Our Nature (2011) 9: 41-48

Does Canopy Height Determine the Pollen Viability and Stigma Receptivity? A Cross-population Observation on *Shorea robusta* Gaertn. f.

A. Bhattacharya

Department of Botany, Krishnagar Govt. College, Krishnagar, Nadia- 741 101, West Bengal, India
E-mail: *drashoke@gmail.com*

Received: 16.04.2011, Accepted: 21.08.2011

Abstract
Pollen viability and stigma receptivity are prerequisite for successful pollination and fruit and/or seed set in flowering plants. The present paper deals with dependence of tree canopy height on pollen viability and stigma receptivity of *Shorea robusta* in a cultivated mature Dipterocarp forest. Pollen and stigma samples were collected from different canopy height and brought to the laboratory for direct assay. Pollen viability were studied following different stain tests using malachite green, acid fuchsin and TTC respectively and also using *in vitro* pollen germination in a nutrient medium consisting of sucrose and different salts of boron, calcium, magnesium and potassium. Stigma receptivity was studied by softening the tissues using sodium hydroxide and stained with water soluble aniline blue. The frequency of pollen viability increased steadily from low to high canopy height. The percentage of viable pollen was different for three different assays showing similar trend of increase. The middle and high canopy height did not show much variation in pollen viability compared to low canopies. The percentage of stigma receptivity increased steadily from low to high canopy height showing minor differences between them compared to pollen viability. So the tree canopy height may attain a vital role in determining pollen viability and stigma receptivity, the major factors for new offspring production in natural/cultivated forest settings.

***Key word*:** Forest, population genetics, modeling process, Dipterocarpaceae.

Introduction

Successful fruit-set generally depend upon viable pollen grains. Modeling processes that control forest tree population genetics may require the basic information on pollen viability and reproductive efforts of forest tree species. A frequent problem with pollen viability studies is that it does not concern with stability, and may ignore the dynamics in the forest. In mature forest, at different heights, sun and wind exposure, and fluctuation in pollen flow between sun-drenched and shaded gaps may impose vital importance into the viability and physiological functioning of pollen grains consequently affecting progeny production. Although some primary botanical rules at anther and pistil level, which may vary among individuals (Kearns and Inouye, 1993; Bhattacharya and Mandal, 2003), might control the physiological functioning of viable pollens. Pollen viability could be assessed by different methods like staining with non-vital dyes, *in vitro* germination test (Heslop-Harrison *et al.,* 1984; Shivanna and Johri, 1985) or by *in vivo* test as analyzing the final seed set (Shivanna and Johri, 1985;

Razora and Zsuffa, 1986). Non-vital stains are useful to determine pollen viability quickly (Kearns and Inouye, 1993). Analyzing the final seed set is the most reliable method (Shivanna and Johri, 1985; Bhattacharya and Mandal, 2004) but it is not useful because it takes so much time to get proper information. According to Barrow (1983) *in vitro* germination method is reliable under assumption that pollens capable of germination would be fertile, although there are some difficulties due to arising problems in pollen tube development in *in vitro* conditions (Mulcahy and Mulcahy, 1988). Viability is considered as a phase in which pollen remains able to germinate on an appropriate (receptive and compatible) stigma (Dafni, 1992; Dafni and Firmage, 2000), which varies from species to species, ranging from minutes after shedding to months (Shivanna and Johri, 1985). The importance of studies dealing with pollen viability has been greatly recognized in pollen biology as a priority for helping to understand species reproductive performance and for successful breeding programme implementation (Dafni and Firmage, 2000).

Stigma receptivity is a crucial stage in flower maturation that may influence pollination rate and success at different stages in flower life cycle. Importance of pollinators, interferences between male and female functions and chances of gametophytic selection have dependence on stigma receptivity (Barrett, 2002). Success in breeding is also dependent on stigma receptive period. Stigma receptivity could be assessed by *in vivo* pollen germination study on the stigma surface (Stone *et al.,* 1995; Bhattacharya *et al*, 2005) and percentage of germinating pollens on stigma (Shivanna and Sastri, 1981). Stigma receptivity is nothing but stigmatic capacity to support pollen germination (Gonzales *et al.*, 1995). It is the readiness to maintain pollen germination (Dafni, 1992). Forest tree improvement depends on the recombination of characters that arises via sexual reproduction. Outcomes are enhanced by knowledge of the reproductive biology and biological processes involved in each plant species of both wild and cultivated forest settings (Bhattacharya, 2005). The basic information on stigma receptivity is important to identify flower age, to determine pollination effectiveness, to study breeding systems and to determine pollinators' effectiveness (Bhattacharya and Mandal, 2000). No documentation on the pollen viability and stigma receptivity at different canopy height of *Shorea robusta* in cultivated forest setting is recorded so far.

The present observation on the pollen viability and stigma receptivity of *Shorea robusta* in a cultivated Dipterocarp mature forest shows its dependence on different canopy heights of same tree.

Materials and methods

Shorea robusta, of the family Dipterocarpaceae, is a large tree, with ovate-oblong leaves. Its bark has deep, vertical fissures. The average height of each tree varies from 30-50 m. The tree flowers from February to April. Harvesting of fruit is from June to August. The fruit is winged and usually has only one seed. It is cultivated in different forests of India for the most important commercial timber used in construction. The seeds are used for fat extraction. The oilcake, though rich in tannins (5-8%), has been used in proportions of up to 20% in concentrates for cattle without detrimental effects. As the protein remains completely undigested, the

oilcake yields energy only. Seed cake can constitute up to 10% of poultry and pig rations without changes in performance. The leaves can be used as roughage for cattle.

Pollen viability studies

The fresh flowers were collected at random from three different canopy heights (ca. 10-15 m; 15-30 m and >30 m) of 14 different trees, randomly selected within forest, at intervals of four hours (08.00, 12.00 and 16.00 h) preceding flower anthesis (07.00-08.00 h). At each time ten flowers from each canopy were collected for study spectrum. The entire period of investigation was for 17 days. The pollen samples were assessed immediately after collection for viability study, using Alexander's stain, Tetrazolium salt and *in vitro* germination test. Ten flowers from each canopy height of 14 different trees were collected and pollen samples taken from all flowers were treated with stain solutions and culture medium for *in vitro* germination, an average value based on 17 days' observation was taken into consideration to minimize the experimental error.

Alexander's stain

Alexander (1969) is a dye containing malachite green and acid fuchsin, which differentially stains aborted and viable pollen; malachite green stains cellulose in pollen grains, and acid fuchsin stains the protoplasm. Thus aborted pollens appear green while pollens with protoplasm appear pink. Pollen samples were placed in a drop of this stain, mounted after gently heating and scored under microscope at 400X magnification following the procedure of Kearns and Inouye (1993).

TTC (2,3,5- triphenyl tetrazolium chloride) test

A modified staining reagent consisting of 0.5% solution of TTC in 20% sucrose was prepared. A drop of this solution was taken on microscope slide and pollens added, mounted immediately to exclude oxygen. The slides were incubated at 60°C for 3 hours, and then scored under microscope at 400X magnification following Shivanna and Tangaswamy (1992). Pollen grains stained red in presence of reductases, indicating the presence of active enzymes. These red grains were considered as viable.

In vitro germination

To ratify the usefulness of stain tests in *Shorea robusta*, data obtained in these assays were compared to *in vitro* germination. A modified 'basic medium' of Brewbaker and Kwack (1963) composed of 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulphate and 100 ppm potassium nitrate was prepared by dissolving all the ingredients in 100 ml of distilled water. Modifying the 'basic medium' with different levels of the following components developed a satisfactory medium for *S. robusta* pollen: sucrose 200 g/l, boric acid 100 mg/l, calcium nitrate 100 mg/l, magnesium sulphate 200 mg/l and potassium nitrate 50 mg/l; pH 7.5. This culture medium was used to evaluate germination. A drop of this medium was taken on each groove of microscopic slides, pollens from immediately dehisced anthers were placed with platinum needle on medium, slides were incubated for 6 hours inside petridishes lined with moist filter paper at laboratory temperature (32°C) and RH (91%). After incubation was over, pollen

cultures were scored according to Shivanna and Tangaswamy (1992). Pollen grains were considered germinated when the length of the tube was more than the diameter of the pollen grain (Shivanna and Tangaswamy, 1992).

Stigma receptivity

To determine the stigma receptivity ten flowers in different canopy height of same tree of 14 different trees were labeled at the time of anthesis and then the stigmas from these labeled flowers were collected on successive times and days for investigation. At every time ten flowers from each height were studied to understand stigma receptivity in relation to *in vivo* pollen behavior over stigma surface. The stigmas along with some portion of styles were taken out of the flowers at different times after anthesis, until the receptive period was over. The stigmas along with the portion of styles were then fixed in acetic-alcohol (1:1) for 24 hours, and cleared with 8N NaOH until the tissue become soft. These were then washed and mounted in 0.05% aniline blue in 0.05 M sodium dihydrogen phosphate (pH 7.5). The tissues got flattened by applying gentle pressure and were preceded for observation under light microscope in 400X magnification. The procedure of stigma receptivity measurement adopted in present paper is slight modification of existing protocol proposed by Shivanna and Tangaswamy (1992). The presence of germinating pollens over stigma surface indicated its receptivity.

Statistical analysis

Statistical analyses were done using SPSS 11.0 version of statistical software. Mean value of 17 days, collecting pollen samples 3 times at 4 hour intervals in each day were considered. Number of trees were considered to be 14 (T1-T14); in 3 canopy heights: L (low), ca.10-15 m; M (middle), ca. 15-30 m; H (high), ca. >30 m. The mean values of pollen viability were calculated by finding the sum of all the individual observations and then dividing the total by the number of observations in each viability test. Standard deviations were obtained from the variance of each test by extracting the square root and were expressed in the units in which the measurements were taken. The standard errors of the mean were calculated from the standard deviation of samples, by dividing it by $\sqrt{n}$ (n is sample size). The correlation coefficient value (R) was calculated by dividing the sum of products of deviations from their respective means by the square root of the products of the sums of squares of deviations from the respective means of the two variables (canopy height vs. stigma receptivity).

Results and discussion

Results of this work mark the importance of canopy height in pollen viability and stigma receptivity of *Shorea robusta*. The pollens are less frequent viable in low canopy height (ca.10-15 m) compared to middle (ca.15-30 m) and high (ca. >30 m) canopy heights. The highest pollen viability (80.6%) was recorded in middle canopy height in Alexnader's stain solution whereas it was found to be minimum (20.6%) at *in vitro* germination (Tab. 1). The percentage of pollen viability in each canopy of 14 trees differs, but the general trends of pollen viability in different canopy heights remained same, showing the sequence of low<middle<high canopy height (Tab. 1). The mean percentages of viability varied in 3 assays. The high frequency of viable pollens was obtained using Alexander's

Table 1. Pollen viability of *Shorea robusta* at different canopy heights.

Canopy height	Viable pollen (% ± SE)*								
	Alexander's stain			TTC			*In vitro* germination		
	L	M	H	L	M	H	L	M	H
T1	50.8±1.92	66.8±6.14	72.2±4.6	44.2±3.27	66.0±2.9	72.2±1.92	11.0±1.58	22.0±2.23	24.8±2.40
T2	51.0±1.58	63.8±2.70	80.4±3.71	41.0±2.23	65.2±5.16	78.0±3.16	12.6±2.07	26.4±3.84	27.8±3.27
T3	53.4± 2.30	66.4±4.82	80.6±2.19	42.2±1.92	67.2±2.58	70.4±2.30	12.2±1.92	22.8±2.17	30.2±1.92
T4	47.2±3.35	63.2±1.48	71.6±1.14	33.6±2.07	61.6±2.07	66.6±2.70	9.2±1.30	21.4±2.07	27.4±1.14
T5	55.2±2.95	68.4±1.67	77.6±1.14	51.6±1.95	72.4±1.67	82.4±2.07	16.2±1.92	29.8±1.30	32.6±1.82
T6	51.8±1.64	70.8±2.17	77.2±1.92	38.8±2.39	61.2±6.30	71.8±4.44	13.4±2.30	26.6±5.02	30.8±2.59
T7	57.6±2.97	66.0±4.0	74.8±2.86	51.6±2.07	70.2±1.64	79.8±1.92	12.6±3.36	23.6±2.40	30.2±5.01
T8	41.2±3.70	57.8±4.66	66.4±4.22	27.4±4.03	57.4±5.02	61.4±3.50	8.4±1.14	18.2±2.59	21.2±1.48
T9	44.8±2.17	58.6±3.36	67.2±2.49	36.2±3.19	54.6±2.30	60.8±3.63	10.8±2.77	22.4±2.97	28.8±3.27
T10	39.2±4.32	60.8±3.70	67.4±6.80	27.4±4.72	51.6±2.61	58.4±3.65	8.2±3.27	18.4±3.97	23.8±1.92
T11	48.0±3.39	67.6±6.80	72.2±4.08	34.2±3.11	56.4±6.02	66.2±3.96	12.2±4.32	21.4±2.50	27.4±3.65
T12	48.2±2.59	65.0±3.08	70.6±6.10	30.6±1.14	51.4±4.92	60.4±3.36	9.4±1.67	18.6±4.72	20.6±3.85
T13	52.6±2.88	71.2±5.72	81.8±5.36	40.0±4.47	62.4±5.32	75.0±3.08	12.6±2.40	22.8±3.03	28.6±4.28
T14	56.6±5.54	72.4±6.87	80.6±4.03	31.4±5.73	55.2±3.56	65.4±3.65	10.6±1.14	21.0±2.74	25.4±2.88

*Mean value of 17 days, collecting pollen samples 3 times at 4 hour intervals in each day. Number of trees 14 (T1-T14); in 3 canopy heights: L (low), ca.10-15 m; M (middle), ca. 15-30 m; H (high), ca. >30 m.

stain, while it was less in TTC and *in vitro* culture medium (Tab. 1). The stigma receptive period and mean percentage of receptivity showed canopy wise variation. The highest percentage (58-70%) of receptive stigmas were noticed in low, middle and high canopy heights at 1500 hrs after flower anthesis where as it was lowest (9-20%) at 2100 hrs after flower anthesis in same canopy position and it showed a strong positive correlation between stigma receptivity and canopy height (Figs. 1, 2). The mean differences of percentage receptivity between three canopy heights were negligible rather than difference of viable pollens. The receptive period attained at following more or less same time, but the percentage of receptivity varied. The trend remained same showing sequence of low<middle<high canopy height considering percentage receptivity (Figs. 1, 2).

Viability of pollen has been defined as having the capacity to live, grow, germinate or develop. It has been reported that pollen viability is so liable that it may differ when pollen is collected at different times of the day (Baez *et al.*, 2002; Davarynejad *et al*., 2008). Pollen collected from flowers in anthesis for one-hour show decreased germination (Shivanna and Tangaswamy, 1992). Pollen viability has a genetic component; results may be different depending on the genetic variability of individuals used as donors (Meo, 1999). The use of Alexander's procedure in present investigation may have led to overestimation of pollen viability since staining capacity depends not on the viability but on protoplasm content of the pollen grains. So, this measure of pollen stain ability may depart considerably from real value of pollen viability (Dafni, 1992). The proportion of viable pollen grains steadily increases with canopy height. Canopy height >30 m exhibited an average proportion of viable pollens 1-1.5 times greater than the one found in trees <30 m. Pollen may express genetically based traits during its development, maturation and free

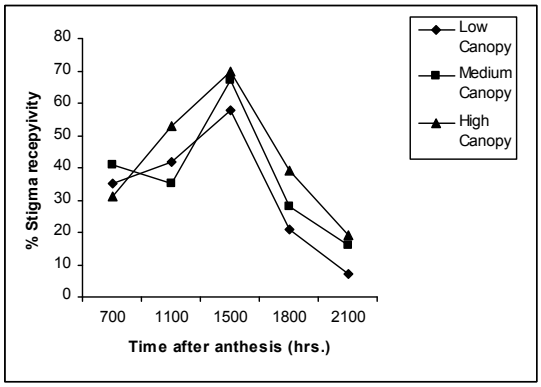

Figure 1. Stigma receptivity at different times after flower anthesis.

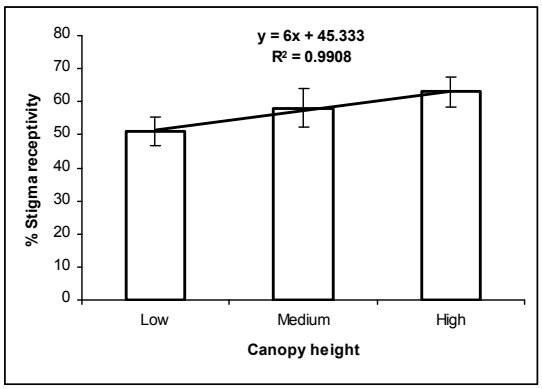

Figure 2. Stigma receptivity at different canopy heights.

dispersal phases. Reproductive effort, physiological stress, resource availability may be the factors for variation in pollen viability. Populations of out crossing plants are far from being genetically uniform (Heywood, 1991; Bhattacharya *et al*., 2005), and may constitute important sources of variability. Accumulation of somatic mutations with increasing heights might be a concept to our understanding of the pollen viability. Regarding germination *in vitro* the culture is dependent on the quality of pollens (Heslop-Harrison *et al*., 1984). Temperature has appeared as a critical factor for *in vitro* germination. The percentage of stigma receptivity increased steadily from low to high canopies showing minor differences between them. Receptivity of the stigma is a critical factor for the successful completion of post-pollination events. Receptivity is generally maximal soon after anthesis. The period of receptivity varies from species to species, and is influenced by temperature and humidity. Alternation in temperature and humidity drastically reduce the period of stigma receptivity. The duration of stigma receptivity varies from a few minutes to two or three weeks (Dafni, 1992; Fohouo *et al*., 2008). The age of the flower, time of the day, and the presence or absence of stigmatic exudates may attain importance in determining stigma receptivity (Dumas and Gaude, 1983).

Conclusion

The tree canopy heights attain a vital role in determining pollen viability and stigma receptivity, the major factors for new offspring production in natural/cultivated forest settings. Appropriate management of pollen viability and stigma receptivity in this tree species is valuable for success of pollination programmes within and between genotypes of forestry interest. Comparisons of the expression of the accumulated genetic load in the gametophytes within single individual or across individuals of same species differing in heights may represent the basic points of making forest tree population genetics models.

Acknowledgements

The author is thankful to the University Grants Commission, New Delhi for providing financial assistance under UGC-CPE scheme (2010-2011) through Krishnagar Govt. College, Krishnagar,

Nadia and also to Dr. M. Das, Officer-in-Charge, Krishnagar Govt. College for providing constant encouragement and facility to carry out the research work.

References

Alexander, M.P. 1969. Differential staining of aborted and nonaborted pollen. *Stain Tech*. ***44*(*3*):** 117-122.

Baez, P., M. Riveros and C. Lehnebach 2002. Viability and longevity of pollen of *Nothofagus* species in south Chile. *Newzealand J. Bot*. ***40*:** 671-678.

Barrett, S.C.H. 2002. Sexual interference of the floral kind. *Heredity* ***88*:** 154-159.

Barrow, J.R. 1983. Comparisons among pollen viability measurements in cotton. *Crop Sci*. ***23*:** 734-736.

Bhattacharya, A. 2005. Does pollen abortion increase with plant age? *Canadian J. Plant Sci*. ***85*(*1*):** 151-153.

Bhattacharya, A. and S. Mandal 2000. Pollination biology in *Bombax ceiba* Linn. *Current Science* ***79*(*12*):** 1706-1712.

Bhattacharya, A. and S. Mandal 2003. Stigma form and surface in relation to *in vivo* pollen germination in *Butea monosperma* (Lamk.) Taub. and *Catharanthus roseus* (Linn.) G. Don. *Phytomorphology* ***53*(*2*):** 179-185.

Bhattacharya, A. and S. Mandal 2004. Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk. *Grana* ***43*(*1*):** 48-56.

Bhattacharya, A., K. Datta and S.K. Datta 2005. Floral biology, floral resource constraints and pollination limitation in *Jatropha curcas* L. *Pak. J. Biol. Sci*. ***8*(*3*):** 456-460.

Bhattacharya, A., S. Mondal and S. Mandal 2005. Pollinating agents of *Eucalyptus citriodora* Hook. Insects or wind? *Asian Journal of Plant Sciences* ***4*(*5*):** 492-495.

Brewbaker, J.L. and B.H. Kwack 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot*. ***50*:** 859-865.

Dafni, A. 1992. *Pollination ecology: A practical approach*. Oxford University Press, New York, ISBN-13: 9780199632992. 250p.

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

Davarynejad, G.H., Z. Szabo, J. Nyeki and T. Szabo 2008. Phenological stages, pollen production level, pollen viability and *in vitro* germination capability of some sour cherry cultivars. *Asian J. Plant Sci*. ***7*:** 672-676.

Dumas, C. and T. Gaude 1983. Stigma-pollen recognition and pollen hydration. *Phytomorphology* ***31*:** 191-201.

Fohouo, F.N.T., D. Djonwangwe and D. Bruckner 2008. Foraging behaviour of the African honey bee (*Apis mellifera adansonii*) on *Annona senegalensis*, Croton macrostachyus, *Psorospermum febrifugum* and *Syzygium guineense* var. *guineense* flowers at ngaoundere (Cameroon). *Pak. J. Biol. Sci*. ***11*:** 719-725.

Gonzales, M.V., M. Coque and M. Herreo 1995. Papillar integrity as an indicator of stigmatic receptivity in kiwifruit (*Actinidia deliciosa*). *J. Exp. Bot.* ***46*:** 263-269.

Heslop-Harrison, J., Y. Heslop-Harrison and K.R. Shivanna 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. TAG *Theor. Appl. Genet*. ***67*:** 367-375.

Heywood, J.S. 1991. Spatial analysis of genetic variation in plant populations. *Ann. Rev. Ecol. Syst.* ***22*:** 335-355.

Kearns, C.A. and D.W. Inouye 1993. *Techniques for pollination biologists.* Colorado University Press, Colorado. ISBN-13: 978-0870812811. 583p.

Meo, A.A. 1999. Impact of pollen and intergenetic crosses between graminaceous (Poaceae) plants. *Pak. J. Biol. Sci*. ***2*:** 809-812.

Mulcahy, D.L. and G.B. Mulcahy 1988. The effect of supplemented media on the growth *in vitro* of bi- and trinucleate pollen. *Plant Sci*. ***55*:** 213-216.

Razora, O.P. and L. Zsuffa 1986. Pollen viability of some *Populus* species as indicated by *in vitro*

pollen germination and tetrazolium chloride staining. *Can. J. Bot*. ***64*****:** 1086-1088.

Shivanna, K.R. and B.M. Johri 1985. *The angiosperm pollen: Structure and function*. Wiley Eastern Ltd., New Delhi.

Shivanna, K.R. and D.C. Sastri 1981. Stigma-surface esterase activity and stigma receptivity in some taxa characterized by wet stigmas. *Ann. Bot*. ***47*****:** 53-64.

Shivanna, K.R. and N.S. Tangaswamy 1992. *Pollen biology: A laboratory manual*. Springer-Verlag, Heidelberg, ISBN-13: 978-0387551708. 119p.

Stone, J.L., J.D. Thompson and S.J. Dent-Acosta 1995. Assessment of pollen viability in hand-pollination experiments: A review. *Am. J. Bot.* ***82*****:** 1186-1197.