

Phenology and Control Pollination Studies in *Casuarina equisetifolia* Forst.

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Abstract

Studies on phenology, floral biology and seed production were conducted in two provenance trials and a clonal hedge orchard of *C. equisetifolia*. Observations in seventeen populations indicate that predominant dioecy with low proportion of monoecy as the commonest sexual strategy. Flowering occurs twice in a year coinciding with the South West and North East monsoons. *C. equisetifolia* exhibits strong anemophilous adaptations such as very high pollen output, reduced flowers with large stigmatic area and light weighing winged fruits. Pollen is viable up to 99%, storable in 4°C up to three months with no loss in fertility. Trees start reproducing within two years of planting. Local land races produce ten to twenty times higher seeds than the recent introduced natural provenances and exotic landraces. Controlled pollination resulted in lower seed set than observed in open pollination. Selfing leads to normal seed set in monoecious trees. Inter specific cross with *C. junguhuhniana* pollen parent results in viable progeny.

Key words: Anemophily, *Casuarina*, cone, landrace, phenology, provenance, pollination, reproduction, seed.

Introduction

Casuarina equisetifolia Forst. is a multi-purpose tree species belonging to the family Casuarinaceae. It is a native of Australia that has naturalized most coastal regions in the tropics and known to grow 20–24 meters in height and 60–80 cm in girth. It is an excellent resource for minor timber, fuel wood, charcoal, fodder and scaffolding. Its fast growth, adaptability to high-density planting, drought resilient nature and nitrogen fixing ability has made it a popular choice in farm and agro-forestry. In India, *C. equisetifolia* is highly preferred for paper pulping and commercial cultivation exceeds thousands of hectares every year (NICODEMUS *et al.*, 2001). In recent times Casuarina growers have started looking forward to improved propagules to obtain greater yield in shorter durations.

The unknown origin of *C. equisetifolia* coupled with the valid assumption of a narrow genetic base has been

a limitation to conventional breeding among the Asian countries. To widen the genetic base systematic domestication programs constituting natural provenances, high yielding exotic and local landraces were initiated during the nineties in India, Vietnam, Thailand and Egypt (PINYOPUSARERK *et al.*, 1996). These programs have helped in screening and selecting populations that significantly vary in growth, form and other economic traits (EL-LAKANY and YUNESS, 1996; PINYOPUSARERK *et al.*, 1996; KIMONDO, 1996; YANG and CHEN, 1996; NICODEMUS *et al.*, 2001). Combining superior selections obtained from the said programs either by orchards or through control pollination programs depend on the knowledge how reproduction is governed in the species (NAGARAJAN *et al.*, 1998). Among tropical trees, reproduction and sexual strategies in hermaphroditic entomophilous species is relatively well documented (BAWA and WEBB, 1984; VEERENDRA and ANANTHAPADMANABA, 1996; PALUPI and OWENS, 1998; NAGARAJAN *et al.*, 1998; STOCK *et al.*, 2004). On the contrary, adaptations and mechanisms involved in anemophilous tree species are relatively unexplored owing to their rarity (JACOBS, 1988; MABBERLEY, 1992). Knowledge on reproduction is a prerequisite to delineate the numeric, gender and genetic composition within breeding programs. Hence, a detailed investigation was conducted in *C. equisetifolia* on the following aspects:

- Phenology
- Floral biology
- Variation in cone and seed production among populations
- Seed set in control pollination

Materials and Methods

Study material

Observations in this study were made in two provenance trials and a clonal hedge orchard from three distinct geographical sites (*Table 1*). The trial at Pondicherry comprises thirty-five populations that include natural provenances, exotic and local landraces (12 trees per plot in 4 replications). It is the first trial in the country tested in a coastal zone (NICODEMUS *et al.*, 2001), of which thirteen populations (48 trees per accession) (*Table 2*) were sampled and observed on a monthly basis for a period of five years (1997 to 2001). The trial at Sadiwayal, an inland wet region consists 6 provenances (12 trees in four replications) (*Table 2*). For conducting floral biology and control pollination ten different clones marked from a hedge orchard in Coimbatore (*Table 1*) was utilized.

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Table 1. – Details on geography and agro-climate in the study sites.

Location	Sadiwayal	Pondicherry	Coimbatore
Year of trial establishment	1992	1995	1997
Specifics	11°00'N, 76°55' E	11°55'N, 79°55' E	11°00'N, 76°58'E
Altitude (m above MSL)	420	10	409
Agro climate type	Inland Wet	Coastal	Inland Dry
Annual Temperature range	20-34°C	20-37°C	20-36 °C
Annual Rainfall (mm)	1200 - 1500	800 -1250	550 - 700

Table 2. – Details of the *C. equisetifolia* seed sources studied.

Seed source	Specifics	Accession Number*	Breeding system
Provenances			
Mariana Island, Guam	13°20' N, 144°40'E	18121	Dioecious
Ranong, Thailand	9°21' N, 98°27'E	18297	-do-
Cotonou Beach, Benin	06°24' N, 02°31'E	18355	-do-
Sarawak, Malaysia	05°02' N, 115°32'E	18161	-do-
Solomon Islands	08°07' S, 157°08'E	18402	-do-
Danger Point, Australia**	11°07' S, 132°20'E	16166	Monoecious
Prachaup, Thailand	11°36' S, 100°37'E	14235	Dioecious
Vanua Levu, Fiji	16°10' S, 177°29'E	18272	-do-
Wangetti Beach, Australia**	16°41' S, 145°34'E	18378	-do-
Seventy Seven, Australia*	24°09' S, 151°53'E	15578	-do-
Illuka, Australia*	30°04' S, 153°12'E	14406	-do-
Landraces			
Montazah, Egypt	31°16' N, 30°05'E	18122	-do-
Beihai, China	21°35' N, 109°00'E	18586	-do-
Orissa, India	21°30' N, 86°54'E	18015	-do-
Tamil Nadu, India	09°05' N, 79°20'E	18119	-do-
Malindi, Kenya	03°05' N, 40°09'E	18135	-do-
Fujian, China*	25°00' S, 119°00'E	14986	-do-

** – Seed lots common to Pondicherry and Sadiwayal; * – Seed lots tested only in Sadiwayal; ♦ – CSIRO, Australian Tree Seed Centre accessions.

Phenology

Observation on frequency and duration of flowering phenology were made in all three sites (Table 1). Study on reproductive maturation was conducted in Pondicherry (Table 1). A comparative study on male expression in monoecious and male trees of dioecious breeding system was conducted in five provenances in Sadiwayal (Table 1). One hundred male inflorescences were harvested randomly from different parts of the crown in each of the 38 monoecious and 60 male trees (12 per provenance). The length of male inflorescences was measured and subjected to T-test.

Floral biology

Investigation on floral biology and control pollination was conducted in a clone hedge orchard (plants pollarded at a height of 2 m) located in Forest Campus, Coimbatore (Table 1). Observations were made in ten clones of superior phenotypes selected across East Coast of India, of which three ramets in each of the five males (TNPP-4, TNKBM-408, APKKD-3, APKKD7 and APVSP-23), four females (PY-119, PY-42, PY-75 and TNRM-2) and a monoecious clone (TNIPT-7) were observed and recorded from floral bud initiation through maturation of cones.

Cone and Seed Production

Estimation on seed (*Casuarina* infructescences are commonly referred as 'cones' and fruits as 'seeds') pro-

duction in individual trees was quantified in 13 provenances (four-individuals per provenance across four replications). The trees were tagged and observed for two consecutive (48 and 54 months after planting) flowering seasons. Cones on individual trees were counted and harvested after maturation using a module access tower (Access Towers, Bangalore, India). One hundred cones per provenance were sampled to quantify size and number of seeds produced. Seed weight (1000 seeds in 8 replications) was quantified on an electronic balance (R200D Sartorius Inc., Germany) using prescribed sampling and germination procedures (100 seeds/accession in four replications) according to standard methods (ISTA, 1993).

Controlled pollination

For pollen collection, male inflorescences in peak anthesis (5.00–6.00 hrs.) were harvested on hot air oven dried glass plates and were allowed to dehisce at room temperature (22–24°C). Using a clean dry razor blade, pollen spread on glass plates was scooped and transferred on to oven dried glass vials and left in a glass desiccation chamber impregnated with silica (for 6 hours) and stored in a refrigerator set to 4°C. Pollen viability was tested periodically according to standard microscopy sampling procedures (RADFORD *et al.*, 1974) using a cytoplasm differential stain (ALEXANDER, 1969).

Controlled pollination was performed in 5 clones (in 3 ramets of each of the clones PY-119, PY-42, PY-75,

TNRM-2 and TNPT-7). One hundred inflorescences (globose heads with 50–70 flowers) were pollinated in each of the female and monoecious clones. An interspecific cross with *C. junghuhniana* (Buot Soer, Kenya; Seed Accession 19491, Australian Tree Seed Center, Canberra) as a pollen parent was also carried out. Young pinkish rosy immature inflorescences resembling a paintbrush head with stigma in size of 2–2.5 mm (in length) were caged in paper bags (12 x 8 cms) and allowed for unwinding for a period of 3–4 days. Stored pollen (in 4°C) after reaching ambient temperature (20–22°C) was dusted on flowers during still mornings (7.30–8.00 hrs.) to avoid strong wind movement. Pollen was applied on the stigma from emergent stage (Day 1) through peak receptivity (Days 3–4) and samples were fixed every one-hour to quantify peak receptivity. Pollen was dusted until the gloss was completely reduced in the treated flowers and was bagged. After confirmation of drying and withering of stigma the inflorescences were debagged. The immature developing cones were tagged and observed during the following eight months until harvest. After recording seed set in individual cones, germination trials (100 seeds in 4 replication in sand media) were conducted according to standard procedures (ISTA, 1993).

Pollen pistil interaction

Open and control pollinated inflorescences were collected and fixed in FAA (Formalin Acetic Alcohol in 1:2:1) for 24–48 hours, washed in running tap water and stored in 70% ethanol. Prior to epifluorescence studies on receptivity and pollen-pistil interaction, inflorescences were treated in 8N Na OH for 3–4 hours to soften the tissues. Treated inflorescences were washed thoroughly in distilled water and then placed in a solution of 0.1% decolorized water soluble Aniline Blue in 0.1N strength K_3PO_4 for staining (MARTIN, 1959). Stained tissues were placed on a clean slide and squashed with a drop of glycerin under a clean dry cover glass using an Epifluorescence microscope (OPTIPHOT-2, Nikon Optical Inc., Japan).

Results

Phenology

The age of attaining reproductive maturation varied among populations. Local land races were the earliest to mature followed by exotic land races and natural provenances. In local land races male trees flowered 10–12 months after planting and females flowered within 12–14 months. In exotic landraces males matured after 12–14 months while females matured in 20–24 months. Among natural provenances reproduction was noticed only after 18–20 months in males and after 22–24 months in females. Observations in Danger Point (30 individuals were tagged and observed) a predominantly monoecious provenance revealed male flowering in 18–20 months followed by female flowering in 24–26 months.

In all the study sites (Table 1) *C. equisetifolia* flowered twice in a year. The first one between late June and early August and the second in late October through

early January in coincidence to South West and North East monsoons respectively. Each flowering season lasted eight to ten weeks. Male and female trees flowered in alternating sequence. Male trees flower 10–14 days ahead of females and were in 3–4 episodes (5–8 days each) per season while females flowered in 2–3 episodes (7–10 days each).

In monoecious trees, male flowers emerge first while the female flowers develop later ensuring temporal and spatial separation. Male flowering is in two (rarely thrice) episodes while females usually flower once (rarely twice). Male inflorescences in monoecious trees are significantly shorter in length than observed in the dioecious males (Table 3).

Table 3. – Variation in size of male inflorescences in monoecy and dioecy breeding systems of *C. equisetifolia*.

Parameter	Monoecy	Dioecy
Inflorescence length (cm)	4.11	6.11
Variance	0.752	0.914
Observations	38	60
T value	10.70	

Intensity of flowering varied considerably among populations. Local land races flowered very profusely. Among the exotic landraces, Beehai, China flowered copiously while Egypt and Kenya were scanty flowerers. Among natural provenances flowering was profuse in Fiji, moderate in Australian accessions and very shy in Thailand.

Floral biology

C. equisetifolia exhibits strong anemophilous adaptations like absence of perianth, enormous pollen output, highly reduced flowers and large stigmatic area. Male flowers are borne terminal and female flowers in axils.

Male flowering

Buds develop and mature within 2–3 weeks and open in episodes. Inflorescences are borne terminally in 10–40 successive whorls, each consisting 7–8 naked flowers (3 mm in length and 2 mm in width). A male inflorescence of 1 cm size contains 36–42 flowers, each producing about 1700–2200 yellowish, powdery, tricolpate, smooth exine pollen in varying range of sizes (24–30 μ m). Male flowers emerge during nights by 19.00 hrs, anthers are completely dehiscent by 6.30 hrs. and ready for dispatch by 7.00 hrs. With increasing day temperature (22–32°C) as the air gets lighter pollen are wind borne.

Female flowering

Female inflorescences are axially borne that consist whorls of bracts each subtending a single naked flower. A fully mature inflorescence has 40–80 flowers, of which those borne in the base mature first. It takes about 4–6 days for flowers within an inflorescence to completely unwind. Inflorescences during the first two days of emergence and development are rosy pink in color that resembles a paintbrush head. At this stage the bilobed

stigma is of 2–2.5 mm in size. On complete maturation the bi-lobed stigma is about 3.5–4.5 mm in length, dark blood red in colour, glossy, 4–5 cells thick at base with a short style in 2–3 mm of length.

Breeding systems

In an overall perspective most provenances observed in this investigation exhibited dioecy with varying proportion of males and females. In Pondicherry twelve out of thirteen accessions and in Sadiwayal five out of six provenances were found predominantly dioecious. The proportion of monoecious individuals among populations was slightly higher or equal to 10%, but never exceeded 16% in both the international provenance trials. The only exception was Danger Point, a natural provenance from Australia that was predominantly monoecious with low proportion of females (less than or equal to 10%) in both locations (Table 2).

Cones and Seed production

Individual trees produce hundreds of cones that mature over 6–8 months. Tamil Nadu, a local landrace produced most number of cones per tree, while Wanghetti Beach, an Australian provenance produced the least (Fig. 1). Local landrace Tamil Nadu produced the largest while Egypt produced the smallest cones (Table 4). Sizes of cone did not vary significantly in populations when quantified across two consecutive fruiting seasons (Table 4). Cones vary in qualitative characteristics such as shape, size and color. India, China, Benin and Fiji accessions produced brownish green linear cones while Solomon Island, Malaysia and Egypt produced greenish yellow globose cones.

Typically a cone consists of a 40–60 seeds oriented radially and vertically to the axis of cone (BOLAND *et al.*,

1996). Individual trees in all populations produce thousands of seeds per tree (Fig. 1). Local land races were the highest in seed production followed by the exotic land race Beehai, China. Seed weight was found to significantly vary among populations (Table 4). Provenance Danger Point produced the heaviest seeds while the lowest weighing ones were recorded in the Egyptian land race (Table 4). Seed did not significantly vary in weight when quantified across two consecutive seasons (Table 4). Seeds are wind dispersed and most fall in close proximity to mother trees. In all clones, open pollinated fruits showed higher seed filling than observed in control pollination (Table 5).

Pollen biology, Pollen-pistil interaction

Pollen is binucleate, highly viable (up to 99 %) and can be stored (in 4°C) with no loss in fertility until three months. Epifluorescent microscopic studies indicate that inflorescences are not receptive during the first two days of emergence. Pollen penetration is noticed in flowers only after three days. Even at this stage despite heavy loading of pollen only a few germinated on the stigma surface and of which only a very few penetrated and gained ovule entry. Pollen penetration patterns were similar in control cross and self-pollinated pistils, they traverse stigma intra-cellular and branching of pollen tube was quite common.

Controlled pollination

About 55–93% of cross-pollinated flowers set fruits and the percentage of seedlings emerging from them vary 38–68% (Table 5). Self-pollination resulted in 63% seed set of which 37% germinated and developed (Table 5). In general seed set and seedling emergence in open pollinated cones was higher than the control pollinated.

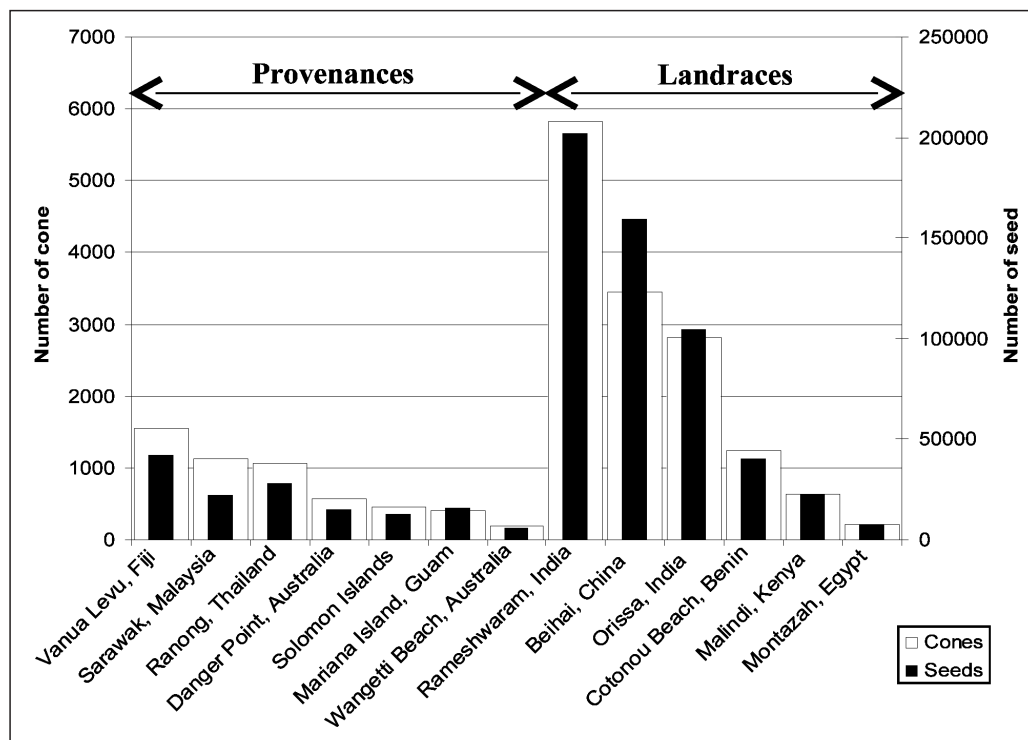


Figure 1. – Cone and seed production in individual trees among different *C. equisetifolia* populations.

Table 4. – Variation in cone size, seed weight and production in *C. equisetifolia*.

Seed source	Cone size (cm)						Seeds /Cone			Seed weight (gm) (1000 seeds)		
	Width				Length		J	O	Mean	J	O	Mean
	J	O	Mean	J	O	Mean						
<i>Provenances</i>												
Danger Point	1.45	1.45	1.45	1.68	1.32	1.50	29.41	29.29	29.35	2.12	2.27	2.19
Sarawak	1.43	1.40	1.41	1.45	1.43	1.44	25.57	27.91	26.74	1.07	1.20	1.13
Guam	1.21	1.16	1.19	1.44	1.39	1.41	33.04	36.91	34.98	1.45	1.10	1.27
Solomon	1.14	1.13	1.14	1.04	1.04	1.04	29.36	31.75	30.55	1.05	1.13	1.09
Fiji	1.14	1.18	1.16	1.22	1.40	1.31	26.53	29.63	28.08	1.01	1.14	1.08
Wanghetti Beach	1.10	1.18	1.14	0.90	0.94	0.92	25.20	26.33	25.77	1.12	1.32	1.22
Thailand	1.03	1.08	1.05	1.22	1.28	1.25	26.35	30.67	28.51	1.07	1.12	1.10
<i>Landraces</i>												
Tamil Nadu	1.49	1.49	1.49	2.05	2.01	2.03	45.40	48.29	46.85	1.36	1.57	1.47
Benin	1.48	1.49	1.48	1.78	1.64	1.70	25.47	25.84	25.65	1.43	1.27	1.35
Orissa	1.28	1.27	1.28	1.50	1.45	1.47	37.17	41.30	39.24	0.50	1.14	0.82
Beechai	1.38	1.39	1.39	1.65	1.67	1.66	46.38	46.08	46.23	1.06	1.33	1.19
Kenya	1.25	1.22	1.24	1.35	1.36	1.36	39.49	36.56	38.02	1.08	1.19	1.14
Egypt	1.07	1.06	1.07	1.21	1.24	1.22	31.68	31.99	31.83	0.78	0.82	0.80
Mean	1.27	1.27	1.27	1.42	1.40	1.41	32.39	34.04	33.22	1.163	1.276	1.219
Provenance Season	SEd	LSD	CV%	SEd	LSD	CV%	SEd	LSD	CV%	SEd	LSD	CV%
	0.073	0.15	10.2	0.11	0.23	13.8	3.64	7.28	19.0	0.14	0.29	20.8
	0.029	0.06		0.04	0.09		1.42	2.86		0.05	0.11	

J – June flowering; O – October flowering.

Table 5. – Seed set and germination in *C. equisetifolia* under open and control pollination.

Clones	Pollination	Cone		Seed set (%)	Germination (%)
		Ovaries	Seeds		
TNIPT7	OP	53.6 ± 4.7	48.40 ± 2.3	90.20 ± 2.1	61.50 ± 6.4
	CP		46.60 ± 3.2	86.60 ± 3.7	38.50 ± 4.8
	SP		34.30 ± 2.6	63.49 ± 1.3	37.25 ± 2.7
PY119	OP	46.0 ± 5.2	42.80 ± 3.4	93.0 ± 2.4	90.17 ± 2.1
	CP		34.30 ± 4.8	74.56 ± 3.1	47.50 ± 8.6
	CP*		28.40 ± 3.8	61.73 ± 2.4	63.44 ± 7.2
PY42	OP	74.40 ± 4.5	67.40 ± 3.2	90.59 ± 1.6	67.50 ± 4.7
	CP		41.10 ± 2.2	55.24 ± 3.4	49.0 ± 5.8
	CP*		46.20 ± 2.1	62.09 ± 1.3	56.34 ± 4.2
PY75	OP	37.30 ± 3.7	34.80 ± 2.4	93.29 ± 2.6	83.50 ± 6.2
	CP		33.0 ± 2.6	88.47 ± 2.7	68.60 ± 4.3
	CP*		24.60 ± 2.8	65.95 ± 3.3	74.30 ± 2.6
TNRM2	OP	51.30 ± 4.8	44.70 ± 3.1	87.13 ± 1.4	78.20 ± 3.6
	CP		34.3 ± 1.7	66.86 ± 2.2	54.30 ± 4.8

OP – Open Pollination, CP – Cross Pollination, SP – Self Pollination, CP* – Crossed with *C. junghuhniana* pollen. Mean values are followed by error.

An interspecific cross *C. equisetifolia* x *C. junghuhniana* also resulted in normal seed set and seedling emergence (Table 5).

Discussion

Plants native to tropics show completely different flowering habits compared to temperate regions (HOFFMANN, 2002). Tropical plants flower once or in sequential episodes within a year, also they are known to flower once in several years (JACOBS, 1988; LITTLE *et al.*, 1974; LONGMAN and JENIK, 1987; WHITMORE, 1992). In case of *C. equisetifolia* it is found adapted to two flowering seasons in a year coincident to the early moisture regimes of monsoons. This is relatively a well-known phenological trend in the tropics (MABBERLY, 1992; MAAS and WEESTRA, 1993).

Owing to the varying levels of naturalization it is noted that reproduction in local land races is prolific

compared to the recent introduced exotic landraces and natural populations (NAGARAJAN *et al.*, 2001). In a breeding perspective the said trend could be useful in determining the proportion of local and exotic selections to be deployed in breeding programs to avert coancestry related inbreeding. With two flowering seasons and seed produce in multiples of thousands in individual trees is an assurance that seedling seed orchard programs would be of great success in *C. equisetifolia*.

It is observed flowering is not of a random pattern in *C. equisetifolia*, male and female trees flower in a sequential alternate manner to maintain sexual symmetry. HOFFMANN (2002) noticed a similar trend in *Symphonia globulifera*, a bird-pollinated taxon. Such a pattern aids in complete randomization of pollen pool (LLOYD and WEBB, 1977; BULLOCK and BAWA, 1981) and in effective utilization of maternal resources (SHARMA *et al.*, 1999). It is interesting to note that reproductive maturation in *C. equisetifolia* occurs within a year of

planting, it is quite an unusual life history trait, perhaps first time being recorded in a tropical tree adapted to anemophily. This is crucial factor that could aid in rapid translation of breeding programs in short duration of time.

Predominance of dioecy with low proportion of monoecy seems to be the common sexual strategy among *C. equisetifolia* populations (VARGHESE *et al.*, 2004). This process is referred to as “leaky dioecy” (COX, 1966), which is frequently recorded among island flora (CARLQUIST, 1966). The sexual symmetry and the levels of selfed progenies contributed by monoecious trees has been a long time curiosity to *Casuarina* breeders (BOLAND *et al.*, 1996). Based on open pollination BOLAND *et al.* (1996) reported that in *C. cunninghamiana* male and female flowering phases were temporally apart and suggested little likelihood of selfing. The trend is similar in *C. equisetifolia*.

It is understood in the past that size variation in inflorescence among individuals influence reproductive output, which in turn have certain spatial and temporal advantages (NAGARAJAN *et al.*, 1998). In *C. equisetifolia* monoecious trees, male inflorescences being significantly shorter than the regular dioecious males is an effective structural advantage to minimize overlap of male and female phases to ensure sexual symmetry. VARGHESE *et al.* (2004) observed that monoecious individuals of *C. equisetifolia* in coastal regions produce longer inflorescences compared to inland populations. Selections from these sites need to be dealt cautiously as it could lead to overlap of sexual phases and consequently promote biparental inbreeding as control selfing indicates normal seed set. Thus, as breeding strategy outstanding monoecious clones can be deployed as functional males and seeds may be harvested if desired. Based on the said facts it can also be inferred that genetic relatedness in populations depends on the proportion of monoecious individuals within them.

Sex expressions in certain *C. equisetifolia* populations are found constant when tested across agro climatic zones (NAGARAJAN *et al.*, 2001). For instance the land race Egypt is predominantly monoecious with low proportion of dioecy (BOLAND *et al.*, 1996) on the contrary the Indian landrace is largely dioecious with negligible levels of monoecy (MAHADEVAN *et al.*, 1994). Such contrasting breeding systems in exotic habitats reflect the sexual strategy of the original seed source. Hence, while introducing genetic resources in to exotic habitat an insight on breeding system is very essential.

Among tropical trees controlled crossing programs are usually reported for higher fruit set than in open pollination (NAGARAJAN *et al.*, 1998; STOCK *et al.*, 2004), however in *C. equisetifolia* it is the reverse. An apt explanation for this phenomenon is that, flowers in *Casuarina* inflorescences unwind over a period of five to seven days. While control pollinating, the early flowers in an inflorescence are in receipt of pollen while those opening after caging are pollen limited and thus produce aborted seeds.

It is suggested that prominent qualitative characteristics such as cone shape and colour can be used as morphological markers in inter-provenance hybridisation

programs. With viable progeny emerging in *C. equisetifolia* x *C. junguhuhniana* crosses tremendous scope exists for breeding new inter-specific hybrids. Also the cross compatibility with other *Casuarina* species needs to be tested in future for production of hybrids.

Being an anemophilous taxon the structural and functional requirements in *C. equisetifolia* orchards could be diabolically different from those of entomophilous tree species. Hence, while designing orchards breeders should not only focus on mere numerical and genetic composition but also on mechanical properties of wind such as its intensity, flow, pattern and periodicity, which could be crucial to the overall reproductive fitness in breeding populations.

Conclusion

Casuarina equisetifolia is an anemophilous obligatory outcrossing species with unique phenology and sexual strategies. Reproductive maturation within one year of planting coupled with prolific seed production twice in a year indicates excellent scope for successful deployment of long-term conventional breeding programs. High rates of success in intra and inter specific control pollination program offers immense scope for producing high yielding hybrids. Significant infra-specific variations in reproductive characteristics such as cone colour, shape, size and seed weight are potential candidate morphological markers to future breeding programs. With clearly understood reproductive processes and controlled pollination techniques, improving the progeny of *C. equisetifolia* should be relatively easier in the future.

References

- ALEXANDER, M. P. (1969): Differential staining of aborted and non-aborted pollen. *Stain Technology* **44**: 117–122.
- BAWA, K. S. and C. J. WEBB (1984): Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. *Am. J. Bot.* **71**: 736–751.
- BOLAND, D. J., M. W. MONCUR and K. PINYOPUSARERK (1996): Review of some floral and vegetative aspects to consider when domesticating *Casuarina*. In: PINYOPUSARERK, K., J. W. TURNBULL and S. J. MIDGLEY (eds.) Recent *Casuarina* Research and Development, Proc. of the Third International *Casuarina* Workshop Da Nang, Vietnam 4–7, March, 1996. pp 17–25.
- BULLOCK, S. H. and K. S. BAWA (1981): Sexual dimorphism and the annual flowering pattern in *Jacartia dolichaula* (D. SMITH) Woodson. (Caricaceae) in a Costa Rican rain forest. *Ecology* **62**: 1494–1504.
- CARLQUIST, S. (1966): The biota of long-distance dispersal IV. Genetic systems in the flora of Oceanic Islands. *Evolution* **20**: 433–455.
- COX, P. A. (1985): Islands and Dioecisms: Insights from reproductive ecology of *Pandanus tectorius* in Polynesia. In: WHITE, J. (ed.) studies of plant demography: A festschrift for JOHN. L. HARPER. Academic Press, London, UK pp 359–372.
- EL-LAKANY, M. H. and M. I. YUNESS (1996): Provenance trials of *Casuarina equisetifolia* in Egypt: Early results of height growth. In: Proc. Recent *Casuarina* research and Development. K. PINYOPUSARERK, J. W. TURNBULL and S. J. MIDGLEY. (Eds.). Third International *Casuari-*

- na Workshop, Da Nang, Vietnam 4–7, March 1996. pp 119–123.
- JACOBS, M. (1988): *The Tropical Rain Forest*. Springer, Berlin. 295 pp.
- HOFFMANN, C. C. (2002): Pollen distribution in sub-recent sedimentary environments of the Orinoco Delta (Venezuela)- an Actuo-Paleobotanical study. *Review of Paleobotany and Palynology* **119**: 191–217.
- ISTA (1993): International rules for seed testing. Supplement rules. *Seed Science and Technology* **21**: 157.
- KIMONDO, J. M. (1996): Two-year growth performance of *Casuarina equisetifolia* provenances in Gede, Kenya. In: *Proc. Recent Casuarina research and Development*. K. PINYOPUSARERK, J. W. TURNBULL and S. J. MIDGLEY (Eds.). Third International Casuarina workshop, Da Nang, Vietnam 4–7, March 1996. pp. 124–130.
- LITTLE, E. L., R. O. WOODBURY and F. H. WOODSWORTH (1974): *Trees of Puerto Rico and the Virgin Island*, Vol.2 Department of Agriculture, Washington, DC, 1024 pp.
- LLOYD, D. J. and C. J. WEBB (1977): Secondary sex characters in seed plants. *Bot. Rev.* **43**: 177–216.
- LONGMAN, K. A. and J. JENIK (1987): *Tropical Rain Forest and its environment* Wiley, New York, 325 pp.
- MAAS, P. J. M. and L. Y. TH. WEESTRA (1993): *Neotropical Plant families*. Koetz Scientific Books, Konigstein 289 pp.
- MABBERLEY, D. J. (1992): *Tropical Rain Forest Ecology*. Blackie, London, 300 pp.
- MAHADEVAN, N. P., K. SHANTHI and B. G. SINGH (1994): Sexual system in *Casuarina equisetifolia*. *Sylva Plus*, IFGTB Newsletter (Biodiversity special) **2**: 17.
- MARTIN, F. W. (1959): Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technol.* **34**: 125–128.
- NAGARAJAN, B., A. NICODEMUS, A. K. MANDAL, R. K. VERMA and N. P. MAHADEVAN (1998): Phenology and controlled pollination studies in tamarind. *Silvae Genet.* **47**(5–6): 237–241.
- NAGARAJAN, B., A. NICODEMUS, M. VARGHESE and V. SIVAKUMAR (2001): Reproductive Biology of *Casuarina equisetifolia*. In: *Casuarina: Improvement and Utilisation* Eds: K. GURUMURTHY, A. NICODEMUS and SIDDAPPA (Eds.) Institute of Forest Genetics and Tree Breeding, India ISBN 81-900346, Pp: 63–68.
- NICODEMUS, A., M. VARGHESE and B. NAGARAJAN (2001): Genetic improvement of *Casuarina equisetifolia* through selection and breeding. In: GURUMURTHY, K., NICODEMUS, A. and SIDDAPPA (Eds.) *Casuarina in India: Improvement and Utilization*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India. pp 3–13.
- PALUPI, E. R. and J. OWENS (1998): Reproductive phenology and reproductive success of teak (*Tectona grandis* L.f.). *Int. J. Plant Sci.* **159**(5): 833–842.
- PINYOPUSARERK, K., E. R. WILLIAMS, V. LUANGVIRIYASAENG and B. PURIYAKORN (1996): Geographic variation in growth and morphological traits of *Casuarina equisetifolia*. In: K. PINYOPUSARERK, J. W. TURNBULL and S. J. MIDGLEY (Eds.). *Recent Casuarina Research and Development*. Proc. of the third International Casuarina Workshop Da Nang, Vietnam 4–7, March 1996. pp 143–151.
- RADFORD, A. E., W. C. DICKINSON, J. R. MASSEY and C. R. BELL (1974): *Vascular plant systematics*. Harper and Row Publishers, New York, USA.
- STOCK, J., M. VAGAS, K. ANAGARITA and R. GONZALEZ (2004): Seed production in *Gmelina arborea* by controlled pollination. *New Forests* **28**: 167–177.
- SHARMA, N., A. K. KOUL and V. KAUL (1999): Pattern of resource allocation of six *Plantago* species with different breeding systems. *Jour. Plant Res.* **112**: 1–5.
- VARGHESE, M., D. LINDGREN and A. NICODEMUS (2004): Fertility and Effective population size in Seedling Seed Orchards of *Casuarina equisetifolia* and *C. junghuhniana*. *Silvae Genetica* **53**: 164–168.
- VEERENDRA, H. C. S. and H. S. ANANDTAPADMANABA (1996): The breeding system in Sandal (*Santalum album* L.). *Silvae Genet.* **45** (4): 188–190.
- WHITMORE, T. C. (1992): *An Introduction to Tropical Rain Forests*, Clarendon, Oxford, 226 pp.
- YANG, J. C. and T. H. CHEN (1996): Provenance trial of *Casuarina equisetifolia* in Taiwan. II. Performance in two years. In: K. PINYOPUSARERK, J. W. TURNBULL and S. J. MIDGLEY (Eds.). *Recent Casuarina Research and Development*. Proc. of the Third International Casuarina Workshop Da Nang, Vietnam 4–7 March 1996. pp 152–158.

Segregation and Linkage Relationships of Allozymes in *Pinus Brutia* Ten.

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Abstract

Female gametophytes of *Pinus brutia* Ten. were analyzed to study Mendelian segregation and linkage among allozyme loci in a seed orchard composed of 28

clones. Isozyme variants of nine enzyme systems encoding 14 loci from megagametophytes were assayed by starch gel electrophoresis. While six of the 14 loci were monomorphic, the remaining eight were polymorphic. Analysis of observed segregation ratios of all polymorphic loci except for *Sdh1*, showed a good fit to the 1:1 ratios expected for Mendelian inheritance. Linkage rela-

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