with the Folin phenol reagent.*Journal of Biological Chemistry.* 1951; 193(1):265-275.

8.Shivanna KR, Rangaswamy NS. Pollen biology: a laboratory manual. Berlin, Heidelberg, New York: Springer-Verlag. 1992

9.Saensouk S,Saensouk P. Palynology of family Convolvulaceae in Thailand. *Research and Knowledge.*2018*;*4:16-33.

10.Mcmuller CK. Pollination biology of a night flowering Galapagos endemic, *Ipomoea habeliana*(Convolvulaceae). *Botanical Journal of Linnean Society.*2009*;*160(1):11-20. 11.Pinto-Torres E, Koptur S. Hanging by a coastal strand: breeding system of federally endangered morning glory of South Eastern Florida Coast, *Jaquenontia reclinata.*Annals of Botany. 2009;104:1301-1331.

12.Anderson, G. J. 1995. Systematic and reproductive biology. *In* P.C. Hoch and A.G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Mongr. *Systamatic Botany* 53: 263-272.

13.Holsinger, K. E. 1991. Mass-action models of mating systems: The evolutionary stability of mixed mating systems. *American Naturalist,* 606-622.

14.Bernardello, G., Anderson, J. G., Lopez, S. P., Cleland, M. A, T. F Stuessy and D. J Crawford, 1999. Reproductive biology of *Lactoris fernandeziana* (Lactoridaceae). *American Journal of Botany* 86: 829-840.

15.Heslop-Harrison, Y. and K. R. Shivanna, 1977. The receptive surface of Angiosperm stigma. *Annals Botany* 41: 1233-1258.