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Reproductive Biology and Variability Studies in *Dalbergia sissoo* (Roxb.)

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ABSTRACT

Dalbergia sissoo, which is an important tree species is native to Indo–Gangetic basin is also found growing naturally in Nepal, Butan, Pakistan and Bangladesh. Studies on variability show that the species is represented by two morphotypes i.e small leaved trees and large leaved trees. Even Bark colour and branching pattern is also variable. Detailed studies on reproductive biology concludes that the species is self pollinated but the occurrence of cross pollination in the species can also not be ruled out due to occurrence of some factors which support it.

INTRODUCTION

Dalbergia sissoo is distributed throughout the sub Himalayan tract usually up to 900m but sometimes ascending to about 1500m (Sharma et al. 2000). It has been cultivated or self sown in the most parts of Indo-Pakistan sub- continent for a long time. *D. sissoo* is a native of Indo-Gangetic basins and grows naturally in parts of Bhutan, Nepal Pakistan and Bangladesh. In this connection, the irrigated and inundation plantations of Punjab now mostly in Pakistan deserve special mention. The tree has been introduced into many other countries like Java, Nigeria, Mauritius, Srilanka, Kenya, etc. It is very likely that sissoo is indigenous to only the sub-Himalayan tract and has been introduced by man elsewhere.

In the natural habitat of shisham, the absolute maximum shade temperature varies from 39c to 49c and the absolute minimum from 4c to 6c. The normal rainfall varies from 760-4570mm most of which is concentrated in 3-4 months during monsoon season followed by a long dry spell. In its natural state, *D. sissoo* grows most typically on alluvial ground either in the beds of river or mostly on the sand or gravel along the banks of rivers often very gregariously. The tree has been introduced over the length and breadth of India in various localities with different kinds of climate and soil and its preference to a porous well irrigated soils with adequate moisture has been its characteristics feature wherever its growth has been a marked success. The tree is however capable of existing in very poor soils, on hilly cliffs even amidst rocky outcrop in extreme cases clinging to the crevices and spreading by means of root suckers. It does not tolerate water logging but is moisture-loving species.

D. sissoo has been widely recognised as an important multipurpose tree species. Its timber is used for making cabinets, veneers, bent wood articles like furniture, superior quality felloes for wheels, in gun carriage, in ordnance factories, etc. It is also used for boat building, brush backs and to a small extent for unshielded bobbins. Considerable use in the sports equipments such as

croquet, mallet heads and balls, as frames of tennis racquets cannot be disparaged. Shisham wood is very hard with specific gravity of 0.78-0.83 with calorific values of the sapwood and heartwood being 4908 and 5181 Kcal/Kg, respectively (Bakshi and Nautiyal 2006). It's stands and scattered trees on farmlands are depleting fast in north-western parts of India as the urban people have developed fancy for its wood. Its wood is hard, heavy, strong, double elastic, seasons well and is decay resistant. Multinational companies are promoting its plantations on farm lands. Sissoo is a deciduous tree often with crooked trunk and light crown. It fixes atmospheric nitrogen to ameliorate the site. Studies on reproductive biology and breeding systems are very important. It is the basic tool to carry out any future tree breeding programme. The breeding system, through the mechanism of reproduction, the method of pollination and the degree of compatibility, regulates the amount of recombination. The efficiency of reproductive system depends upon our understanding of factors affecting reproductive biology. Studies on variation in flowering, pollination, seed set, seed quality, seed viability and germination are of practical importance. To carry out any improvement programme, it is desirable to collect all the information regarding the variability, which is existing in the species with respect to the tree, flower, fruit, seed and even chromosomal level. Thus, after getting the variability pattern in the species, requisite steps should be taken to exploit these variations for any improvement programmes.

MATERIAL AND METHODS

Surveys were carried out in different agro-climatic zones of Punjab during flowering and fruiting period for consecutive three years to record various observations. Trees growing along the roadside were taken under observations to carryout the present studies. Morphological variations were recorded during surveys through visual studies while cytological variations were known through preparation of meiotic slides by fixing the flowering buds in carnoy's fixative. The complete data on the flowering chronology was recorded by visiting the marked fifty trees daily as soon as the deciduous phase was over. Data on floral biology covering floral initiation, development, maturation, anthesis, receptivity, structure of flower, size of floral organs, pollen size, pollen structure, pollen germination were recorded. Anthesis time of the flowers were noted by tagging fully mature 100 buds per tree, on sunny day. These marked buds were visited after every hour. The number of buds that opened was noted for 24 hours. Receptivity period was known through visual observations. In this case, the stigma was kept under constant observations with the help of hand lens and the stigma on which the shinning surface was seen was called as receptive stigma. Pollination behavior was known through carrying out the pollination experiments separately for cross and self-pollination.

Fruit biology covering parameters like fruit set percentage, time taken for full development of fruit, fruit shape, weight, size, colour, number of seeds formed per pod were also recorded. These studies were carried out separately for the fruits collected from the individuals, which were self pollinated under controlled conditions and also from the naturally pollinated individuals. Seed biology covered seed set percentage, seed shape, size, weight, colour, germination (fresh and after storage). These parameters were also analysed separately from individuals with self pollination done manually and also from individuals where pollination occurred naturally. These results were statistically analysed for comparison at 5% level of significance.

RESULTS AND DISCUSSION

Morphological Variations

A survey revealed that there existed a wide variation in the phenotypic characters of individuals. During survey two morphotypes have been identified. Large variation was observed in leaf size, very small leaf size and large leaf size, Average size of leaves was recorded with scale manually. Leaves are imparipinnate, alternate with swollen base, rachis is 3.4- 9cms long. Leaflets are 3-5 in number with petiole 3.5-6mm in length.

Small leaves have 2.69±0.27cm length and 2.19± 0.26cm breadth. The average length and breadth of larger leaves were observed to be 5.44 ± 0.28cm and 4.91± 0.22cm, respectively. These two types of trees were identified and marked.

A wide range of variability was also observed for stem straightness and other morphological characters. Stem form ranged from branched or forked to completely straight trees. Large variations were also seen in total tree height, clear bole height, girth at breast height and crown thickness. Average total tree height was 12.28±2.25 m. Clean bole height 4.08±1.12 m, girth at breast height 105.67± 28.90 cm and crown thickness ranged from very light to very thick. Branching pattern in *D. sissoo* ranged from upright branching to looping type pattern. Variability in bark colour was also noticed. The bark colour was recorded variable between brown to black.

Phenological variations including fruit and seed variability

Trees of *D. sissoo* largely varied for flowering intensity. Trees with very dense flowering and very light flowering were also identified. Some differences in the flowering period of the trees was also observed. Some trees flowered earlier than others, which can be due to change of environmental factors at different sites. Due to large variation in flower bearing capacity of trees, large variation in pod setting of trees was also observed. Trees which bear less flowers produced very less pods or even had no pods and tree with very heavy flowering showed very heavy pod setting. Seeds in the pods ranged from 1 to 4. This variation can be attributed to genetic makeup of the species along with different age and growing environment of the different trees.

Cytological variations

The cytological studies carried out on the species revealed that the diploid chromosome number in the species is 2n = 20. Similar variations have also been reported in species like *Pinus taeda*, *Populus ciliata* and *Prosopis cineraria* by Zobel et al (1960), Khosla et al (1980) and Kckar et al (1986), respectively.

Reproductive Biology

Tree remains leafless during December-January. The leaflets fall separately and before falling they turn brown. In the month of March-April, flowering starts along with the appearance of leaves. Flowering is influenced by temperature and rise in temperature hastens flowering. The whole process from bud initiation to flower opening was completed in 15-20 days (flowering initiated during 2nd week of March, peak during 1st week of April and decline during 4th week of April). It took 7-8 months from bud initiation to fully ripened pods. It was also seen that buds and flowers occur at the same time in different stages of development, even on the same branch of the tree.

Floral Biology

It is observed that the inflorescence of *D. sissoo* was an axilliary panicle, composed of several short spikes with sessile to sub sessile flowers ranging from 7-14 in number per inflorescence. Length of inflorescence was recorded to be 3-8cms. Flowers of *D. sissoo* were small pea shaped, slightly fragrant with a fine toothed calyx, five petals (including two narrow wings and a narrow keel) and were yellowish white in colour. Flower colour changed from yellowish white to orange after pollination. Androecium was composed of nine stamens that are united into a broad stalk, monoadelphous, stamens were in two sizes five large and four small. Length of all floral organs stamen, ovary, petals and sepal were measured to understand the pollination mechanism. It was found that average length of flower petals was 0. 9± 0.07cm and lengths of stamens 0.79± 0.2cm were slightly less than hairy pistil, which was measured to be 0.85± 0.06cm. The size of floral organs was recorded after the anthesis, when all the parts were fully exposed. The stalked hairy pistil had a narrow ovary containing 5-6 ovules, a short style and a dotlike stigma. In the bud stage, the ovary is in bent form and surrounded by anthers.

Data on flower opening was recorded and it was found that maximum number of flower

opening took places between 10.00 to 14.00 hours with a peak between 11.30-13.30 hours. It was also found that dehiscence of anthers also took place in morning hours in the bud stage, just before flower opened.

Receptivity: Receptivity of stigma was noted during reproductive biology study. Data were recorded on the opened flowers and unopened flowers. It was found that stigma becomes receptive few hours before flower opening and it was also noted that it remained receptive for few hours even after flowers opened. The stigma, which showed shiny and sticky surface, was considered as receptive, which was noted with the help of magnifying lens.

Pollen structure: Size and structure of pollen grains from different flowers was measured with the help of microscope using micrometer and occular. Pollen grains were three zonocolpate, thin walled, spherical in shape having size 10.2 m± 0.11 m. This variation could be attributed to genetic makeup of the species along with different age and growing environment of the different trees.

Pollen viability: Viability of pollen grains was measured by the acetocarmine test. Two per cent acetocarmine solution was used to stain pollen grains on slides. Viability of pollen grains was studied at different stages of flower development. Hundred per cent pollen viability was found at bud stage before flowers opening, which decreases considerably with storage. This gives important information about the pollination pattern of the species. When the viability of the pollen grains was checked from the already opened flowers, it was recorded quite low i.e., 56 per cent. This highlights that the chances of the pollen to effect the fertilization are considerably low after the opening of the flowers.

Pollen germination: Pollen germination studies *in vitro* highlighted that the pollen grains germinate in two per cent sucrose solution made in agar- agar.

Mode of Pollination

To know about the mode of pollination in the species, trees were kept under constant observations for the visit of the insects and birds. It was seen that birds do visit the flowers but do not participate in the pollination. Similarly the insects that were found to visit the trees also had no role in the pollination mechanism, insects were found when pollination had already taken place and flowers were in the stage of withering.

Pollination Pattern

Self pollination. Under this experiment single bud inflorescence was selected before opening on each tree. 100 buds were bagged for selfing and data were recorded on pod setting. It was found that 16.25 per cent pod setting occured under controlled self-pollination. Some inflorescences were selected and tagged after counting the buds for recording pod setting percentage in nature and 35 per cent pod setting was recorded. Less pod setting under controlled self pollination with bagging may be due to covering the buds with bags, which may have changed the environment of the bud. Effective pollination was found to take place in the bud condition.

Artificial crossing For this study, young buds in which just corolla become visible were selected on the marked trees. These selected buds were emasculated early in the morning before 8.00AM. This process exposed the gynoeciums surrounded by nine stamens. After emasculation each bud was bagged to prevent contamination. On the same day at 11.00 to 12.00 hour, the emasculated buds were pollinated with the pollens from other trees and again bagged. Data on pod setting was recorded and found that only 5-7 per cent pod setting had taken place. Thus, a conclusion can be drawn from the present study that the trend of pollination in the present species is towards selfing though the occurrence of cross-pollination cannot be ruled out.

Fruit Biology

It has been seen that flowering and fruiting occurs simultaneously as young pods can be seen along with flowers on the inflorescences. Pods matured in November-December. Pods were light brown and flat in shape. In natural pollinated inflorescences, the number of pods/inflorescence

ranged from 4-7.5 with a mean of 6.01, while in selfed ones, it ranged from 3.2-4.5 with mean of 4.0. It was noticed that the pods took about 6-7 months for complete maturity. The difference for mean number of pods per inflorescence between natural and self-pollinated inflorescence was significant.

Pod length: The average pod length in naturally pollinated inflorescence was 5.132 cm while 4.542 cm in self-pollinated inflorescence. The differences between and within the natural and self-pollinated tree for pod length were found significant.

Pod breadth: In natural pollinated inflorescences, the mean pod breadth was observed 7.42mm while in selfed inflorescences, it was measured to 7.40mm. Statistically self and natural pollinated individuals were found at par for this parameter, however, within the natural and self pollinated trees the differences were found significant.

100 pod weight: The mean 100 pod weight in natural pollinated inflorescence was recorded and the range was found between 6.459-8.315gm with an average of 7.055 gm and in self-pollinated pods, it was found between 5.100-6.425gm with the average of 5.647 gm. These average values were found significantly different. The reason for significant differences for various pod characteristics can be attributed to the change in the microclimate of the self pollinated individuals as they were bagged.

Fruit and seed development: Pod is indehiscent in nature and remains on the tree for months together even after attaining the maturity. While this pod development was taking place, seed development also occur inside the pod simultaneously. There were 5-6 ovules/ovary. Thus depending upon the number of ovules fertilized, the corresponding number of seeds also developed inside the pod. The number is usually 1 to 4 seeds per pod. It is observed that the size of pod with one seed is smaller than the pod with 3 or 4 seeds. The seeds are initially green and the cotyledons are fully endospermic, they have the funicle attached to the pod wall. As they attain maturity, the testa becomes hard and brown in colour and the dehydration of the seed takes place.

Seed Biology

Seeds of *D. sissoo* were usually light brown in colour, flat and thin reniform in shape. A sample of 10 seeds was taken from natural pollinated pods for the measurement of seed length. The ten seeds were placed in line lengthwise and with the help of scale the length of ten seeds was measured. The mean length of one seed was observed to be 0.83 cm in natural pollination trees and 0.82 in self pollinated individuals, however, these differences were found non-significant. To measure seed breadth same sample of ten seeds were taken and placed in a line breadth wise and measured with scale. The mean seed breadth of one seed was recorded 0.42 cm in self as well as naturally pollinated individuals. A sample of hundred seeds was taken to measure the seed weight and the average hundred seed weight of *D. sissoo* was recorded with 1.53 gms individual seed weight, which were selfed as well as naturally pollinated.

Seed Germination

No problem/barrier i.e., dormancy in seed germination was recorded and the germination percentage was very high in the fresh seeds i.e., up to 83.6 per cent in some trees. However, the mean seed germination percentage in naturally pollinated individuals was found to 73.68 per cent and in self pollinated individuals the value was 73.99 per cent with no significant difference in the seed germination behavior of either naturally or self pollinated individuals.

Germination behavior: Germination began on the third day of keeping the seeds on the moist petriplate and ended on the 6th day. Maximum germination was on the 4th day of setting up of the experiment. The germination was found to begin slowly i.e., 5-28% on 3rd day while 40-55% germination on the 4th day and after this there was decrease in the germination of the seeds. It was observed that the germination percentage remained very high even after one year of collection (85%-73%) i.e., in the pods collected in the month of January, the seeds retained

germination capacity up to next one year, but after this the germination percentage decreased considerably in the pods, which were stored under open conditions. The differences in germination of seeds in the natural and self pollinated individuals was found statistically significant among the trees itself, which can be due the genetic constitution, age and the environment of the trees.

Factors favouring self pollination in the species

- Ovary in the bud stage is enclosed in the whorls of stamen.
- Dehiscence of anthers takes place at the bud stage.
- Anthesis takes place after the dehiscences of the anthers.
- Receptivity takes place inside the bud.
- Pollen fertility to be very high in the bud stage.

  Controlled pollination experiments led to good amount of pod formation. Very few significant differences were found among the pod characteristics of the self pollinated and naturally pollinated individuals.
- As for the seed characteristics are concerned no significant differences could be found. There is apparently no difference in the seed germination behaviour of the self pollinated and naturally pollinated individuals.

Factors favouring cross pollination in the species.

- Receptivity of the style persisting even after the opening of the flowers. Viability of the pollen grains though decreased but still exists even after the opening of the flowers such that they can effect the pollination.
- Though the role of insects and birds acting as agents in dispersal of the pollen grains has not been found in the present studies but chances of anemophily in the species cannot be ruled out.
- Ovary coming out of the whorls of stamen in receptive stage after the opening of flower may increase the chances of cross pollination as there is no hindrance of other floral organs and chances of any pollen grains in the air can effect the pollination.
- Though very less, but still there is formation of

pods on artificial cross pollination.\ Thus, it can be concluded that there is predominantly self pollination occurring in the species but the chances of cross pollination occurring in the species simultaneously cannot rule out.

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