The Breeding System in Sandal (*Santalum album* L.)

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Abstract

Studies on artificial selfing, natural out-crossing and obligatory selfing showed that sandal is a predominantly outbreeding species though its flower structure was designed for self-pollination. However Sandal does produce seeds by selfing. Self incompatibility was observed to some extent. Heterostyly was noticed in some genotypes. The pollinating agents are Bees, Butterflies and Beetles. Apomixis and parthenocarpy were not noticed.

Key words: Artificial selfing, self incompatibility, outbreeding, heterostyly, pollinating agents.

FDC: 165.41; 176.1 *Santalum album.*

Introduction

Before initiating a rational tree breeding programme of any plant species a knowledge of its breeding system is necessary as it affects not only the pattern of group variation but also adoptive potentialities of the group concerned. The breeding system determines the spread of genetic variability in the population and this in turn is reflected to some extent in the morphology of the population. The importance of open pollination in *Sandal* (*Santalum album*) in relation to seed setting was dealt by BAGCHI and VEERENDRA (1987) but a further detailed study on the precise nature of the breeding system was necessary.

Santalum is a large genus with more than 18 species mostly distributed in south Asia, Australia and Hawaii. In India, Sandal (*Santalum album*) grows profusely in southern parts of the country. No specific varieties or ecotypes of sandal have been identified so far, although a large number of morphological variants exist in the population. PARTHASARATHI *et al.* (1985) reported some differences in isoenzyme patterns in different morphological variants of sandal and mentioned that this could be possibly due to genetic differences. SRIMATHI (1983), while discussing flowering behavior, mentioned that flowering in sandal may appear once, twice and throughout the year in a single population. Detailed studies on all these variabilities however have not been documented. Hence, this study was undertaken on 7 different genotypes and it involves open cross pollination, artificial selfing and seed setting so that the relative importance of pollination mechanisms in elucidating intergenotypic relationships can be assessed.

Materials and Methods

Sandal trees were selected for pollination studies on the Institute of Wood Science and Technology campus, Bangalore. All trees are well established, originated from a single seed source, but details of the seed source are not known. The flowering season varies from ecotype to ecotype. In the studied material, flowering was from July to September. During 1993 and 1994 5 trees each were selected for artificial selfing. Artificial pollination has been done by selecting mature buds on inflorescences which were to open in 2 days, from all sides of the crown. All those buds were emasculated in the evening and the whole inflorescences were bagged for the next 2 days until stigma matures. Then, pollen from same tree was smeared by brush on the stigma and the buds were bagged.

To determine obligatory selfing, 5 trees each were selected during 1993 and 1994. After removing immature buds, the whole inflorescences were bagged and observed for seed setting.

For free out-crossing 5 trees were selected during 1993 and 6 trees during 1994. Mature buds were emasculated from all sides of the crown and left open without bagging. For studying self compatibility, mature buds were kept under bagged condition without emasculation. For the flower studies of the sandal population on the campus, 10 trees were selected randomly and from each tree 10 flowers at different heights were observed for floral variation, as well for relative anther and style position.

Results and Discussions

Floral biology

Most of the trees show flowers of different types. They may be classified as: flowers possessing stigma above anther level designated pin (1 tree), flowers possessing stigma below anther level called thrum (2 trees) and stigma and anther at same level, homostylous (remaining 7 trees). This floral variation varies from tree to tree in the population.

Sandal flowers are minute and light yellowish in colour. They are bisexual, actinomorphic and epigynous. The flower is not differentiated into calyx and corolla. The number of tepals varies 4 to 6 with length and breadth of 1.5 mm to 3 mm and 1.2 mm respectively. Stamens number varies in accordance with the number of tepals. They may or may not possess staminal hairs. The length of stamens varies from 1.5 mm to 2.5 mm. The perianth tube varies in shape from tubular to conical. Gynoecium is epigynous and differentiated into style, stigma and ovary. Style is simple, stigma is lobed, ovules are 1 to 2 in number. The total length of flower varies from 6 mm to 7 mm and breadth from 1.5 mm to 2 mm.

Pollination and seed setting

Sandal flowers are mainly entomophilous. It has been observed that about 15 different species of insects visit sandal flowers. Small quantity of nectar present in the perianth serves as an attractant for insects. Ants and bees are regular visitors.

Pollination mechanism

The pollination studies made were as follows:

1. 500 flowers were bagged in August 1994 and the number of fruits formed were counted.
2. Artificial selfing was done during August and September of 1993 and 1994 involving 5 trees and 5822 flowers.
3. Flowers were subjected to free outcrossing during August and September of 1993 and also in 1994 involving 5 and 6 trees respectively with 5645 flowers.

Hand pollination with pollen belonging to the same plant was done only after proper emasculation. In case of free out-crossing, emasculated flowers were kept open for pollination by pollinating agents. For obligatory self pollination, the buds were bagged without emasculation. The observations made are shown in the *tables 1* to *2*.

Table 1. – Free outcrossing in sandal during 1993 and 1994.

Year	No. of flowers emasculated	No. of flowers setting seeds	% of flower setting seeds	No. of fruits matured	% of fruits matured	% of seeds collected from flower setting seeds
1993	1316	78	5.9	69	5.2	88.5
± SE			± 1.36		± 1.3	
CV (%)			50.99		55.78	
1994	4329	258	5.9	229	5.3	88.7
± SE			± 1.37		± 1.3	
CV (%)			56.03		61.84	

Table 2. – Artificial selfing in sandal during 1993 and 1994.

Year	No. of flowers emasculated	No. of flowers setting seeds	% of flowers setting seeds	No. of fruits matured	% of fruits matured	% of seeds collected from flower setting seeds
1993	2428	174	7.1	84	3.46	48.3
± SE			± 1.91		± 1.04	
CV (%)			53.65		59.92	
1994	3394	238	7.02	58	1.7	24.4
± SE			± 1.81		± 0.40	
CV (%)			51.73		43.87	

From *table 1*, it may be seen that in both flowering seasons there are no significant differences between the percentage flowers setting seeds and matured fruits, close to 6% flowers setting seeds and 5.2% produced fruit. After artificial selfing the mean percent of flowers setting seeds was higher, 7.1% in 1993 and 7.02% in 1994 but the final harvest of seeds during 1993 and 1994 was substancially lower, 3.46% and 1.66% respectively (*Table 2*). This difference between initial fruit formation and final harvest in both flowering seasons may indicate some degree of self incompatibility in the population. It was less in 1993 than in 1994, possibly due to climatological conditions. Variation in the genetic composition and age structure of the population, as well as edaphic conditions are also expected to contribute to the observed heterogeneity of estimates (XIE *et al.,* 1991). The 500 flowers bagged in a separate experiment for obligatory selfing gave only 0.8% success.

Discussion

From the study of floral structure and variations, it can be assumed that sandal is a predominantly out-breeding species. Flowers of sandal are very small and not very showy. The presence of a nondifferentiated perianth, nectar, and small amount of sticky pollen are characteristic features of sandal flowers (SINDHU VEERENDRA and SARMA, 1991). Small size of the flower, nondifferentiation of the perianth, small amount of pollen production and presence of nectar are the characteristic features of autogamous plants (ORUDUFF and MOSQUIN, 1970; ROLLINS, 1963). HAQUE and GHOSHAL (1981) stated that "large flowered varieties are out-breeders and small flowered varieties are inbreeders". Therefore, sandal flowers have a general tendency towards inbreeding. But in view of the differential pollination success of free outcrossing and the relative differences in anthers position and stigmatic level, it can be inferred that sandal is a predominantly out-breeding species.

Floral variations also reveal that the species possess homostylic, pin and thrum type of flowers. Such positional differences of anthers relative to stigmatic level were only observed for individual trees so far. Though, the role of relative position of anthers and stigma was not studied here, it may be that this is a factor reducing pollination success in the case of obligatory self pollination. HAQUE and GHOSHAL (1981) showed that the position of anthers and stigma do affect seed setting in some heterostylic flowers of ornamentals which was increased by hand pollination. Hence, it can be assumed that heterostyly is a mechanism to avoid self pollination in general. It is also important to note that mechanisms exclusively designed to assure self pollination may not necessarily achieve it. KEVEN (1972) reported in *Saxifraga oppositifolia* that movement of filaments and anthers on stigmas did not result in fertilization, even in the absence of cross pollination. Hence, it can be presumed that even though flowers are designed to achieve selfing, sandal is adapted to cross pollination as the predominant method.

In the present study 6 genotypes were considered and all genotypes showed success in both artificial selfing and natural outcrossing. It may be observed that the magnitude of fertilization success, which was larger in the case of artificial pollination than in obligatory selfing, indicated that pollinating agents are responsible for a better success.

BAGCHI and VEERENDRA (1987) reported high pollen fertility in sandal (88.4%). When all flowers were hand pollinated, chances of pollen not reaching the stigma was much less. Good percentage of fruit initiation indicated better success in fertilization rate, but the lower percentage of matured fruits indicated presence of genotypic barriers to embryo development. Hence, it can be inferred that self-incompatibility is floating in the population. Keeping in view the self incompatibility in the case of artificial pollination and by pollinating agents, it can be assumed that the magnitude of fertilization success following natural outcrossing may depend on the source of pollen (EL-KASSABY *et al.,* 1993). It was reported that germination of sandal seeds is around 55% to 60% (BAGCHI and KULKARNI, 1985). Trees with a high percentage of embryonic lethals have higher apparent outcrossing estimates (XIE *et al.,* 1991). This differential success for open pollination indicated that sandal is a predominantly out-breeding species and this is in conformity with our earlier investigations (SINDHU VEERENDRA and SUJATHA, 1989). Its reproductive strategy prevents selfing through genotypic barriers such as non-success of fertilization, through heterostyly and by exudation of nectar to attract insects.

Sandal is rich in variations. Variation in flower structure, heart wood and oil content, morphological characters and their intermediate forms and above all heterostyly can be explained by the differential success of pollination (DORAN and BURGERS, 1993). The selection pressure in tropical tree species always favours predominance of out-breeding to facilitate maximum genetic diversity (BAWA, 1976). A thorough study of the breeding system of each variety of sandal may indicate a continuum of strategies from inbreeding to out-breeding.

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Realized Genetic Gains from Slash Pine Tree Improvement

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Summary

Realized genetic gains of slash pine (*Pinus elliottii* ENGLEM. var. *elliottii*), were estimated for tree volume and rust resistance using data from 2051 controlled-pollinated (CP) families grown in 175 CP tests. All CP families were divided into 5 groups for volume (groups V1 to V5) and 5 groups for rust (groups R1 to R5). For each variable the 5 groups represent the expected performance range from best to worst of the CP families based on parental breeding values (BVs) predicted by best linear prediction (BLP) from an independent data set of 367 open-pollinated (OP) progeny tests.

Using simple linear regression, realized genetic gains were estimated by comparison among these groups and also by comparison of improved materials to unimproved materials (called CHECK) included in most tests. On a region-wide basis, the ranking of realized gain ratios (in percent above unimproved material) for volume and rust of the 5 groups corresponded exactly to the order predicted by *a priori* classification based on the BLP predicted BVs. For example, for volume the realized gain increased linearly for the 5 volume groups.

Regression analyses were used to examine how age and site characteristics (e.g., site index, rust hazard) and their interactions with the 2 types of genetic groupings (V1 to V5, R1 to R5) affected genetic gains in the 2 traits, volume and rust. Age was almost never significant for volume or rust suggesting that the percentage gain above unimproved material is constant over the range of ages 5 to 11. The results also suggest that superior families for tree volume express increased gain in volume (on a % basis) compared to the unimproved material on better quality sites, and that genetic differences among genetic groups are greater on better sites (SIclass 3, 4 and 5) relative to poorer sites (SIclass 1 and 2). Also, genetically superior families for rust resistance have more tree volume on the high hazard sites, and on these sites the most resistant families also had the highest volume gains relative to the CHECK.

Key words: Realized genetic gain, best linear prediction, breeding value.

FDC: 165.4; 165.3; 56; 443; 174.7 *Pinus elliottii*).

Introduction

Tree improvement programs produce genetic gains in specific traits of interest, and even small genetic gains can have enourmous economic returns in a large scale tree improvement program (WEIR, 1973). It is the genetic progress achieved which justifies the costs incurred in the selection and breeding of superior phenotypes (PORTERFIELD, 1975; ROW and DUTROW, 1975). Thus, estimates of realized genetic gains are an important means of evaluating the performance of a tree improvement program (HODGE *et al.*, 1989). However, precise estimates of realized genetic gain are difficult to obtain, and are infrequently found in the tree improvement literature.

Generally, realized gains from a selection or breeding program are determined by a comparison of performance of improved materials with unimproved materials (ZOBEL and TALBERT, 1984; LA FARGE, 1993). Two different genetic test designs can be used to assess the performance of the improved and unimproved materials: 1) Row or single-tree plots, and 2) large block plots (LOWERTS, 1986). Large block plots of improved and unimproved materials provide unbiased estimates of realized genetic gain for growth traits on a per unit area basis (LOWERTS, 1986); however, large block plots suffer from large block (replication) sizes which contribute to low statistical precision. Therefore, a large number of experimental locations are needed to determine statistically significant differences (TANKERSLEY *et al.,* 1983). There are many statistical and logistical advantages of using row or single-tree plots; however, they can provide biased estimates of realized gain for growth traits if dominant phenotypes gain early competitive advantage (WRIGHT, 1975; CANNELL, 1982). Studies on estimating