Phenology and Controlled Pollination Studies in Tamarind

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Abstract

Reproductive biology and breeding system were studied in five Tamarind clones. Considerable vegetative and reproductive phenological variations were observed. Flowers showed strong entomophilous adaptations, open pollination fruit setting was between 1% to 2%. Controlled pollinations indicate that tamarind is a predominantly out crossing species with extremely low level of selfing, apomixis was absent. Long term pollen storage was possible, variation in pollen size was observed and pollen sterility was very low. Fruits showed colour dimorphism.

Key words: Breeding system, clone, controlled pollination, crossing, dimorphism, phenology, pollen storage, selfing, Tamarind.

FDC: 165.41; 181.52; 181.8; 176.1 *Tamarindus indica;* (540).

Introduction

Tamarindus indica L. commonly known as tamarind is a monotypic genus belonging to the family Leguminosae. It is widely distributed in Africa, Asia and West Indies. In India it is found distributed continuously in the Southern and Central regions and occurs as sparse patches up in the North. Tamarind grows up to fifteen meters in height with a dense spreading crown and clear trunk. It is an excellent multipurpose tree species which is used as food, food preservatives (TSUDA, 1995), fodder (KAITHO, 1996), drug (MUSTAPHA, 1996), minor timber and as fire wood. Tamarind fruit pulp is rich in tartaric and ascorbic acids and is used as an important natural preservative in pickle industry. It is a highly drought tolerant species and is found to be very useful in afforestation of marginal lands. Tamarind shows extensive variations in characteristics such as foliage and flower production, flower colour, fruit size, fruit pulp yield and wood quality.

Genetic improvement activities in tamarind were initiated in India almost a decade ago. Domestication was mainly aimed for fruit pulp yield, hence candidate plus trees were selected based on reproductive traits such as pod size and fruit pulp to seed ratio. At present in India a few good germplasm banks and single parent progeny trials are available. Tamarind can also be easily propagated by grafts, very recently tamarind clonal plantations are becoming popular because of their uniform growth and performance. Though tamarind is being domesticated for fruit production still its reproductive biology is not thoroughly understood, hence a detailed study was conducted in five high yielding clones with the following objectives:

- to know the floral biology and phenological variations;
- to understand the breeding system and develop controlled pollination techniques.

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Materials and Methods

Phenology recording

Studies were conducted from April to October during 1996 with five tamarind clones NBN1, NBN2, NBN3, RDB Patna and JRK (here in after referred to as C1,C2,C3,C4 and C5) in India (*Table 1*). From each clone five ramets were selected for observations. Phenological parameters such as terminal and axillary shoot elongation, leaf production, inflorescence length and flower production per inflorescence and per branch were recorded. Five hundred measurements or counts were made in a genotype (hundred per ramet) depending upon the characteristic.

Table 1. – Specifics and agroclimatic details of the study site.

Location name	Gottipura, Karnataka State, India
Specifics	13°06'N 77°47' E
Elevation	900 M
Annual Low Mean Temperature range	20° - 43° C
Annual Mean Rainfall	625 mm
Soil Type	Red Sandy Loam

Controlled pollinations

Controlled pollinations were done in four clones (C1, C2, C3 and C4) using a full diallel mating design (ZOBEL and TALBERT, 1984), apomixis treatments were also done. Flowers were emasculated using a clean fine tip forceps (from 15.00 hrs to 20.00 hrs) and flowers were dusted with pollen with a dry paint brush or needle from 6.00 hrs to 11.00 hrs. In apomictic treatments the emasculated flowers were not dusted with pollen. In an inflorescence only the treated flower was retained. In each clone 100 flowers were operated per day per treatment and all treatments were repeated for seven days. Operated flowers were caged in paper covers (in size of 12 cm x 7 cm) and tagged properly. Bags were removed on the seventh day for recording fruit setting.

Pollen biology

Anthers were collected in clean and dry 1.5 cm diameter Petri plates during early mornings and were allowed for air drying in open sunlight (25 °C to 32 °C) until dehiscence. By gently shaking and tapping the plates pollens were removed from the dried anthers. Pollen collections were stored in ambient (37 °C) and cold (5 °C) conditions. Pollen viability was assessed in weekly intervals using a cytoplasmic differential stain, in which viable pollens stained pink while dead pollen stained green (ALEXANDER, 1969). Slides were prepared and analysed according to the procedures described by RADFORD et al. (1974).

Data analysis

ANOVA, T-Test (WALLER and DUNCAN, 1969) and coefficient of correlation of means were done using SAS package, 6.09E version.

Results

Floral biology

Flowers are cream yellow in colour, bisexual, herkogamous (*Fig.1*), five sepals, five petals with odd petal being showy, three stamens fused at the base with filaments incurved towards the ovary base (*Fig.1*), style simple, stigma is unbranched and pappillate, ovary is superior with 12 to 14 ovules. Ovary base has numerous uniseriate hairs with copious nectar. Pollination is mostly by honey bees, which is most common among Leguminosae members (ARROYO, 1978). Insect visitation peaks between 08.00 hrs and 11.00 hrs. Anthesis starts at 20.00 hrs and flowers are completely open by 02.00 hrs, anther dehiscence is only by 08.30 hrs in the morning. Stigma is receptive nearly for 48 hours with peak receptivity on the day of anthesis. Fruits are green in colour except in clone C2 that produced pink fruits. It was observed that clone C2 attracted more number of insect visitors as its anther filaments and styles were also pink in colour.

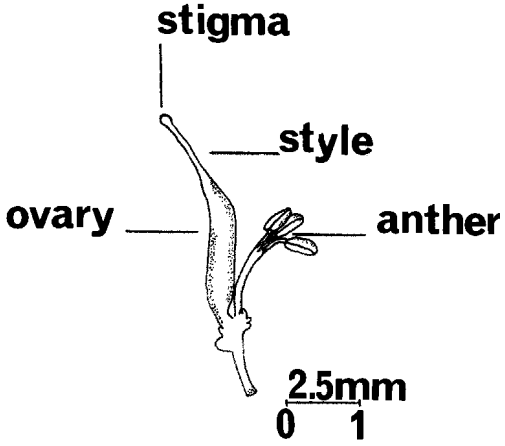

Figure 1. – Dissected flower (diagrammatic) of Tamarind showing herkogamous adaptation where androecium and gynoecium are placed in different angles and heights to avoid self pollination.

Vegetative phenology

Terminal vegetative shoots are produced annually which bear flowers only in the next immediate flowering season. Two types of terminal shoot production could be observed clearly, clones C1 and C2 produced shorter terminal shoots while C3, C4 and C5 produced long shoots (*Table 2*). Due to which in habit appearance clones C1 and C2 looked erect while clones C3, C4 and C5 appeared drooping. Also clones with lengthy terminal shoots produced more foliage, clone C5 showed the maximum foliage production (*Table 2*). Terminal shoot length and foliage production were found to be highly correlative (*Table 6*). Clones varied considerably in axillary shoot length, and leaf length (*Table 2*).

Reproductive phenology

In an inflorescence, flowers bloomed only every alternative days. Production of flowers significantly varied between clones (*Table 3*). Clones with longer vegetative terminal shoots were found to produce more flowers (*Table 6*). Style and ovary length also varied between the clones (*Table 3*).

Pollen biology

Pollen sterility was found to be very low in all clones (*Table 4*). Under ambient conditions (37 °C to 40 °C) pollen viability was 88% until 3 days, while pollen stored in 4 °C remained 97% viable up to 100 days. Pollens were produced in two distinct sizes (40 µm to 42 µm and 22 µm to 25 µm), of which the count of smaller pollens increased during late flowering seasons (*Table 4*) possibly due to deficient nutrition (MULLER, 1979).

Breeding system

Fruit setting in open pollination ranged 1% to 2% among the clones, clone C2 showed significantly higher fruit set than other clones (*Table 5*). In controlled cross pollination fruit set was more than 75% in all clones (*Table 5*), in contrast only negligible fruit set could be observed in self pollination, highest rate of selfing occurred in C2 (*Table 5*). Controlled pollinations clearly indicated that tamarind is a self incompatible species. No fruit set could be observed in apomixis treatments.

Discussion

Understanding the biology of reproduction in trees is important before attempting their genetic improvement, such basic studies are crucial especially in lesser understood tropical species (ZOBEL and TALBERT, 1984). Reproductive biology studies help in estimating the genetic variations (COSTICH, 1995) and also they reveal about the quality and quantity of seeds produced by a species (NAGARAJAN *et al.*, 1996). In tropical trees many ecology and evolution based reproductive biology works are available (BAWA, 1974; BAWA and WEBB, 1984; CHAN, 1981), in contrast only a very few applied studies have

Table 2. – Variation in vegetative phenology in Tamarind clones.

Characteristic	Clone							
	C1	C2	C3	C4	C5	SEM	LSD	CV %
Terminal shoot length (cm)	15.42_D	14.38_E	19.12_C	19.41_B	21.45_A	0.039	0.084	0.347
Axillary shoot length (cm)	6.75_D	6.18_E	8.41_B	7.54_C	9.34_A	0.008	0.017	0.167
Terminal shoot leaf length (cm)	8.24_C	8.24_C	8.15_D	9.37_A	9.12_B	0.021	0.043	0.376
Leaves per terminal shoot	8.78_D	8.55_E	13.76_A	12.18_C	13.13_B	0.016	0.034	0.224
Leaves per axillary shoot	5.34_A	4.45_B	4.06_C	4.05_C	3.67_D	0.067	0.140	2.450

Means with same subscript are not significantly different by DUNCAN's Multiple Range Test (p = 0.05).

Table 3. – Variation in flowering and floral organ in Tamarind clones.

Characteristic	Clone							
	C1	C2	C3	C4	C5	SEM	LSD	CV %
Inflorescence length (cm)	4.38_D	4.85_C	5.7_A	5.07_B	4.91_B	0.11	0.234	3.5
Inflorescences per branch	7.56_D	13.69_B	13.59_B	16.32_A	11.92_C	0.174	0.368	2.18
Flowers per inflorescence	13.28_C	8.05_E	15.57_B	17.09_A	12.21_D	0.024	0.053	0.296
Flowers per branch	100.47_E	109.12_D	212.70_B	279.8_A	145.61_C	2.769	5.871	2.583
Style length (mm)	4.57_B	4.62_A	4.74_A	4.30_C	4.64_A	0.064	0.137	2.237
Ovary length (mm)	6.6_B	6.74_A	6.61_B	6.13_D	6.21_C	0.038	0.08	0.927

Means with same subscript are not significantly different by DUNCAN's Multiple Range Test (p = 0.05).

Table 4. – Sterility, dimorphism and viability of Tamarind pollen.

Characteristic	Clone							
	C1	C2	C3	C4	C5	SEM	LSD	CV(%)
Pollen sterility (%)	1.18_B (1.08)	1.13_B (1.06)	0.79_C (0.88)	1.80_A (1.33)	1.93_A (1.38)	0.038	0.081	5.262
Pollen dimorphism (%)	11.03_C (3.32)	13.39_A (3.65)	12.08_B (3.47)	9.63_E (3.1)	10.23_D (3.19)	0.039	0.083	1.856
Pollen viability in ambient storage (%)	88.00_A (9.38)	88.20_A (9.39)	84.80_A (9.2)	85.60_A (9.25)	86.60_A (9.3)	0.145	NS	2.464
Pollen viability in cold storage (%)	97.40_A (9.87)	96.80_A (9.84)	97.00_A (9.85)	96.60_A (9.83)	97.00_A (9.85)	0.032	NS	0.523

Means with same subscript are not significantly different by DUNCAN's Multiple Range Test (p = 0.05). The values in parenthesis are transformed means (square root transformation).

Table 5. – Fruit setting in tamarind under open and controlled pollinations.

Treatment	Clone					SEM	LSD	CV(%)
	C1	C2	C3	C4	C5			
Open pollination (%)	1.65_B (1.28)	2.32_A (1.51)	1.15_C (1.07)	1.47_B (1.21)	1.44_B (1.19)	0.082	0.174	10.379
Self pollination (%)	2.40_C (1.51)	6.80_A (2.59)	2.60_C (1.59)	4.60_B (2.13)	NA	0.107	0.234	8.683
Cross pollination (%)	84.20_A (9.16)	88.20_A (9.39)	87.40_A (9.34)	75.80_B (8.70)	NA	0.182	0.396	3.145

Means with same subscript are not significantly different by DUNCAN's Multiple Range Test (p = 0.05). The values in parenthesis are transformed means (square root transformation). NA: Data not available.

been made so far (MUKHERJEE *et al.*, 1968; VENKATESH and SHARMA, 1975; EGENTI, 1976; VEERENDRA and ANANTHAPADMANABA, 1996). With many tropical tree improvement programs progressing from base populations to breeding populations and parents being selected for hybridisation the need for applied reproductive biology is felt acute by tropical tree breeders.

Short and rapid flowering periods, delicate floral organs, difficulty in harvesting pollen, poor controlled crossing techniques (BAWA and WEBB, 1984), and genetic relatedness (HABER and FRANKIE, 1982) make hybridisation and other related studies difficult among tropical trees. Though generally plants show increased fruit set in crosses (JOHNSTON, 1991; YOUNG

Table 6. – Correlation coefficient of vegetative and flowering phenologies in Tamarind.

	1	2	3	4	5	6	7
1	0						
2	0.670**	0					
3	0.927**	0.424*	0				
4	0.462*	-0.029	0.716**	0			
5	0.342	0.465*	0.433*	0.615	0		
6	0.566**	0.401*	0.633**	0.384	0.255	0	
7	0.579**	0.564**	0.667**	0.594**	0.761**	0.815**	0

* significant at 5% level; ** significant at 1% level
1 – Terminal shoot length 2 – Terminal shoot leaf length
3 – Leaves per terminal shoot 4 – Inflorescence length
5 – Inflorescence per branch 6 – Flowers per inflorescence
7 – Flowers per branch.

and YOUNG, 1992) it has not been so in most tropical trees (EGENTI, 1976; BAWA *et al.,* 1985) this can be overcome if the phenology is properly understood and exploited. A simple manipulation in number of flowers crossed per inflorescence and their proximity to the maternal axis seems to determine successful fruit setting. It has also been observed that early initiated fruits abort in very low levels (AKER and UDOVIC, 1981; UDOVIC and AKER, 1981) and develop better due to position effect (BAWA and WEBB, 1984; MCNEILAGE, 1991).

In earlier studies legume breeders have considered characteristics such as internode numbers and leaf production as quantitative traits (ASHIR, 1970; SANDHU and KHEHRA, 1983). In such a case the vegetative phenology patterns and their high correlation to reproductive behavior seen in tamarind is of immense value. In general genotypes with longer inflorescences (INOUE, 1985) and increased floral duration (SCHEMSKE *et al.,* 1978; MOTTEN, 1986) show higher fruit setting ratio, thus while establishing commercial tamarind orchards selection of clones with long vegetative terminals should be an advantage for higher fruit yield.

Very low fruit setting noticed in tamarind under open pollination is not a rarity, such a condition is quite common in many tropical trees (BAWA, 1974). A low fruit set mean value is normally an indicator of pollinator limitation (CALVO, 1990). This is severe especially when monoculture is practiced, it can be overcome by introducing captive bee hives (MONCUR *et al.,* 1995). While specialised anther arrangement makes pollinator interaction effective (HARDER and BARRETT, 1993; NILSSON, 1988) herkogamy promotes a strongly out crossing breeding system in tamarind under natural conditions. High fruit setting in controlled crosses and negligible fruit set in selfing are ample evidences of selfincompatibility (MUKHERJEE *et al.,* 1968) however we observed a slightly increased selfing in one genotype. As pollen tripping mechanism is common in many legumes (ARROYO, 1978) the cytogenetics behind this needs to be further investigated. Colouration in reproductive organs, a qualitative trait has been earlier recorded in temperate trees (COPES, 1972; STEINHOFF, 1974; STURGEON and MITTON, 1980; FARRIS and MITTON, 1985) in comparison no such inheritance has so far been reported in tropical trees. Fruit colour dimorphism in tamarind reported in this study could be of immense breeding value and can be effectively used as a morphological marker in progeny testing programmes.

Conclusion

Tamarind is an out crossing species, low fruit setting observed in natural conditions is mainly due to pollinator limitation. Unique floral adaptations in this species promotes out crossing under natural conditions. Hybridisation is relatively easier with controlled crossing resulting an average of 84% fruit set. Monoculturing a single high yielding genotype is likely to result in fruiting failure due to extremely low levels of compatibility with self pollen, thus while developing commercial clonal orchards at least five genotypes should be included. Since storage of pollen is quite simple samples can be easily exchanged or transferred across locations for conducting controlled crossing programmes. With clearly understood breeding system and standardised controlled pollination techniques tamarind needs to be exploited further for its genetic improvement.

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Seed Storage Protein Size Variations and Their Significance in the Evolution and the Systematics of *Acacia* and *Prosopis (Mimosaceae)*

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Abstract

Phylogenetic relationships among 20 species (38 accessions) of *Acacia* and ten (13 accessions) of *Prosopis* were estimated using data from seed storage protein size variations. Thirty-two different bands were detected (245 to 29 kD). The studied tetraploid, hexaploid and octoploid accessions of subgenus *Acacia* had almost the same numbers of bands. WAGNER parsimony analysis revealed six equally parsimonious trees of 72 steps with consistency index = 81.5% and retention index = 78%. The 50% majority rule consensus tree revealed four clades consistent with the three subgenera of *Acacia* and the genus *Prosopis*. Subgenus *Aculeiferum* appeared ancestral to subgenera *Acacia* and *Heterophyllum*. *Prosopis africana* appeared ancestral to the other studied *Prosopis* species and *Acacia polyacantha* ancestral to the other studied species of *Aculeiferum*. The two accessions of *A. albida* clustered within the ten accessions of the subgenus *Aculeiferum*. The results indicated that hexaploidy and octoploidy in *Acacia* were geologically more recent than tetraploidy, and that the evolution in *Acacia* proceeded towards a reduction in the concentration and number of variable protein molecules in the seed.

Key words: parsimony, phylogeny, SDS-PAGE, seed storage proteins.

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Introduction

The genus *Acacia* (TOURN.) MILLER comprises 1200 known species with a large number of subspecies and varieties (ROSS, 1979). BENTHAM (1875) subdivided the genus *Acacia* into five series *(Phyllodineae, Pulchelae, Botryocephalae, Vulgares* and *Gummiferae).* Later, VASSAL (1972) classified *Acacia* into three subgenera *(Heterophyllum, Aculeiferum* and *Acacia)* and six sections *(Monocantha, Aculeiferum, Acacia, Pulchelloidea, Heterophyllum,* and *Uninervata)* excluding *A. albida.* The genus *Prosopis* (L.) BURKART includes 45 species, 41 of which are indigenous to tropical America (HUNZIKER *et al.,* 1986). The genus *Acacia* fits in the tribe *Acacieae* and *Prosopis* in *Mimoseae* (BENTHAM, 1875). Although these two genera belong to

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