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Reproductive biology of *Solanum viarum* Dunal (Solanaceae) in Northeast India

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Abstract

Solanum viarum Dunal (Solanaceae), is a prickly and economically important medicinal herb. Alkaloid solasodine obtained from its fruits are used to synthesize medicinally much useful steroid hormones. The present article recorded different reproductive parameters of this species including floral morphology; anthesis; pollen production, germination, viability; foraging behaviour of flower visitors and the meiotic system. The species generally flowers year-round with the peak during March - April and mature fruits during May - June. Flowers open in between 4.00 to 6.00 hrs One flower produces an average of 2,98,350 pollen grains. The maximum (90 %) pollen germination along with 187µm tube development was found in 35 % sucrose solution supplemented with 100 ppm boric acid. Pollen viability was determined by using Tetrazolium test. Chromosome counts revealed that *Solanum viarum* is diploid i.e., n = 12.

Key words: *Solanum viarum*, Reproductive biology, Floral morphology, Meiotic system.

INTRODUCTION

Reproduction is a natural means of increasing the number of individuals of the same species and is vital for its survival and evolution. For successful cultivation and conservation of plants the detail knowledge of the plant's reproductive biology is required (Moza & Bhatnagar 2007). Reproductive biology mainly focuses on flowering phenology, floral biology, pollen-pollinator interaction, pollen-pistil interaction, breeding systems and gene flow through pollens and seeds. According to Shivanna (2003) pollen-pistil interaction is unique to flowering plants and it covers all sequential events those take place in the pistil starting from pollination until pollen tube entry into the embryo sac. The environment in which an organism live affects its reproductive success of a plant (Sedgley & Griffin 1989). The physiology of reproduction in most of the flowering plants is closely under the control of environmental factors (Taiz & Zeiger 2003). Environment exerts considerable influence on flowering, pollen fertility, *in vitro* pollen germination and fruit setting in plants (Shivanna 2003). The knowledge of floral biology, including pollination is prerequisite for any rational breeding programme and determination of extent of seed and fruit setting. Good fruit set and high crop yield generally depends on viable pollen grains. Seeds and fruits are the economic products for more than 90 % of flowering plants.

Solanum viarum Dunal (Solanaceae), which belongs to the section Ancanthophora, and subgenus Leptostemonum (Nee 1991), commonly referred as Tropical soda apple, is a serious invasive weed for pastures, vegetable crops, row crops, forests, urban and natural areas (Bryson 1996; Coile 1993; Hall *et al* 1998). This plant has invaded both agricultural

and natural ecosystems (Mullahey & Colvin 1993; Mullahey & Akanda 1996). Though the species is a native to Brazil and Argentina, but has become a weed of other areas of South America, Africa, India, Nepal, West Indies, Honduras, and Mexico (Nee 1991). However, *Solanum viarum* is an economically important medicinal plant with a rich source of solasodine, an alkaloid used in thesynthesis of steroid hormones for treating cancer, Addison's disease, rheumatic, arthritis, and for producing contraceptives (Chandra & Srivastava 1978; Hendique 1986; Nayak & Patil 2001; Satyabrata *et al* 2000). According to Budavari (1989), the glycoalkaloid present in the fruits of *Solanum viarum* is a precursor for the steroid diosgenin and is used as contraceptive. In spite of its importance and wide distribution the reproductive biology of *Solanum viarum* has so far not been worked out by any worker. The objective of the present investigation is to understand the floral biology, anthesis, pollen production, pollen viability, *in vitro* pollen germination and meiotic study of this important medicinal plant. The detail insights on its reproductive biology will be helpful for the proper management of this economically important as well as an invasive weed.

MATERIAL AND METHODS

Floral Biology: Two populations of *Solanum viarum* Dunal were selected for the present investigation in their natural conditions. Ten healthy plants were selected from each population and observations were made on a day-to-day basis on flowering phenology which includes phenology, anthesis etc. Floral morphology was also studied in the laboratory. Flowering period, flower colour, odour and other floral characters were visually observed through extensive field exploration. Anthesis and other phenological characters were studied following the method of Reddi & Janaki Bai (1981) and Mathur & Mohan Ram (1986). Foraging behaviour of insect visits was recorded and observed visually at 2 hrs intervals every day Shivanna & Rangaswamy (1993).

Pollen germination: *In vitro* Pollen germination was carried out in different concentration of sucrose (2 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 % and 40 %) alone and in combination with Boric acid [100 ppm + 2 %, 100 ppm + 5 %, 100 ppm + 10 %, 100 ppm + 15 %, 100 ppm + 20 %, 100 ppm + 25 %, 100 ppm + 30 %, 100 ppm + 35 %, 100 ppm + 40 %]. The experimental set up was done as per the method of Shivanna & Rangaswamy (1993). Pollen grains, which had germinated and produced pollen tubes in the medium, were recorded. Lengths of pollen tube in different concentrations were recorded 45 minutes after the commencement of germination and percentage of pollen germination was calculated and the average length of pollen tube was calculated.

Pollen morphology: Pollen morphology was studied following Acetolysis method as proposed by Erdtman **(1960)**. The size of the pollen grains (for radio symmetric ones the diameter in the polar view, and for bilateral ones the polar and equatorial axes) was measured in glycerine jelly (Wodehouse 1935) using standard ocular micrometer. For each taxon under study, pollen grain size was measured for 50 randomly chosen grains. The terminology used is in accordance with Erdtman (1952), Faegrie & Iversen (1964), Walker & Doyle (1975), Nair (1964).

Pollen Production: The pollen production study was carried out following the standard methods (Nair & Rastogi 1963; Mandal & Chanda 1981). In determining pollen productivity mature and undehisced anthers were collected while they were in peak blooming season. One anther was crushed and dispersed it in 50 drops 50 % glycerine. One drop of the mixture was put on a slide and covered with a 22 × 22 mm cover glass. The number of pollen grains in this area was counted with an average of five drops for each species. This average was multiplied by 50 to get the number produced per anther. The pollen count was made for ten anthers randomly collected from different flowers and from different indivuals of *Solanum*

viarum. Five countings were made, and the average number of pollen contained in an anther was derived.

Viability of Pollen: The pollen viability was assed using 1 % TTZ test. In TTZ test, 1 g triphenyltetrazolium chloride (TTC) and 1.5 g sucrose were dissolved in 100 ml distilled water **(Norton, 1966).** One or two drops of TTC solution was put on a clean micro slide and pollen grains were sprinkled on these drops with a brush. Then, the drop was carefully covered by a cover glass without trapping air and kept for 2 h at ambient conditions. Pollen grains were examined using a light microscope. The viability of pollen was scored according to stainability i.e., pollen with bold red colour as viable and colourless as non-viable.

Reproductive Success: The pollen : ovule ratio is the more accurate measure of reproductive success than the total pollen per flower or per plant (Shivanna & Johri 1985).

By dividing the number of pollen grains produced per flower by the number of ovules in the flower, the pollen: ovule ratio was obtained (Cruden 1977). For determining the pollen-ovule ratio, an average number of ten flowers were taken.

Meiotic chromosome study: Young flower buds were fixed in 1 : 3 acetic alcohol for 24 hours. Anthers were teased out on slides, stained with drops of 1 % acetocarmine and smeared gently. Slides were observed under a compound microscope for chromosome counts and meiotic configurations.

RESULTS AND DISCUSSION

Floral biology: Flowers open early in the morning from 4.00 – 6.00 hrs. (Table 1 and Plate I, a & b) after that pollen presentation time starts and the cream coloured oblong anthers are then dehisced by apical pores (Table 1).

Cymes in small extra-axillary clusters; pedicels 6.8 - 7.5 mm; flowers 14.2 ± 2.0 mm in diameter, complete, actinomorphic, bisexual, hypogynous, pentamerous; sepals-5, spiny, persistent, green; petals-5, white, recurved; stamens-5, filament short (1.0 – 1.5 mm), stout, slightly swollen at the base, whitish, anthers more or less oblong (8 – 9 × 1 – 2 mm), 2-celled, basifixed, dehisces by apical pores, creamish white; carpels-2, syncarpous; ovary (2.5 – 3.0 mm), style in two different forms, - long (9.0 – 9.5 mm), and short (2.0 – 2.5 mm), slender stigma green, wet, partially lobed (0.5 – 1.0 mm),; ovary 2-chambered with many ovules in each; berries globose with a persistent calyx, 20 – 30 mm wide, green with dark speckled, when immature, dull yellow when ripe (Plate 1, e); seeds numerous, compressed, golden yellow.

The biological potentiality of individual flowers in an inflorescence could be calculated by counting the total pollen grains per flower, but it is very difficult to estimate absolute pollen production (Mandal & Chanda 1981). Flowering and anthesis time [Table-1] may depend upon physical, physiological and biochemical factors of a plant as well as on climate conditions (Hamilton 1959; Davies 1969; Reis & Kostic 1976). A single flower produces an average of 2, 98,350 (Table-1) pollen grains. After flower opening *Xylocopa* sp., *Amegilla* sp., few thrips and some members of Diptera visit the flowers for foraging. The thrips are capable to enter the flower even in bud stage (Plate I, d). They feed on pollen and stigmatic exudates. They crawl through anthers to stigma and help in pollination. *Amegilla* sp. is the main pollinator.

***In vitro* pollen germination:** The effect of sucrose on *in vitro* pollen germination of *Solanum viarum* showed that the taxa required comparatively high sucrose concentration (35 %) for their optimal germination (Plate I, f) and boric acid to some extent also influence the percentage

Table 1. Floral biology of *Solanum viarum*

Parameters	**Observations**
Flowering period	Throughout the year
Flower shape	Regular
Flower colour	White with cream coloured anthers
Odor	Present
Nectar	Absent
No. of Stamens	5, creamish white
Flower opening time	Early morning (4.00 – 6.00 hrs.)
Number of pollen per flower	2,98,350
Anther dehiscence time	Just after flower opening
Mode of anther dehiscence	By apical pores
Stigma	Simple, Wet
Style	Long and short, Terminal
Ovary	Superior
No. of ovules/flower	Numerous

of pollen germination. But, the best result was obtained in 35 % sucrose solution supplemented with 100 ppm boric acid. *In vitro* pollen germination method is rapid, reasonably simple and most commonly used for assessing pollen viability (Bhowmik & Datta 2012). In the present study, it is observed that the addition of boric acid and sucrose solution results in the increase in the rate of pollen germination as well as pollen tube development. In *Solanum viarum*, the germination percentage was 57.5 % in 100 ppm boric acid with 35 % sucrose solution and maximum tube length was 187µm (Figure 2) but it was also observed that both germination percentage and tube length decreases at 40 % sucrose solution alone as well as in combination with boric acid (Figure 1 and 2). This is attributed to the fact that sucrose is necessary for proper pollen nutrition, osmotic control and in combination with boric acid promoted pollen germination. According to Gauch & Duggar (1953) boron combines with sugar to make a

Table 2. Values of different floral attributes of *Solanum viarum* determining its absolute and ecological reproductive potentials

Floral Attribute	**Value (Mean ± SD)**
Number of inflorescence per plant	8.2 ± 0.68
Number of flowers in an inflorescence	3.2 ± 0.75
Number of fruits per plant	21 ± 5.83
Number of seeds per fruit	179.6 ± 18.64

sugar-borate complex which is translocated with greater facility rather than non borate sugar molecules. The role of boron has been confirmed in germinating pollen and growing pollen tubes by Sidhu & Malik (1986). Boron may enhance the sucrose uptake and stimulate germinating ability. This observation gets support from Pal *et al* (1989), Gupta *et al* (1989), Mandal *et al* (1982), Bhattacharya *et al* (1997), Mohi-ud-din *et al* (2007), and Biswas *et al* (2008). Several workers also supported the fact that boric acid in combination with sucrose enhances both germination as well as pollen tube development (Mandal *et al* 1982; Pal *et al* 1989). Dafni *et al* (2005) also stated that germination success in sucrose medium might depend on the humidity at which the pollen grains were exposed prior to the germination test and on the age of pollen grains.

Pollen viability: All the dyes used in the experiment for pollen viability of *Solanum viarum* showed good colour to differentiate between fertile and sterile pollens viz., Muntzing's mixture (61.15 ± 10.55) and Acetocarmine (48.96 ± 7.50). In the TTZ test the percentage of viable pollen was 73.20 ± 6.28 (Plate I, c). Nyine & Pillay (2007) also found similar results in their experiments, emphasizing that pollen grain viability assessment through the staining method seem to express the germination potential, but not its occurrence. It may be explained by the fact that this technique overestimates the percentage of pollen tubes formed. Pollen viability is considered as an important parameter of pollen quality (Dafni & Frirmage 2000). Pollen size and viability are good markers of the course of microsporogenesis. Normal meiosis produces pollen grains regular in size and highly viable, and disturbed meiosis reduces pollen viability and causes variability of pollen grain size (very small and giant pollen are formed in addition to those normal in size); the latter can result from inbreeding depression, autopolyploidy, segmental allopolyploidy, hybridization, mutations, and also environmental effects (Stace 1991).

Pollen morphology and pollen production: Pollen grains radially symmetrical, heteropolar, prolate. Polar axis P (37.62-) 33.96 ± 1.25 (-34.52) µm and Equatorial diameter E (24.12-) 25.36 ± 1.47 (-26.12) µm.Pollen grains tricolpate, colpi (29.23) 30.25 ± 1.79 (-31.42) µm. Exine (1.85-) 2.17 ± 0.76 (-2.54) µm thick.

The number of pollen produced per flower varies from 2,96,700 - 3,00,000 (Mean: 2,98,350). It is evident that pollen production in terms of number per anther varies rather widely from

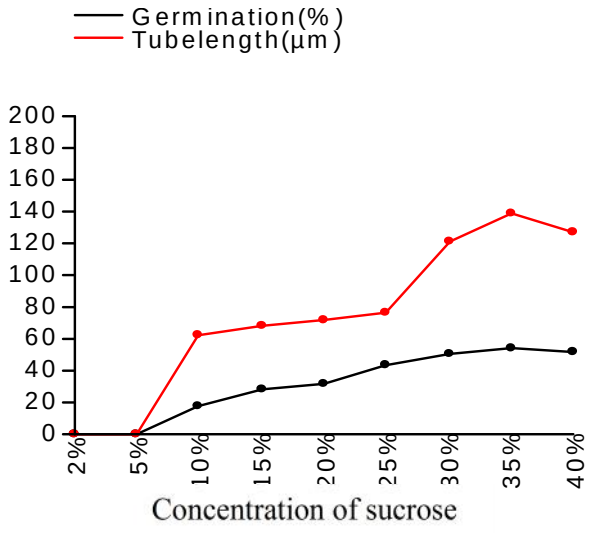

Fig. 1. Pollen germination and tube length at different concentration of sucrose

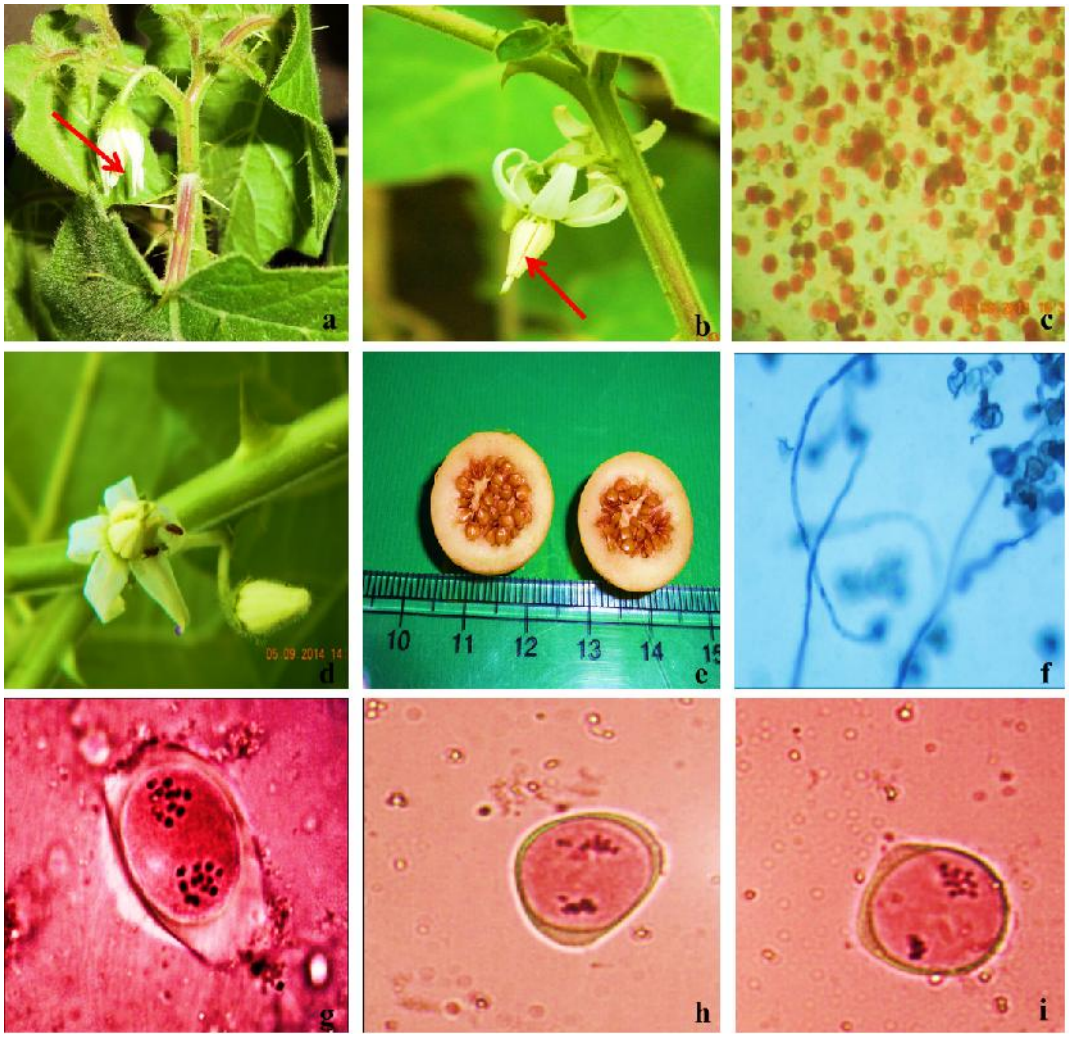

PLATE – I: *Solanum villosum* Dunal: (a) Flower opening, 4.00 hr.**; (b)** Full bloom flower**,** 6.00 hr.; **(c)** Pollen viability; **(d)** Pollinator; **(e)** Dissected fruit; **(f)** Pollen germination at 35 % sucrose solution; **(g)** Metaphase II; **(h)** & **(i)** Metaphase II showing irregular chromosomal division. [In **a & b** arrow indicates: down- short style and up-long style]

family to family; genus to genus; species to species; even within the different flower of the same plant. The pollen production is a characteristic of all plants, and is by definition, an integral part of the pollination process. In *Solanum viarum*, anther dehisces by means of apical pores and facilitate the pollinators to come touch with their body. Nair & Kapoor (1973) stated that the studies on pollen production helped to locate the differences in the biological potential of individual flower in an inflorescence. Smart *et al* (1979) showed that variation in pollen production could be related to the mode of reproduction.

Reproductive Success: The mean Pollen: Ovule ratio (P/O) is 2984 : 1. There was a significant effect of taxonomic position (genus), reward type and pollination mechanism on P/O. Species offering only nectar as a floral reward (where species with brush mechanism) had a significantly lower P/O than species offering pollen or pollen and nectar. Species with

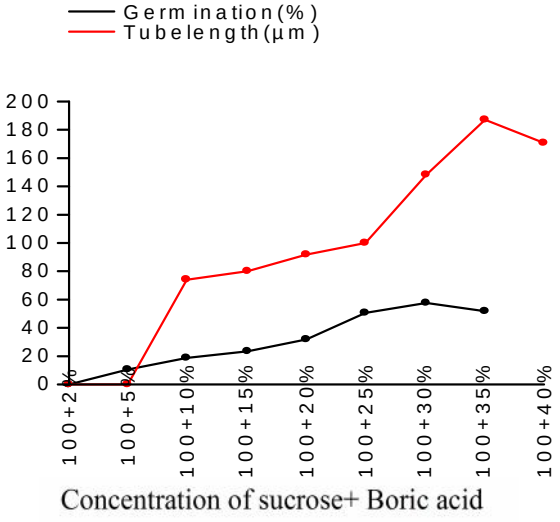

Fig. 2. Pollen germination and tube length at different concentration of sucrose and boric acid

the brush pollination mechanism had the lowest P/O, while species with valvular and pump mechanism had the highest P/O. However, pollen presentation (primary and secondary) and flower size did not have a significant effect on P/O. The high P/O ratio along with high pollen production in *Solanum viarum* attributes to its high seed set.

Meiotic study: The chromosome number of *Solanum viarum* is counted to n = 12, i.e. with 12 bivalents. Different chromosomal stages were observed during meiotic division. In *Solanum viarum*, the normal pollen meiosis (Plate I, g) is indicating the normal pollen development, however, in some cases though the frequency is very low they show some meiotic abnormalities in anaphase I, viz., abnormal separation of the bivalents and irregular distribution of chromosomes (Plate I, h and i).

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LITERATURE CITED

Bhattacharya, A.; Mondal, S. & Mandal, S. 1997. *In vitro* pollen germination of *Delonix regia* (Boj.) Raf. *Sci. Cult.* 63(5-6): 143 – 144.

Bhowmik S, Datta BK 2012. *In vitro* pollen germination in *Eichhornia Crassipes* (Mart.) Solms: An insight into its preferred mode of clonal reproduction. *Notu. Sci. Biol.* 4(2): 65 – 71.

Biswas, K.; Mondal, S. & Mandal, S. 2008. Studies on *in vitro* pollen germination of *Solanum surrattense* Burm. f. and *Solanum nigrum* L. *Sci. Cult.* 74(3-4): 149 – 152.

Bryson, C.T. 1996. The role of United States Department of Agriculture, Agriculture Research Service in the control of introduced weeds. *Castanea.* 61: 261 – 270.

Budavari, S. 1989. The Merck index 11th ed, Merck and Co., Inc., Rahway NJ. Pp. 1606.

Chandra, V. & Srivastava, S.N. 1978. *Solanum viarum* Dunal syn. *Solanum khasianum* Clarke, a crop for production of solasodine. *Ind. Drugs.* 16: 53 – 60.

Coile, N.C. 1993. Tropical soda apple, *Solanum viarum* Dunal: the plant from hell. Greenville, FL: Florida Department of Agriculture and Consumer Services, Division of Plant Industry, *Bot. Circular*. vol. 27, pp- 4.

Dafni, A. & Firmage, D. 2000. Pollen viability and longevity practical, ecological and evolutionary implications. *Plant Syst. Evol*. 222: 113 – 132.

Dafni, A.; Pacni, E. & Nepi, M. 2005. Pollen and stigma biology. In: Dafni, A.; Kevan, P.G. and Husband, B.C. (eds.). *Practical Pollination Biology*. Cambridge, Ontario, Enviroquest Ltd. Pp. 83 – 142.

Davies, R.R.1969. Climate and topographyin relation to aeroallergens at Davos and London. *Acta Alerg*. 24: 432 – 496.

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy- Angiosperms*. Almqvist and Wiksell, Stockholm.

Erdtman, G. 1960. The acetolysis method – A revised description. *Sv. Bot. Tidsskr.* 54: 261 – 264.

Faegri, K. & Iversen, J. 1964. *Textbook of pollen analysis*. Munksgaard, Copenhagen, 2nd ed, 237pp.

Gauch, Hg. & Dugger, W.M. Jr. 1953. The role of boron in the translocation of sucrose. *Pl. Physiol*. 28: 457 – 466.

Gupta, S.; Bhattacharya, K.N. & Chanda, S. 1989. *In vitro* pollen germination of *Solanum sisymbriifolium*. *J. Palynol*. 25: 65 – 72.

Hall, D.W., Currey, W.L. & Orsennigo, J.R. 1998. Weeds from other places: the Florida beachhead is established. *Weed Tech*. 12: 720 – 725.

Hamilton, E.D.1959. Studies on the airspora. *Acta allergy*.13: 143 – 175.

Hendique, A.K. 1986. Breeding behaviourof *Solanum khasianum* Clarke. *Euphytica*. 35: 631 – 632.

Kelly, J.K.; Rascha, A. & Kalisz, S. 2002. A method to estimate pollen viability from pollen size variation. *Am. J. Bot.* 89(6): 1021 – 1023.

Klein, M. 2000. Pollen studies in plant breeding and selection. *Bot. Guidebooks.* 24: 171 – 193.

Mandal, S. & Chanda, S. 1981. Aero-allergens of West Bengal in the context of environmental pollution and respiratory allergy. *Biol. Membr*. 6: 1 – 61.

Mandal, S.; Barui, N.C. & Ganguly, P.N. 1982. *In vitro* pollen germination in *Arachis hypogoea*. *Sci. Cult*. 48: 115 – 116.

Mathur, G. and Mohan Ram, H. Y. 1986. Floral biology and pollination of *Lantana camara*. *Phytomorphology*. 36: 79 – 100.

Mohi-ud-din, G.; Nawchoo, I.A. & Wafai, B. 2007. Reproductive biology of *Picrorhiza kurrooa* Royle Ex Bnth. – An endangered medicinal plant of the north west Himalaya. *Phytomorphology*. 57: 109 – 116.

Moza, M.K. & Bhatnagar, A.K. 2007. Plant reproductive biology studies crucial for conservation. *Curr. Sci*. 92: 243 – 244.

Mullahey, J.J. & Akanda, R.U. 1996. Reproductive biology and control of tropical soda apple (*Solanum viarum*). *Proceed. South. Weed Sci. Soc.* 49: 146 – 147.

Mullahey, J.J. & Colvin, D.L. 1993. *Tropical soda apple: A new noxious weed in Florida*. Gainesville, FL: Florida Cooperative Extension Service Initiative for Future Agriculture and Food Systems, University of Florida, Fact Sheet WRS- 7.

Nair, P.K.K. & Rastogi, K. 1963. Pollen production in some allergenic plants. *Curr. Sci*. 32 (12): 566 – 567.

Nair, P.K.K. 1964. *Adv Palynol*. National Botanical Garden, Lucknow, India.

Nair, P.K.K. & Kapoor, S.K. 1973. Pollen morphology and production in *Daucas carota* L. *J. Palynol*. 9: 152 – 159.

Nayak, B.G. & Patil, S.K. 2001.Variation of solasodine in *Solanum viarum* Dunal with maturity stages. *Karnataka J. Agri. Sci*. 14: 185 – 186.

Nee, M. 1991. Synopsis of *Solanum* Section *Ancanthophora*: a revision of interest for glyco-alkaloids. In: Hawkes, J. G., Lester, R. N., Nee, M. and Estrata, N. (eds.). *Solanaceae III: Taxonomy, Chemistry, Evolution*. Kew, Richmond, Surrey, UK: Royal Botanic Gardens. Pp. 258 – 266.

Norton, J.D. 1966. Testing of Plum pollen viability with tetrazolium salts. *J. Am. Soc. Hort. Sci*. 89: 132 – 134.

Nyine, M. & Pillay, M. 2007. Banana nectar as a medium for testing pollen viability and germination in *Musa*. *Afr. J. Biotech.* 6: 1175 – 1180.

Pal, J.K.; Datta, B.K.; Mandal, S. & Bhattacharya, G.N. 1989. Studies on the *in vitro* pollen germination of *Cassia fistula* L. *Mendel*. 6(3-4): 311 – 315.

Reddi, C.S. & Janki Bai, A. 1981. Floral Biology of *Mimusops elengi* L. *J. Bomb. Nat. Hist. Soc*. 77: 471 – 475.

Reis, N.M. & Kostic, S.R. 1976. Pollen season severity and meteorologic parameters in central New Jersy. *J. Allergy Clin. Immun*. 57: 609 – 614.

Satyabrata, M.; Chandra, R.; Geetha, K.A. & Mandal, K. 2000. Production technology of some important medicinal and aromatic crops developed under the All India Coordinated Research Project. *Indian J. Arecanut, Spic. Med. Pl.* 2: 88 – 98.

Sedgley, M. & Griffin, A.R. 1989. *Sexual Reproduction of Tree Crops*. Academic Press, London.

Shivanna, K.R. & Rangaswamy, N.S. 1993. *Pollen Biology – A laboratory manual*. Narosa Publishing House, New Delhi.

Shivanna, K.R. 2003. *Pollen Biology and Biotechnology.* Oxford and ISH publishing Co. Pvt. Ltd., New Delhi.

Sidhu, R.J.K. & Malik, C.P. 1986. Metabolic rate of boron in germinating pollen and growing pollen tubes. *Biotechnology and ecology of pollen* (ed. Mulcahy *et. al*.), Springer, New York. Pp. 373 – 378.

Smart, I.J., Tuddenham, W. G. & Knox, R.B.1979. Aerobiology of grass pollen in the city atmosphere of Melbourne: effects of weather parameters and pollen sources. *Aust. J. Bot*. 27: 335 – 342.

Stace, C.A. 1991. *Plant Taxonomy and Biosystemetics*. 2nd Ed. Cambridge University Press, Cambridge.

Taiz, L. & Zeiger, E. 2003. *Plant Physiology* 3rd edn. Panima Publishing Corporation, New Delhi.

Walker, J.W. & Doyle, J.A. 1975. The bases of Angiosperm Phylogeny: palynology. *Ann. Missouri Bot. Gard.* 62 (3): 664 – 723.

Wodehouse, R.P. 1935. *Pollen grains*. McGraw Hill and Co. New York.