

Twenty-Five Years of Research on Casuarinas at IFGTB



Editors

Kannan C.S. Warriar

B. Gurudev Singh

N. Krishna Kumar



Institute of Forest Genetics and Tree Breeding
(Indian Council of Forestry Research and Education)
Coimbatore, Tamil Nadu, India



Twenty-Five Years of Research on Casuarinas at IFGTB

© ICFRE, 2014

All Rights Reserved

ISBN 978-93-82387-08-4

Editors

Kannan C.S. Warriier

B. Gurudev Singh

N. Krishna Kumar

Citation : Warriier, K.C.S., Singh, B.G. and Kumar, N.K. (Eds.), 2014. *Twenty-Five Years of Research on Casuarinas at IFGTB*. Institute of Forest Genetics and Tree Breeding (Indian Council of Forestry Research and Education), Coimbatore, Tamil Nadu, India, 144p.

Published by

Institute of Forest Genetics and Tree Breeding

P.B. No. 1061, R.S. Puram – 641 002

Coimbatore, Tamil Nadu, India

Printing

Prdag Print

Coimbatore - 641 012.

Ph : 0422 3059034

E-mail : prdagprint@gmail.com

1.0

INTRODUCTION

Casuarinas are widely planted in the tropics, subtropics and Mediterranean countries because of their ready adaptability to a variety of environmental conditions and also for their rapid growth performance. Among the 96 species of trees and shrubs in the family Casuarinaceae (Turnbull, 1990), *Casuarina equisetifolia* has gained much attention due to its multiple end-uses. Another species which is gaining momentum is *C. junghuhniana*.

In India, *C. equisetifolia* was introduced from Australia in the second half of nineteenth century mainly to fuel the steam locomotives. It was originally planted in Karwar during 1868-69 (Kaikini, 1937), Nilgiris of the then Madras Presidency (Kondas, 1983) and Chengalpet and South Arcot districts of Tamil Nadu state (Gurumurthi and Subramanian, 1998). The species later spread along the coasts to different parts of Tamil Nadu, Andhra Pradesh, Orissa and West Bengal. It was also introduced in the coastal areas of Kerala, Maharashtra and Karnataka. India is the largest *Casuarina* growing country in the world with an estimated 800,000 ha of plantations (Pinyopusarerk and Williams, 2000). Nicodemus (2009) estimated that about 500,000 ha are planted with *Casuarina* in the coastal states of Andhra Pradesh, Orissa, Tamil Nadu and the Union Territory of Puducherry.

The nitrogen fixing ability and adaptability to grow in a wide range of soil and climatic conditions including moisture and nutrient limited sites makes *Casuarina* a preferred choice for commercial and environmental planting programme. The short-rotation period of 3-4 years suits the average Indian farmer with small landholding (Rawat, *et al.*, 2011). Its nitrogen fixing ability, desirable stem form, fast growth and light crown characteristics make it an ideal tree for agroforestry systems (Saravanan *et al.*, 2012; Viswanath *et al.*, 2001). It has also gained importance as a major pulpwood species (Amanulla *et al.*, 2001; Jain and Mohan, 2001). Its usefulness

in environmental protection has been fully realized after the tsunami and is now a major component in any coastal afforestation programme in India.

Growing *Casuarina* is also steadily increasing in inland areas where it is not traditionally grown. Shortage of farm labour, insufficient water availability for agriculture, non-remunerative prices for farm produce and growing tendency of absentee farming are the major reasons for farmers shifting to *Casuarina* cultivation (Nicodemus, 2009).

Since casuarinas play an important role in meeting the domestic, industrial and environmental needs, the research and development activities should always match the demand. Though *C. equisetifolia* has multiple end-uses, it is mainly planted for fuelwood, mitigation of strong winds and scaffolding. Therefore, *Casuarina* improvement programme is now focused on improving tree growth, yield and form. Abundant phenotypic variations are reported in this species throughout its distribution range in India. Striking phenotypic variations were observed in shape of crown, branch angle, length of branchlets, size and shape of cones and seeds in the east coast of Tamil Nadu (Kondas, 1983). This species is reported to show tremendous variation in growth parameters also (Warrier and Venkataramanan, 2011; Prasad and Dieters, 1998). Individual trees often vary a great deal from one another even when growing in the same stand. This is the major type of genetic variation the geneticist uses in a selection and breeding programme.

Wood production in *Casuarina* varies greatly across locations, cultivation techniques adopted and age at which harvested. Plantations with irrigation and fertilizer application yield 100 to 150 tonnes of air dried wood (up to 20 cm girth) per hectare (40 to 60 tonnes per acre) in 4 years at a spacing of 1m x 1m. Under rainfed conditions an average yield of 75 to 100 tonnes per hectare is obtained in 6 years (30 to 40 tonnes per acre) depending upon soil quality and amount of rainfall during the cultivation period. An additional 5 to 7 tonnes of miscellaneous wood is produced per acre in the form of branches, tops and roots. At the time of harvest the average height of the tree is 12 m and girth is 25 cm. The best trees may measure 20 m in height and 50 cm in girth. The average net income from irrigated plantations is Rs. 2,27,000 per ha with four years' rotation period. Under rainfed conditions the

same income is realized in 6 to 7 years depending on soil type and rainfall received during the rotation period.

Realising the potential of *Casuarina* for yield improvement a written research programme with support from CSIRO Australia was prepared and systematic tree improvement programme has been undertaken at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, for nearly two decades. Today IFGTB is a focal point for *Casuarina* research in India.

2.0

GENETICS AND TREE BREEDING

2.1 Land Races of *Casuarina equisetifolia*

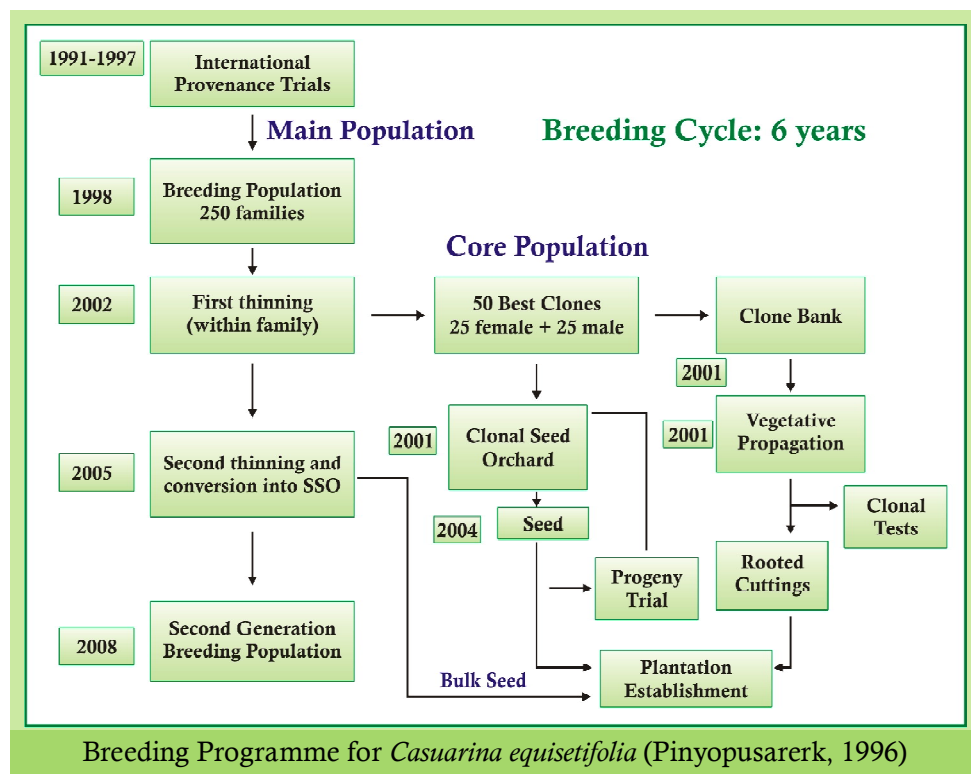
The Institute of Forest Genetics and Tree Breeding carried out a selection programme from 3-5 year plantations of *Casuarina equisetifolia* from 1992 to 1995 aimed at production of high yielding clones. The survey was carried out in Chidambaram (Latitude 11° 24' N, Longitude 79° 44' E) Chengalpeta (Latitude 12° 42' N, Longitude 80° 01' E) and Tiruchendur (Latitude 8° 30' N, Longitude 78° 11' E) in Tamil Nadu. 106 clones thus selected were assembled in a clone bank and multiplication garden for mass multiplication. Later, another selection programme in 8-10 year old plantations located on stressed sites under conditions of salinity, alkalinity, drought and diseased and pest-infested plantations was taken up. Fifty one CPTs were selected at a high selection intensity of 1 in 10,000 and 45 of them were assembled in the Clone bank at Coimbatore. Presently 229 clones of *C. equisetifolia* are available in the Clone Bank of IFGTB which includes selections by other stakeholders also.

2.2 Systematic Tree Improvement of *Casuarina equisetifolia*

With the understanding of geographic variation in *Casuarina equisetifolia* from the International Provenance Trials at Neyveli (1992) and Puducherry (1995), a detailed breeding programme was prepared for implementation by IFGTB with technical collaboration of CSIRO in 1996. The objective of the breeding programme was to maximize the yield and quality of poles and pulpwood in casuarina plantations. The breeding strategy relies on open-pollinated management of breeding populations together with the use of vegetative propagation for deploying rooted cuttings for clonal planting and to establish clonal seed orchards. The two-tier breeding populations include a 'Main Population' established as large provenance-family trials (100+ open pollinated families) and a 'Core Population' consisting of clones from the best 25 families selected from the Main Population.

Twenty-Five Years of Research on *Casuarinas* at IFGTB

New germplasm in the form of provenances were assembled from 40 locations in 15 countries spread in three continents (Africa, Asia and Australia). Four Main Populations were established at Kakinada, Rajahmundry, Nellore (Andhra Pradesh) and Sadivayal (Tamil Nadu) in 1998. They underwent two thinnings after ranking the trees based on growth and form traits at four and six years of age. Provenances from Thailand, Malaysia and Solomon Islands showed up to 40% faster growth than local casuarina seedlots in the coastal regions. In areas away from coast (inland region) provenances from Kenya and Thailand showed fast growth. Similar provenances with many desirable traits like stem form to increase pole value, freedom from diseases and insects were also identified. Provenances from Kenya and Solomon Islands provenances had 30 to 50 kg more wood per cubic metre compared to the local seedlot. These thinned trials now function as Seedling Seed Orchards to supply seeds for planting programmes. The Core Population comprised 50 outstanding clones (25 male and 25 female) selected from the Main Population based on superiority in growth and stem form. They were drawn from the best



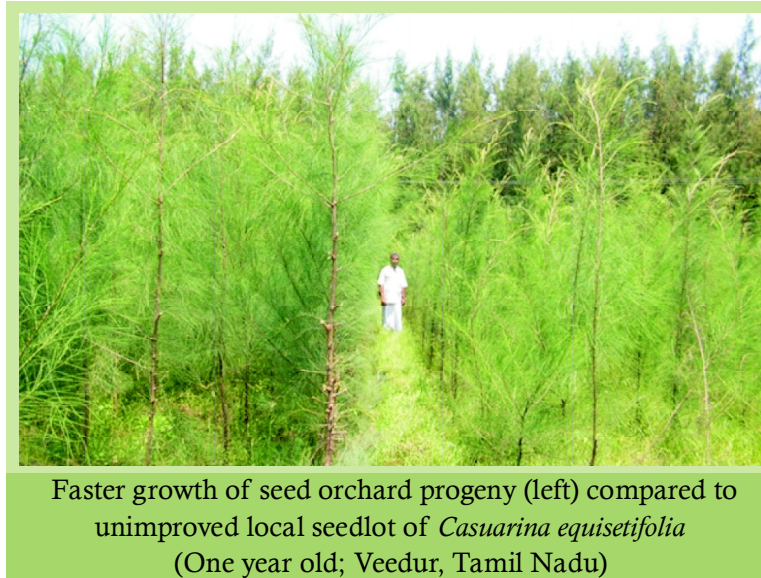
provenances and assembled in a clone bank through rooting of cladode cuttings. Two clonal tests were established with these clones at Sadivayal (Tamil Nadu) and Hosekote (Karnataka). Clones selected from outstanding provenances like those from South East Asia outperformed selections from unimproved plantations. These clonal tests were subjected to two thinnings to remove inferior clones and converted into Clonal Seed Orchards. Second generation breeding populations were established in different locations with progeny of outstanding individuals from the first generation orchards. Seeds from these orchards are now collected and supplied to different user groups like forest departments, wood-based industries and farmers. On-farm gain tests showed that seeds from seed orchards provided 13% more wood under rainfed conditions and 28% with irrigation than unimproved local seed. Four clones of *C. equisetifolia* which showed consistently outstanding growth when subjected to field tests at multi-locations have been released for commercial planting in Tamil Nadu, Karnataka and Puducherry in 2010.



Locations of field testing of *Casuarina* germplasm



A second generation breeding ('Main') population of *Casuarina equisetifolia* (21 months old; TNPL Campus, Tamil Nadu)



2.3 Selection of *Casuarina junghuhniana* Provenances

Casuarina junghuhniana is native to the Indonesian islands of Java, Timor and Wetar. In order to widen the genetic base of this potential species, IFGTB established two field trials at Puducherry and Panampally to select superior provenances. These field trials comprised of 10 provenances of *C. junghuhniana* ssp. *junghuhniana* from Indonesia, six provenances of subspecies *timorensis* from Indonesia and seven land race seedlots from Kenya and Tanzania. Provenances from Timor and Wetar Islands performed better than those from Java and land race seedlots of Kenya and Tanzania. Provenances from East Timor grew 38% faster than the local seedlot with almost equal wood density. Studies indicated that *C. junghuhniana* has more photosynthetic efficiency, drought and salinity tolerance than *C. equisetifolia*. Wood density of provenances ranged from 0.54 to 0.73 g/cc at the age of 4 years in Puducherry. The overall mean density of *C. junghuhniana* was found to be less than that of *C. equisetifolia*. The highest density among *C. junghuhniana* provenances was recorded for the Camplong provenance (0.73) which is comparable to the local seedlot of *C. equisetifolia* at Puducherry (0.7). Seed orchards have been established for this species and seeds are supplied to tree planters. Identification of high yielding varieties (provenances, families and clones) and mass production of their seeds in seed

orchards have contributed to increase wood production of casuarina plantations significantly.

2.4 Estimation of Gene Diversity, Breeding Efficiency, Population Size and Genetic Gain in Seedling Seed Orchards

Two provenance trials each of *Casuarina equisetifolia* and *C. junghuhniana* that were converted to seedling seed orchards after early evaluation and thinning to remove inferior provenances and trees within provenances were studied for their breeding efficiency. Bulk seed from more than 25 parent trees in each seedlot was used for establishing three provenance trials in randomized complete block design in coastal and inland regions. One of the provenance trials of *C. equisetifolia* located at Sadvayal in Tamil Nadu was established to maintain pedigree information as a provenance – progeny trial. Distinct coast to inland trends were evident in both the species of *Casuarina*. Fertility variation was more in inland locations though the gender pattern was more or less similar in both locations. There were many non-flowering trees in inland orchards compared to coastal site. The pollen and seed production per tree was however higher in inland trees resulting in a skewed fertility pattern in inland orchards. Unpedigreed orchards maintained adequate diversity in both *Casuarina* species compared to the pedigree identified progeny trial. There was no significant correlation between tree height and fertility. Thus selection without considering fertility would not promote gain in the next generation.

Two seedling seed orchards each of *C. equisetifolia* and *C. junghuhniana* established by thinning provenance trials in coastal (Puducherry) and inland locations (Karunya and Panampally) in South India were evaluated for sex expression and fertility variation. More than 80% of the trees in *C. equisetifolia* orchards were fertile in both sites with a similar pattern of more (almost 2 times) female trees and equal proportion of monoecious and non-flowering trees. In *C. junghuhniana*, the coastal orchard had twice the proportion of fertile trees as that of the inland. Orchards established in coastal environment had less fertility variation and hence maintained higher diversity in both species. Coastal site had more trees contributing effectively to seed production than inland locations. Measures like constrained seed collection from large number of trees and promoting representation of superior provenances with low fertility would be useful in checking diversity loss during domestication.

Two genetic gain trials were established for *Casuarina equisetifolia* and *C. junghuhniana* in different locations to test the performance of seed collected from seedling seed orchards. *C. junghuhniana* seeds from both orchards were superior to other seedlots in the casuarina trial at one location. The differences were not significant in the second location. Thus the early trends indicate that low diversity resulting from poor flowering and related mating in pedigreed orchards would be manifested in the inferior performance of the progeny. Unpedigreed orchards generally maintain adequate diversity and hence do not produce inbred progeny.

2.5 Reproductive Biology of *Casuarina equisetifolia*

Flowering clearly occurs in two seasons the first during late September - December which is a major one and the second one during late May and July is shorter. There is considerable variation in flowering patterns between different seed lots. The naturalised seed lots such as South Arcot and Orissa from India were the first to start flowering 12-14 after field planting. Most seedlots were reproductively mature after two years. Excepting the Indian seedlots there were considerable number of individuals in different seed lots that did not show any signs of flowering even after 4 years.

Male flowering: In both monoecious and dioecious conditions male flowering preceded the female phase. Male flowering is lengthy with an initial episode lasting 7-14 days followed by 5-6 intermittent episodes lasting 2-3 days. These episodes are spread over 2-3 months.

Female flowering: Female flowering is mostly one time flowering lasting 7-10 days. Seldom they may flower one or two short episodes lasting 3-4 days. Usually such flowering happens with fully mature buds that remain dormant. Individuals that reached maturity showed a gradation in their reproductive output from few to profuse.

Fruiting Phenology: The seedlots varied considerably in terms of fruit yield per tree. Accessions South Arcot and Orissa from India and Beechai, China showed very heavy bearing of fruits from 3000 – 5000 per tree. The seedlots Benin, Thailand, Sarawak and Fiji showed a moderate bearing of 1200 – 1500 fruits. In comparison to the above said accessions seedlots from Kenya, NT Australia, Solomon Islands

and Guam showed low fruiting (200 - 500 cones). The lowest fruit set was observed in Egypt seedlot. In terms of fruit sizes once again the Indian seedlots showed the highest width and length immediately followed by Beechai, China. The smallest of matured fruits were produced by Solomon Islands and Ranong, Thailand seedlots.

Seed yield: Highest seed yield per cone was recorded in Beechai, China. Almost similar values were recorded in the naturalised Indian seed lots. In terms of seed filling percentage Benin showed the lowest seed fill (35.7%) while Egypt recorded the highest (71%). In terms of seed yield per tree which was extrapolated from the seed yield per cone and number of cones Indian seed lots were the highest of about 20,200 seeds per tree while the lowest was produced by Northern Territory Australia which was 5900 per tree.

Controlled pollination: Pollen viability is quite high upto 95% with low sterility on the day of collection. Normal seed set could be obtained with 14- 21 days stored pollen. Seed set was recorded in clone TNIPT 7 with its own pollen. The pollen pistil interaction was normal with pollen tube penetrating till the ovary end.

2.6 Inter-Provenance and Inter-Specific Hybrids in Casuarinas

Forty families of inter-provenance and inter-specific hybrids of *Casuarina equisetifolia* and *C. junghuhniana* were produced through control pollination. Three field tests



were established in 2007 at Veedur (Tamil Nadu), Panampally (Kerala) and Sriharikota (Andhra Pradesh) to test the performance of F1 progeny. All the tests were assessed for height and survival. Survival was more than 90% in Veedur and Panampally and 85% in Sriharikota. Inter-specific hybrid families showed better height growth than local seedlings and seed orchard progenies of pure species. The best inter-specific hybrid family showed 35 to 53% better height growth than local seedlot and 17 to 21% over orchard progenies. The average height of the best 5% of trees was 49 to 68% more than that of local seedlot and 29 to 32% over orchard progeny. Periodic assessment of growth and form traits were gathered for further ranking of families and individuals.

2.7 Variation in Early Growth and Stem Straightness among Open-Pollinated Families of *Casuarina equisetifolia* in Second Generation Progeny Tests

First generation breeding populations were established using provenance-family seedlots of outstanding provenance and landrace seedlots in many locations in Andhra Pradesh, Puducherry and Tamil Nadu. These progeny tests were converted into seedling seed orchards after culling the inferior families and individuals based on growth, form and tolerance to wilt disease and borer attack. Three second generation progeny tests were established in Tamil Nadu and Andhra Pradesh using open-pollinated seeds of 207 outstanding female trees selected from seven first generation orchards. Variation in growth and stem straightness of these families at the age of one (Chellanchery) and two years (TNPL and Tirupati) were recorded. Significant differences in height, diameter and volume index were observed among the families and the first generation orchards which contributed the families in all three locations. Stem straightness of families and orchards measured in the first year at Chellanchery also varied significantly. The irrigated farmland conditions in Chellanchery resulted in the best growth among the three locations recording a mean values of 5.08 m for height, 4.22 cm in diameter and a volume index of 103 followed by TNPL with mean values of 4.23 m, 3.58 cm and 73 for height, diameter and volume index respectively in the first year. During the second year growth was more in TNPL compared to Tirupati recording 8.9 m height, 6.45 cm diameter and volume index of 475. Families originating from the Karunya and Puducherry

orchards recorded faster growth than other sources in Chellanchery and TNPL while those from Rajahmundry were the best in Tirupati. Narrow sense heritability estimates for all traits assessed were low to moderate ranging from 0.03 to 0.38. Trait-trait and age-age correlations for the two growth traits- height and diameter were positive and strong while weak correlation was observed between growth and stem straightness. The large variation present among the second generation progeny and the orchards they originated from offers scope for both forward and backward selections. Additional thinning in first generation orchards based on progeny test results and restricted seed collection from female trees with high breeding value will enhance the currently realized genetic gain from these orchards. Outstanding trees of the best families may be selected for the next generation breeding and clonal propagation populations. Since there is no adverse correlation between growth and stem straightness genetic improvement of both the traits can be improved with one breeding population.

2.8 Variability Studies in *Casuarina equisetifolia*

Selection of clones: 106 phenotypically superior trees of *C. equisetifolia* were selected through extensive survey of 3 to 4-year-old *C. equisetifolia* plantations in the coastal belt of Tamil Nadu raised by the State Forest Department (Tamil Nadu) and private planters at Chidambaram (CH) Chengalpet (CP) and Tiruchendur (TCR) and assembled in the clone bank of IFGTB. Index selection method was followed for selection of clones.

Variability with respect to biometric characteristics: Crown length exhibited the highest degree of variation followed by diameter at breast height (DBH) or collar diameter (CDM) among all the primary characters (total height, DBH, CDM, crown length, cladode length, cladode diameter and number of primary branches) in both CH / CP and TCR clones. Diameter at breast height showed higher degree of variation than total height in both the groups of clones. Among the CH / CP clones, the best clone was 454 per cent superior than the average of all the clones when volume index was examined and the difference between the best and the worst clone was 3192 per cent. Within TCR clones, the best clone had 308 per cent more volume growth than the grand mean for volume growth and 1282 per cent more value than the lowest ranking clone.

Genetic parameters responsible for variability: Volume index recorded the maximum genotypic coefficient of variation (GCV) followed by crown length in both CH / CP and TCR clones at 8 years of age. Diameter at breast height, frustum volume and CDM also registered higher values for GCV in both the groups of clones. Number of primary branches registered the minimum GCV. Number of primary branches, cladode length, cladode diameter and total height showed narrow difference between the values of phenotypic coefficient of variation (PCV) and GCV indicating that these traits were less influenced by environment. Total height recorded the maximum value for broad-sense heritability in CH / CP clones whereas, cladode length and cladode diameter registered the maximum value for broad sense heritability (H^2) in TCR clones at age 8. Volume index and crown length recorded high values for broad-sense heritability coupled with high values for GCV and genetic gain in CH / CP clones. High GCV indicated that these traits had considerable genetic variability, thus offering good opportunity for improvement through selection. Height recorded moderately high value for genetic advance. For all the other characters, though heritability values were high or moderately high, GCV and genetic advance were low only. In TCR clones, though both cladode length and cladode diameter registered high heritability values, GCV and genetic advance were low.

When heritability was studied over 6 years, marginal reduction in values was noticed for total height during ages 4 and 5 in CH / CP clones. However, from sixth year onwards, it was found stabilized. For all the other characters, a decreasing trend in values of broad-sense heritability was observed. Similar was the response in TCR clones with respect to total height whereas, the heritability values exhibited an increasing trend over the years for DBH and volume index.

Association studies: The genotypic correlation coefficients were higher in magnitude than the phenotypic correlation coefficients for most of the traits in both CH / CP and TCR clones indicating that the association between these traits was genetically controlled. Among the primary characters, diameter at breast height recorded the strongest positive correlation with volume index in both the groups of clones, followed by CDM. Crown length in CH / CP clones and total height in TCR clones also exhibited high values for genetic correlation coefficient when

associated with volume index. Strong positive correlations were observed between total height, DBH and CDM at the genetic level, indicating that an improvement in one character could bring considerable improvement in the other characters also.

When path analysis was performed, only crown length showed positive direct effect on volume in CH / CP clones. However, cladode length, cladode diameter and number of primary branches exerted positive indirect effects through crown length. In TCR clones, though the direct effects of all the four characters on volume index were positive, crown length only could register a value of higher magnitude. Number of primary branches exerted positive indirect effect (which was higher than its direct effect) on volume index through this character.

Studies on age-age correlations resulted in significant and positive correlations for total height, DBH, CDM, frustum volume and volume index among all combinations of ages (age 3 to age 8) at phenotypic and genotypic levels in both CH / CP and TCR clones. Genetic correlation coefficients were higher than the phenotypic correlation coefficients. Though the maximum values of correlation were recorded between ages 7 and 8, genetic correlation coefficients obtained between ages 3 and 8 were also very high in magnitude (>0.90) for all the characters indicating that selection at an early age (age 3) may be practised to maximize the gain per unit time.

Genetic divergence : Application of Mahalanobis' D^2 statistics and Tocher's clustering method grouped the CH / CP clones into 11 clusters and the TCR clones into seven clusters. Among the various characters, volume index contributed the maximum towards genetic divergence. Based on the inter and intra-cluster distances, male clones CH 3004, CH 0401, CP 0207, CP 3903, CH 2604 and CP 1501 and female clones CH 2703, CH 2803, CP 3703, CP 0301 and CH 3002 are recommended for further breeding programmes from the CH / CP group. From the TCR group of clones, TCR 110202, TCR 090201 and TCR 030101 (male) and female clones, TCR 040204, TCR 120102, TCR 080201, TCR 120203 and TCR 090102 are recommended.

Stability analysis over growth periods: In the CH / CP group, CP 4202, CH 3002, CH 2803 and CP 3903 were identified as the most stable clones (over five growth periods) with respect to total height. Clones CP 0207, CP 3903 and CH

0401 exhibited stability for both frustum volume and volume index. Two clones (CH 3004 and CH 2703), which exhibited excellent growth characteristics, could not register favourable values for stability parameters. Among the 15 superior TCR clones with respect to total height, 10 were found to be highly stable over the growth periods. Select clones were observed to be stable for DBH (7), CDM (9) and frustum volume (10) also. Clones TCR 060101, TCR 030202 and TCR 030101 exhibited high stability for all the four traits. No clones were found suitable for stress or favourable growth phases.

Evaluation of clones based on point grading method: Most of the clones in the CH / CP group (around 80%) were growing almost straight, vertical and possessed thin limbs. Among the TCR clones, 37 per cent of the clones had very crooked stems and around 50 per cent carried thick branches. When straightness, verticality and branch thickness were considered, 20 clones each from the two groups (61 and 47% respectively) were found to be suitable for pulp production. Around 67 per cent of the CH / CP clones and 30 per cent of the TCR clones were found ideal for agroforestry (Wide-row intercropping) purposes. Based on the stem straightness and axis persistence, 61 and 13 per cent clones from the CH / CP and TCR groups respectively were identified for the purpose of scaffolding. Eighteen CH / CP clones were observed to be free from pest / disease problems whereas almost all the TCR clones were found susceptible to any one (or in combination) of the major pest / pathogens. The rooting percentage was satisfactory in general for CH / CP and TCR clones.

2.9 Evaluation and Characterization of Clones of *Casuarina* with Reference to Yield, Tree Form, Biomass and Pulping Characteristics

Eighty-seven clones of *Casuarina equisetifolia* selected and assembled by IFGTB were tested in multilocal field trials at Mayiladumparai, Karur district (Latitude: 10° 52.080' N and Longitude: 78° 27.376' E, Inland red soil), Moorthipalayam, Karur district (Latitude: 11° 01.933' N and Longitude: 78° 01.121' E, Sodic soil) and Sirugramam, Cuddalore district (Latitude: 11° 47.390' N and Longitude: 79° 27.339' E, Inland casuarina growing region).

Results reveal that observations recorded on tree height, DBH and volume index at age 1 could be reliable indicators for the growth at age 3 and selection at an early age may be practised to maximize the gain per unit time. Based on the divergence analysis, five male clones namely, TNVM 2, CE 2003/4, CE 2003/3, CE 219 and CE 347 and ten female clones TNPP 2, TN



A clonal trial at Moorthipalayam, Tamil Nadu



A clonal trial at Mayiladumparai, Tamil Nadu



Another view of the clonal trial

111, CE 2002/1, CE 220, CE 268, CE 243, CE 9, CE 303, CE 281 and CE 2003/5 can be selected for further breeding programmes. Five monoecious clones namely, CE 2002/2, CE 329, CE 327, CE 224 and CE 83 which were found to be productive and divergent, could be used as pollen parents in breeding programmes. Twenty-eight clones have been identified for wide row intercropping and 27 clones were identified for the purpose of poles and scaffolding. Lignin content varied widely from 11.21 to 29.25%. Clones CE 398, CE 327, CE 276, TNVM 3, CE 2003/3, CE 2003/4, TNVM 2, TN 111, TNCS1 and CE 9 with low lignin content (< 18 per cent) are ideal for pulping. CE 268, CE 303, CE 224, TNIPT 16, CE 329, TNRM 5, CE 2002/1, TNPV 4 and TNPP 2 with a lignin content of more than 25 per cent are suitable for poles. The clones were also tested for different pulping traits.

2.10 Clonal Seed Orchards for Quality Seed Production in *Casuarina equisetifolia*

Seed orchard is a plantation of selected clones or progenies which is isolated or managed to avoid or reduce pollination from outside sources to produce quality seeds on a large scale. Clonal Seed Orchard is the immediate option for quality seed production for species that are amenable for vegetative propagation like *Casuarina equisetifolia*. It can easily be propagated using the technique of rooting of cutting, one of simplest and cost effective methods of vegetative propagation. Moreover, the advantage of early flowering can fully be utilized in this species as mature cladode cuttings are also amenable for rooting. Viable seeds can be obtained even from six months of age. *Casuarina* being a polygamous species, preventing the monoecious clones from entering into the orchard can avoid the danger of selfing.

An experimental Clonal Seed Orchard (CSO) of *C. equisetifolia* was established at Panampally, Kerala in February, 1996. Twelve superior clones (8 males and 4 females) identified from 12 genetically divergent clusters were planted at a spacing of 2 x 2 m in a Randomized Complete Block Design with 4 replications and 40 ramets per clone. Randomized Block Design was followed to avoid ramets of a single clone occurring together or in clusters in the design. This approach is a variation from the traditional concept of CSO where untested clones form the first generation orchard and rouging the inferior ones leads to the development of advanced generation production orchards.

The clones in the CSO started flowering from six months after planting. Synchronisation in flowering was observed during September and fruit set was noticed in all the four female clones later. Seeds extracted from mature cones collected (clone wise) during December-January were tested for germination parameters. Seed collection and germination studies were conducted for four years from 1997 to 2000. Details of production of cones and seeds from the CSO were also worked out.

Twelve genetically divergent clones with synchronised flowering habit in a seed orchard of 0.768 ha can produce 22 million plants annually.

Production of cones and seeds from the Clonal Seed Orchard of *Casuarina equisetifolia*

Total Area of the CSO	0.768 ha
Total number of female trees	640
Average production of cones per tree	1.20 kg
Total production of cones	768 kg
Average yield of seeds / 1kg of cones	30 g
Total yield of seeds	23.04 kg
Seed rate	8,05,000 seeds per kg
Total number of seeds	1,85,47,200
Average germination percentage	75 %
Total number of plants that can be raised using the seeds produced in one season	1,11,28,320 (survival rate taken as 80%)
Total number of plants that can be produced during one year (2 harvests)	2,22,56,640
Total area that can be planted (2 x 1.5 m spacing)	742 ha
Total area that can be planted (1 x 1 m spacing)	2,225 ha

Maintaining the orchards as hedge garden of reachable height is recommended to enhance quick and maximum seed collection. However, flowering behaviour of clones should be carefully recorded as continuous hedging may result in change of sex. Prophylactic control measure for the blister bark disease should be undertaken. Periodic inspection and removal of the infected ramets if any, is essential.

2.11 Adaptive Variations in Wild and Naturalized Genetic Resources of *Casuarina equisetifolia*, Linking Breeding Systems, Reproduction and Domestication

Wild, exotic and local land races of *Casuarina equisetifolia* in tropics have revealed significant intra-specific variations in terms of reproduction, morphology and growth. The local land races of *C. equisetifolia* were early maturing and prolific in reproductive output compared to their shyly reproducing late maturing provenance relatives. Thus to meet large volumes of quality seeds deploying high yielding local

selections could be a very practical strategy. Clonal seed orchards can be planted with late maturing provenances in the first year and early maturing local land races can be accessed in the second or third year for greater panmixis. A study conducted on six provenances indicated that sexual symmetry and monoecy levels did not vary significantly when tested across coastal and inland conditions. Since dioecy is a major sexual system quantifying combining ability in male selections is of obligatory and of high genetic value in future domestication programmes. Pollen-pistil interaction is very unique in that only very few pollen gain entry and their growth is very slow. Also branching of pollen tube is very common. Monoecious individuals produce viable offspring when selfed, however they do not show any morphological signs related to inbreeding depression. In comparison to regular males the significantly short male inflorescences could be used as a morphological marker. Also, qualitative reproductive characteristics such as cone colour, shape and architecture could be potential markers in inter-provenance hybridization programmes.

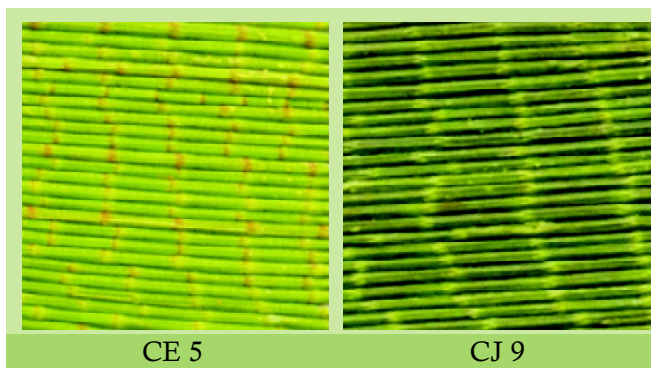
2.12 Developing Descriptors for *Casuarinas* for Species and Clonal Identification

The Protection of Plant Varieties and Farmers Rights Authority (PPVFRA) has developed a *sui generis* system for protection of plant varieties by integrating the rights of breeders, farmers and village communities, and taking care of the concerns for equitable sharing of benefits. The Act covers all categories of plants, except microorganisms. Forestry crops which are in various stages of domestication also find a place in this category. Unlike in the case of agricultural crops, tree species are more complex, and a different set of rules had to be adopted for them. In this context, as an attempt to initiate the process for tree species, the Authority entrusted the task of development of descriptors for two major plantation species, popular among farmers, Eucalypts and Casuarinas, to the Institute of Forest Genetics and Tree Breeding, Coimbatore. Draft descriptors were developed for Eucalypts and Casuarinas using a worldwide germplasm available with the Institute. National level meetings involving stakeholders were conducted to finalize the draft guidelines. The descriptors have been notified in 2013 and IFGTB has been declared as the DUS testing Centre of Casuarinas.

In Casuarinas, two species, *Casuarina equisetifolia* and *C. junghuhniana*, the two main species cultivated by the farmers, were taken up. Forty-seven descriptors were identified in all for discriminating species and clones. Morphological variations in vegetative and reproductive parts were considered for the development of descriptors. Among these, descriptors of bark features, needle characteristics, flower and fruit descriptions are the main descriptors which can be deployed to discriminate variations in the genera.

Cladode ('needle') Characters:

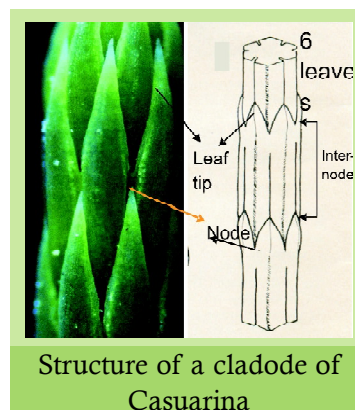
Cladode colour : As a xerophytic adaptation, the leaves of *Casuarina* species are fused along the stem with only teeth-like projections of leaf tips at every node. This gives a needle-like appearance. Two major classes are dark green (*C. equisetifolia*) and fluorescent yellowish green (*C. junghuhniana*). Grouping of the intermediate forms was done based on shades available with RHS colour codes.



Cladode thickness: Thickness of the cladode is grouped in 3 classes viz. thick (>1 mm), intermediate (0.5 to 1 mm thick) and thin (<0.5 mm thick). *C. equisetifolia* has a thick needle whereas *C. junghuhniana* has thin needles.

Leaf Characters:

Number of leaves per node: The number of leaves per node of matured deciduous branchlets was short listed as one of the morphological trait that can be unambiguously assessed in the field to distinguish at species and clone level. The foliage of *Casuarina* species consists of long needle-like deciduous branchlets. These branchlets have regularly spaced nodes. At each of these nodes is a ring of teeth, which are the tips of the reduced leaves. The leaves arise in

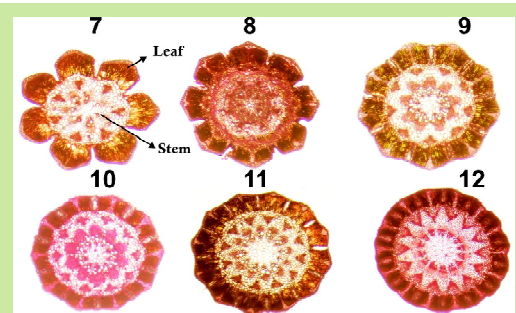


a whorl at each node, and are fused along the next internode, forming longitudinal ridges on the branchlet, and become free at the next node, where they terminate in the teeth. The number of leaves per node is diagnostic at species level and to an extent within species among provenances.

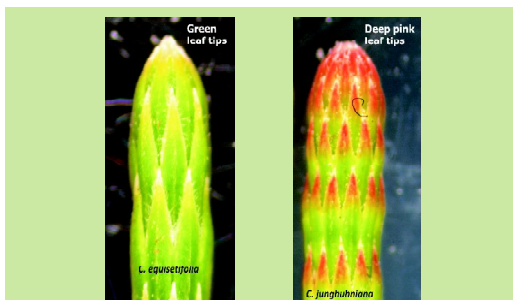
C. equisetifolia has 6 to 8 leaves per node while *C. junghuhniana* has 9 to 12 leaves. Within *C. equisetifolia*, the number of leaves per node was found to be similar among clones selected from different populations. Since the number of leaves per node can easily be counted using a field lens, it makes a convenient descriptor for Casuarina.

Colour of leaf tip: The leaf tip possesses different colours in the two species of Casuarina. It is a distinct pink in *C. junghuhniana* but colourless or translucent in *C. equisetifolia*. The hybrids between the two species show varying degrees of pink colour which can be assigned to different shades of pink using a Royal Horticulture Chart.

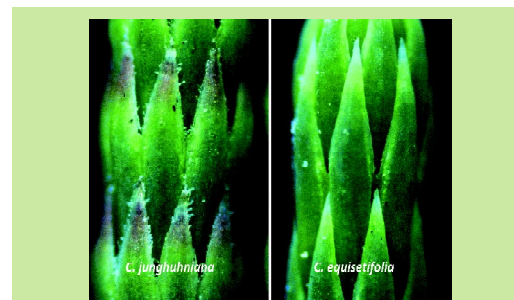
Pubescence on leaf tips: Leaf tips of *C. junghuhniana* have hairs on the margins whereas they are glabrous in *C. equisetifolia*. The size and intensity of hairs differ among clones of *C. junghuhniana*.



Cross section of branchlets showing variation in number of leaves per node in *Casuarina* species (7&8: *C. equisetifolia*; 9-12: *C. junghuhniana*)



Variation in leaf tip colouration and pubescence in *Casuarina* species (A: *C. equisetifolia*; B: *C. junghuhniana*)



Presence of trichomes on leaf tips of *Casuarina* (left: *C. junghuhniana*; right: *C. equisetifolia*)

2.13 Outputs from Breeding Programmes

Although the breeding programme prescribed collection and supply of seeds from SSOs from the fifth year (i.e. after the first thinning in the fourth year), commercial supply was deferred till the eighth year to have adequate flowering and panmixis in the orchards. Flowering in the natural provenances especially those from the South East Asia was quite low up to the age six whereas heavy flowering occurred in the Indian landrace seedlots. Since these provenances are among the best in both coastal and inland sites, low reproductive output from them will affect the genetic gain that can be realized from the SSO progeny. Genetic gain tests involving seeds collected from SSOs at age four revealed they were not significantly different from natural provenance (Thailand) and CSO seed of Indian landrace.

At around the age six, the overall proportion of flowering trees increased to over 80% in particular that of the outstanding provenances like Thailand, Malaysia and PNG. Commercial seed supply was started from the eighth year (2006) after starting the breeding programme. Between the years 2006 and 2009, more than 100 kg of seeds have been collected from the orchards of the two species and about 90% of it was supplied to different user groups. Approximately one third of the seed supplied was that of *C. equisetifolia* and the rest *C. junghuhniana*. Since both seed rate and germination of *C. junghuhniana* are far higher than that of *C. equisetifolia*, the seed supplied could cover planting in six times more area. More research is needed to increase seed production in *C. equisetifolia* orchards and seed germination. Till such a time better orchard management and nursery techniques are available the seed demand could be met through establishing more orchards and clonal propagation.

Generally seeds were supplied to a particular planting site from an orchard located in a site similar in soil type, rainfall and distance from the sea coast. Growth of orchard progeny was better than that of the local unimproved seedlot in all planting sites. In the coastal site the *C. equisetifolia* orchard progeny recorded 50% more volume growth over the unimproved *C. equisetifolia* at 3 years age. Similarly the *C. junghuhniana* orchard seedlot produced more than twice the volume growth than the local *C. equisetifolia* seedlot at 5 years age. A minimum of 20% better growth was obtained in low input plantations raised under rainfed conditions.

2.14 Release of High Yielding Clones of Casuarina

The Institute of Forest Genetics and Tree Breeding (IFGTB) established three clonal tests of *Casuarina equisetifolia* in the year 2000 with 124 entries (115 clones and 9 seedling controls). The locations of these tests are Hosekote, Karnataka (inland dry), Coimbatore, Tamil Nadu (inland semi-moist) and Puducherry (coastal). All three tests were planted in incomplete block designs with a minimum of 5 replications and with one to two trees of a clone per replication. All the trees were assessed for height, dbh, stem straightness, incidence of insect and disease attack and reproductive behaviour annually up to 7 years age (up to 5 years in Puducherry).

The clonal entries were drawn from the following sources:

- (i) Provenance-progeny tests: These populations were developed with a large genetic base of nearly 40 provenances / landraces of the species drawn from 15 countries of 3 continents to serve as breeding populations. All trees in the tests were assessed and outstanding trees were selected through index selection method.
- (ii) Selections from farmers' plantations: Extensive surveys were made in the farmers' plantations and superior trees were selected based on growth and tree form through check tree method.
- (iii) Assembling of clones selected by other agencies: Clones selected by other agencies like forest departments and wood-based industries were collected and included in the trial and their original names were retained.

All trees planted in the clonal tests were developed through rooting of cladode cuttings collected from 2 to 6 year old trees. Cuttings rooted in the mist chamber / poly tunnels were grown in polybags for 3 months before field planting. All trials were managed under rainfed conditions without addition of any fertilizers. Annual weeding was undertaken till the seventh year after planting.

All trees were assessed annually for height and diameter at breast height. Stem straightness was scored at the age of 7 years and observations on flowering and fruiting was initiated from third year onwards. Incidence of pest and disease attack was monitored once in two months. Statistical analysis for growth and tree form

traits were carried out and the clones were ranked according to their superiority based on growth traits. Four clonal entries viz., IFGTB- CE-1, IFGTB-CE-2, IFGTB CE-3 and IFGTB CE-4 were found to be superior in their growth performance over the seed control. The salient features of these clones are given in the table.

As per the “Guidelines for testing and releasing of tree varieties and clones” of the Indian Council of Forestry Research and Education, the entire selection programme was scrutinized by an independent Implementation Team which submitted a report to the Regional Variety Testing Committee (RVTC). The RVTC deliberated on this report and unanimously recommended releasing of the four clones. Following the RVTC’s recommendation, the Variety Releasing Committee (VRC) constituted by ICFRE under the chairmanship of Director General of Forests and Special Secretary,

Clone	Sex	Wood density (kg m ⁻³)	Recommended cultivation location	Descriptors
IFGTB CE 1	Monoecious	689	Inland	1. Triangular branch scar. 2. Brownish purple bark with uniformly and scatteredly distributed lenticels.
IFGTB CE 2	Female	685	Coastal & inland	1. Grey bark with patchily distributed lenticels. 2. Secondary branches arise from protrusions on primary branches.
IFGTB CE 3	Female	684	Coastal & inland	1. Paired primary branches. 2. Brownish purple bark with uniformly and densely distributed lenticels
IFGTB CE 4	Male	687	Inland	1. Fissured bark up to 1 m from base. 2. Sparsely distributed secondary branches.

Twenty-Five Years of Research on Casuarinas at IFGTB

Ministry of Environment and Forests, Government of India discussed the merits of the clones. The VRC unanimously approved the release of the above clones of *Casuarina equisetifolia* in the States of Tamil Nadu and Karnataka and the Union Territory of Puducherry.

Ten clones of *Casuarina* (two *C. junghuhniana* clones for their growth superiority, three *C. equisetifolia* clones for growth superiority in inland conditions and five *C. junghuhniana* clones as windbreaks) are slated for release soon.

Efforts for popularization of the clones: The clones of *Casuarina* were released for commercial cultivation by farmers. These clones were mass multiplied and supplied to farmers free of cost under National Agriculture Innovative Project (NAIP) funded project on A value chain on Industrial Agroforestry in Tamil Nadu with Forest College and Research Institute (FCRI), Mettupalayam as the lead partner. IFGTB took efforts to popularize these clones by way of establishing Model Clonal Plantations (MCPs) and Clonal Demo Plantations (CDPs) in farmer's lands free of cost. In addition, the clonal stock plants were also transferred to Forest Departments, Forest Corporations, paper mills and other private nurseries.



Model Clonal Plantations (MCPs): MCPs are clonal plantations established in clusters. About 10 to 15 farmers clusters were formed at Coimbatore for *Casuarina* in association with Tamil Nadu Newsprint and Papers Limited (TNPL) and Seshasayee Paper and Boards Limited (SPB). Each farm of about 1 ha area was planted with about 3 to 4 best performing clones, given free of cost, for establishment of block plantations. Around 400 to 500 tree blocks were made.

The cost of planting and the maintenance is borne by the farmers. IFGTB provides them the technical know-how and advice as and when required. The plantations are frequently visited and growth observations are taken regularly. These MCPs serves as the model for the other farmers in the cluster. Every year two trainings are conducted in the cluster and the farmers are taken to these plantations and explained

the advantage of the clonal plantations established with superior clones of IFGTB along with released clones. The Industries also conduct training programmes and the farmers are taken to these MCPs.

Clonal Demo Plantations (CDPs): The released clones of IFGTB were planted in blocks of about 400 trees side by side with other commercially planted clones. These CDPs were established to demonstrate the superiority of the released clones viz the other commercial clones. Clonal blocks of specific clones are established sequentially. Farmers can walk through the plantations and see the superiority of the released clones. The commercial clones and IFGTB released clones are planted alternatively.

Transfer of clones through Material Transfer Agreement (MTAs): The clonal stock plants were also transferred to Tamil Nadu Papers and Newsprints Ltd (TNPL) free of cost. The plants were given on MTA with an agreement for mass multiplication and supply to farmers. The clonal stock was also given to Ballarpur Industries Limited (BILT) for mass multiplication and supply to farmers.

Pamphlets: The information on the clones released was printed in the form of pamphlets in vernacular and English for popularization.

3.0

SILVICULTURE

3.1 Rehabilitation of Mine Spoils with *Casuarina equisetifolia*

Casuarina equisetifolia was grown on sieved mine spoils of bauxite, magnesite and lime stone under nursery conditions. The beneficial microbes particularly Arbuscular mycorrhizal fungi and Nitrogen fixing bacteria *Frankia* were isolated and inoculated to *C. equisetifolia* and transplanted at bauxite, magnesite and lime stone mine spoils. This technology reduced the cost of top soil, manures and fertilizers. Successful plantations of *C. equisetifolia* results in improvement of soil and environment and this technology could save the cost of top soil and chemical fertilizers.



Rehabilitation of magnesite mine spoils with *Casuarina equisetifolia*



Rehabilitation of lime stone mine spoils with *Casuarina equisetifolia*

3.2 Suitable Clones of *Casuarina equisetifolia* for Sodic Soils

Sodicity, the presence of a high proportion of sodium ions relative to other cations in a soil, directly reduces plant yield through its effect on soil properties. High levels of sodium in surface soils cause increased crusting and decreased water entry. In subsoil, sodicity reduces soil water storage, aeration and leads to increased soil strength. Susceptibility to erosion under rainfall impact or flowing water is increased in sodium affected soils. A field experiment was established at Anbil Dharmalingam Agricultural College and Research Institute (Tamil Nadu Agricultural University, Lat. 10° 45' N; Long. 78° 36' E; Alt. 85 msl; Annual rainfall: 800 mm), Tiruchirapalli, India to screen salt tolerant clones of *C. equisetifolia* selected from Chengalpet, Chidambaram and Tiruchendur in Tamil Nadu. The soil is sodic with pH 9.2, Exchangeable Sodium Percentage (ESP) 28.5 and Electrical Conductivity (EC) 0.24 dSm⁻¹.

Explicit inter clonal variation with respect to growth and physiological traits was observed. Among the 73 clones, two (08 and 44) proved their intolerance and dried within six months of growth. Eight clones (24, 31, 64, 26, 70, 52, 23 and 13) exhibited superior growth characteristics at age 3. Clone 24 recorded the maximum value for volume index (23010.69 cm³) and 15 other clones were found on par with it. The minimum value (283.74 cm³) for volume index was recorded by clone 48. Fifty-five clones were found on par with it. Instantaneous water use efficiency (WUE), measured as the ratio of net photosynthesis to transpiration varied from 0.19 to 1.59 $\mu\text{mol mmol}^{-1}$ and two clones registered significantly superior values when compared to the other clones. Clones 31 and 18 possessed excellent water use efficiency coupled with superior growth traits. However, no correlation was noticed between productivity and WUE in general.

3.3 Screening of *Casuarina equisetifolia* for Problem Soils of Tamil Nadu

Surveying over more than 1800 hectares in the east coast of Tamil Nadu, lateritic soils of inland and the magnesite mine spoils, 182 trees were assessed by Point Grading Method and 51 trees were selected from the districts of Tiruvallur, Chengalpet, Villupuram, South Arcot, Thanjavur, Pudukottai, Nagapattinam,

Ramanathapuram and Salem. Sprigs collected from the selected trees were rooted and assembled in clone banks/vegetative multiplication gardens at Neyveli (Tamil Nadu), Kulathupuzha (Kerala) and Port Blair (Andaman and Nicobar Islands) and were used for establishment of first generation clonal seed orchards at Neyveli, Pudukottai and Coimbatore in Tamil Nadu and Nilambur in Kerala.

The seeds of many of the selected trees were also collected and the families were included in the base populations that have been established in the states of Tamil Nadu, Andhra Pradesh and Orissa as a part of the National level breeding programme of *Casuarina equisetifolia* prepared by CSIRO under UNDP/FAO Regional project on Improving Productivity of the Man – made Forests through Application of Technological Advances in Tree Breeding and Propagation (FORTIP). They were also used in the establishment of Progeny trial – cum – Seedling Seed Orchard of *Casuarina equisetifolia* under the Planting Stock Improvement Programme. Out of the 51 trees selected 45 trees have been vegetatively multiplied successfully and assembled at a clone bank at IFGTB. This clonal assemblage serves as a source of clonal material for supply to forest departments and wood based industries as well as various trials being established by the Institute.

3.4 Effect of Potting Media in Quality Seedling Production of *Casuarina junghuhniana* ssp. *timorensis*

Casuarina equisetifolia x *C. junghuhniana* hybrid propagated through vegetative propagation is popular amongst farmers in Tamil Nadu. For the first time, seed based *C. junghuhniana* was introduced into the country by IFGTB. A study was conducted to understand the effect of different potting media formulations on quality seedling production of *Casuarina junghuhniana* ssp. *timorensis*. 30 potting media mixtures developed by various Institutes and forest departments for quality planting stock production of various species including *Casuarina equisetifolia* were shortlisted. seedling quality parameters viz., sturdiness quotient, biomass index and seedling quality index for each potting media mixture was calculated at the end of six months. Based on seedling quality index (SQI) and ease of availability, sand : farmyard manure in the ratio 2:3 is recommended for raising quality planting stock of *Casuarina junghuhniana* ssp. *timorensis*.

3.5 Effect of Containers on Quality Seedling Production of *Casuarina junghuhniana* ssp. *timorensis*

Planting of bare root seedlings is the most favoured method of raising plantations of *Casuarina equisetifolia* by farmers in Tamil Nadu. However container raised plants have been reported to survive better than bare rooted seedlings in all planting sites for *C. equisetifolia*. As *C. junghuhniana* ssp. *timorensis* is being attempted for inland sites with less moisture availability, use of container raised planting stock becomes inevitable while raising plantations. A study was carried out to understand effect of various types and sizes of containers on quality seedling production of *C. junghuhniana* ssp. *timorensis*. Ten types of containers (polybag – 3 sizes; single cell root trainers – 4 sizes; hyco pots – 2 sizes and thermocol) commonly recommended for use in containerized nurseries were tested in Sand: Red soil: FYM in the ratio of 2:1:1. Seedling quality parameters viz., sturdiness quotient, biomass index and seedling quality index for each type of container were calculated at the end of four months. Planting stock of 40 cm height was produced in 10 x 20 cm bag in 60 days after transplanting while most containers produced seedlings with average height of more than 30 cm in 90 days after transplanting except 150 cc hycopots.

3.6 *Casuarina* as Bioshields: Need for Salt Tolerant Genotypes

The Indian coast line stretches approximately 10,000 km of which Tamil Nadu coast is the longest in the east coast extending to 1076 km in length. The tsunami in 2004 has caused serious damage to the coastal ecology and economic resources. The coastal vegetation such as mangroves and *Casuarinas* played a pivotal role in mitigating the impact of tsunami wherever present. Tamil Nadu Forest Department established bioshield experiments in 2005-06 and 2006-07 near Marakkanam in Villupuram district and near Cuddalore. The major tree species planted included *Casuarina equisetifolia* and *C. junghuhniana*. *Thespesia populnea*, *Azadirachta indica*, *Acacia auriculiformis*, *Madhuca longifolia* and *Cocoluba uvifera* were the miscellaneous tree species introduced. The grass and shrub species included were *Spinifex littoralis*, *Agave* and *Pandanus*. Input of various biofertilizers including VAM, Azospirillum, Phosphobacteria; Biomanures like poultry manure, tank silt, vermicasting and different espacements were the treatments attempted. Eight shelter belt models were also established by the Tamil Nadu Forest Department during 2006-07. A linear

distance of 1600 m with 50 m width was identified for the experiment and the area was divided into 8 blocks of 200 x 50 m each. The various combinations of plant species attempted were (Model 1) *Spinifex littoralis*, *Tephrosia purpurea*, *Sanseveria roxburghiana*, *Zizyphus* spp, *Phoenix sylvestris*, *Anacardium occidentale*, *Leucaena leucocephala*, *Polyalthia longifolia* and *C. junghuhniana*, (Model 2) *Ipomea pescaprae*, *Dodonea* spp, *Pongamia pinnata*, *A. auriculiformis* and *C. equisetifolia*, (Model 3) *Agave* species, *Thespesia populnea*, *Polyalthia longifolia* and *Eucalyptus tereticornis*, (Model 4) *Pandanus odoratissimus*, *Simarauba glauca*, *Peltophorum pterocarpum* and *Albizia richardiana*, (Model 5) *Cassia marginata*, *Kigelia pinnata* and *Polyalthia pendula*, (Model 6) *Acacia senegal*, *Calophyllum inophyllum*, *Muntingia colubra*, *Cassia siamea* and *Bambusa tulda*, (Model 7) *Caesalpinia coriaria*, *Wrightia tinctoria*, *Albizia amara* and *Holoptelia integrifolia*, (Model 8) *Dodonea viscosa*, *Aloe vera*, *A. senegal*, *Zizyphus jujuba*, *A. indica*, *C. equisetifolia* and *E. camaldulensis*. The plant species were planted in such a way that the tiers were represented by grass, knee level shrub, small tree, medium tree and tall tree. Observations recorded during February 2011 revealed that only *Casuarinas* could establish as bioshields in the coastal belt with a survival percentage of about 70 per cent. *Cocoluba uvifera* established reasonably well with a survival percentage of about 45. However, its growth was stunted. *A. auriculiformis*, *A. indica* and *Pandanus* established poorly with a survival of less than 10 per cent. *Pongamia*, *Polyalthia*, *Thespesia*, *Peltophorum*, *Albizia*, Bamboos, *Caesalpinia*, *Wrightia*, *Cassia* and *Madhuca* could not establish in the seashore. Salt stress, wind velocity and other adverse site conditions could be attributed to the poor survival and establishment of tree species other than *Casuarinas*. Among the *Casuarinas*, *C. equisetifolia* established well in comparison to *C. junghuhniana* under these harsh environmental conditions suggesting the need for identification of better salt tolerant genotypes of *Casuarinas* for coastal shelterbelt afforestation.

3.7 Time Trend Studies on Biomass Production in High Density Plantations of *Casuarina equisetifolia*

In Tamil Nadu a number of private planters are growing *Casuarina equisetifolia* on 5 to 7 years rotation. The output is sold as a pole crop used mainly for scaffolding and subsequently as fuel, with the result its value increases. Its wood has relatively higher energy content and also yields excellent charcoal. Biomass studies on contribution by different girth class of trees on hectare basis and biomass assessment at different

periods of time were carried out for development of equations for biomass prediction. Foliage constituted maximum biomass in lower GBH trees and wood being dominant in trees of higher GBH category. Generally branch biomass rapidly declined when the GBH of trees crossed 10 cm. There was sharp increase in tree biomass from 42 months of age, much of the dry matter being channelized to bole biomass. Alternate rhythm between the rate of increase of foliage and branch or root exists suggesting periodicity in allocation of photosynthates. Mean annual yield for both utilizable and bole biomass showed increasing trend. Increase in mean annual yield of the bole biomass is an indication of tree-form amenable to close planting. The percent contribution of root to total biomass remained steady at all periods of study, suggesting constant dry matter allocation to roots. D²H, a measurable parameter can be used to predict the biomass at almost all the components, individually and totally with high degree of accuracy. Estimates of utilizable biomass are also possible with data on D²H. Thus equations have been developed to predict biomass non-destructively.

Regression equations derived for prediction of various components

Variables y (g.tree ⁻¹)	x(cm)	Regression equation	Coefficient of correlation “r”	SE of regression coefficient
Bole wood	D ² H	$\text{Log } y = 0.314 + 0.767 \text{ Log } x$	0.990***	0.314 ± 0.0196
Bole bark	D ² H	$\text{Log } y = 0.016 + 0.614 \text{ Log } x$	0.986***	0.016 ± 0.0185
Bole (W + B)	D ² H	$\text{Log } y = 0.443 + 0.748 \text{ Log } x$	0.991***	0.443 ± 0.0178
Branch	D ² H	$\text{Log } y = 0.536 + 0.594 \text{ Log } x$	0.878***	0.536 ± 0.0430
Utilizable dry matter	D ² H	$\text{Log } y = 0.809 + 0.685 \text{ Log } x$	0.980***	0.809 ± 0.0240
Foliage	D ² H	$\text{Log } y = 1.298 + 0.439 \text{ Log } x$	0.915***	1.298 ± 0.0340
Root	D ² H	$\text{Log } y = 0.507 + 0.654 \text{ Log } x$	0.966***	0.507 ± 0.0300
Total dry matter	D ² H	$\text{Log } y = 1.316 + 0.618 \text{ Log } x$	0.973***	1.316 ± 0.0269

*** P 0.001

3.8 Yield Table and Carbon Table for Plantations of *Casuarina equisetifolia* in Tamil Nadu

Regional yield table has been developed for estimating yield of *Casuarina equisetifolia* plantations from easily measurable growth parameter viz. girth and carbon table for assessing carbon sequestration potential of the plantations. Representative sample trees were felled from *C. equisetifolia* plantations in three agroclimatic zones viz., north eastern zone, cauvery delta zone and southern zone covering three major soil types viz., alluvial and sandy soil (Soil order: Inceptisol), red loamy soil (Soil order: Alfisol) and black clay and clay loam soil (Soil order: Vertisol).

Data on dry weight of all the biomass components of sample trees were used to develop prediction equations by using easily measurable growth parameters like diameter at breast height (dbh), height (h) and d^2h (Spurr's combined variable). Best-fit equations have been developed for prediction of dry matter accumulation in various biomass components - needle, twig, branch, stem, bark, agb, root and grand total- using diameter measurement.

Yield Tables: These tables are based on actual measurements of sufficiently large samples and have been prepared on the assumption that the trees of the same species with the same dimensions will have the same biomass or volume. For preparation of yield, diameter and height were considered. D^2H was used as the independent variable for developing prediction equation.

The prediction equation used for construction of biomass table for stem wood of *C. equisetifolia* on green weight basis was $y = 3.187 X^{1.013}$ (Annexure –I) while on dry weight basis $y = 1.458 X^{1.034}$ was used (Annexure –II).

Carbon tables: All biomass components (needle, twig, branch, stem and bark) are separately converted into carbon with the help of separate prediction equations. The following equations are used for different biomass components:

$$\text{Needle} : y = 0.008 X^2 + 0.234 X + 0.132$$

$$\text{Twig} : y = 0.127 X^{0.682}$$

$$\text{Branch} : y = -0.004 X^2 + 0.229 X + 0.586$$

$$\text{Stem} : y = 1.458 X^{0.787}$$

$$\text{Bark} : y = 0.246 X^{0.787}$$

In all the prediction equations, d^2h was used as independent variable. The carbon content of all biomass components were added to present the total carbon for above ground biomass in *Casuarina equisetifolia* for Tamil Nadu (Annexure –III). Carbon values for below ground biomass was calculated using the prediction equation $y = 0.616 X^{0.832}$ (Annexure –IV).



Method of yield estimation in Casuarina plantation for developing Yield Table

3.9 Casuarina Yield Calculator Utility Software - CYCUS v1.0

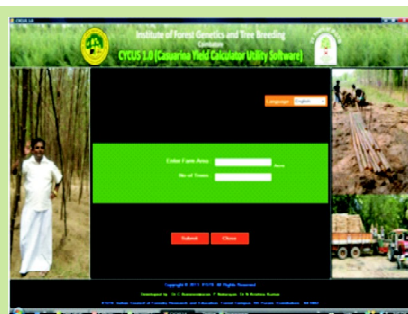
IFGTB has developed a software to facilitate farmers and other user agencies who go in for large scale plantations to assess the yield using observations on the girth of the trees to the tune of 100 trees per acre of plantation. This software will be very useful to empower the Casuarina growers in assessing the yield potential of their plantations and to have hold in marketing of the wood produce. The advantages of this software over manual calculations are:

- ◆ No need for working out frequency distribution of girth classes
- ◆ No need for height measurements of standing trees
- ◆ No manual calculations for estimating expected yield

The software was released during National workshop on 'Tree Seed Science and Silviculture' held at IFGTB, Coimbatore on 27-28 November 2013.



Release Function



Front page view of the CYCUS software

3.10 Response of *Casuarina equisetifolia* and *Casuarina junghuhniana* to Elevated CO₂ Levels

Three months old seedlings of *Casuarina equisetifolia* and *C. junghuhniana* were exposed to elevated CO₂ levels in automated open top chambers at 600 ppm and 900 ppm for a period of three months. Simultaneously, seedlings were also kept as control in open top chambers without CO₂ enrichment and also in the ambient



A view of Automated Open Top Chamber facility at IFGTB, Coimbatore

environment. *Casuarina equisetifolia* registered greater mean growth increment under high CO₂ concentrations (900 ppm). Further, the mean growth increment was the least when the temperature was regulated at 4 °C plus ambient temperature. In *C. junghuhniana*, the mean growth increment was highest under exposure of seedlings to highest level of CO₂ (900 ppm) and the least growth increment was recorded for seedlings grown in ambient environment. Carbonic anhydrase activity varied at different CO₂ levels and brought corresponding differences in growth and dry matter accumulation. These species may be considered for greater carbon sequestration under elevated CO₂ and temperature levels. There exists huge intra-specific variation, in both *C. equisetifolia* and *C. junghuniana*, which could be exploited for future breeding programme in developing climate ready genotypes having greater potential to sequester more CO₂.

4.0

CLONAL FORESTRY

4.1 Developing Cloning Techniques for Raising High Yielding Clonal Plantations of *Casuarina equisetifolia*

Clonal forestry programmes of *Casuarina* is in its early stages of development. Unlike eucalypts clonal plants, growth of casuarina clones is not rapid and good growth is generally observed only after 2 to 3 years resulting in extended rotation period. Rooting of cladode cuttings is the widely practised method of vegetative propagation in *C. equisetifolia*. Different experiments were undertaken to develop clonal propagation method using juvenile propagules. Attempts were made to induce coppice shoots by providing congenial conditions like adequate moisture, mulch, fertilizers, application of growth regulators, induction of root suckers and girdling. Pollarding at various heights from the ground level was also carried out. Possibility of rooting of individual needles was explored for scaling up of vegetative propagation from the available juvenile shoots. Hydroponic technique for rooting of individual branchlets and sprigs was also attempted.

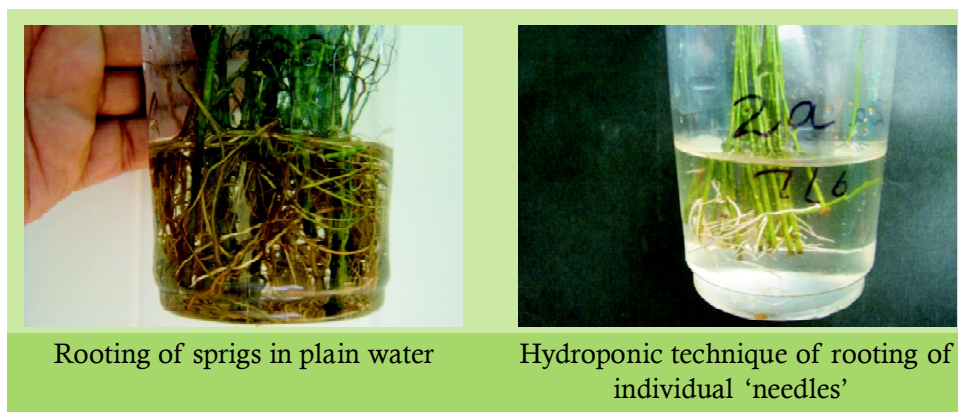


Profuse coppice growth from a stump, 4 months after coppicing at a height of 45 cm from the ground level



Profuse coppice growth from a stump, 4 months after coppicing at a height of 60 cm from the ground level

Stumps cut at 45 and 60 cm from the ground level produced the maximum number of sprouts with high rootability. A cost effective hydroponic technique of rooting could be developed by rooting in plain water. The success of rooting was up to 100 per cent for 'needles' and 80 per cent for sprigs. Plantlet development from rooted sprigs and needles was 95 and 65 per cent respectively. Plantlets were developed from individual branchlets which helped in mass multiplication of the limited rejuvenated plant material thereby scaling up the production of plantlets.



4.2 Coppicing Ability of *C. junghuhniana*

Casuarina junghuhniana has the potential to produce a coppice crop. When 2.5 year old trees were cut at ground level, a tuft of coppice shoots developed within a month's time. There were 2-7 stems per tree after 3 years. Each of the coppice stools had height and diameter growth similar to that of uncut trees at 2.5 years' age. Trees of five outstanding provenances were tested for coppicing potential and all of them produced good coppice growth.

4.3 Differential Rooting Responses of Juvenile and Adult Tissues of *Casuarina equisetifolia*

An attempt was made to elucidate the difference between the juvenile and adult tissues of *Casuarina* with respect to rooting using cladode cuttings collected from four different positions of trees. A decreasing trend in rooting ability was observed as cuttings are collected progressively from lower to upper parts of the tree. The juvenile material responded fully to rooting whereas in mature tissues, the rooting response was reduced but not completely suppressed.

S. No.	Positions	Flowering pattern	Rooting per cent
1.	Upto 60 cm from the ground level	No flowering in any branch	80.74
2.	Between 60 cm and 95 cm from the ground level	Occasional flowering in some branches	80.68
3.	Between 95 cm and 145 cm from the ground level	Many branches showing flowering	68.16
4.	Between 145 cm and 165 cm from the ground level	All branches showing flowering	46.15

4.4 Early Growth Comparison between Seedlings and Clones of *Casuarina equisetifolia*

Difference in growth performance between seed raised and clonally propagated plant materials derived from nine 6-year old randomly selected female trees grown in a clone bank of *Casuarina equisetifolia* was examined upto 3 years under controlled conditions. Half yearly observations on total height and collar diameter and data on shoot length, root length, collar diameter, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, biomass index and total biomass (fresh weight and dry weight basis) at age 3 were recorded. The results of this early growth comparison clearly indicated the superiority of seedlings over rooted cuttings. This could be attributed to the fact that seeds were obtained as a result of random mating of the superior casuarina selections in the clone bank. Being a polygamous species, possibility of using clone bank as a research seed orchard exists in this species. Flowering and fruit set were observed in clones from 8 months onwards. However, no flowering was noticed in any of the seedlings. As mature branchlet cuttings from higher up the stem can be rooted which will flower at a very early stage, clonal route will be the most promising option to raise orchards for quick and quality seed production in *C. equisetifolia*.

Clone banks maintained to preserve and test a large number of genotypes are of great importance for long-term tree improvement programmes. Clone banks can also be considered as Research Seed Orchard and seeds can be obtained. Though

they are not meant to produce massive quantities of seed for operational planting, they will have a definite role to play in case of non-availability of seeds from conventional seed production systems (like seed orchards / seed production areas). Clone banks thus need a broad genetic base to avoid inbreeding in future generations and to preserve genes and genotypes that might be useful as the tree improvement programme develops. Being a polygamous species, excluding the monoecious individuals for seed collection will eliminate the problem of selfing in casuarina. Though clonal forestry is not widely practiced in this species for large scale plantation programmes, the blessings of easy rootability and early flowering in rooted cuttings from mature materials can be efficiently exploited in raising clonal seed orchards for quality seed production.

4.5 Clonal Variation in the Adventitious Rooting Behaviour of *Casuarina equisetifolia*, *C. junghuhniana* and their Interspecific Hybrids

Ten clones each of *Casuarina equisetifolia*, *C. junghuhniana* and interspecific hybrids between these two species were studied for their rooting behaviour in the nursery. Coppice shoots from the selected clones were subjected to rooting in polytunnels for a period of one month. The highest rooting of 98.5% was observed in the hybrids followed by *C. junghuhniana* clones with 97% rooting. *C. equisetifolia* clones widely differed in the rooting ability ranging from 30 to 100% with a mean of 67%. Hybrid clones also took the shortest time to initiate rooting (8 days) whereas clones of the pure species started rooting from 18 to 20 days. Highly significant ($p < 0.001$) differences were found among the three groups of clones for root parameters like root length, number of roots, fresh and dry weight of root and shoot. Hybrid clones possessed better rooting characters than that of the two pure species indicating that they are suitable for large scale propagation to develop clonal plantations.

5.0

SEED TECHNOLOGY

5.1 Improved Cone and Seed Handling Procedures for *Casuarina equisetifolia*

The greatest early losses in seed quality result from collecting cones before seeds are fully mature. In addition, in the tropics, seed collection is manual and not equipment driven hence the chances of product quality and efficiency decreasing are very high. In addition, bulking of cones from orchards brings in an assortment of cones of varied sizes which has also found to have a bearing on seed quality. A study was conducted to determine if cone characteristics and seed quality in *Casuarina* vary by cone size, since a better understanding of this will be useful during the harvesting process and will provide indicators for quality of the seedlots. Cones of *Casuarina equisetifolia* collected from single tree collections were graded into large, medium, small and ungraded cones and seeds extracted. The cones showed variations in relation to size, weight and germination percent, large cones exhibiting higher percentage of germination followed by ungraded, medium and small cones. The cones were cut into 3 parts namely upper, middle and lower portion and time taken for release of seeds studied. Upper portion exhibited faster release and higher percentage of germination followed by middle portion and lower portion. Bulkied cones could be subjected to grading based on size followed by segregation of seeds based on time release to obtain quality seeds in the species.

5.2 Seed Quality Assessment of *Casuarina equisetifolia* through X-ray Radiography

Seed quality in terms of germination capacity is generally low in *Casuarina equisetifolia*. Often only half of the sown seeds germinate. Though many reasons contribute for the poor germination, presence of shriveled, insect damaged and empty seeds is one of the noticeable factors. A non-destructive and quick method

of seed quality assessment makes the seed testing simple for efficient management of large seedlots. Hence an attempt was made to fractionate the sound, shriveled, insect damaged and empty seeds using x-ray radiography. Nineteen single tree seedlots were collected from seed orchards at Panampally, Kerala. Samples were drawn from these seedlots and subjected to x-ray radiography. Similar samples from the respective seedlots were tested for germination. The x-ray images were examined for the presence of sound, shriveled, insect damaged and empty seeds and their corresponding counts recorded. The results showed that there is no significant difference between the sound seed fraction identified using x-ray image (49.9%) and the actual germination percentage (48.2%). Hence x-ray radiographic technique can be effectively used as a tool for assessing the germination percentage of *Casuarina equisetifolia* seedlots.

5.3 Petroleum Flotation Technique to Upgrade the Germinability of *Casuarina equisetifolia* Seedlots

The germination of *Casuarina equisetifolia* seedlots is low (40–50%) even when freshly collected. This study aimed at upgrading the germination performance by removing non-germinable seeds using petroleum flotation technique. Seeds collected from 19 individual trees in seedling seed orchards in India were assessed using X-ray to determine the proportion of different seed lot fractions. The feasibility of petroleum ether as a separation medium was tested. Morphological characters of floating and sunken seeds were further studied using image analysis technique to examine their effects on the separation efficiency. The X-ray analysis revealed a large quantity of shrivelled, empty and insect-damaged seeds, altogether accounting 50%, which were the causes of low percentage germination of un-graded seed lots (48%). Petroleum flotation resulted in 90% germination in the sunken fraction and 4% in the floating fraction. The separation was distinct for most seed lots, except few seed lots that had relatively low germination in the sunken fractions and more than 10% germination in the floating fractions. As a whole, petroleum flotation appears to be a feasible technique to upgrade the germination of *C. equisetifolia* seedlots, and its efficacy is influenced by wing surface area, seed density and wing quotient of filled and empty seeds.

5.4 Relationship of Cone and Seed Traits on Progeny Growth Performance in *Casuarina equisetifolia*

The influence of cone and seed traits on juvenile and adult growth of *Casuarina equisetifolia* was studied. Cones and seeds were collected from 18 selected trees. Samples of 10 cones and 25 seeds were taken in 4 replications to measure 2D surface area, length, breadth, weight, roundness and aspect ratio. Five seedlings in 4 replications from each seedlot were planted in randomized block design at 2 m spacing. Progeny height and diameter were recorded at juvenile (18 months after sowing – 18 MAS) and mature (60 months after sowing – 60 MAS) stages. Significance test and estimation of variability, heritability and genetic advance were carried out. The seed and cone morphological characters were found to have high heritability and genetic advance values than tree growth characters. Correlation of cone or seed traits on progeny growth characters and path analysis of seed traits on diameter at 60 MAS were carried out. The 100 seed weight was found to have significant correlation consistently on progeny growth characters both at juvenile and mature stages. Shape characters like roundness and aspect ratio also showed significant correlation on progeny growth. Juvenile-adult correlation was established with diameter growth of juvenile on both height and diameter growth at 60 MAS. Seed 2D surface area had high direct and indirect effect on diameter at 60 MAS. Identification of better seed sources or grading of seeds may be carried out based on seed weight and/or size and/or shape to get better progenies.

5.5 Variation in Cone and Seed Yield amongst Individual Trees of *Casuarina junghuhniana* ssp. *timorensis*

A study was undertaken to study seed parameters of select trees of *C. junghuhniana* ssp. *timorensis*. Cones were collected from 57 phenotypically superior and good cone bearing trees selected from IFGTB seed orchards at Panampally (23 trees), Sadivayal (11 trees) and Puducherry (23 trees). Fresh weight of cones and weight of seeds obtained after shade drying were maintained for individual trees. Fresh weight of 100 cones and weight of 100 seeds of individual trees was recorded. Number of seeds per cone was recorded for 23 seed sources collected from Puducherry. Based on fresh weight of cones collected and seeds obtained, quantity of cones to be

collected for obtaining one kilogram of seed was calculated. Variation in weight of 100 cones was noticed amongst individual trees and it ranged from 10.31g to 28.92g. Average weight of 100 seeds ranged from 0.038 g to 0.079 g amongst individual trees. Number of seeds per cone ranged from 42 to 71.

5.6 Meeting Demand of Improved Seed through Community and Industrial Seed Orchards

Systematically implemented tree breeding programmes for nearly two decades helped IFGTB to develop highly productive seeds and clones of Casuarinas (*Casuarina equisetifolia* and *C. junghuhniana*). On-farm tests clearly showed that the genetic gain from the new improved planting stock can be converted into substantial economic gain to tree growers like farmers, forest departments and wood-based industries. To make this happen up-scaling of genetically improved seeds/clones is essential to make the benefits accessible and affordable to all those involved in plantation development. The demand for genetically improved planting stock is so huge that no single organization can fulfill it on its own. Hence networking among all stakeholders and decentralizing the planting stock production are prerequisites for making the research output useful to tree growers.

Industrial seed orchards: Since the wood-based industries have to meet their wood requirement outside the forests, almost every such industry is undertaking an active farm forestry programme. They strive to make available high quality seedlings at subsidized price to farmers so that the plantation productivity per unit area is increased benefitting both the farmer and the industry. Since the industries need large quantities of seeds every year, buying seeds from IFGTB is expensive and the seeds may not be available to match local nursery season. Under such circumstances, IFGTB encouraged the industries to grow their own improved seeds to reduce the cost of seeds and also to get guaranteed seed supply every year. IFGTB has transferred the seed orchard technology to The Andhra Pradesh Paper Mills, Rajahmundry, Andhra Pradesh through consultancy and to Tamil Nadu Newsprint and Papers Limited, Karur, Tamil Nadu through collaborative research programme. These orchards have already started producing seeds for the farm forestry programmes of the respective industry. Other paper industries have also expressed

their willingness to establish and manage their own orchards with IFGTB's technical support. Planting stock and orchard designs have also been made available to State Forest Departments of Andhra Pradesh and Gujarat for establishing seed orchards.

Community seed orchards: IFGTB has introduced a new concept of developing "Community Seed Orchards" through which farmers and self-help group members are encouraged to establish their own seed orchard in government / village land with inputs from IFGTB in the form of planting material and technical support. The cost of maintaining the orchards for two years is also taken care of by the Institute in addition to capacity building



One year old Casuarina plantation in farmer's land raised with IFGTB seed (Chellanchery, Cuddalore District, Tamil Nadu)

of farmers in orchard management. The seeds produced will be used by the farmers themselves and any surplus will be sold to others like wood-based industries with quality assurance from IFGTB. The model orchards are already established in Tamil Nadu and Puducherry and several more are proposed to be established in the near future.

It is the long term goal of IFGTB to use community seed orchards and the genetic gain from their seed output as a means of enhancing livelihood opportunities for traditional nursery operators. At present the traditional low-cost nurseries are operated by landless agricultural labourers who hail from socially deprived communities with minimal livelihood security. Since they are not trained in modern nursery techniques and lack the means to provide additional inputs in their nursery operations the planting stock is priced low and the per capita income is meager. IFGTB is working with this highly skillful group of nursery operators to impart training in latest nursery techniques like use of genetically superior seeds, replacing

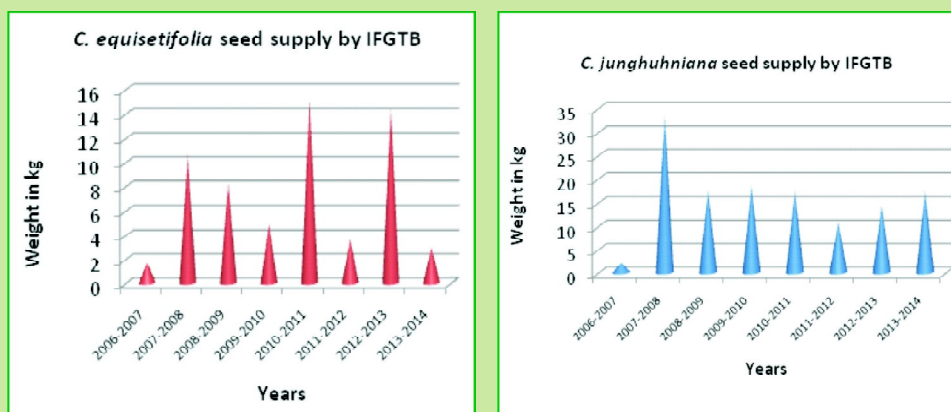
chemical fertilizers with biofertilizers, pest management and clonal propagation technology. One of the community seed orchards of Casuarina is established adjacent to the nurseries at Valluvamedu in the Union Territory of Puducherry. Once the orchard



starts yielding seeds, they will be deployed in the nursery under the supervision of Scientists from IFGTB and the seedlings will be sold at a premium price compared to the other seedlings raised from unimproved seed sources. If this model of decentralized seed production and enhancing genetic quality of planting stock from traditional nurseries leading to livelihood enhancement to landless agricultural labourers is successful it will be extended to other tree species and locations in Tamil Nadu and Puducherry. It can support afforestation and reforestation programmes like Green India Mission and also Trees Outside Forests in a big way.

5.7 Improved Seed Supply by IFGTB

Due to growing interest in Casuarina cultivation, the number of Casuarina nurseries are increasing day by day. In order to cater to the growing demand for Casuarina



The seeds supplied by IFGTB have been appreciated for its good quality by various stakeholders

seeds, IFGTB has established seeds orchards of both *Casuarina equisetifolia* and *C. junghuhniana*. The Seed Technology Division of IFGTB regularly collects, processes, tests and supplies quality seeds to various stakeholders. The present cost of *C. equisetifolia* is Rs.6000/ kg while it is Rs.8000/- for *C. junghuhniana*. Since 2006, improved seeds of Casuarina is being supplied by the Institute to stakeholders namely, State Forest Departments, paper and pulpwood industries, farmers and NGOs. The trend of Casurina seed supply has been varying every year depending upon various factors like climate and rainfall, seed demand and quantity of seed collected.

6.0

AGROFORESTRY

6.1 Casuarina Based Agroforestry Models for Higher Income to Farmers

Casuarina enriches the soil with nitrogen, prevents soil erosion and improves other physico-chemical properties of the soil. Investigations carried out on different agroforestry models have shown that a density of 325 trees/ha can be used for developing Casuarina-Maize model (alley cropping) and the trees can be harvested at 3 years age without any significant reduction in crop yield. The model can be replicated in other regions wherever the climatic conditions are favourable to the species and also provides fuel, food, fodder and small timber to the farmer. The net income from the model amounts to Rs. 10,805 /ha/yr which is more than one and half times that of sole agriculture.

Economic return (Rs/ha as on 2001) from Agri-silviculture System (model, Casuarina-Maize)

Tree species : <i>Casuarina equisetifolia</i> (espacement 7 X4 m; density 325trees/ha, age 3yrs)	
No of poles /ha	325
Income from poles @ Rs 45/pole	14,625
Fuel wood (t/ha)	1.3
Income from fuel wood @ Rs 700/ton	910
Total income	15,535
Expenditure	1,869
Net income	13,666
Average annual net income (Rs/ha/yr)	4,555

Agricultural crop : Maize	
Grain yield (t/ha/yr)	2.0
Stover yield (t/ha/yr)	1.5
Income from grain @ Rs 4000/t	8,000
Income from stover @ Rs 500/t	750
Expenditure (Rs/ha/yr)	2,500
Net income from Maize (Rs/ha/yr)	6,250
Average annual net income from the model(Rs/ha/yr)	10,805

6.2 Management of *Casuarina equisetifolia* in Agroforestry Systems for Sustainable Economic Returns

Casuarina equisetifolia when closely integrated in farming systems needs to be tended. Under wide row intercropping system, mechanical removal or pruning of side branches is necessary for ensuring smooth farming operations and also to reduce shading in agricultural crops close to the tree line. Pruning of lower branches up to $\frac{1}{4}$ to $\frac{1}{2}$ the size of full canopy is recommended to get a better bole without adversely affecting stem height and has to be done at the end of first and second year. The branch pruning so obtained can be used as firewood or for staking vegetable crops like tomato.

In wide-row inter cropping, pruning of lateral roots in initial year has been found to minimize competition for resources especially under rainfed conditions. Pruning of lateral roots by digging trenches up to 50 cm at about one feet on either side of tree row and inserting thick guage (500 μ) polythene strips on either side of tree row in the trenches, can effectively curtail spread of lateral roots for at least 4-5 years and thereby minimize competition. However, this will incur an additional cost of Rs 2000/ha (as on 2003) by way of labour and material costs in the first year. But the benefits by way of enhanced crop yields especially if high value crops like turmeric are taken far outweigh the additional cost incurred. The encouraging LER (Land Equivalent Ratio) values obtained are also an additional incentive to adopt this system. Though the returns to soil by way of nitrogen fixation has not been

quantified, the overall enhancement in soil chemical properties is an additional incentive to practice wide row intercropping systems with Casuarinas.

A word of caution is that stress conditions may increase the tree's susceptibility to pest and disease attack. Under unirrigated conditions, pruning of side branches may make Casuarina susceptible to attack of the deadly blister blight disease caused by *Trichosporium vesiculosum*. Hence, selection of such sites needs to be avoided in planting programmes. Even in wide row interplanting, occurrence or outbreak of this disease has to be monitored. Proper care and attention including timely phytosanitary measures may be adopted for checking spread of this disease which even devastates older trees.



A view of root spread of *Casuarina equisetifolia* without root management treatment. The lateral roots on either side of tree row extended up to 4 -5m.



Casuarina lateral root spread contained under root management treatment (insertion of polythene barriers) as viewed at time of final harvest (4 years).

6.3 Windbreaks of Casuarina to Tailor Growth of Teak Trees in Farm Bunds

Agroforestry system: Windbreaks of Casuarina on either side of teak tree row in boundary planting tailors the growth of teak by minimizing the desiccating effect of strong wind on teak terminal shoot growth and by providing competition for shade to enhance height increment in teak being a strong light demander species.

Establishment of the Casuarina + Teak based agroforestry model: After land preparation by ploughing and formation of three channels (rows) at distance of 1 m all along the boundary, superior clones of Casuarina are planted at 1 m interval within the rows in the two outer rows. Teak is planted in the middle row at 2 m spacing. Thus the tree density will be around 800 trees per hectare for Casuarina and around 200 trees per ha for teak (The tree density may vary depending on actual perimeter of the boundary). After planting of tree seedlings, agriculture and horticulture crops can be cultivated, as per standard package of practices.



Growth of teak in the middle row (Age: 2 years) with windbreaks of Casuarina in Puthinampatty village, Trichy district of Tamil Nadu



Growth of *Casuarina junghuhniana* X *C. equisetifolia* hybrid clone (Age: 2 years) as windbreaks in Puthinampatty village, Trichy district of Tamil Nadu

Growth of tree components: Girth at breast height and total height of teak and Casuarina were measured row-wise in all the four directions of the boundary of the farm field in Puthinampatty village in Trichy district of Tamil Nadu. After a period of two years after planting, Casuarina recorded mean height of 5.2 m and mean girth of 14.1 cm while teak registered mean height of 4.5 m and mean girth of 13.8 cm.



Growth of teak in inner row with profuse branching in Puthinampatty village, Trichy district of Tamil Nadu

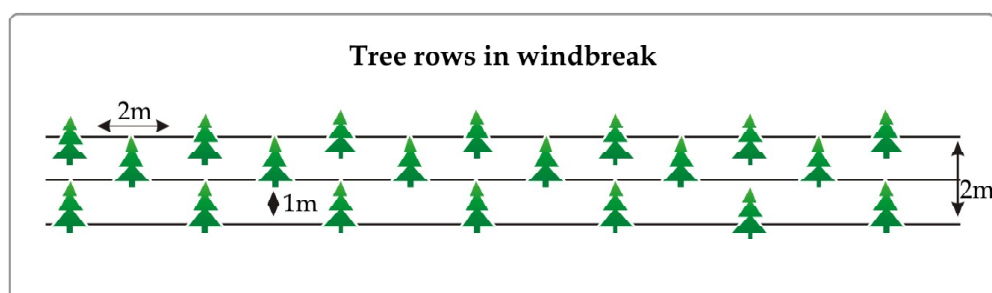
6.4 Casuarina and Banana based Windbreak Agroforestry Systems with Superior Clones of *Casuarina junghuhniana*

A windbreak agroforestry system with Casuarina and banana crop has been developed at IFGTB to minimize the deleterious effect of wind on banana crop production as well as to make agro-ecosystems more climate change resilient through i) enhanced productivity, ii) reduced evapo-transpiration and in turn increased water use efficiency of the agro-ecosystem.

Five productive clones of *Casuarina junghuhniana* viz. IFGTB-WBC 6, IFGTB-WBC 8, IFGTB-WBC 9, IFGTB-WBC 17 and IFGTB-WBC 18 exclusively suitable for windbreak agroforestry system have been identified. These clones will have more number of branches, greater branch thickness, wider branch angle along with greater

Twenty-Five Years of Research on Casuarinas at IFGTB

growth rate and biomass productivity and are suitable clones for windbreaks. Farmer's experience of cultivating plantain under windbreak agroforestry system with these superior clones of IFGTB showed that there exist good compatibility of Casuarina and banana for co-cultivation of the both crops. Three channels are formed at a distance of 1 m all along the boundary. Within each channel, superior clones of Casuarina are planted at 2 m interval in 'Quincunxs' pattern resulting in 600 trees per hectare. Rhizomes of plantain are then planted inside the field in the 'Quincunxs pattern with 4 m distance within a row and 2 m distance between rows (plantain density 2500 per ha.).



Observation on growth parameters of top ranking superior clones of *Casuarina junghuhniana* under this windbreak agroforestry system in Kovilpalayam in Coimbatore district, Tamil Nadu revealed that the girth at breast height was 12.0 cm and total height was 6.0 m at the end of first year of the plantation. At this rate of growth, volume production will be 12.0 cubic metre per hectare. Estimated wood yield on fresh weight basis at harvest age of three years is 8.0 metric tonnes per

Expected additional income from the tree component

Location : Kovilpalayam, Coimbatore District	Year:2013
Particulars	Values
i) Cost of cultivation of tree component:	Rs.6,000/-
ii) Benefits from tree component after three years:	
a) Income from pulpwood (@ Rs. 2300 per MT)	Rs.18,400/-
b) Income from branch wood –(@ Rs.1000 per MT)	Rs.2,500/-
iii) Net Additional income from tree component	Rs. 14,900/-

hectare. The average yield of plantain (Variety-Kadali) under windbreak agroforestry system obtained in the farm field in Kovilpalayam is 7.0 kg per bunch (after excluding the weight of fruit stalk (rachis)-which is around 2.5 kg in kadali variety). Fruit yield from plantain adjoining to the tree rows was not affected by co-cultivation of superior clones of *Casuarina junghuhniana* and Kadali variety of plantain crop.



One year old growth of superior clones for windbreak agroforestry system for protecting plantain crop from wind damage



One year old growth of superior clone selected exclusively for windbreak agroforestry system by IFGTB in the farm field in Coimbatore district, Tamil Nadu



Six months growth of Casuarina and Banana crop in Rayarpalayam



Normal growth of Banana crop adjoining to tree rows in windbreak

7.0

BIOTECHNOLOGY

7.1 Development of Post-transcriptional Gene Silencing Approaches as a Tool for the Functional Analysis of Symbiotic Genes in the Tropical Actinorrhizal Tree *Casuarina glauca*

Through a study carried out at the *Institut de Recherche pour le Développement* (IRD), Montpellier, France, composite transgenic strategy was optimised and used for functional characterisation of the *CgCCaMK* gene in the actinorrhizal tree *Casuarina glauca*. Effect of temperature, light conditions and suitable explant sources were evaluated on *Agrobacterium rhizogenes* mediated transformation. The full length cDNA and genomic sequence of *CgCCaMK* were cloned, and the *CgCCaMK* expression studied using qRT-PCR. To enable GFP based screening of transformed RNAi roots, the gateway cassettes of pHellsgate 12 containing the inverted repeats of calmodulin / untranslated regions of *CgCCaMK* were spliced in the *SacI-SpeI* site of pHKN29. The dsRNA expression vectors thus generated were used to generate composite *C. glauca* plants with transformed RNAi roots in which the *CgCCaMK* expression was downregulated. The RNAi roots showed perturbation of the Frankia infection process, delayed nodulation, decreased size and number of nodules, and inhibition of arbuscular mycorrhizal interaction. The study contributed to the understanding that *CgCCaMK* plays a central role during actinorrhizal symbiosis, as well as in arbuscular mycorrhizal symbiosis in *C. glauca*.

7.2 DNA Extraction Protocol for *Casuarina equisetifolia*

A simple protocol was developed for DNA isolation in *Casuarina equisetifolia* without using liquid nitrogen. Higher amounts of polysaccharides, polyphenols particularly tannins, fibrous nature and xerophytic adaptations of *Casuarina* needles pose hindrance in DNA isolation. The yield of the DNA ranged from 178-287 µg/g fresh weight after fixing the needles in absolute alcohol. The spectral quality of

genomic DNA isolated using this method as measured by the A260/A280 absorbance ratio ranged from 1.828 to 1.955. The purity of the isolated DNA was further confirmed by RAPD-PCR analysis. The DNA samples prepared by this method were consistently amplifiable in the RAPD reaction and gave reproducible profiles. This method does not require fixation or grinding in liquid nitrogen, making it advantageous over common protocols.

7.3 Genetic Variability Studies in *Casuarina* and *Allocasuarina* Species Using DNA Markers

Eight provenances of *C. equisetifolia* belonging to Thailand, Malaysia, Egypt, Papua New Guinea, Kenya, China and Philippines were analyzed for the genetic diversity using RAPD markers. Primer OPB 04 estimated high within provenance variability (0.7416) whereas OPE 06 estimated the least (0.5246). On an average most of the diversity (60.32%) existed within provenances and 39.68% of variability occurred between *C. equisetifolia* provenances. Shannon's index of phenotypic diversity was partitioned into within and between provenance components and mean within provenance variability (H_{pop}) for all provenances was estimated as 0.2481. The average total genetic diversity that existed in provenances was 0.4187. The results suggest that the provenances harbour high genetic diversity and may be retained as gene resource population, which would play a major role in genetic resource conservation for the long term breeding of *C. equisetifolia*.

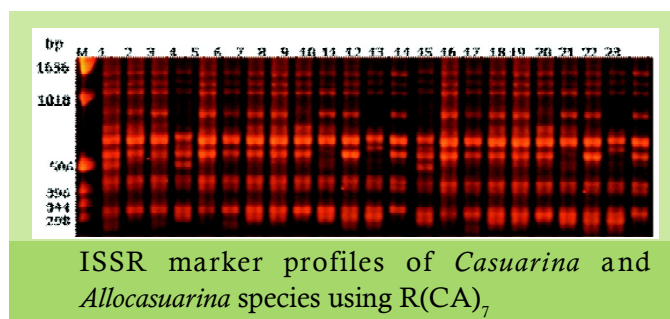
Inter simple sequence repeat polymerase chain reaction (ISSR-PCR) was used for the genetic analysis of six species of *Allocasuarina*, five species of *Casuarina* and 12 superior performing selections of *C. equisetifolia*. *C. equisetifolia* selections were also fingerprinted using Fluorescent-ISSR-PCR (FISSR-PCR), an improvised ISSR-PCR assay. The ISSR analysis provided information on the frequency of various simple sequence repeats in the casuarina genome. The di-nucleotide repeats were more common, among which (CA)_n and its complementary nucleotide (GT)_n, repeat motifs amplified relatively higher number of bands with an average of 6.0 ± 3.5 and 6.3 ± 1.8 respectively. Eleven species of casuarinas were amplified with 10 primers anchored either at 5' or 3' end. A total of 253 PCR products were obtained and all were polymorphic, out of which 48 were specific to *Allocasuarina* and 36

were specific to *Casuarina* genus. Genetic similarity among the species was 0.251. A UPGMA dendrogram grouped all the *Casuarina* species together. The 12 superior performing selections of *C. equisetifolia* produced 57 polymorphic ISSR markers while the FISSR assay revealed 105 polymorphic markers. The primer CRR(ATT)₄ distinguished all the selections. DNA profiles obtained with ISSR and FISSR assays would serve as a reference library for the establishment of clonal identity in casuarinas.

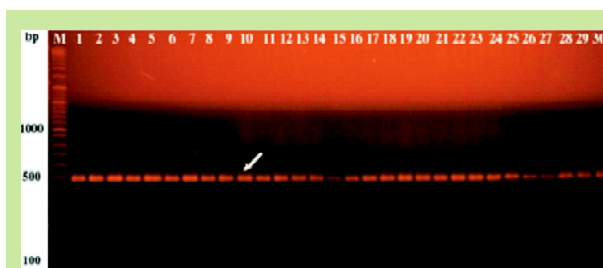
Efforts on development of Simple Sequence Repeat (SSRs) markers for casuarinas were made using publicly available ESTs and genomic sequences. A total of 34,893 sequences downloaded from NCBI and analyzed for the presence of minimum 6, 5 and 5, di, tri and tetra nucleotide motifs respectively. One thousand two hundred and seventy five (1275) SSRs were identified in total with suitable flanking sequences, which included 736 dinucleotides, 501 trinucleotides and 38 tetra nucleotide motifs. Among the dinucleotide motifs the AG/GA/TC/CT motifs were represented more as reported in many other plant species. These SSRs would immediately serve as base collection to initiate advanced genetic analysis of casuarina populations.

7.4 Development of Species Specific Markers in *Casuarina equisetifolia*

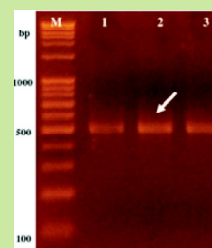
Seven ISSR primers were amplified in 120 individuals belonging to three species of *Casuarina* (*C. equisetifolia*, *C. glauca* and *C. junghuhniana*) and two *Allocasuarina* species (*A. littoralis* and *A. huegeliana*). One species-specific amplicon at 650 bp amplified in all individuals of *C. equisetifolia* was cloned, sequenced and primer pairs were developed and designated as IFGTBCE01. The sequence characterized amplified region (SCAR) marker was multiplexed with internal SSR primer pair (positive control) and amplified in ten randomly selected individuals of *C. equisetifolia*, *C.*



junghuhniana, *C. glauca*, *A. littoralis* and *A. huegeliana*. The species-specific band amplified only in *C. equisetifolia* at 500 bp. Subsequently, the SCAR marker was validated in 30 individuals collected from 10 provenances belonging to 9 countries and in 10 locally selected clones. The marker was validated in the natural male hybrid of *C. equisetifolia* X *C. junghuhniana* which is widely planted in India for fast growth, wide adaptability and good form. This marker can be used for accurate and rapid identification of the species during certification and determination of putative hybrids in breeding programme.



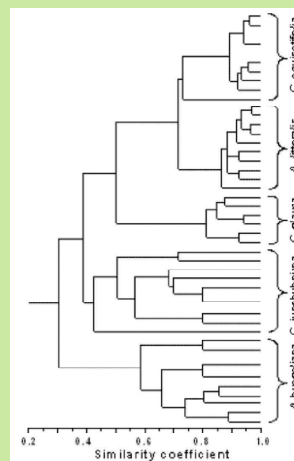
ISSR –SCAR marker profile of *C. equisetifolia* populations



ISSR –SCAR marker profile of natural male hybrid of *C. equisetifolia* X *C. junghuhniana*

7.5 Comparison of Morphological and Molecular Diversity in Casuarina Species

Morphological and genetic diversity among three species of *Casuarina* (*C. equisetifolia*, *C. glauca* and *C. junghuhniana*) and two species of *Allocasuarina* (*A. huegeliana* and *A. littoralis*) were studied with eighteen morphometric parameters and seven ISSR primers. The morphometric parameters that differentiated most species included plant height, branch length, internode length and teeth length and revealed a significant diversity among the

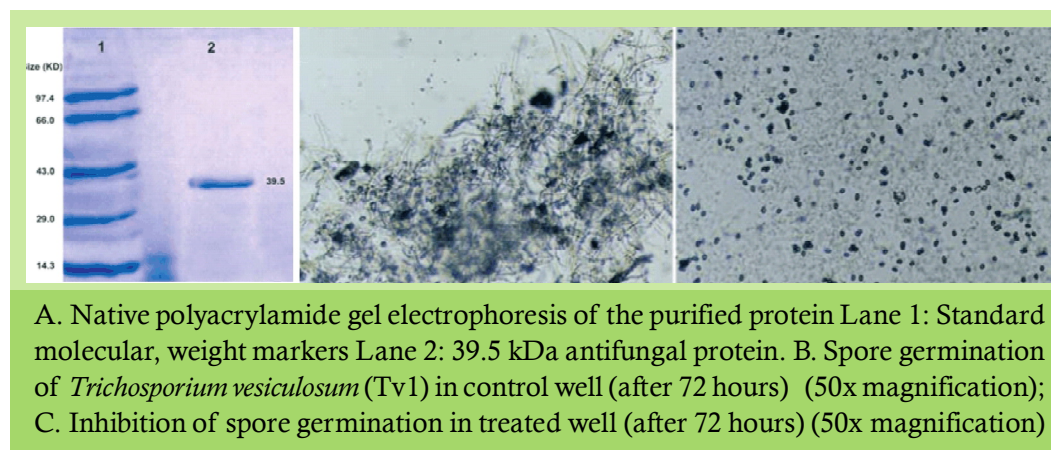


Dendrogram showing the similarity among the different species of *Casuarina* and *Allocasuarina* based on morphometric data.

species studied. ISSR-PCR analysis was also conducted to estimate the genetic relationship among and within the species. The dendrogram generated from ISSR and morphometric markers clustered *C. equisetifolia*, *C. glauca* and *A. littoralis* into one group while *C. junghuhniana* and *A. huegeliana* grouped separately. Thus diversity estimation by conventional method using morphometric parameters and advanced technique utilizing molecular data can be used synergistically in diversity estimation studies for population/parent selections in breeding programs of tree species.

7.6 Purification of Antifungal Protein from *Andrographis paniculata*

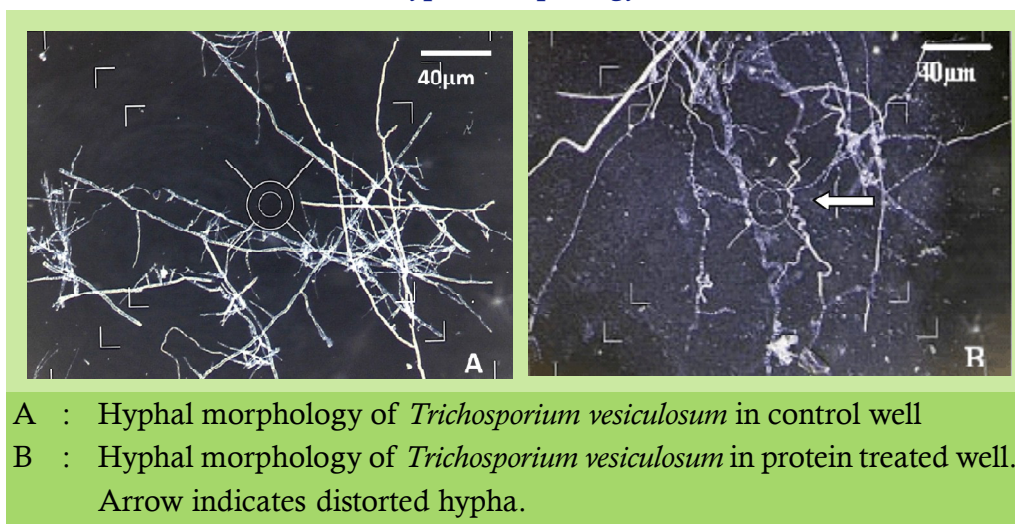
A protein extract from the leaves of *Andrographis paniculata* belonging to family Acanthaceae, was found to inhibit the spore germination and hyphal extension of *Trichosporium vesiculosum*, the blister bark pathogen of *Casuarina equisetifolia*. The antifungal protein component was further purified from the crude extract and the molecular mass of the toxic protein was estimated to be 39.5 kDa.



7.7 Purification of Antifungal Peroxidase from *Andrographis paniculata*

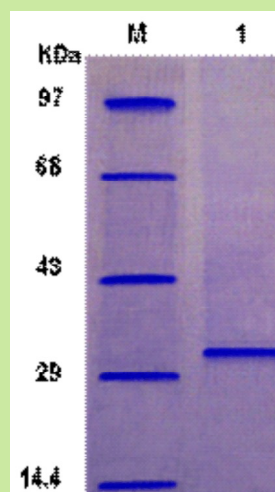
A constitutively expressed 35 KDa peroxidase with pI 6.0 was purified from the leaves of *Andrographis paniculata* and showed antifungal activity against *Trichosporium vesiculosum*.

In vitro hyphal morphology studies



7.8 Purification and Characterization of Antifungal Protein from *Acorus calamus*

Leaf proteins from *A. calamus* were fractionated by cation exchange chromatography and gel filtration and the fraction inhibiting the hyphal extension of phytopathogens was characterized. The temperature stability and pH optima of the protein were determined and its presence was localized in the leaf tissues. The purified protein was identified as a class III haem peroxidase with a molecular weight of approx. 32kDa and pI of 7.93. The temperature stability of the enzyme was observed from 5°C to 60°C with a temperature optimum of 36°C. Maximum enzyme activity was registered at pH5.5. The pH and temperature optima were corroborated with the antifungal activity of the enzyme. The enzyme was localized in the leaf epidermal cells and lumen tissues of xylem, characteristic of class III peroxidases.



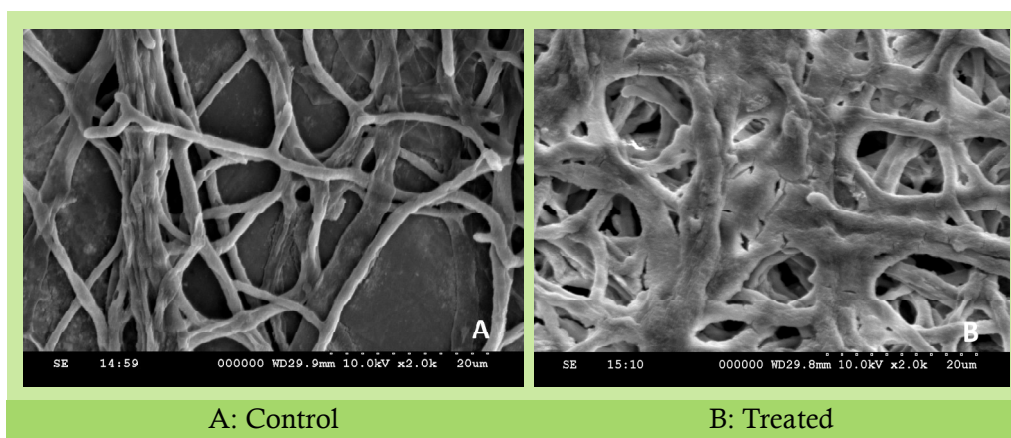
SDS-PAGE of purified protein
 Lane M: Molecular weight marker
 Lane 1: Purified protein

The toxic nature of the enzyme which inhibited hyphal growth was demonstrated against phytopathogens such as *Macrophomina phaseolina*, *Fusarium moniliforme* and *Trichosporium vesiculosum*. Microscopic observations revealed distortion in the hyphal structure with stunted growth, increased volume and extensive hyphal branching.

7.9 Purification of Antifungal Protein from *Withania somnifera*

A 30 KDa monomeric acidic lectin-like protein was purified from the leaves of an important medicinal herb, *Withania somnifera* (L.) Dunal (Solanaceae), by a series of gel filtration and affinity chromatography methods. The inhibitory concentration of the protein ranged from 7 µg to 11 µg against major phytopathogens like *Trichosporium vesiculosum* under *in vitro* conditions. The peptide sequence showed similarity to concanavalin A like lectin from *Canavalia ensiformis* and caused distinct cell wall adhesion of the protein treated hyphae under SEM. Further, the antifungal activity of the protein was compared with standard lectins like concanavalin A, phytohemagglutinin and wheat germ agglutinin.

Hyphal morphology studies of lectin treated *Trichosporium vesiculosum* using SEM



7.10 Isolation and Characterization of Class I Chitinase from *Casuarina equisetifolia*

A cDNA clone encoding class I chitinase (*CeChi1*) belonging to PR-3 family was cloned and characterized from the needle tissues of *C. equisetifolia* challenged with the toxic exudate of the fungal pathogen *T. vesiculosum*. The *CeChi1* open reading

frame comprised 966 nucleotides that encoded 321 amino acid residues with the molecular mass of mature protein being approximately 34 kDa. Analysis of the predicted amino acid sequence revealed the similarity of *CeChi1* protein to class I chitinase from other plant species containing a hydrophobic signal peptide domain and hinge domain. The sequence also harboured a cysteine-rich chitin-binding domain and lysozyme like domain. A hydrophobic C-terminal domain similar to the vacuole targeting sequences of class I chitinases isolated from other plants was also detected. The genomic sequence of *CeChi1* indicated that the coding region contained three exons and two introns. *In silico* analysis of the untranslated regions revealed the presence of several cis-acting regulatory elements associated with hormonal regulation and stress responses. Quantitative real-time PCR analyses at different time points showed upregulation of the transcript during pathogen elicitation and salicylic acid signalling. However, no significant expression of *CeChi1* was observed during other abiotic stress condition including wounding, water deficit, salt and heat stress revealing the specific expression of the gene during pathogenesis. Detailed functional analyses of *CeChi1* will help in understanding its specific role in defence against pathogens in this tropical tree species.

7.11 Characterization of Transcripts Up-regulation during *Casuarina equisetifolia*- *Trichosporium vesiculosum* Interaction

Transcript profiling during elicitation induced by cell wall components of *T. vesiculosum* revealed expression of resistance genes; cytochrome oxidase; trans membrane proteins; genes involved in programmed cell death like 26S proteasome and ubiquitin activating enzyme; early nodulin gene, wound inducible metalcarboxy peptidase inhibitor, glucanase, metal binding protein and signal recognition particle. The fold expression of selected transcripts including glucanase, 26 S proteasome, signal recognition particle, cytochrome oxidase and the metal binding protein using RT-qPCR revealed 12-59 fold increase in expression after 48 hours of elicitor treatment. The expression of these transcripts during abiotic stresses like heat, mechanical wounding, salt (NaCl) and drought (PEG) was also analyzed. Glucanase was up-regulated significantly during wounding and heat stress while proteasome was up-regulated 1-4 fold during NaCl, PEG and wounding stress. The novel transcript *CeHMA* was up-regulated under all the stress conditions. This is the

first report on molecular defense in *C. equisetifolia* and has provided a pool of candidate genes for detailed molecular dissection to further broaden the knowledge on the response of woody perennials during pathogen cell wall elicitation.

7.12 Comparison of PAL Activity during Salicylic Acid Signalling in *Casuarina equisetifolia* and *C. junghuhniana*

Casuarina equisetifolia and *C. junghuhniana* were analyzed for their differential expression phenyl alanine ammonia lyase (PAL) and phenol during acetyl salicylic acid (ASA) stress. The rooted cuttings of both species were incubated in ASA containing media and the (PAL) and phenol content in the root and needle tissues of both species were estimated for 24 hours. A substantial increase in PAL activity and phenol content was observed in both species during signaling, however in *C. junghuhniana*, both PAL and phenol content were found to sustain at higher levels even after 24 hours while in *C. equisetifolia*, the levels of both PAL and phenol decreased by 16 hours of treatment. The results indicate that the higher level of biotic stress tolerance in *C. junghuhniana* in comparison to *C. equisetifolia* may be attributed to the sustenance of high level of phenol and PAL in tissues during stress conditions.

7.13 Standardisation of Protocol for Isolation of Protoplasts in *Casuarina equisetifolia*

Protoplast culture provides an alternative method of improving plants and is one of the few systems that can utilise somaclonal variation, somatic hybridisation, cybridisation and genetic engineering. The determination of source materials and preculture conditions, plating densities, culture procedures and conditions are all important in obtaining viable and regenerable protoplasts. Needles, callus and cotyledons of *Casuarina* were used as source material for the isolation of protoplasts. Cellulase in association with pectinase and hemicellulase mixture was required for the efficient release of protoplasts from the explants having mannitol as osmoticum. Protoplasts were successfully isolated from cotyledons of young seedlings but not from needles or callus. Protoplasts were then purified using mannitol gradient. Exclusion staining with 0.2% Evans blue and 0.1% methyl blue suggested the viability of the isolated protoplasts.

7.14 Isozyme Studies in *Casuarina equisetifolia*

Isozyme analysis is a simple, efficient, and inexpensive powerful biochemical technique with numerous applications in forestry. Twelve isozymes viz., Aspartate Amino Transferase (AAT), Peroxidase (POD), Esterase (EST), Glutamate Dehydrogenase (GDH), Superoxide Dismutase (SOD), Alcohol Dehydrogenase (ADH), Isocitrate Dehydrogenase (IDH), Glucose -6-phosphate Dehydrogenase (G-6-PDH), Malate Dehydrogenase (MDH), Malic Enzyme (ME), Polyphenol Oxidase (PPO) and Lactate Dehydrogenase (LDH) were studied. Most of the isozymes revealed with two loci and two alleles. Five enzymes (Alcohol dehydrogenase (ADH), Malate dehydrogenase (MDH), Lactate dehydrogenase (LDH), Peroxidase (POD), Isocitrate dehydrogenase (IDH,) showed more stable expressions towards gender specificity among the twelve different enzymes studied. Peroxidase was identified as biochemical marker for gender discrimination in *Casuarina equisetifolia*.

8.0

BIOCHEMISTRY

8.1 Biochemical Marker Assisted Screening of *Casuarina equisetifolia* Clones for Saline Tolerance

Experiments were conducted to identify suitable biochemical parameters as markers which enables screening of clones of *C. equisetifolia* at nursery stage for saline tolerance. Salinity induction on 25 rooted clones of *C. equisetifolia* was carried out using Hoagland solution as growth medium under controlled conditions in the nursery. At the end of three months, based on the survival percentage the clones were classified into 4 groups namely, tolerant (withstands upto 250mM), medium tolerant (upto 200 mM), less tolerant (upto 150 mM) and sensitive (upto 100 mM). Biochemical analysis for soluble proteins, phenols, proline, peroxidase, ascorbic acid, chlorophyll a, Chlorophyll b, total chlorophyll, anthocyanin, anthocyanin: chlorophyll, root and shoot sodium and root and shoot potassium ions were conducted in the clones before and after saline induction. Physiological parameters such as membrane injury index, relative water content, chlorophyll stability index and morphometric parameters were also studied. Threshold levels for the biochemical parameters were also identified to classify the saline tolerant and sensitive clones. Validation of the results on a new set of clones, revealed that proline successfully grouped the clones to 50%, protein to the level of 60% and both phenol and phenol + protein to the scale of 80%. Biochemical markers could be used for screening *C. equisetifolia* clones at nursery stage for saline tolerance in order to support Casuarina improvement program. Appropriate clones can be recommended for suitable areas and problematic sites.

8.2 Screening for Salinity Tolerance in *Casuarina equisetifolia* Clones under Laboratory Conditions

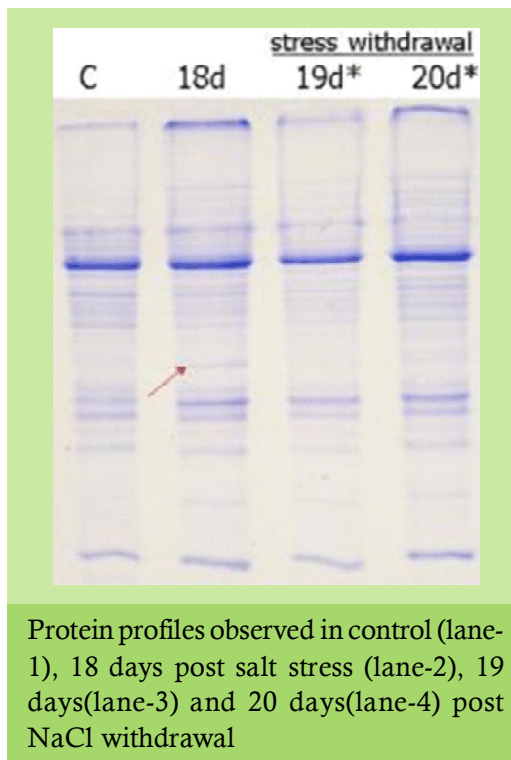
Four salt tolerant clones of *C. equisetifolia* (CH-03-02, TCR-11-02-02, TCR-02-01-01 and TCR-08-01-02) and two salt sensitive clones (TCR-08-02-01 and TCR-03-

01-06) were subjected to salt stress in a hydroponic system. One of the clones *viz.*, TCR 11-2-2, was found to tolerate 340 mM (2 %) NaCl stress for a period of 30 days in a hydroponic system while others failed to survive beyond 8 days. Protein profile studies carried out in this clone showed accumulation of a 41-kDa polypeptide on the fourth day after salt stress. Withdrawal of salt stress on the 18th day resulted in immediate reduction in the levels of this polypeptide.

In another separate study, eighty-four clones of *C. equisetifolia* were assessed for their salt stress response under gradually increasing concentrations of sodium chloride ranging from 50 mM to 550 mM in Hoagland's solution, and highly tolerant (TNIPT-4, TNKBM-407, APKKD-10, APVSP-14 and TNMT-2) and sensitive clones (PYN, JKCE-8, APVJM-33, TNPP-4, TNVM-3 and TNPV-2) were identified. The identified clones could be used for understanding the molecular basis of salt tolerance and development of association based molecular marker approaches in *C. equisetifolia*. The selected tolerant clones could also be good candidates for further testing in flooded and coastal saline tracts. Sodium estimation in the roots and branchlets of these clones revealed that sodium accumulation in roots or shoots could not be correlated with salt tolerance. However, the shoot to root ratio of sodium could be correlated. While the most sensitive clones had a shoot to root [Na⁺] ratio of 1.58 ± 0.27 , it was 0.88 ± 0.11 for the tolerant clones, indicating that shoot to root ratio of sodium could be considered as one of the markers for screening of salt tolerant casuarina clones.

Proline helps to preserve structural integrity and cellular osmotic potential within different compartments of the cell. The effect of short term salt stress on growth and proline levels in the most susceptible (PYN) and the most tolerant (TNIPT4) *C. equisetifolia* clones were evaluated. Three months old ramets of these clones maintained in the glass house conditions were subjected to incremental NaCl concentrations from 50 mM to 650 mM over a period of 4 months. The clones showed visible symptoms of yellowing and drooping of branchlets at 300 mM NaCl and browning at 500 mM for PYN and at 600 mM for TNIPT4. It was also observed that PYN could not withstand salt concentration above 550 mM while TNIPT4 was able to survive upto 650 mM salt concentrations. The number of branchlets showed a marked increase with increasing NaCl concentrations upto 200 mM in

PYN and 500 mM in TNIPT4, after which they decreased gradually. There was a progressive increase in proline content with the increasing NaCl concentration upto 450 mM after which there was a decline. Clone TNIPT4 showed a higher accumulation of proline at 450 mM salt concentration as compared to clone PYN. These results indicate that salt tolerance in *C. equisetifolia* may be due to faster and higher accumulation of proline in response to the elevated Na⁺ concentration in the cells. Altering expression of genes controlling proline metabolism may therefore, enhance tolerance of this species to salt.



8.3 Differential Expression of Biochemicals with Reference to the Degree of Juvenility and Sex in *Casuarina equisetifolia*

Biochemical / anatomical studies were conducted to understand the tissue characteristics between the juvenile and adult tissues of *Casuarina equisetifolia*. Phylloclad cuttings collected from four different positions (position 1 to 4 as mentioned under 4.3) of 9 year old male, female and monoecious trees were subjected to biochemical analyses including total phenol content, peroxidase activity, total chlorophylls, total crude protein content and DNA content. Total phenol content and peroxidase activity exhibited an increasing trend when tissues from lower to upper positions were examined (7.43 mg g⁻¹ to 14.30 mg g⁻¹ and 27.35 enzyme units (mg protein)⁻¹ to 39.31 enzyme units (mg protein)⁻¹ respectively) whereas, chlorophylls, total crude proteins and DNA content recorded a decreasing trend (3.95 mg g⁻¹ to 2.85 mg g⁻¹, 37.05 mg g⁻¹ to 33.99 mg g⁻¹ and 354.09 mg g⁻¹ to 292.59 mg g⁻¹ respectively). Degree of juvenility plays an important role in successful

clonal propagation of this species. These biochemicals could be used as indicators of juvenility. Among the various anatomical parameters, pith diameter and thickness of phloem tissue varied among the stem cuttings obtained from the four positions.

Significant differences among the male, female and monoecious trees were observed with reference to total phenols, chlorophylls, peroxidase activity, total crude proteins and DNA content. Male trees registered higher values for chlorophylls and peroxidase activity whereas, the monoecious individuals estimated more phenols and proteins when compared to the other sexes. The DNA content was higher for female trees. With reference to the anatomical studies, (a) pith diameter, (b) diameter of the area excluding pith and phloem, (c) diameter, area, roundness and aspect ratio of the xylem vessels varied significantly among the male, female and monoecious trees.

8.4 Wood characteristics of *Casuarina equisetifolia* Provenances

Since Casuarina wood is used for its physical (e.g. poles and fuel wood) and pulp qualities, separate selections need to be made for these different end uses. Significant provenance differences were noticed for wood density and other wood properties of *Casuarina equisetifolia*. At Sadivayal (Tamil nadu) the wood density of seven provenances ranged from 0.59 to 0.71 g/cc. The Australian provenance Seventeen Seventy recorded the highest and South Arcot seedlot from India recorded the lowest wood density. Further, fibre characteristics like fibre lumen width and fibre double wall thickness also varied significantly among the provenances tested. At Puducherry wood density of *Casuarina equisetifolia* seedlots at the age of five years ranged from 0.66 to 0.8 g/cc (mean=0.71). Considerable tree to tree variation was also found within a population. Seedlot from Northern Territory (Australia) has significantly higher wood density (0.8 g/cc) than rest of the seedlots. The lowest wood density was recorded by the seedlots from Sarawak, Malaysia (0.66 g/cc), Thailand and China (0.68 g/cc). The local South Arcot seedlot recorded intermediate density of 0.7 on par with the mean density 10 provenances tested. These results indicate that appropriate provenances can be selected for strength and pulp qualities.

8.5 Wood Property Variation in Selected Clones of *Casuarina equisetifolia* for Pulp and Paper Making

Variation in wood physical (specific gravity), anatomical (fibre, vessel and ray morphology) and chemical (cellulose and lignin per cent) properties of 46 *Casuarina equisetifolia* clones grown in Karur district, Tamil Nadu was studied to assess their suitability for pulp and paper making. Wood property analysis was conducted at the department of Wood Science, Kerala Agricultural University, Thrissur. Transverse discs representing each clone which were collected from the base of billets were converted to smaller specimens for undertaking studies on wood physical, chemical and anatomical properties. Nested analysis of variance was carried out to find out inter and intra-clonal variation of clones. All the physical and anatomical properties except fibre lumen width, runkel ratio, rigidity coefficient, flexibility coefficient and shape factor, showed significant difference between clones. Within clone variation was also significant for all the physical and anatomical parameters except specific gravity (oven dry). In order to assess the suitability of clones for pulp and paper making, specific gravity (oven dry), fibre length, Runkel ratio, shape factor, slenderness ratio, flexibility coefficient, rigidity coefficient, and cellulose and lignin content were considered. Among these, fibre length, slenderness ratio, flexibility coefficient, shape factor and cellulose and lignin content of clones were found to be within the acceptable range for pulp and paper making. For selecting the best clones suitable for pulp and paper making, clones were grouped to four clusters by carrying out hierarchical cluster analysis on the basis of all physical, anatomical, chemical and growth parameters. Cluster 4 (one clone) and cluster 2 (11 clones) were found to be better for pulp and paper making in comparison to other clusters.

9.0

TREE PHYSIOLOGY

9.1 Photosynthesis and Drought Adaptation in *Casuarina equisetifolia*

Ten seedlots of *C. equisetifolia* planted in the Puducherry trial were subjected to biochemical analyses. Seedlots from Northern Territory (Australia), Solomon Islands and Papua New Guinea, Orissa (India), South Arcot (India), and China showed higher rates of photosynthesis (CO₂ fixation) compared to those from Guam, Fiji, and Egypt. These seedlots were also found to have high foliar nitrogen content and nitrate reductase activity. These results were consistent with the growth data recorded in the trial. The physiologically superior seedlots of Orissa and Northern Territory (Australia) recorded higher height and diameter growth than the rest of the seedlots tested. They also possessed nearly twice the amount of proline, superoxide dismutase activity compared to other seedlots in the trial suggesting these are better adapted to dry conditions than the rest. Such physiological and biochemical parameters can be useful for early selection of provenances and clones for propagation and further improvement.

9.2 Gas Exchange Characteristics in *Casuarina* Clones

Thirty-three clones of *C. equisetifolia* selected from Chidambaram / Chengalpet (CH/CP) region in Tamil Nadu and maintained in the clone bank of IFGTB were subjected to physiological studies at 4 years of age. Studies revealed considerable variation with respect to physiological parameters including WUE in 33 casuarina clones. Seven clones which exhibited superior growth performance coupled with favourable physiological characteristics including high photosynthesis, carboxylation efficiency and water use efficiency have been identified suitable for planting in drought prone areas. They could be used as potential candidates for special purpose clonal seed orchards for quality seed production.

10.0

TREE HEALTH

10.1 Insect Pests of *Casuarina equisetifolia*

Casuarina equisetifolia is free from major insect pest problems in its native land. However this species is encountered by several severe problems in countries where it has been introduced and India is not an exception. Studies carried out during 1998 to 2013 in the *Casuarina* growing tracts of Tamil Nadu, India have revealed that this species is infested by 40 species of insects. They include 3 species of stem borers, 2 species of bark feeders, 23 species of needle feeders, 11 species of sap suckers and 1 species of seed feeder. The maximum number of insects was found to feed on needles. The insects attacking *C. equisetifolia* in Tamil Nadu falls under 6 orders viz. Coleoptera (12 species), Hemiptera (11 species), Hymenoptera (1 species), Isoptera (1 species), Lepidoptera (9 species) and Orthoptera (6 species).

Among the 40 species of insects recorded during this study, four species are of concern in *Casuarina* growing tracts of Tamil Nadu. Others may not pose any serious threat. In general, except *Indarbela quadrinotata*, *Icerya purchasii*, *Nipaecoccus vastator* and *Eumeta crameri* most of the other insects reported are of no major economic consequence. As the plantation programme of *Casuarina* expands, the possibility of some of the minor pests attaining major pest status cannot be ruled out.

List of insects recorded on *C. equisetifolia*

Insects (Order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
Coleoptera			
Bostrychidae			
<i>Sinoxylon</i> sp.	Coimbatore	Bores into stem and branches	Minor

Insects (Order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
Buprestidae			
<i>Psiloptera</i> sp.	Neyveli	Gnaws bark of young shoots and needles	Minor
Cerambycidae			
<i>Batocara rufomaculata</i> De Geer	Ramanathapuram	Bores into stem	Minor
<i>Niphona malaccensis</i> Breuning	Mahadanapuram	Feeds on bark of stem and young branches	Minor
Curculionidae			
<i>Myllocerus discolor</i> Bohem.	Coimbatore	Feeds on needles	Minor
<i>M. improvidus</i> H.L. Andrews	Neyveli	Feeds on needles	Minor
<i>M. undecimpustulatus</i> Faust.	Mahadanapuram, Pirappanvalasai and Coimbatore	Feeds on needles	Minor
<i>M. viridanus</i> Fab.	AC Zone 1, 4 and 5	Feeds on needles	Minor
<i>Myllocerus</i> sp.	Coimbatore	Feeds on needles	Minor
Mylabridae			
<i>Mylabris pustulatus</i> Thunb.	Neyveli	Feeds on young needles and flowers	Minor
<i>Mylabris</i> sp.	Pudukkottai	Feeds on needles	Minor
Scarabaeidae			
<i>Oxycetonia versicolor</i> Fab.	Neyveli	Feeds on needles	Minor
Hemiptera			
Cicadidae			
<i>Platyplura hampsoni</i> Dist.	Rameswaram	Makes incision in branches for egg laying	Minor

Insects (Order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
<i>Eurybrachys tomentosa</i> Fab.	Coimbatore	Sucks sap from young shoots	Minor
Margarodidae			
<i>Icerya purchasi</i> Maskell	Pudupattinam, Mahadanapuram, Rameswaram and Coimbatore	Sucks sap from needles and young branches	Moderate
Membracidae			
<i>Otinotus oneratus</i> Walker	AC Zones 1, 4 and 5	Sucks sap from young portion of stem / branches	Minor
<i>Oxyrachis tarandus</i> Fab.	AC Zones 1, 4 and 5	Sucks sap from young portion of stem / branches	Minor
Pentatomidae			
<i>Chrysocoris purpureus</i> Westwood	Mahadanapuram	Sucks sap from young portion of stem / branches	Minor
<i>Chrysocoris stocherus</i> L.	Pudukkottai	Sucks sap from young portion of stem / branches	Minor
<i>Halys dentatus</i> Fab.	Coimbatore	Sucks sap from young portion of stem / branches	Minor
<i>Nezara viridula</i> L.	Pudupattinam and Coimbatore	Sucks sap from young portion of stem / branches	Minor
Pseudococcidae			
<i>Ferrisia virgata</i> Cockerall	Coimbatore	Sucks sap from needles	Minor

Insects (Order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
<i>Nipaecoccus vastator</i> (Maskell)	Puduppattinam	Sucks sap from needles	Moderate
Hymenoptera			
Torymidae			
<i>Bootanellus orientalis</i> Mathur and Hussey	Coimbatore	Feeds on seeds	Minor
Isoptera			
Termitidae			
<i>Odontotermes obesus</i> Rambur	Tirupporur and Mahadanapuram	Feeds on outer bark	Minor
Lepidoptera			
Cosmopterigidae			
<i>Microlepidopteran needle miner</i>	Rameswaram and Killai	Mines into needles	Minor
<i>Ascotis selenaria</i> Denis, ssp. <i>imparata</i> Walker	Coimbatore	Feeds on needles	Minor
Metarbelidae			
<i>Indarbela quadrinotata</i> Walker	AC Zones 1, 4 and 5	Bores into stem and feeds on bark	Major
Lasiocampidae			
<i>Metanastria hyrtaca</i>	Neyveli	Feeds on needles	Minor
Cramer			
<i>Taragama siva</i> Lefebvre.	Neyveli	Feeds on needles	Minor
Lymantridae			
<i>Dasychira mendosa</i>	Coimbatore	Feeds on needles	Minor
Heubner			
<i>Lymantria detersa</i> Walker	Coimbatore	Feeds on needles	Minor

Insects (Order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
Psychidae			
<i>Chalioides</i> sp., nr. <i>vitrea</i> Hampson	Killai	Feeds on needles	Minor
<i>Eumeta crameri</i> (Westwood.)	AC Zones 1, 4 and 5	Feeds on needles	Moderate
Orthoptera			
Acrididae			
<i>Orthacris maindroni</i> Bolivar	Coimbatore	Feeds on needles	Minor
<i>O. ruficornis</i> Bolivar	AC Zones 1,4 and 5	Feeds on needles	Minor
<i>Oxya fuscovittata</i> (Marschall)	Puduppattinam	Feeds on needles	Minor
<i>O. nitidula</i> (Walker)	Puduppattinam	Feeds on needles	Minor
<i>Xenocatantops</i> sp.	Puduppattinam	Feeds on needles	Minor
Pygomorphidae			
<i>Neorthacris auticaps</i> Singh and Kevan	Mahadanapuram	Feeds on needles	Minor



Infestation of *Nipaecoccus vastator* (Maskell)



Cottony Cushion Scale, *Icerya purchasi* Maskell



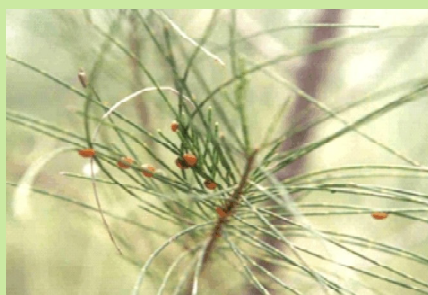
Feeding symptom of *Niphona malaccensis* Breuning



Hairy caterpillars of *Lymantria detersa* Walker



Larva of *Taragama siva* Lefebvre



Micraspis sp. - A coccinellid predator of *Nipaecoccus vastator* (Maskell)



Bark eating caterpillar infected by *Beauveria bassiana* (Balsamo) Vuillemin



Mortality of bark eating caterpillar after application of *Pongamia pinnata*



Mortality of bark eating caterpillar after application of Neem oil



Mortality of bark eating caterpillar after application of 'Formulation-A'

10.2 Bark Eating Caterpillar in Casuarina

Studies on incidence and intensity of attack of bark eating caterpillar: The bark eating caterpillar (*Indarbela quadrinotata*) is the most serious pest of *C.equisetifolia*. The caterpillar feeds on the outer as well as inner bark of trees. It bores a short burrow downwards into the wood, which is used by the larva as a day shelter. The larva eats the bark during night, excavating broad irregular patches and paths. These portions are roofed with silk and fragments of bark and faecal pellets.

The full grown larva is 3 cm to 5 cm long, blackish, with chitinated patches on the segments. Pupation takes place in the tunnel. The pupa is able to move to the opening of the larval tunnel by means of rows of teeth or hooks on abdominal segments. The pupal period is 3 weeks.

Life cycle of the pest is annual, with moth emergence taking place during May – July and larval period from June – April. The moth has forewing with rows of dark rusty red spots; wing expanse is 35 mm to 50 mm. It is a polyphagous pest and attacks trees belonging to *Bombacaceae*, *Euphorbiaceae*, *Leguminosae*, *Loganiaceae*, *Myrtaceae*, *Rhamnaceae* and *Verbenaceae*. Due to the infestation of the pest, secondary invasion of pathogens takes place and severely affected plants succumb to the attack. *Casuarina* plantations of 2 years age and above are usually attacked by the pest.

An assessment of the incidence and intensity of attack of the bark eating caterpillar in plantations grown in different agro-climatic zones of Tamil Nadu State was made. Wide variation was noticed in the infestation levels of bark eating caterpillar in the four agro-climatic zones, with higher levels of infestation in the North-eastern Zone (AC I, 39.78%) and the Cauvery Delta Zone (AC IV, 33%). In plantations of agro-climatic zone IV, mortality of trees caused by the bark eating caterpillar ranged from 3 to 5 per cent annually. The plantations raised in Southern Zone (AC V) had very low pest infestation (2.66%), while the ones established in the High Rainfall Zone were not attacked by the pest. Plantations of younger age suffered from higher levels of infestation, compared to that of older ones. The espacement of trees in the plantations / density of trees did not influence the pest infestation. The infestation was more in the plantations raised in sandy soil, compared to those grown in sandy-clay type. The pest attack was also found to be higher in plantations

raised in acidic soils than alkaline soils. Among the climatological parameters, the minimum temperature was observed to have profound influence on the pest infestation.

Assessment of loss in growth increment due to bark eating caterpillar: An assessment of loss in growth increment due to the attack of bark eating caterpillar was made in a *C. equisetifolia* plantation raised in 2006 at T.S. Pettai (a hot spot area of bark eating caterpillar incidence), Cuddalore Forest Division, Tamil Nadu. Growth parameters of trees in the protected (pest attack prevented by periodical application of insecticide) and unprotected plots (allowed for natural infestation of the bark eating caterpillar) were collected for 2 years. The study showed that, the bark eating caterpillar infestation was able to cause a loss of 6.66 per cent in terms of diameter growth and 7.31 per cent in terms of height growth, per annum. A positive correlation was found between the infestation level of the bark eating caterpillar and the growth reduction of the trees, with significantly higher impact on diameter increment.

Studies on host plant resistance against the bark eating caterpillar: Screening of 55 seed sources of *C. equisetifolia* available in two International Provenance Trials established at Neyveli (Tamil Nadu, India) and Puducherry (India), against the bark eating caterpillar (*Indarbela quadrinotata*) was carried out. The studies showed that, the seed sources 'Kilifi' from Kenya, '15958, QL Australia' and '18141, Kenya' were completely free from bark eating caterpillar infestation. The seed sources 'Wangetti Beach' from Queensland and 'Mamorah Alexandria' from Egypt, '16166, NT Australia', '18008, NT Australia', '18122 Egypt', '18271, Vanua hen from Fiji', '18143 Kenya' and '18135 Kenya' were found to be moderately susceptible to the bark eating caterpillar. Some of the Malaysian seed sources like '18244 Sarawak' though highly susceptible to the bark eating caterpillar, exhibited good growth performance. The Indian seed sources including the 'local' one were found to be highly susceptible to the bark eating caterpillar.

The anatomy of the bark of resistant and susceptible seed sources of *C. equisetifolia* revealed that, the bark of resistant seed sources contained large number of tanniferous cells, compared to that of susceptible ones. Analysis of secondary

metabolites in the bark of resistant and susceptible seed sources of *C. equisetifolia* showed that, there was significant negative correlation of susceptibility with total tannin content.

Management of bark eating caterpillar: The bark eating caterpillar does not have many natural enemies. The entomopathogenic fungus, *Beauveria bassiana* was causing large scale mortality of bark eating caterpillar larvae, in *Casuarina* plantations raised in some pockets of Tamil Nadu, India. Evaluation of this fungus against the bark eating caterpillar in the laboratory showed that a concentration of 4×10^8 spores/ml can cause 100 per cent mortality of test larvae. The same spore concentration when tested under field conditions resulted in 60 to 70 per cent larval mortality.

A number of botanicals (oils of *Azadirachta indica*, *Jatropha curcas*, *Pongamia pinnata*, *Hydnocarpus pentandra*, seed kernel extract of *Melia azedarach*, Vilvekam, Formulation-A and Formulation-B) and a few entomopathogenic fungi (*B. bronginartii*, *Verticillium lecanii* and *Metarrhizium anisopliae*) were evaluated against the bark eating caterpillar, in the laboratory condition and among them, the materials found suitable/ promising were tested in the field to know their efficacy. Seed kernel extract of *Melia azedarach*, oils of *Azadirachta indica* (Neem oil), *Pongamia pinnata* and *Hydnocarpus pentandra* and the Formulation-A developed by the Bioprospecting Division of IFGTB, were the botanicals adjudged as very effective in the field condition.

Considering the adverse impact of hazardous chemical insecticides, the new paradigm of bark eating caterpillar management in *Casuarina* plantations need to be based on environmental management, with a thrust on biological methods.

Clones of Casuarina resistant / tolerant to bark eating caterpillar: IFGTB has identified provenances of *Casuarina* which are tolerant / resistant to *I. quadrinotata*. Comparison of growth performance of *Casuarina* clones and seedlings in a trial at Sirkali, Nagapattinam district, Tamil Nadu showed that seedlings of *C. junghuhniana* performed significantly better than all accessions of *C. equisetifolia*. Among the *C. equisetifolia* clones, NT Australia and PNG showed better performance than others in terms of growth.

Twenty-Five Years of Research on Casuarinas at IFGTB

Significant variation in the larval, pupal and the adult duration was observed when the bark eating caterpillars were raised on different clones of *C. equisetifolia*. While the total larval-to -adult duration was minimum for insects raised on local *C. equisetifolia*, it was observed to be extended in clones like Queensland Australia and Vietnam. The larval and pupal periods of the life cycle were extended and the adult period shortened.

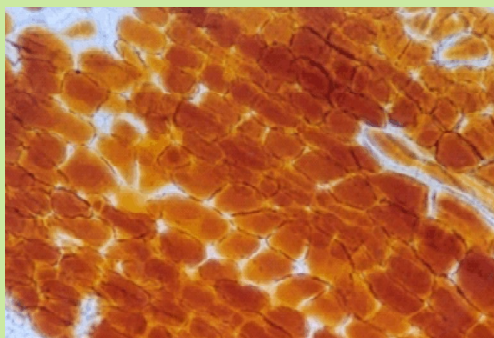
Maximum feeding by larvae under no choice situation was observed on local *C. equisetifolia* trees. Lowest area of bark feeding by the third instar larva was on the clones Kenya, *C. junghuhniana*, Chandipur, NT Australia and Fiji. Biochemical analysis of bark and wood for phenol, tannin, lipids, free fatty acids, phenolic acids, free fatty acids showed quantitative and qualitative differences in the resistant and susceptible clones.



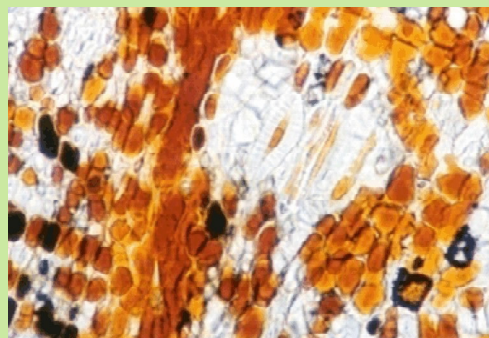
Indarbela quadrinotata



Injury in pole caused by the bark feeder



Section of bark of tolerant seed source 'Kenya' showing numerous tanniferous cells



Section of bark of susceptible 'TN-India' seed source, showing less number of tanniferous cells

These tested casuarina varieties with better growth and wood traits can be utilised by farmers for planting at their inland or coastal sites. In areas where bark feeder incidence is prevalent such clones can be deployed to avoid economic loss to farmers.

10.3 Screening for Key Nursery Insect Pests in *Casuarina equisetifolia*

Two hundred and twenty selected clones were maintained in the nursery at IFGTB in replicated experiments and screening carried out at periodical intervals for natural infestation of two targeted pests, *Icerya purchasi* (sap sucker) and *Eumeta crameri* (needle feeder). The targeted insects were also be reared in the lab/insectary and stock culture maintained for controlled condition studies in respect of determination of the resistance / susceptible nature of the clones.

Population dynamics of these pests on the clones during different seasons were studied. Incidence and infestation of Myllocerus beetle, a species of mealy bug and a species of scale insect during different seasons on different clones were also recorded. Mass multiplication of the targeted pests using their original hosts as well other alternative hosts like potato, pumpkin, *Acacia nilotica* were attempted.

Clones free from attack of these pests were short listed and controlled condition studies in respect of determination of true and pseudo resistance with *E. crameri* carried out for the 88 short listed clones. Analysis of biochemical parameters such as phenol and tannin was completed for all the short listed clones showing different levels of tolerance for *Eumeta crameri* and *Icerya purchasi*. Based on the mean number of individuals of *I. purchasi* and *E. crameri* colonized and multiplied on ramets of each clone, the 220 clones were categorized into 5 types namely resistant, very less susceptible, less susceptible, moderately susceptible and highly susceptible. The study resulted in detection of 10 apparently resistant clones, five for scale insect, *I. purchasi* (PY-157, APKKD - 11, TNKP - 2, APKKD - 3 and TNIPT- 6) and five for the bagworm, *E. crameri* (TNKBM - 403, JKCE - 13, CE - 112, CE - 2003/4 and TNIPT - 6).

10.4 Blister Bark Disease

Blister bark is a lethal disease of *Casuarina equisetifolia* caused by *Subramanianospora vesiculosa* (= *Trichosporium vesiculosum*). This disease affects more than two and half years old trees in plantations. The conducive factors of the disease are not known.

Hence IFGTB investigated on the factors influencing the disease under controlled conditions. It is essential to study the factors influencing the disease for its management.

The conidia of the pathogen from cultures were inoculated on the seedlings of *C. equisetifolia* (3 months to 6 months old) and the seedlings were raised in three different soil types (alfisol, vertisol and ultisol). The pathogenecity study showed that the infection of *S. vesiculosus* started at the age of 5 months. Temperature between 25°C and 30°C favoured the development of conidial formation in seedlings. The soil pH 5.5 to 6 favoured the disease where as soil pH<7.5 did not favour the disease. Relative humidity between 45 and 60 % favoured the pathogen to multiply in the seedlings. It was also found that regular watering of seedlings inoculated with pathogen did not favour the development of the disease. The pathogen proliferated in the soil and further spread to healthy seedlings through air. The disease showed severity in the seedlings raised in vertisol soil type than those raised in alfisol and ultisol soil types.



A plantation affected by blister bark disease



Trichosporium vesiculosus

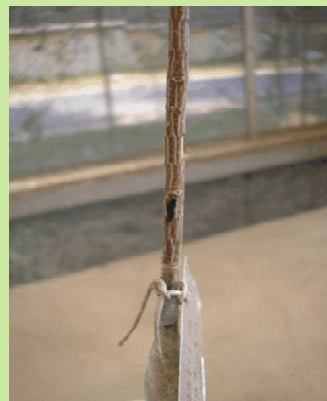
10.5 Identification of Resistant Clones of *Casuarina equisetifolia* to Blister Bark Disease

Cultured *T. vesiculosus* was inoculated into *C. equisetifolia* clones and assessed for disease resistance through disease severity score and phenol content in the clones. 100 clones were vegetatively propagated and inoculated with the pathogen *T. vesiculosus*. The clones TNIPT 7 and TNIPT 11 showed no symptom of infection and APSKLM 30, TNRM 8, TNRM 2, TNIPT 1, TNIPT 10, TNIPT 20 showed

fewer symptoms. Two clones were fully resistant and six clones were moderately resistant. The rest of the clones were susceptible. Analysis of total phenols for all the 100 clones showed that the clones TNIPT 7, TNIPT 11, APSKLM 30, TNRM 8, TNVM 2, TNIPT 1, TNIPT 11 and TNIPT 20 had higher content of total phenols. It is deduced that these clones showed resistance under controlled conditions to *T. vesiculosum* due to the higher content of total phenols.



Symptom of blister bark disease
in field



Symptom of blister bark disease
in nursery



Resistant clone (TNIPT 7) and susceptible clone
(APVSP 22) of *C. equisetifolia* to blister bark disease

10.6 Ecology and Distribution of Stem Wilt or Blister Bark Disease of *Casuarina equisetifolia* in South India

Incidence of the blister bark disease caused by *Trichosporium vesiculosum* was recorded in different plantations and experimental trials of state forest departments, IFGTB

and also in farmers' field in different agro-ecological zones of Tamil Nadu, Puducherry and Kerala. Variation in the level of incidence of blister bark disease in different plantations/experimental trials was observed. Maximum incidence (40-100%) was noticed in the Progeny Trial cum Seedling Seed Orchard at Panampally. This is followed by International Provenance Trial (20-80%) at Neyveli and International Provenance trial (2-65%) at Puducherry. It was interesting to note that no incidence of the disease was recorded in farmer's field at Chengalpet, Cuddalore, Puducherry and Sirkazhi.

Progeny trial cum seedling seed orchard (SSO), Panampally (Kerala): The individuals of 50 families in the Progeny trial cum SSO at Panampally, Kerala were screened for the incidence of the blister bark diseases since 1997. It was observed that out of 50 families, 35 families were affected by the blister bark. However, the intensity of attack of this disease varied among the families. The individuals of the family number 39 were totally (100%) affected by the blister bark, while few other families 63, 59, 56, 49, 44, 40, 28, 26, 24, 23 and 11 recorded more than 80% of the attack of the disease. The family numbers 2, 4 and 38 registered less than 50% attack. However, the variation observed on the level of susceptibility among the families requires detailed study to confirm host resistance.

International provenance trial, Puducherry: Field survey in the International Provenance Trial at Puducherry revealed that out of 35 provenances, 17 provenances were affected by blister bark disease. Of these, the provenances from Sarawak (Malaysia) (Number 24) registered the highest (65%) attack followed by Egypt (Number 11) (26%). The provenances of NT Australia (Numbers 2, 3) showed low incidence of the disease (2-4%).

Management strategies: Many recommendations for control or management of blister bark disease have been reported in the literature. Recommendations are based on acceptance that the disease is initiated by infection of wounds by spores of *T. vesiculosum*; secondary spread of the fungus to neighbouring trees is by root to root contact, disease spread is exacerbated where the fungal spore inoculum is allowed to buildup within a plantation, and trees weakened by inadequate growing conditions and environmental stress. Hence, severely affected trees should immediately be removed and burnt for preventing the spread of the disease. Initiation of the disease

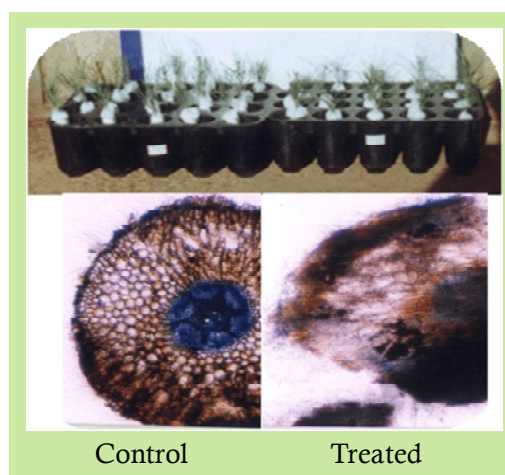
could also be observed to occur on stem and branches other than the roots. It is, therefore, suggested to avoid pruning and lopping of branches to minimize the incidence of the disease in plantations.

10.7 Biological Control

The effect of bio-control agents of the fungal species viz., *Trichoderma harzianum* and *T. viride* against blister bark disease pathogen, *Trichosporium vesiculosum* was investigated under laboratory conditions. Different isolates of *T. vesiculosum* collected from three different agro-climatic zones of Kerala, Tamil Nadu and Puducherry were subjected to antagonistic study. The bio-control agents effectively inhibited the mycelial growth of all the isolates of the pathogen. Among the different isolates of the pathogen, the Rameswaram isolate of Tamil Nadu showed least colony growth by the bio-control agents under laboratory condition.

Laboratory bioassay test: The consistency of the level of tolerance of different clones was tested against blister bark disease through a bioassay method using rooted cuttings under controlled conditions. Pure culture of the blister bark pathogen, *T. vesiculosum* was raised, culture filtrate was prepared and used. Rooted cuttings of five different clones of *C. equisetifolia* viz., TNRM2, TNRM3, TNRM5 (Rameswaram, Tamil Nadu), TNBS1 and TNBS2 (Salem, Tamil Nadu) were screened and the performance was observed for levels of tolerance and susceptibility.

Rooted cutting Bio-assay method



Biological Control



Among the five clones tested, TNBS1, TNBS2 (Salem) were susceptible, TNRM3 (Rameswaram) was highly susceptible and TNRM2 and TNRM5 were comparatively tolerant.

10.8 Testing and Evaluation of Selected Existing Control Methods for Key Diseases of *Casuarina* spp. with Reference to Blister Bark and Root-Rot

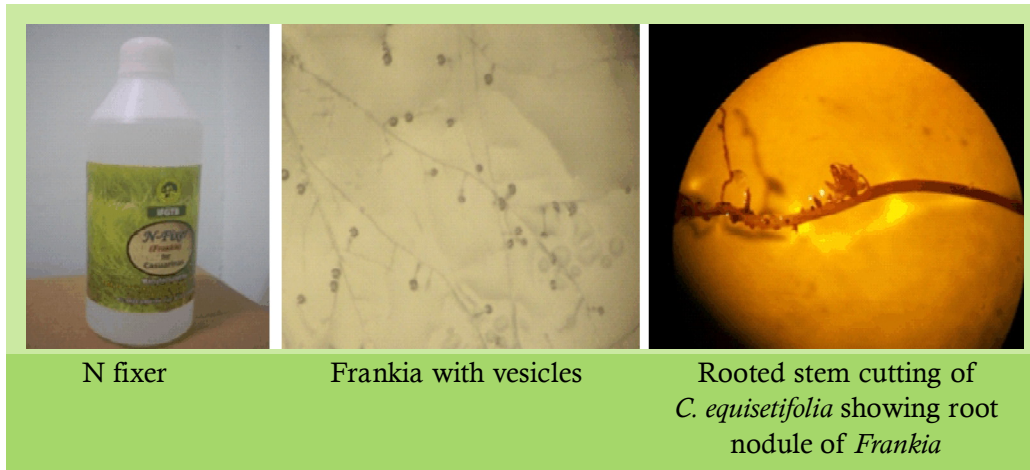
Attempts were made to test and evaluate the efficacy of different mycorrhizal fungi viz., ectomycorrhizal (*Pisolithus albus*) and Arbuscular Mycorrhizal fungi (*Glomus* spp.) and bio-control agent (*Trichoderma viride*) for diseases of *Casuarina equisetifolia* and *C. junghuhniana* with specific reference to blister bark disease caused by *Trichosporium vesiculosum* and root-rot disease due to *Ganoderma lucidum* in plantations. Seedlings of *C. equisetifolia* and *C. junghuhniana* were raised in nursery and inoculated with different combination of mycorrhizal biofertilizers and bio-control agent. Experimental trials were laid in respect of developing integrated methods of management of these pathological problems and maintained in two different locations viz., IFGTB Research Station, Panampally (Kerala) and Farmer's field, Karikalampakkam (Puducherry) where the incidence of these diseases are common.

No incidence of these targeted diseases was recorded in both the tree species up to the age of 2 years. An attempt was made to artificially introduce the blister bark disease pathogen *Trichosporium vesiculosum* to the trees of *C. equisetifolia* in the experimental trial at Panampally (Kerala) and monitored for the incidence of symptoms of the blister bark disease at regular intervals. After a period of inoculation, few trees of *C. equisetifolia* in various treatments expressed the symptom of blister bark disease on *C. equisetifolia* in the experimental trial. Maximum infection of blister bark disease was recorded on uninoculated (control) trees. But there was no infection/symptom of blister bark disease in the trees inoculated with mycorrhizal biofertilizers (AM and ECM fungi) and bio-control agent (*Trichoderma viride*) individually as well in combination during the period of observation.

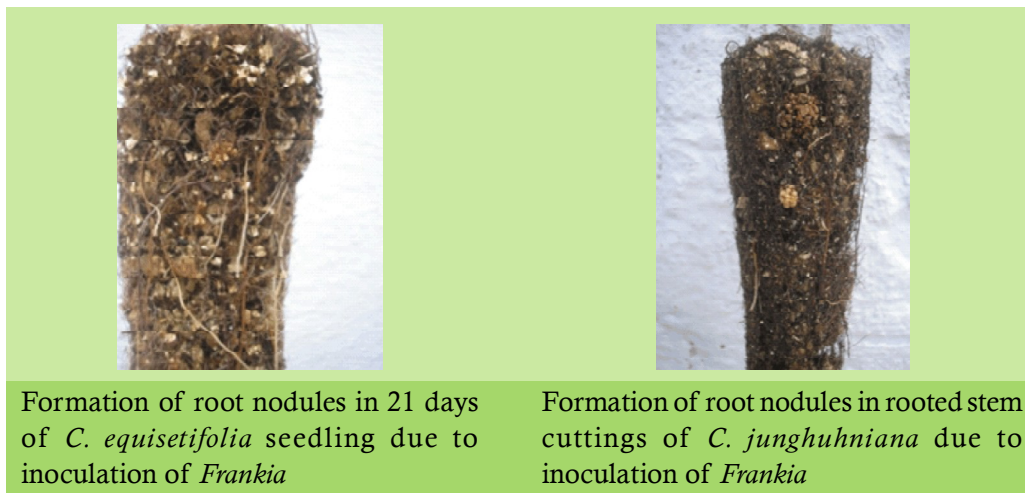
Studies on the persistence of the mycorrhizal association with *Casuarina equisetifolia* and *C. junghuhniana* trees revealed that the root samples screened at different intervals of time had prominent vesicular, arbuscular and hyphal structures of AM fungi. In general, it was noted that higher per cent colonization of AM fungi was observed in all the AM treated plant root samples as compared to uninoculated (control) plants. It was also recorded that the maximum percent colonization was found during the period July to September, followed by October to December and January to March. Less percent colonization was seen in the root samples of uninoculated (control) plants during the entire period of observation. The root samples of both the tree species also revealed the presence of the ECM fungal structures like fungal mantle, emanating hyphae and Hartig-net during different periods of observations. The rhizosphere of all the samples had AM fungal spores but varied in spore population among different treatments. Maximum number of AM spores was found during the period July to September, followed October to December and January to March. In general, the spore population of AM fungi was found greater wherever AM inoculation was done in the rhizosphere of *C. equisetifolia* and *C. junghuhniana*.

10.9 Frankia : The Symbiotic Nitrogen Fixing Bacteria of Casuarinas

Casuarinas fix atmospheric nitrogen through a symbiotic relationship with Frankia, a soil bacterium of the actinobacteria group. The roots of *C. equisetifolia* and *C. junghuhniana* produce root nodules where the bacteria fix atmospheric nitrogen, which is an essential nutrient for all plant metabolic activities. To enhance root



nodulation under nursery and field conditions in Casuarinas, superior strains of *Frankia* have been identified and applied. *Frankia* inoculated rooted stem cuttings were propagated under nursery conditions and transplanted in the nutrient-deficient soils of Karaikal, Puducherry. Under nursery experiments the growth and biomass of *C. equisetifolia* and *C. junghuhniana* rooted stem cuttings inoculated with *Frankia* showed three times higher growth and biomass than uninoculated control. These stocks were transplanted and monitored for their growth and survival for one year in the nutrient-deficient farm land. The results showed that the rooted stem cuttings significantly improved growth in height, stem girth and tissue nitrogen content than uninoculated control. The soil nutrient status was also improved due to inoculation



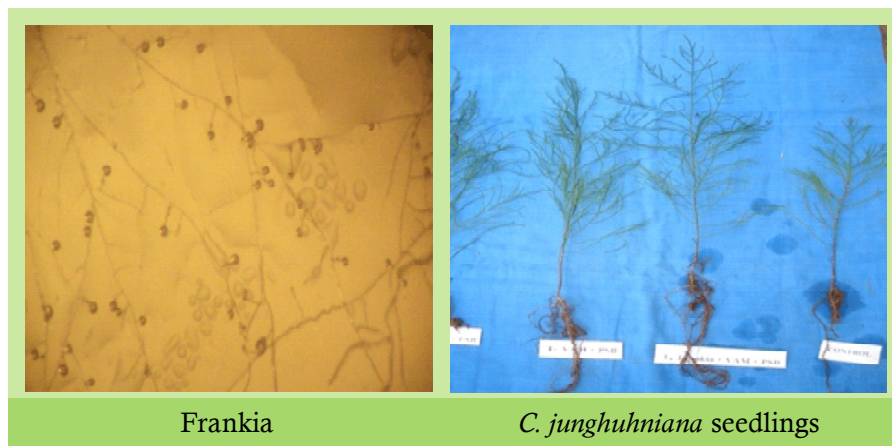
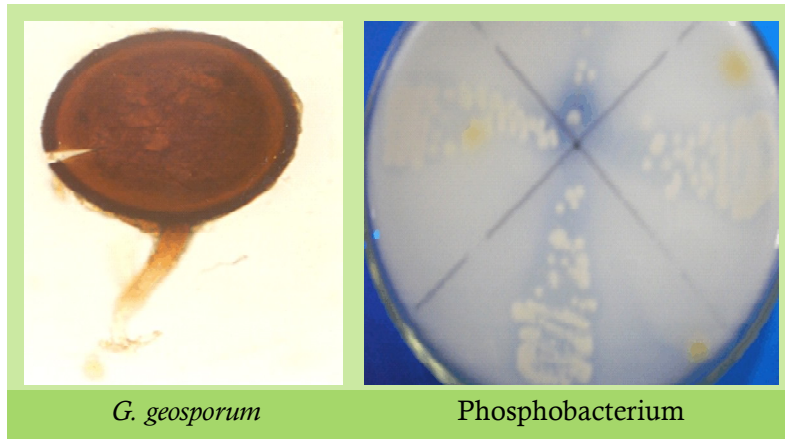
of Frankia. The superior strains were identified based on the nitrogenase activity through acetylene reduction. A product called N fixer was also developed and released for the benefit of Casuarina growers.

Performance of Frankia under field conditions: Seedlings of *C. equisetifolia* and *C. junghuhniana* inoculated with *Frankia* strains showed increased growth, numerous numbers of root nodules and increased nitrogen content under field conditions. The soil nutrients were also improved upon inoculating Casuarinas with *Frankia* strains.

10.10 Growth Responses of Casuarinas to Beneficial Microbes

Indigenous AM fungi *Glomus geosporum* (Nicol. & Gerd.) Walker. and Phosphobacterium (*Pseudomonas fluorescens*) were isolated from the rhizosphere of mature trees of *C. junghuhniana* and used to inoculate the seedlings of *C. junghuhniana* individually and in combinations. The results at the end of nine months showed that co – inoculation of *Frankia*, AM fungi and PSB increased the growth, biomass, root nodule numbers, root nodule biomass and nutrient uptake of *C. junghuhniana* seedlings than single inoculation. The growth and biomass were increased three folds in *Frankia*, AM fungi and PSB inoculated seedlings. Inoculation of seedlings of *C. junghuhniana* with these microbial symbionts facilitated early association thereby improving the quality of seedlings.

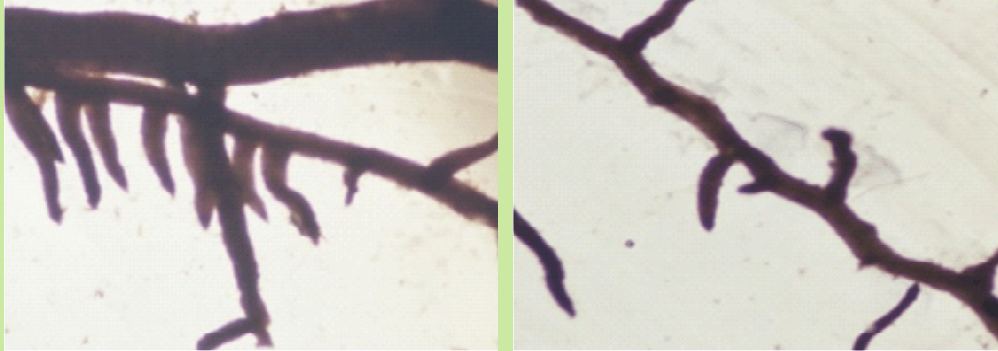




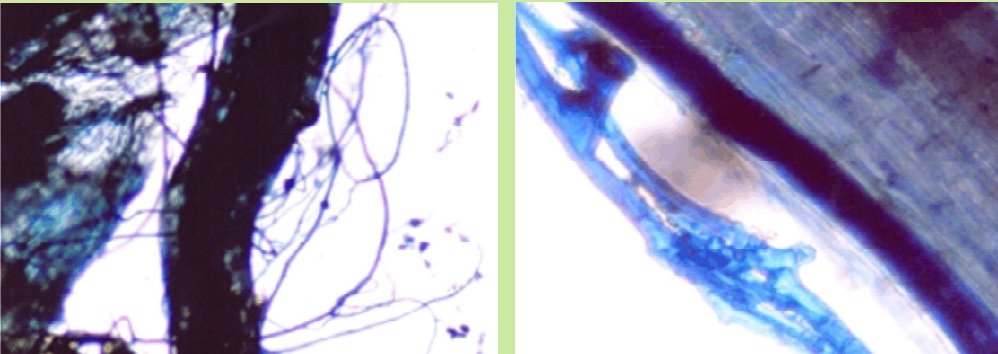
10.11 Screening and Identification of Potential Isolates of Ectomycorrhizal Fungi for Increased Productivity of Casuarina Species in Nursery

An attempt was made to select suitable Ectomycorrhizal (ECM) inoculum for growth improvement of *Casuarina equisetifolia* and *C. junghuhniana* in nursery. The ECM fungal isolates viz., *Laccaria fraterna*, *Pisolithus albus* and *Scleroderma* sp. were used.

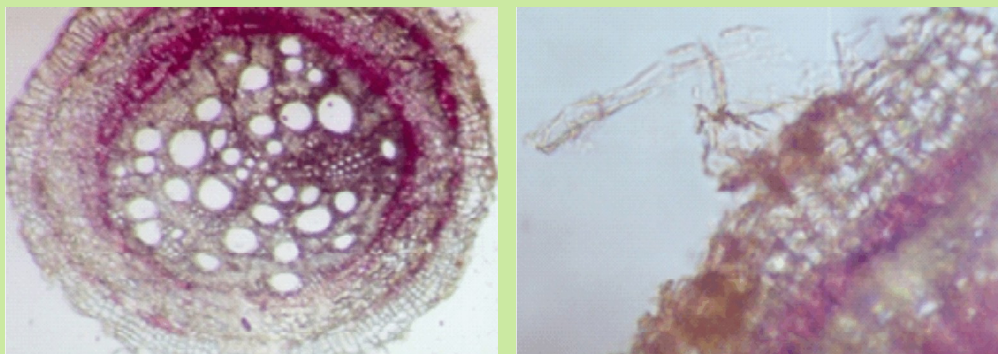
Seedling growth in terms of height increment was comparatively higher in all ECM fungal treatments than the uninoculated (control) seedlings. Vegetative mycelial inoculum of *P. albus* was found to be the most efficient inoculum which gave maximum per cent of Mycorrhizal Inoculation Effect (MIE) in both the *Casuarina* species. ECM application in seedling stage may assist the seedlings to withstand



ECM colonized root tips



Extramatrerial hyphae with clamp connections (x 200)



T.S. of root (myco tip) (x 200) Mantle with radiating hyphae in root (x 400)

dry land conditions and support the seedlings when out planted under agro-forestry and plantation systems.

10.12 Biotransformation of Some Secondary Metabolites as Growth Regulators in Nodulation and Biomass of *Casuarina equisetifolia*

From tropical climate regions to Iceland actinorhizal plants are being more frequently used in reforestation and soil reclamation. However, these attempts may fail, as introduced plants occasionally are unable to form nitrogen-fixing symbiotic structures with *Frankia* present in soil, even when *Frankia* strains infecting the utilized actinorhizal species are introduced.

Frankia strains cover the production of many secondary products. They are a source of secondary metabolites of potential interest and thus play an important role in the regulation of plant communities in terms of growth and resistance to their herbivores. The long list of secondary metabolites includes pigments such as carotenoids and glutamine, hapanoids, lipids and a host of other compounds. However, no literature is available on the bioconversion of secondary plant products by bacterial spores of *Frankia* strains. Esterases are being increasingly recognized as useful for stereo specific manipulation of esters. Several aromatic compounds related to lignin (including ferulic acid, vanillin, vanillic acid, p-hydroxybenzoic acid and protocatechuic acid), abundantly present in the rhizosphere are important for signaling in gene expression between plants and some bacteria, and in *Agrobacterium tumefaciens*, these compounds play a major role in the communication between plants and beneficial rhizosphere bacteria. Citrinin, a broad-spectrum antibiotic, which has limited pharmaceutical applications due to its nephrotoxic effects on experimental animals, when transformed selectively into a product (decarboxycitrinin) using microbial enzyme has retained its antibiotic activity and at the same time is not toxic to test animals. Microbial enzymes are relatively more stable than corresponding enzymes derived from plants or animals. Microbial secondary metabolites also play an important role in the field of industry, agriculture, medicine, human health and as unique biochemical tools. Their role in plant-pathogen and plant-insect interactions is receiving increasing attention because of their potential use in pest control. However, little is known about their physiology and the regulation processes of the plant-endophyte interaction especially during sporulation and the bioconversion of bioactive compounds for nodulation capacity with multiple applications in Casuarinas.

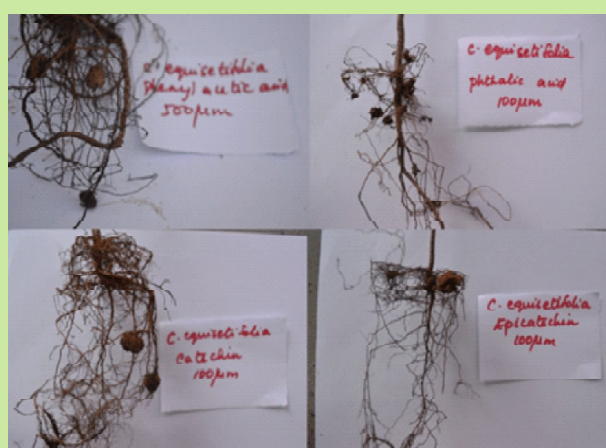
Biochemical budgeting of Frankia cultures: The details of biochemical budgeting in different days of Frankia culture and its influence on biomass in casuarina were estimated. The chemical composition of the Frankia cultures was found to be a mixture of mono- and sesqui-terpenoids, and fatty acids. Over 56 compounds were detected from different day culture fractions of frankia using GC-MS-MS. The terpene derivatives (Hopanoids) Viminalol and 2- Amyrine present in 15th day cultures were found essential for nodulation and nitrogen fixation in *C. equisetifolia*. The 15th day frankia culture induced significant increase in plant height compared to other inoculants, control and crushed root nodules due to the presence of mono-sesquiterpenoids, fatty acid and hopanoid derivatives.



C.equisetifolia cuttings treated with Frankia culture



C.equisetifolia seedlings treated with identified biosignalling compounds



C.equisetifolia seedlings treated with biosignalling molecules

11.0

NEW RECORDS

11.1 Studies on Sexual Variation in Clones of *Casuarina equisetifolia*

Three to four year old plantations in Chengalpet and Chidambaram in Tamil Nadu were surveyed and superior trees were selected and cloned using the cladodes. Ramets of these clones which were clearly identified as male, female and monoecious were established in the clone bank of IFGTB for selection and breeding in the year 1992. Observations on various biometric and physiological parameters were recorded at intervals of three months. The observations recorded during the month of September, 1997 and March, 1998 on flowering behaviour in the clones provided proof of change in sex expression in this species.

Constant males accounted for 59 per cent, constant females 26 per cent and constant monoecious individuals 4 per cent of the population. Six clones (11 per cent of the population) changed their sex in various ways. Four clones viz., CHCE 1003, CHCE 2903, CPCE 0109 and CPCE 3702 were females originally and changed their sex to bisexuals. Clones CHCE 0401 and CPCE 3501 were originally males and transformed into monoecious plants by producing female cones approximately five years after introduction.

Studies were initiated to understand this rare phenomenon at the biochemical level. Protein profiles of the inconstant individuals were compared with those of the constant males, constant females and monoecious individuals during the flowering season. Further studies were taken up during the non-flowering season also to test for variations in protein levels. Total crude protein levels were analyzed during the flowering (January to March) and non-flowering (July to August) seasons during the year 2000. It was observed that the two types of plants namely the constants and the inconstants differed remarkably with respect to their protein levels.

Protein levels were as low as 9.62 and 8.68 mg/g fresh tissue in males and females respectively during the non-flowering seasons which increased drastically to 39.4 and 32.0 mg/g fresh tissue during flowering (almost 4 fold increase over the initial values). In monoecious individuals, the protein levels were comparatively higher during the flowering season, but the variation in protein levels between the flowering and non-flowering seasons were marginal.

In case of the inconstants, there was a remarkable change in protein levels. During flowering, the male plants, which changed their sex to inconstant individuals showed a 15-fold increase in protein levels when compared to the non-flowering season, while the female plants, which changed to inconstants showed a 10-fold increase. A reduction in protein content was noticed in the transformed plants when compared to the constant individuals at the time of flowering. But the rate of increase in protein levels in the transformants during flowering was almost thrice that of the normal plants. This may be effected as a result of the change in the sex expression in the transformants from either male or female to monoecious where it is required to produce female or male inflorescence additionally.

The only external influence was continuous hedging of plants. It may however, be pointed out that though all the clones were maintained as hedge orchards for planting stock production, only 11 per cent of the population showed change of sex termed as inconstants which may have evolutionary significance. Plant health and the successional stage of the stand need to be studied along with resource allocation to explain the proximate mechanisms of sex expression.

11.2 A New Record of Abnormal Phylloclad Modification in *Casuarina equisetifolia*

Phenotypic variants have been reported in *Casuarina equisetifolia* throughout the distribution range in India on crown shape, branch angle, phylloclad length, size and shape of infructescence and seed morphology. Abnormality in the phylloclad of this species was reported from IFGTB. As a part of the tree improvement programme of the Institute of Forest Genetics and Tree Breeding, Coimbatore, clones identified for high productivity were tested in a sodic region in Tamil Nadu. Out of 87 clones tested in replications, one clone revealed an abnormal phylloclad modification at the end of one year. The phylloclad which should have been

cylindrical was observed to open out resembling a hand fan. The width at mid region of the modification varied from 2.5 to 7.0 cm with the mid diameter ranging from 1.79 to 3.47 mm, almost 3-6 times the normal measurements. The length of the structure varied from 4.6 to 12.8 cm. There were six such modified phylloclads in the clone. No such observations have been recorded earlier, nor has there been any report in the same field in other clones following this occurrence. The reasons for this abnormality need to be investigated. It could be attributed to physiological stress in the plant.



Abnormal phylloclad modification in
C. equisetifolia

12.0

NETWORK ON CASUARINAS

12.1 National Seminar on Casuarinas

First National Seminar (1989): The First National Seminar on Casuarinas was organized jointly by IFGTB, Tamil Nadu Forest Department and Tamil Nadu Forest Plantation Corporation at Neyveli on December 18 & 19, 1989. Twenty four papers were presented in three sessions namely Status of Casuarina cultivation in the States and the future programme, Silviculture including different aspects of Casuarina cultivation management for different end use, Genetics and Pests & Diseases and Utilization, Marketing and Economics. It was attended by Forest Departments of Casuarina growing States, research and academic institutions, forest based industries and NGOs.

Second National Seminar (2011): The Second National Seminar on Casuarinas was held at Coimbatore, in IFGTB, on 3rd and 4th of March, 2011. This two day seminar consolidated the research upto date on the species of Casuarina in the country, and also provided a platform for the Casuarina researchers for exchange of information and ideas. This helped in forming the network required to implement the All-India Coordinated Programme on Casuarina.

12.2 Peer Review on Casuarina (1997-2001)

The Institute of Forest Genetics and Tree Breeding, Coimbatore, brought together the Casuarina researchers in the county and created a network which annually met every year from 1997 to 2001 with Dr. K. Gurumurthi, functioning as the Chief Technical Adviser. The full length proceeding for every year was also brought out for circulation.

Five annual workshops were convened. The first three workshops were held at the Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu where as

the fourth was held at the Institute of Wood Science and Technology, Bangalore, Karnataka and the fifth at the Regional Forest Research Centre, Rajahmundry, Andhra Pradesh. The network had participants from various state forest departments, universities, research institutes, NGOs and forest based industries. The working papers presented in the first workshop outlined the need for developing different research areas, their interlinking to develop information base and integrate to deliver results and sustainable strategies so that the Casuarina improvement programme is capable of running its own planning cycles. Subsequently, comprehensive international provenance trials were established by the Andhra Pradesh Forest Department and the Institute of Forest Genetics and Tree Breeding.

Multilocal trials identified superior provenances of *Casuarina equisetifolia* and *C. junghuhniana* for growth, tree form and wood properties. Over 500 clonal accessions were selected by the network and extensive clonal test plots were laid out. Clonal Seed Orchards were established using genetically divergent clones. Clone banks and vegetative multiplication gardens were also positioned in various parts of the country. Physiological studies on inter-clonal variability with respect to photosynthesis, drought and salinity tolerance were completed and stress tolerant clones identified. Techniques were standardized for DNA based polymorphism and clones of economic importance fingerprinted. Agroforestry models were developed using casuarina as one of the components and the economic analysis proved the potential of the species in agroforestry practices. The experiments conducted reveal that casuarina can be profitably raised combined with teak and *Leucaena* to provide poles, fuelwood, fodder and timber. Periodic survey of pest and disease problems and screening for disease, pest tolerance was undertaken and preventive strategies have been provided to farmers and planters. Prophylactic approach to controlling blister bark disease was also developed.

The network brought together the industries requiring tree based raw materials in the country namely ITC Bhadrachalam Paper Boards Ltd, Sarapaka, Andhra Pradesh, J.K. Paper Corporation, Rayagada, Orissa, West Coast paper Mills, Dandeli, Karnataka, Andhra Pradesh Paper Mills, Rajahmundry, Andhra Pradesh, Seshasayee Paper and Boards, Erode, Tamil Nadu and SIV Industries Ltd, Sirumugai, Tamil Nadu. Farmer cooperatives also sought advice, solutions and

quality propagules from the network. The network provided leads in research, industrial requirements and commercialization in addition to developing institutional partnership with industries and farmers. It functioned as a strong voice for casuarina species.

12.3 IFGTB-IPMA Collaborative Project (2012-2014)

A project between IFGTB and Indian Paper Manufacturers Association (IPMA) on Rapid Improvement of *Casuarina* and *Leucaena* to Enhance Pulpwood Production from Farm Forestry Plantations is the first collaborative venture between IPMA and ICFRE. International Paper APPM Ltd., JK Paper Ltd, Ballarpur Industries Limited (BILT), Tamil Nadu Newsprint and Papers Limited (TNPL) and West Coast Paper Mills (WCPM) are the organizations involved in the network.



Interaction between IFGTB and IPMA members

12.4 All India Coordinated Programme for Genetic Improvement of Casuarinas (2012 to 2022)

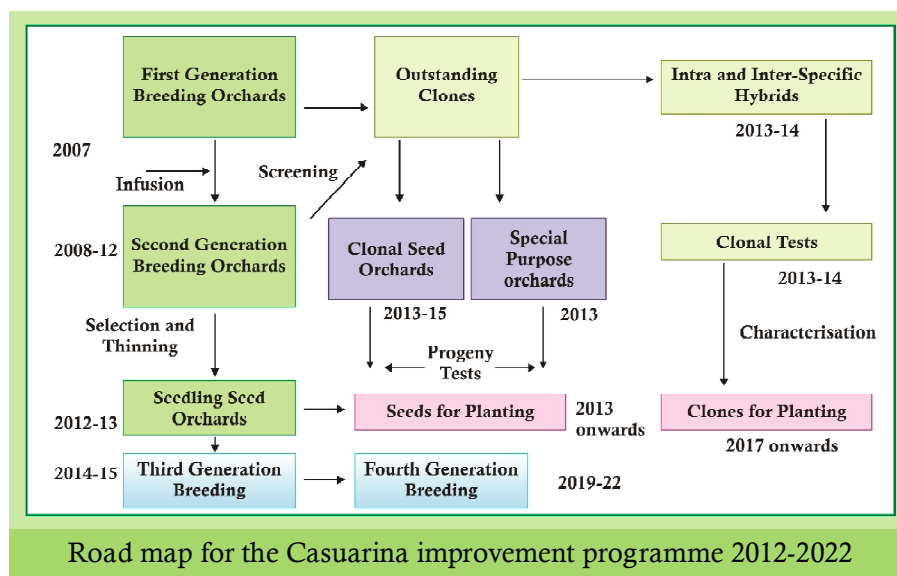
ICFRE Institutes and Forest Departments of their jurisdiction States, Wood based Industries and Universities are partners in this nationally coordinated project on

Twenty-Five Years of Research on Casuarinas at IFGTB

Casuarinas. With inputs received from the partners, a programme has been prepared for the improvement of Casuarina for ten years. The objectives include.

- To develop advance generation breeding populations for *Casuarina equisetifolia* and *C. junghuhniana* with adequate infusion populations in multilocations.
- To develop a core population of superior individuals from breeding populations and select outstanding clones through multilocal clonal testing.
- To develop inter-provenance and interspecific hybrid clones through control pollination, progeny and clonal testing.

To characterize selected clones for growth, morphology and pulp characters and release for large scale commercial cultivation



13.0

GENETIC RESOURCES OF
CASUARINAS ASSEMBLED BY IFGTB

The Planting Stock Improvement Programme (PSIP) component under the World Bank aided FREE Project (1994-2000) aimed at improving the quality of planting stock in three ways - firstly, by improving the genetic quality of the seeds produced, through the establishment of certified seed sources such as the Seed production Areas (SPAs), Seedling Seed Orchards (SSOs) and Clonal Seed orchards (CSOs); secondly, by improving the quality of harvesting, handling, testing and storage practices of such genetically improved seeds and thirdly, by carrying out improvements in the nursery practices using these seeds for production of quality planting stock. Under the systematic tree improvement programme implemented by IFGTB, many more resources were added.

13.1 *Casuarina equisetifolia*

S No.	Type of assemblage	Year	Area (Ha)	Location	Remarks
1.	SSO (IG)	1995	1	Puducherry	25 provenances
2.	SSO (IG)	1998	2	Karunya	34 provenances
3.	CSO	2000	1	Karunya	50 clones
4.	SSO (II G)	2008	2	TNPL	150 families
5.	SSO (II G)	2008	2	Tirupati	150 families

S No.	Type of assemblage	Year	Area (Ha)	Location	Remarks
6.	SSO (IIG)	2009	1	Chellanchery	150 families
7.	CSO	2009	1	Marakkanam	24 clones
8.	CSO	2010	1	Puducherry	24 clones
9.	Clone Bank	1995+	0.5	IFGTB Campus	229 clones
10.	Species Trial	2000	0.6	Karunya	6 species
11.	Hybrid Progeny Trial	2008	0.5	Panampally	30 hybrid progeny
12.	Hybrid Progeny Trial	2008	0.5	Sriharikota	30 hybrid progeny
13.	Hybrid Progeny Trial	2008	0.5	Veedur	30 hybrid progeny
14.	Seed Production Area	-	0.48	Nayashar, Andamans	Natural population

13.2 *Casuarina junghuhniana*

S No.	Type of assemblage	Year	Area (Ha)	Location	Remarks
1.	SSO (I G)	1996	2	Panampally	21 provenances
2.	SSO (IG)	1996	2	Puducherry	21 provenances
3.	HSO	2000	1	Karunya	6 provenances
4.	SSO (IIG)	2009	2	Salem	90 families
5.	SSO (IIG)	2009	2	Nellore	90 families
6.	SSO (IIG)	2010	1	Veedur	90 families
7.	SSO (IIG)	2010	2	Madurai	70 families
8.	Clonal Test	2009	1	TNPL, Karur	6 clones
9.	Clonal Test	2009	1	SPB, Erode	6 clones
10.	Clonal Test	2010	1	Tirupati	35 clones
11.	Clonal Test	2010	1	Bharathiar University Campus	70 clones
12.	Clone Bank	2001+	0.2	IFGTB Campus	110 clones

14.0

ECORESTORATION SERVICES

14.1 Eco Restoration for Tsunami Devastated Coastline of Andaman Group of Islands

This was a developmental project involving transfer of technology from IFGTB to create shelterbelts in Tsunami-affected Andaman group of islands through which livelihood opportunities were created for the people especially women during and after the project period. The project was jointly implemented by IFGTB, Coimbatore and the Department of Environment and Forests, Andaman and Nicobar Administration with financial support from the Department of Biotechnology, GoI.

It involved mass multiplication of species and provenances of *Casuarina* identified for Andaman Islands and enhancing the tree cover. Best strains of *Frankia* and mycorrhiza were identified and inoculated for improving seedling quality and field performance. Local field staff and workers were trained in production of quality planting stock of *C. equisetifolia* and *C. timorensis* with suitable beneficial microbes and composting of locally available bio wastes.



Frankia inoculation in
2006



Fully grown *Frankia*-inoculated
seedlings ready for planting (2007)



Embankment plantation raised
in 2007



Embankment plantation raised
in 2009



C. equisetifolia plantation at Casuarina bay (2007)

14.2 Supply of Quality Planting Material to Farmers Affected by the Cyclone 'Thane'

The cyclone 'Thane' caused extensive damage to crops and properties in the Districts of Cuddalore and Villupuram in Tamil Nadu and Puducherry region of U.T. of Puducherry apart from claiming several lives. Large areas planted with agricultural crops like paddy (1,47,000 ha), sugarcane (12,326 ha) and banana, cotton and oilseeds (39,939 ha) were destroyed. This region is also well known for cultivation of tree crops like casuarina, jackfruit and cashew which usually provide alternative

income to farmers when their agriculture crops fail or the income from them is insufficient.

Hon'ble Minister, MoEF announced a grant of Rs.1 crore to produce 15 lakhs of quality planting stock of Casuarina for supply to the Thane' cyclone affected farmers of Cuddalore, Villupuram and Puducherry areas. Seedlings were raised using seeds from IFGTB seed orchards. These seedlings were supplied free of cost to the affected farmers in Cuddalore and Villupuram Districts of Tamil Nadu and Puducherry. This has helped in reviving the Casuarina plantations in the areas.

15.0

CONSULTANCIES

15.1 The Andhra Pradesh Paper Mills, Rajahmundry (2007-09)

IFGTB was involved in a technical consultancy with the APPM, Rajahmundry for establishing clonal and seedling seed orchards of Casuarina using improved material developed at IFGTB for improving productivity under its farm forestry programme. The activities included assessment of seed and seedling production systems, selection of suitable clones / families for inclusion in seed orchards, establishment of new seed orchards and training of staff for orchard management.

15.2 Forest and Ecological Management Plan for Sriharikota Island (SHAR), Satish Dhawan Space Centre (2007-08)

The Department of Space requested IFGTB to prepare an Ecological Management Plan for Sriharikota Island. Accordingly a management plan was prepared for the period from 2009 to 2019 for delineating the island into protection, plantation, development, coastal zone and enrichment working circles and introduction of new species like Casuarinas for coastal afforestation, and strengthening of infrastructure like setting up a modern nursery and shade house.

15.3 BILT Tree Tech Limited (2012-14)

A Technical Consultancy Project has been awarded by BILT Tree Tech Limited for establishing Seed Orchards and Demonstration Plots of Casuarina in Andhra Pradesh, Orissa and Maharashtra. IFGTB will provide planting material, site-specific designs and technical guidance for establishing four demonstration plots and three seed orchards.

PUBLICATIONS ON CASUARINAS

- Anandalakshmi, R., Sivakumar, V., Singh, B.G., Mahadevan, N.P. and Kumar, A.M. 2002. Seed handling in Casuarina improvement programmes. In: Gurumurthi, K., Nicodemus, A. and Siddappa (Eds.), *Casuarina in India: improvement and utilization*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India. pp. 69-80.
- Balasubramanian, A. 2000. Screening for salinity resistance in clones of *Casuarina equisetifolia* Forst. Thesis submitted for the degree of Doctor of Philosophy in Forestry to the Forest Research Institute (University), Indian Council of Forestry Research and Education, Dehradun, India.
- Balu, A. and Rajarishi, R. 2007. Native entomopathogenic fungi as biocontrol agents of teak and casuarinas stem borers, *Sahyadrassus malabaricus* (Moore) and *Indarbela quadrinotata* (Walker). In: The proceedings of the National symposium on “Emerging trends of bioinoculants in forestry” held at Tropical Forest Research Institute, Jabalpur in April, 2007.
- Balu, A. Rajarishi, R., Prasanth Jacob, J. and Murugesan, S. 2001. Integrated methods of management of the bark feeder, *Indarbela quadrinotata* (Lepidoptera: Metarbelidae). In: The second National Symposium on *Integrated Pest Management in Horticultural Crops* held at IIHR, Bangalore –Oct.17-19, 2001.
- Balu, A., Sasidharan, K.R. and Rajarishi, R. 1998. A research report on “A new record of the promising biocontrol agent, *Beauveria bassiana* on the bark feeder/stem borer, *Indarbela quadrinotata*”. *Sylva Plus*, vol.VI, No.2.
- Balu, A., Sasidharan, K.R., Rajarishi, R. and Deeparaj, B. 2001. Insect pest of *Casuarina equisetifolia* and their management. In: Gurumurthi, K., Nicodemus, A. and Siddappa (Eds.), *Casuarina in India: improvement and utilization*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India, 161-167.
- Barathwal, S., Warriar, K.C.S., Kumar, A. and Paliwal, K. 2001. Photosynthesis and water relations in *Casuarina equisetifolia*. In: Gurumurthi, K., Nicodemus, A., and

- Siddappa (Eds), *Casuarina: Improvement and Utilization*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India pp 97-102.
- Chezhian, P., Yasodha, R. and Ghosh, M. 2009. Genetic Diversity Analysis in *Casuarina* and *Allocasuarina* Species Using ISSR Markers. *Madras Agric. J.*, 96: 32-39.
- Daniel, A. and Shanthi A. 2010. Isolation of genomic DNA from *Casuarina equisetifolia* L. without liquid nitrogen suitable for RAPD analyses. *International Journal of Integrative Biology*, 10(2): 82-85.
- Dasgupta, M., Radha, V. Yasodha, R. and Mohan, V. 2010. Genomic bioprospecting of uncharacterized gene pools for biotic and abiotic stress tolerance: a case study from *Casuarina equisetifolia*. *The International Forestry Review*. Vol. 12 (5): 388.
- Dasgupta, M., Veluthakkal, R. and Sundari, K.R.B. 2013. Characterization of genes expressed in *Casuarina equisetifolia* in response to elicitation by cell wall components of *Trichosporium vesiculosum*. *Silvae Genetica*, (Accepted).
- Diagne, N., Karthikeyan, A., Ngom, M., Mathish, N.V., Franche, C., Krishna Kumar, N. and Laplace, L. 2013. Use of Frankia and actinorhizal plants for degraded lands reclamation. *Bio med Research International*. ID. 948258. 9.
- George, M., Peddappaiah, R.S. and Manivachakam, P. 1998. Productivity of *Casuarina* in agroforestry systems in the semi-arid regions of Tamil Nadu. *Workshop cum peer review on Casuarina* on October, 26, 1998.
- George, M., Viswanath, S. and Manivachakam, P. 2001. Role of MPTs (*Tectona grandis*, *Casuarina equisetifolia* and *Acacia auriculiformis*) in natural resource management through agroforestry: an experience from south India. International Conference on *Nature Farming and Ecological Balance (ICNFEB)*. Haryana Agricultural University, Hissar, 7-10th March 2001.
- Gherbi, H., Nambiar –Veetil, M., Zhong, C., Félix, J., Autran, D., Girardin, R., Vaissayre, V., Auguy, F., Bogusz, D. and Franche, C. 2008. Post-transcriptional gene silencing in the root system of the actinorhizal tree *Allocasuarina verticillata*. *Molecular Plant Microbe Interactions*, 21: 518–524.
- Ghosh, M. 2009. Purification of a lectin-like antifungal protein from the medicinal herb, *Withania somnifera*. *Fitoterapia*, 80: 91–95.

- Ghosh, M. 2006. Antifungal Properties of Haem Peroxidase from *Acorus calamus*. *Annals of Botany*, 98 (6): 1145-1153.
- Ghosh, M. 2010. Purification of Peroxidase from the medicinal herb *Andrographis paniculata* causing fungal hyphal distortion. *Herba Polonica*, 56 (2) 69-78.
- Ghosh, M., Chezian, P., Sumathi, R. and Yasodha, R. 2011. Development of SCAR marker in *Casuarina equisetifolia* for species authentication. *Trees*, 25:465–472.
- Ghosh, M., Thangamani, D., Thapliyal, M., Yasodha, R., Gurumurthi, K. 2004. Purification of antifungal protein against blister bark pathogen of *Casuarina equisetifolia* J.R. Forster et G. Forster. *Acta Botanica Croatica*, 63 (2): 75- 81.
- Gurumurthi, K. and Kumar, A. 1996. Establishment of Clone bank of *Casuarina equisetifolia* for tree improvement In: Pinyopusarerk, K., Turnbull, J.W. and Midgley, S.J. (Eds.), *Recent Casuarina Research and Development*, Third International Casuarina Workshop, Da Nang, Vietnam. Published by CSIRO Australia. pp 159-161.
- Gurumurthi, K. and Rawat, P.S. 1992. Rooting of *Casuarina equisetifolia* cladode cuttings as influenced by season and auxins. *Nitrogen Fixing Tree Res. Assoc.* 10: 137-140.
- Gurumurthi, K. and Warriar, K.C.S. 2000. Four years of casuarina network in India In: *Proceedings of the 4th Annual Workshop on Casuarina* (Compiled by K. Gurumurthi). September 18, 2000. Institute of Wood Science and Technology, Bangalore. pp.7-11.
- Gurumurthi, K., Nicodemus, A., and Siddappa 2001. *Casuarina: Improvement and Utilization*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India. 218 p.
- Jacob, J.P., Ramasubramanian, N. and Naveenkumar, A. 2012. Resistance in Casuarina clones against bark feeding caterpillar *Inderbela quadrinotata* (Lepidoptera: Metarbelidae). In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A and Kumar, N.K. (Eds.), *Advances in Casuarina Research in India –Proc. of the 2nd National Seminar on Casuarina* 2012, India.
- Jayaraj, R.S.C., Nicodemus, A., Balu, Sasidharan, K.R., Savio, M.D., Bachpai, V.K.W., Ponraj, G., Hegde, R. and Kamalakannan, R. 2001. Selection Programme of *Casuarina equisetifolia* for afforestation of problem soils of Tamil Nadu, In: Rao,S.K., Sawant, S.G. and Aggarwal, P.K. (Eds.), *Forestry, Forest Products and Coastal Population*, Institute of Wood Science & Technology, Bangalore, pp 59-67.

Twenty-Five Years of Research on Casuarinas at IFGTB

- Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds). 2012. *Advances in Casuarina Research in India*, Proceedings of the 2nd National Seminar on Casuarinas, Institute of Forest Genetics and Tree Breeding Coimbatore.
- Kamalakannan, R., Barthwal, S., Chezhian, P., Balasaravanan, T., Yasodha, R., Gurumurthi, K. and Ghosh, M. 2006. Morphological and Molecular Diversity among *Casuarina* and *Allocasuarina* Species. *Biotechnology*, 5: 301-307.
- Karthikeyan, A. and Kumar, N.K. 2012. *Principles and application of Frankia*. Indian Council of Forestry Research and Education, 26p.
- Karthikeyan, A. 2011. Establishment of nodulation and nitrogen fixation in *Casuarina junghuhniana* Miq. rooted stem cuttings with *Frankia* under aseptic conditions, In: 1st Indian Forest Congress held at New Delhi from 22-25 November 2011. pp.184-185.
- Karthikeyan, A. 2013. Nodulation and Nitrogen fixation in rooted stem cuttings of *Casuarina junghuhniana* Miq. by *Frankia* inoculation. In: Velu, R.K. (Ed), Microbiological Research in Agro ecosystem management, Springer India pp289-296.
- Karthikeyan, A. and Kumar, N.K. 2012. Rehabilitation of bauxite mine spoils with beneficial microbes and fast growing tree species. In: Ang, L.H., Ho, W.M., Lee, C.T. and Sim, H.C. (Eds). *Proceedings of the International Symposium on Reclamation, Rehabilitation and Restoration Towards a Greener Asia*, 3-5 July 2012, Kulalampur Malaysia. Concept Press, Batu caves, Malaysia pp.72-82.
- Karthikeyan, A. and Kumar, N.K. 2013. *Eco Friendly Approaches for Rehabilitation of Mine Spoils*. Indian council of Forestry Research and Education, 59p.
- Karthikeyan, A. and Nepolean, P. 2011. Influence of edapho climatic factors on blister bark disease in *Casuarina equisetifolia* In: Singh, O. and Singh, V.R.R. (Eds), *Proceedings of 4th National Forestry Conference*, F.R.I. Dehradun. pp 295-299.
- Karthikeyan, A., Chandrasekaran, K., Geetha, M. and Kalaiselvi, R. 2013. Growth response of *Casuarina equisetifolia* Forst. rooted stem cuttings to *Frankia* in nursery and Field conditions *Journal of Biosciences*. 38; 741-747.
- Karthikeyan, A., Muthukumar, T. and Sureka, I. 2011. Etiology of *Trichosporium vesiculosum* Butl. causing blister bark disease in *Casuarina equisetifolia* Forst. *Plant Pathology Journal*. DOI: 10.3923/ppj/2011, 10:85-88.

- Karthikeyan, A., Sathishkumar, S.R. and Sakthivel, K.M. 2011. *Frankia* - As growth promoting source for Casuarina plants. *Agrobios*: 10; 15-16.
- Karthikeyan, A., Sathishkumar, S.R. and Sakthivel, K.M. 2010. A New technique for Casuarina rooted stem cuttings. *Science and Technology/Agriculture, The Hindu* Dt. 19.8.2010.
- Karthikeyan, A., Geetha, M. and Chandrasekaran, K. 2012. Analysis of Nitrogenase activity of *Frankia* using Acetylene reduction assay. In: *National Seminar on Forest Health Management*, IFGTB, Coimbatore on 21 & 22 March.2012. p. 143.
- Karthikeyan, A., Rajendran, P., Chandrasekaran, K. and Geetha, M. 2013. Identification of resistant clones of Casuarina blister bark disease. In: Balu, A., Jayaraj, R.S.C., Regupathy, A., Mohan, V., Warriar, R.R., Raghunath, T.P. and Krishnakumar, N. (Eds.), *Forest Health Management*. Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education. Coimbatore, pp.581-586.
- Karthikeyan, A., Savio, M.D. and Nepolean, P. 2011. Growth response of *Casuarina junghuhniana* to indigenous *Frankia*, Arbuscular mycorrhizal fungi and Phosphobacterium under nursery conditions In: In: Zhong, C., Pinyopusarerk, K., Kalinganire, A. and Franche, C. (Eds), *Improving Smallholder Livelihoods through Improved Casuarina Productivity*, Proceedings of the 4th International Casuarina Workshop, Haikou, China, 21-25 March 2010. China Forestry Publishing House, pp. 131-136.
- Karthikeyan, A., Chandrasekaran, K. and Geetha, M. 2012. *Frankia* sp CeCo1 16S Ribosomal RNA gene partial sequence. NCBI, USA., Accession No JQ 412181.
- Karthikeyan, A., Deeparaj, B. and Nepolean, P. 2009. Reforestation in Bauxite mine spoils with *Casuarina equisetifolia* Forst. and beneficial microbes. *Forests, Trees and Livelihoods*. 19: 153-165.
- Karthikeyan, A., Sathishkumar, S.R and Sakthive, K.M. 2011. Nodulation in *Casuarina equisetifolia* rooted stem cuttings by inoculation of *Frankia*. *Indian Forester*: 1130-1131.
- Krishnakumar, N., Jainalaudeen, A. and Warriar, K.C.S. 2011. Casuarina as bioshields: need for salt tolerant genotypes. In Jayaraj, R.S.C., Warriar, R.R. and Nicodemus A.(Eds) *Proceedings of the 2nd National Seminar on Casuarina*, 3-4 March 2011, Institute of Forest Genetics and Tree Breeding, Coimbatore, pp 54.

- Krishnakumar, N. and Karthikeyan, A. 2013. Frankia for improving productivity of *Casuarina equisetifolia* Forst. In: 17th International conference on Frankneae and Actinorhizal plants (April 10-12 2013) Shillong. Abstract p.16.
- Kumar, A. 1995. Genetic Improvement of *Casuarina equisetifolia*. Doctoral Thesis. Forest Research Institute Deemed University, Dehra Dun, India, 169 p.
- Kumar, A. and Gurumurthi, K. 1996. Path Coefficient studies on morphological traits in *Casuarina equisetifolia*. *Indian Forester*. 122 (8): 727-730.
- Kumar, A. and Gurumurthi, K. 1996. Selection method for identifying superior performers of *Casuarina equisetifolia* In: Pinyopusarerk, K., Turnbull, J.W. and Midgley, S.J. (Eds.), *Recent Casuarina Research and Development*, Third International Casuarina Workshop, Da Nang, Vietnam. Published by CSIRO Australia.pp 131-135.
- Kumar, A. and Gurumurthi, K. 1998. Genetic assessment of clonal material of *Casuarina equisetifolia*. *Indian Forester* . 124 (3): 237-242.
- Kumar, A. and Gurumurthi, K. 1999. Effect of Frankia on growth and nodulation in *Casuarina equisetifolia*. *Indian Forester*. 125 (5): 490-495.
- Kumar, A. and Gurumurthi, K. 2000. A technique to transport cladode cuttings of *Casuarina equisetifolia* for cloning. *Indian Forester*. 126 (8): 901-902.
- Kumar, A. and Gurumurthi, K. 2000. Genetic divergence studies on clonal performance of *Casuarina equisetifolia*. *Silvae Genetica* 49 (2): 57-60.
- Kumar, A. and Gurumurthi, K. and Patel M. 1996. Pulp and Paper making properties of fifteen clones of *Casuarina equisetifolia*. *IPPTA*. 8 (4): 13-17.
- Kumar, A., Jayachandran, C.K., Venkataramanan, K.S., Ganesan, M., Balasubramanian, A., and Gurumurthi, K. 1994. Production of high yielding varieties of *Casuarina equisetifolia* ICFRE Technical Bulletin. p.11.
- Kumar, A., Verma, R.K. and Gurumurthi, K. 1993. Selection method for Vegetative Propagules Production in *Casuarina equisetifolia*. In: Vivekanandan, K., Subramanian, K.N., Jabala, N.Q. and Gurumurthi, K. (Eds.), *Proceedings of Workshop on Production of Genetically Improved Planting Material for Afforestation Programmes*, 18-25 June, Coimbatore. Food and Agriculture Organization of United Nations Los Banos, Philippines. pp 189-195.

- Mahadevan, N.P., Sivakumar, V. and Singh, B.G. 1999. Relationship of Cone and Seed Traits on Progeny Growth Performance in *Casuarina equisetifolia* Forst. & Forst. F. *Silvae Genetica* 48, 6:273-277.
- Mohan, V. 2008. Efficacy of Mycorrhizal Fungi and Bio-Control agent on Management of blister bark and root-rot diseases of *Casuarina equisetifolia* and *C. junghuhniana* in the National Seminar on Advances in Plant Pathology for Sustainable Agriculture” held at TNAU, Coimbatore on 24th and 25th November, 2008.
- Mohan, V. and Manokaran, P. 2012. Studies on blister bark or stem wilt disease of *Casuarina equisetifolia* in south India. In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds), *Advances in Casuarina Research in India*. Proceedings of the 2nd National Seminar on Casuarinas. IFGTB, Coimbatore, Tamil Nadu, India. pp. 95-104.
- Mohan, V., Manimekalai, M. and Manokaran, P. 2007. Status of Arbuscular Mycorrhizal (AM) association in seedlings of important tree species in Tamil Nadu. *Kavaka*. 35: 45-50.
- Mohan, V., Narayanan, C. and Manokaran, P. 2002. *Lasiodiplodia* Collar-Rot Disease on *Casuarina junghuhniana* MIQ. – A New Record. *Indian Forester*. 128: No. 9. 1045-1046.
- Mohan, V., Vinodhini, R.K. and Gayathiri, P.S. 2012. Status of pathological problems of *Casuarina* spp. and their management. In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds), *Advances in Casuarina Research in India*. Proceedings of the 2nd National Seminar on Casuarinas. IFGTB, Coimbatore, Tamil Nadu, India. pp.173-192.
- Munusamy, S., Mohan, V. and Kumar, S.R. 2010. Studies on beneficial microflora and their effect on growth improvement of *Casuarina* species. *Journal of Pure and Applied Microbiology*. 4(1): 125-138.
- Murugesan, S., Sumathi, R., Senthilkumar, N., Rajeshkannan, C. and Karthikeyan, A. 2012. Bio transformation of some secondary metabolites of Frankia – *Casuarina* relationship. In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds), *Advances in Casuarina Research in India*. Proceedings of the 2nd National Seminar on Casuarinas. IFGTB, Coimbatore, Tamil Nadu, India. pp 271-276.

- Murugesan, S., Sumathi, R., Karthikeyan, A., Senthilkumar, N. and Rajeshkannan, C. 2012. Biotransformation of some secondary metabolites of Frankia - Casuarina relationship, In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N,K. (Eds), *Advances in Casuarina Research in India*. Proceedings of the 2nd National Seminar on Casuarinas. IFGTB, Coimbatore, Tamil Nadu, India. 283-288.
- Muthukumar,T., Karthikeyan, A. and Udaiyan, K. 2012. Evaluation of the growth and nutrient uptake of Casuarina equisetifolia on Alfisol soil inoculated with different arbuscular mycorrhizal fungi In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N,K. (Eds), *Advances in Casuarina Research in India*. Proceedings of the 2nd National Seminar on Casuarinas. IFGTB, Coimbatore, Tamil Nadu, India. pp. 47-53.
- Nambiar-Veetil, M., Prabhu, J.S., Roopesh, M., Selvakesavan, R.K., Bachpai, V.K.W., Balasubramanian, A., Venkatachalam, R., Jayaraj, R.S.C., Franche,C. Gherbi,H. Swistoonoff,S., Florence, A., Hocher, V., Bogusz, D. and Vivekanandan, R. 2010. Development of an *In silico* Gene Bank for Plant Abiotic stresses: towards its utilization for molecular analysis of salt tolerant and susceptible *Casuarina equisetifolia* clones, In Proceedings of the 4th International *Casuarina* Meeting on Improving smallholder livelihoods through improved *casuarina* productivity, 22-25 March 2010, Haikou, China, pp 144-151.
- Nambiar -Veetil, M., Vaissayre, V., Svistoonoff, S., Zhong, C., Hocher, V., Auguy, F., Franche, C., Bogusz, D. and Gherbi, H. 2008. Study of early events in actinorhizal symbiosis: isolation and functional analysis of *CCaMK* gene from *Casuarina glauca*. In 15th International Frankia and Actinorhizal Plants Meeting, Argentina, October 19-23, 2008.
- Narayanan, C. and Nicodemus, A. 2005. Incidence of wilt (blister-bark) disease of *Casuarina junghuhniana* in India. *Indian Forester*, 131:257-258.
- Narayanan, C., Sasidharan, K.R. and Sampath Kumar, A.S. 1998. Occurrence of blister bark disease in *Casuarina* hybrid (*C.equisetifolia* x *C.junghuhniana*) *Indian For.* 124 (2): 167-168.
- Nicodemus, A. 2007. Genetic improvement of *Casuarina equisetifolia* through selection and breeding. *ENVIS Forestry Bulletin*, 7(1):91-99.

- Nicodemus, A. 2009. *Casuarina – A Guide for Cultivation*. Institute of Forest Genetics and Tree Breeding (Indian Council of Forestry Research and Education) Coimbatore, India, 16 p.
- Nicodemus, A., Kannan, K., Christi Sagariya, Y., Pauldasan, A., Karthikeyan, A. and B. Gurudev Singh 2013. Variation in blister bark disease incidence among *Casuarina equisetifolia*, *C. junghuhniana* and their interspecific hybrids and its implications for disease resistance breeding In: Balu, A., Jayaraj, R.S.C., Regupathy, A., Mohan, V., Warriar, R.R., Raghunath, T.P. and Krishnakumar, N. (Eds.). *Forest Health Management*. Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education. Coimbatore, pp.552-563.
- Nicodemus, A., Jayaraj R.S.C. and Bennett, S.S.R. 1996. A National level breeding programme for *Casuarina equisetifolia*. In: Proc. VII Regional meeting of Silviculturists and Forestry Research workers of Central region, Bhubaneshwar, Feb. 6-8, 1996. p 15.
- Nicodemus, A., Varghese, and Nagarajan, B. 2001. Genetic improvement of *Casuarina equisetifolia* through selection and breeding. In: Gurumurthi, K., Nicodemus, A. and Siddappa (Eds.), *Casuarina Improvement and Utilization*, Institute of Forest Genetics and Tree Breeding, Coimbatore, India. pp 3-13.
- Nicodemus, A., Varghese, M. and Nagarajan, B. 2003. Coppicing ability of *Casuarina junghuhniana* Miq. provenances. *My forest*, 39(4):359-363.
- Nicodemus, A., Varghese, M., and Nagarajan, B. 2005. Selection of *Casuarina junghuhniana* Miq. provenances for multiple end uses in India. In: Tewari, V.P. and Srivastava, R.L. (Eds.), *Multipurpose trees in the tropics: management and improvement strategies*. Proceedings of the IUFRO international conferences held between 22-25 November 2004 at Jodhpur, India. Arid Forest Research Institute, Jodhpur, India. pp. 650-657.
- Nicodemus, A., Varghese, M., Narayanan, C., Nagarajan, B. and Chaudhri, K.K. 1996. Variation in growth and wood traits among *Casuarina equisetifolia* provenances. In: Pinyopusarerk, K., Turnbull, J.W., Midgley, S.J. (Eds.), *Recent Casuarina Research and Development* Proc. Third International Casuarina Workshop, Da Nang, Vietnam, 4-7, March 1996. pp 136-142.
- Perrine-Walker, F., Gherbi, H., Imanishi, L., Hocher, V., Ghodhbane-Gtari, F., Lavenus, J., Benabdoun, M., Nambiar –Veetil, M., and Svistoonoff, S. and Laplaze, L. 2011.

Twenty-Five Years of Research on Casuarinas at IFGTB

- Symbiotic signaling in actinorhizal symbioses. *Current Protein and Peptide Science*, 12: 156-164.
- Pillai, M.S.R., Rajarishi, R. and Khan, S.A.M. 1999. A new Lymantrid defoliator of *Casuarina equisetifolia*. *Indian Journal of Forestry*, 22(4) 381-383.
- Ponnambalam, A., Sugavanam, V., Devaraj, P. and Jayaraj, R.S.C. 1998. Growth response of *Acacia auriculiformis* and *Casuarina equisetifolia* in quartz sand dumps from cement factories. *Journal of Arid Land Studies*, 7S:315-318.
- Radha, V., Sumathi, R., Yasodha, R., Mohan, V. and Dasgupta, M. 2008. Identification of defense – related transcripts in *Casuarina equisetifolia* during pathogenesis. In: National Symposium on Plant Biotechnology for Conservation, Characterization and Crop Improvement & 29th Annual Meeting of Plant Tissue Culture Association (India). Department of Biotechnology, Mohanlal Sukhadia University, Udaipur, India. pp. 105.
- Radha, V., Yasodha, R., Mohan, V. and Dasgupta, M. 2009. Expression profiling *Casuarina equisetifolia* response to *Trichosporium vesiculosum* toxin reveals up – regulation of defense related and signaling genes in pathogen defense. In: *IV National Forestry Conference*, Forest Research Institute, Dehra Dun, India. pp. 57-58.
- Radha, V., Yasodha, R., Mohan, V. and Dasgupta, M. 2009. Transcriptome analysis of *Casuarina equisetifolia* during pathogen elicitation. In: *National Seminar on Frontiers in Biotechnology*, 22-24 July, 2009 held at Bharathiar University, Coimbatore, Tamil Nadu, India. pp.25.
- Ramakishnan, M., Shanthi, A., Cesar, S.A., Melvin., Daniel, A and Ignacimuthu, S. 2011. Genetic pairwise distance matrix analysis in the clones of *Casuarina equisetifolia* using RAPD markers. *Elixir BioTech*. 38: 4143-4149.
- Rasmi, R.R., Daniel, A., Shanthi, A and Suguna, P. 2011. Analysis of genetic relationships in superior individuals of *Casuarina equisetifolia* L. using ISSR markers. *International Journal of Integrative Biology*, 10(2): 82-85.
- Santhi, S., Sumathi, R., Murugesan, S., Karthikeyan, A., Senthilkumar, N., Manivachagam, P., Rajeshkannan, C., Monisha, M. and. Vijayalakshmi., K.B. 2012. Impact of Bio chemical signals of *Frankia* on *Casuarina equisetifolia* improvement In: National Seminar on Forest Health Management held at IFGTB, Coimbatore on 21 & 22 March.2012. Abstract p. 153.

- Santhi, S., Sumathi, R., Rajeshkannan, C., Manivachakam, P. and Murugesan, S. 2012. Profiling metabolites in different day cultures of a root endophyte, *Frankia Brunchorst* from *Casuarina equisetifolia* L. using GC-MS-MS, *European Journal of Experimental Biology*, 2 (3):539-542.
- Sasidharan K.R. and Deeparaj, B. 1997. Severe Cottony Cushion Scale attack on *Casuarina*. *Sylva plus* 5 (2).
- Sasidharan, K. R. and Varma, R.V. 2007. Field evaluation of *Beauveria bassiana* (Balsamo) Vuillemin, Neem Seed Kernel Extract and Neem oil against the bark caterpillar, *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae). *Annals of Forestry* 15 (1):101-105.
- Sasidharan, K. R. and Varma, R.V. 2008. Insects associated with nurseries and plantations of *Casuarina equisetifolia* L. in Tamil Nadu, India. *Indian Journal of Forestry* 31 (3): 389-393.
- Sasidharan, K. R. and Varma, R.V. 2008. Seasonal population variations of the bark eating caterpillar (*Indarbela quadrinotata*) in *Casuarina* plantations of Tamil Nadu. *Tropical Ecology* 49 (1): 79 - 83.
- Sasidharan, K. R., Balu, A., Deeparaj, B., Nicodemus, A. and Varma, R.V. 2005. Screening *Casuarina equisetifolia* provenances against the bark caterpillar, *Indarbela quadrinotata* and possible biochemical factors determining resistance. *Journal of Tropical Forest Science* 17 (4): 625 – 630.
- Sasidharan, K. R., Varma, R. V. and Sivaram, M. 2010. Impact of *Indarbela quadrinotata* on the growth of *Casuarina equisetifolia*. *Indian For.* 136 (2):182-186.
- Sasidharan, K.R. and Ramesh, G. 2013. Laboratory evaluation of the fungus, *Beauveria bronginartii*, oils of *Jatropha curcas* and *Pongamia pinnata* against the bark eating caterpillar, *Indarbela quadrinotata*. *J. Ecobiol.* 32 (1) 69-75.
- Sasidharan, K.R. and Varma, R.V. 2005. Laboratory evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Indarbela quadrinotata* Walker (Lepidoptera : Metarbelidae) – a key pest of *Casuarina equisetifolia* L. in Tamil Nadu. *J. Biol. Control.* 19 (2): 197 –200.
- Sasidharan, K.R., Balu, A., Deeparaj, B. and Varma, R.V. 2007. Screening of *Casuarina* seed sources for pest resistance and the role of bark chemistry in conditioning the resistance. *Journal of Ecobiology* 21 (2): 157 -162.

- Selvakesavan, R. K., Nair, D.N. Abraham, S.M. Jayaraj, R.S.C., Balasubramanian A., Deeparaj, B., Venkatachalam, R., Rani, S.K.S., Hassen, G., Valérie, H., Claudine, F., Didier, B. and Nambiar –Veetil, M.. 2011. Identification of *Casuarina equisetifolia* clones contrasting for their salt stress tolerance and sodium uptake, 43: In proceedings of the 2nd National Seminar on *Casuarinas*, 3-4 March 2011, Institute of Forest Genetics and Tree Breeding, Coimbatore.
- Shanthi, A., Gunasekaran, T., Manjula, S., Suguna, P and Geetha, M. 2010. DNA polymorphism in two different genetic resources of *Casuarina equisetifolia*. Abstracts of National seminar on Tropical Ecosystems: Structure, Function and Services. (TESFES). held at IFGTB on 28 & 29th Dec. pp.80.
- Shanthi, A., Suguna, P., Dasgupta, M., Warriar, R.R. and Geetha, M. 2011. Gender identification in *Casuarina equisetifolia*. *Elixir Applied Botany* 41: 5912-5913.
- Shanthi, A., Suguna, P., Geetha, M and Bachpai, V. K. W. 2011. Wood proximate and DNA marker analyses in *Casuarina equisetifolia*. In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds) *Advances in Casuarina Research in India*, Proceedings of the 2nd National Seminar on Casuarinas, Institute of Forest Genetics and Tree Breeding Coimbatore, pp-227-233.
- Shanthi., A., Daniel., A., Dasgupta, M., Yasodha, R and Ramya, M. 2013. Development of new Microsatellite markers in *Casuarina equisetifolia*. In: Dasgupta, M., Yasodha, R. and Warriar R.R. (Eds). Proceedings of National seminar on Tree Biotechnology. Institute of Forest Genetics and Tree Breeding, Coimbatore.
- Singh, R., Sasidharan, K.R. and Salarkhan, A.M. 1997. Blister bark disease- A serious threat to *Casuarina equisetifolia* cultivation in Ramanathapuram and Rameshwaram areas (Tamil Nadu) and strategies for disease management. *Sylva plus* 5: 25-26.
- Singh, R., Sasidharan, K.R., Salarkhan, A.M. and Mahalakshmi, R. 2001. *Batocera rufomaculata* (Coleoptera: Cerambycidae) – A new insect record on *Casuarina equisetifolia* L. in India. *Indian For.* 127 (6): 723-724.
- Sivakumar, V., Anandalakshmi, R., Warriar, R.R. Singh, B.G., Tigabu, M. and Ode'n, P.C. 2007. Petroleum flotation technique upgrades the germinability of *Casuarina equisetifolia* seed lots. *New Forests* (2007) 34:281–291.

- Subramanian, K.N., Bedell, P.E., Gurumurthi, K., George, M., Mandal, A.K., Pillai, S.R.M. and Singh, B.G. 1992. *Casuarinas: Trees of Multiple Utility*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India, 115 p.
- Suguna, P., Shanthi, A., Warriar, R.R. and Geetha, M. 2011. Study of Different Isozymes in *Casuarina equisetifolia*. In: Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds) *Advances in Casuarina Research in India*, Proceedings of the 2nd National Seminar on Casuarinas, Institute of Forest Genetics and Tree Breeding Coimbatore, pp-235-237.
- Suvega, T., Hemalatha, V., Radha, V. and Ghosh, M. 2009. Variation of phenylalanine ammonia lyase and phenol content in two species of *Casuarina* during salicylic acid signalling. *Journal of Genetics and Evolution* II: 7-19.
- Svistoonoff, S., Benabdoun, F.M., Nambiar –Veetil, M., Imanishi, L. and Vaissayre, V., et al. 2013. The Independent acquisition of plant root nitrogen-fixing symbiosis in Fabids recruited the same genetic pathway for nodule organogenesis. *Public Library of Science ONE*, 8(5): e64515. doi:10.1371/journal.pone.0064515.
- Svistoonoff, S., Gherbi, H., Nambiar –Veetil, M., Zhong, C., Michalak, Z., Laplaze, L., Vayssaire, V., Auguy, F., Hoher, V., Dumas, P., Bonneau, J., Bogusz, D. and Franche, C. 2009. Contribution of transgenic *Casuarinaceae* to knowledge of the actinorhizal symbiosis. *Symbiosis*, 50: 3-11.
- Thushara P., Abraham, S.M. Selvakesavan, R.K., Aravindhakumar, V., Balasubramanian, A., Sangeetha, M., Sowmiyarani, K.S., Venkatachalam, R. and Nambiar –Veetil, M. 2011. Effect of salt stress on growth and proline levels in tolerant and susceptible clones of *Casuarina equisetifolia*. In Conference proceeding of the Indian Forestry Congress (IFC-2011), 22 -25 November 2011, New Delhi, pp 239-240.
- Tripathi, S. B., Nambiar –Veetil, M., and Gurumurthi, K. 2001. Changes in protein profiles in *Casuarina equisetifolia* during salt stress. 126- 134: In Proceedings of the 5th Annual workshop on *Casuarina*, 8-9 October 2001, Regional Forest Research Center, Rajahmundry.
- Varghese, M. and Nicodemus, A. 2001. Fast growing and high yielding tree cultivars of *Casuarina*. In: Proc. of a workshop on *Fast Growing and High Yielding Selected Tree Cultivars for Southern States*. Regional Centre, National Afforestation and Eco-Development Board, Bangalore.

- Varghese, M. and Sivaramakrishna, D. 1996. Wood properties of *Casuarina equisetifolia* in relation to ecological factors. In: Pinyopusarerk, K., Turnbull, J.W., Midgley, S.J. (Eds.), *Recent Casuarina Research and Development* Proc. Third International Casuarina Workshop, Da Nang, Vietnam, 4-7, March 1996. pp 218-222.
- Varghese, M., Lindgren, D. and Nicodemus, A. 2004. Fertility and effective population size in seedling seed orchards of *Casuarina equisetifolia* and *C. junghuhniana*. *Silvae Genetica*, 53(4-5): 164-168.
- Varghese, M., Nicodemus, A., Gothandam, K.M., Kannan, R., Hegde, R. and Siddappa. 1999. Evaluation of a one parent progeny trial of *Casuarina equisetifolia* using incomplete analysis. In: the National Symposium on *Forestry Towards 21st Century*. Tamil Nadu Agrl. University, 27-28 September, 1999.
- Varghese, M., Nicodemus, A., Nagarajan, B. and Palanisamy, K. 2002. Selection and Breeding of multipurpose tree species for Agroforestry. In: National Workshop on Agroforestry- Prospects and Challenges held at IFGTB, Coimbatore, on November 22, 2002.
- Veluthakkal, R. and Dasgupta, M. 2012. Isolation and characterization of pathogen defence-related class I chitinase from the actinorhizal tree *Casuarina equisetifolia*. *Forest Pathology*, 42: 467–480.
- Venkataraman, K.S., Warriar, K.C.S., Ajith Kumar, K.G. and Gurumurthi, K. 1998. Difference in rooting response between the juvenile and adult tissues of *Casuarina equisetifolia* Forst. *Ann. For.* 6 (2): 156-158.
- Viswanath, S. 2003. Growing 'casuarinas' in Southern India. *Asia Pacific Agroforestry News* 21: 12-14.
- Viswanath, S., George, M. and Manivachakam, P. 2001. *Casuarina equisetifolia* in agroforestry practices in Tamil Nadu: Opportunities and Limitations. In: Proceedings of the *Fifth Annual Workshop of Casuarina Network*. IFGTB, pp 202-206.
- Viswanath, S., Peddappaiah, R.S., Subramoniam, V., Manivachakam, P. and George, M. 2003. Canopy and Root Management in *Casuarina equisetifolia* for Optimizing Performance in Wide-row Intercropping Systems in Tamil Nadu. In *Proceedings of National Symposium on Agroforestry & Sustainable Production*. NCRA, Jhansi. pp 60-62.

- Warrier, K.C.S., Venkataramanan, K.S., Kumar, A.K.G., Warrier, R.R. and Gurumurthi, K. 2001. Variability studies in *Casuarina equisetifolia* Forst. In: *Proceedings of the 5th Annual Workshop on Casuarina* (Ed. K. Gurumurthi). October 8-9 2001 at Regional Forest Research Centre, Rajahmundry, Andhra Pradesh. Institute of Forest Genetics and Tree Breeding, Coimbatore. pp.31-47.
- Warrier, K.C.S. 2013. Clonal technology in Casuarina. In: Dasgupta, M. and Warrier, R.R. (Eds). *Nursery and Clonal Technology*, Training Manual. Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education. Coimbatore, pp 34-36.
- Warrier, K.C.S. 2009. A note on the correct nomenclature of *Casuarina equisetifolia*. *Indian Forester* 135(5):721-722.
- Warrier, K.C.S. 2010. Improving productivity of casuarina through clonal forestry. In: Palanisamy, K. and Kumar, N.K. (Eds), *Training Manual on Clonal Forestry*, Institute of Forest Genetics and Tree Breeding, Coimbatore, pp. 22-30.
- Warrier, K.C.S. 2011. Clonal forestry. In: Warrier, K.C.S., Sivakumar, V., Gurudev Singh B. and Kumar, N.K. 2011 (Eds.), *Planting Stock Improvement*, Training Manual. Institute of Forest Genetics and Tree Breeding, Coimbatore, pp 127-136.
- Warrier, K.C.S. 2011. Developing Cloning Techniques for Raising High Yielding Clonal Plantation of *Casuarina equisetifolia* L. Project Completion Report. Institute of Forest Genetics and Tree Breeding. Indian Council of Forestry Research and Education. Coimbatore, 64p.
- Warrier, K.C.S. 2013. Clonal forestry. In: Palanisamy, K., Savio, M.D. and Kumar, N.K. (Eds), *One Week Training Course on Forest Genetic Resource Management for Indian Forest Service Officers*. Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education. Coimbatore, pp 116-123.
- Warrier, K.C.S. and Ganesan, M. 2010. Early growth comparison between seedlings and clones of *Casuarina equisetifolia* L. *Indian Forester* 136(7): 877-883.
- Warrier, K.C.S. and Gurudev Singh, B. 2010. Selection of genetically divergent clones of *Casuarina equisetifolia* for further breeding programmes. *Silvae Genetica* 59(5): 242-248.

- Warrier, K.C.S. and Gurumurthi, K. 2005. Age-age correlation studies in clones of *Casuarina equisetifolia* Forst. Accepted for presentation in the XXII IUFRO World Congress, Brisbane, Australia 8-13 August 2005.
- Warrier, K.C.S. and Singh, B.G. 2013. Stability analysis in clones of *Casuarina equisetifolia*. In: Chauhan, J.A., Singh, C., Singh, R. and Mamgain, S.P. (Eds), Compendium of the First Indian Forest Congress, November 22-25 2011, Volume II. Indian Council of Forestry Research and Education, Dehradun, pp. 367-375.
- Warrier, K.C.S. and Venkataramanan, K.S. 2003. Screening for salt tolerance in clones of *Casuarina equisetifolia* In: Abstracts, National Seminar on Management of Degraded Forests for Productivity Enhancement and Carbon Sink Expansion. Tropical Forest Research Institute (Indian Council of Forestry Research and Education), Jabalpur. January 15-16, 2003.
- Warrier, K.C.S. and Venkataramanan, K.S. 2004. Casuarina for rehabilitation of salt affected areas. In: Ray, M. and Panwar, V.P. (Eds.), Abstracts (National Seminar on Rehabilitation of Lands under Anthropogenic Stress and Degradation. Institute of Forest Productivity (Indian Council of Forestry Research and Education), Ranchi. January 20, 2004. pp.26.
- Warrier, K.C.S. and Venkataramanan, K.S. 2005. Selection of clones of *Casuarina equisetifolia* for agroforestry in semi-arid locations. In: Abstracts, Workshop on Agroforestry for Attappady Wastelands: Potential and Prospects. January 8-9, Attappady Hills Area Development Society, Agali, Palakkad, Kerala, pp 9.
- Warrier, K.C.S. and Venkataramanan, K.S. 2011. Suitable Clones of *Casuarina equisetifolia* for Sodic Soils in India. In: Zhong, C., Pinyopusarerk, K., Kalinganire, A. and Franche, C. (Eds), Improving Smallholder Livelihoods through Improved Casuarina Productivity, Proceedings of the 4th International Casuarina Workshop, Haikou, China, 21-25 March 2010. China Forestry Publishing House, pp. 194-198.
- Warrier, K.C.S. and Venkataramanan, K.S. 2012. Clonal propagation of *Casuarina* and *Thespesia*. In Venkataramanan, K.S. and Krishnakumar, N. (Eds.), Training Manual on Role of Micro and Macro Propagation for Large Scale Production of Quality Planting Stock with Focus on Economically Important Tree Species. Institute of Forest Genetics and Tree Breeding, Coimbatore, pp 41-42.

- Warrier, K.C.S. Suganthi, A., Subramani, S.P. and Singh, B.G. 2013. Clones of *Casuarina equisetifolia* for improving soil health in salt affected area. In: Balu, A., Jayaraj, R.S.C., Regupathy, A., Mohan, V., Warrier, R.R., Raghunath, T.P. and Krishnakumar, N. (Eds.). *Forest Health Management*. Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education. Coimbatore, pp 77-83.
- Warrier, K.C.S., Ajith Kumar, K.G., Warrier, R.R. and Gurumurthi, K. 2000. Change in sex expression in clones of *Casuarina equisetifolia* Forst. In: Jandl, R., Devall, M., Khorchidi, M., Schimpf, E., Wolfrum, G. and Krishnapillay, B. (Eds.), *Forests and Society: The Role of Research, Poster Abstracts Volume III XXI IUFRO World Congress*, Kuala Lumpur, Malaysia, 7-12 August, 2000 pp 71.
- Warrier, K.C.S., Anandalakshmi, R. and Warrier, R.R. 2006. Biochemical indicators for juvenility in *Casuarina equisetifolia* Forst. In Abstracts: APSI Scientists Meet 2006 – National Conference on Plant Sciences Research and Development, PSG College of Arts and Science, Coimbatore, 11-12 January 2006.
- Warrier, K.C.S., Anandalakshmi, R., Warrier, R.R. and Singh, B.G. 2013. Differential expression of biochemicals with reference to the degree of juvenility in *Casuarina equisetifolia*. *International Journal of Advanced Life Sciences*, 6(3):216-224.
- Warrier, K.C.S., Ganesan, M. and Venkataramanan, K.S. 2007. Gas exchange characteristics in *Casuarina* clones. *Indian J. Plant Physiol.* 12(1): 83-87.
- Warrier, K.C.S., Gurumurthi, K., Barthwal, S., Warrier, R.R. and Venkataramanan, K.S. 2005. *Variability Studies with Special Emphasis on Physiology, Biometry and Biochemistry in Selected Tree Species for Tree Improvement*. Project Completion Report. Institute of Forest Genetics and Tree Breeding (Indian Council of Forestry Research and Education), Coimbatore, India, 130 p.
- Warrier, K.C.S., Kumar, A. and Balasubramanian, A. 2004. Assessment of clones of *Casuarina equisetifolia* Forst. using point grading method. In: Souvenir cum Abstracts, IUFRO International Workshop on Multipurpose Trees in the Tropics: Assessment, Growth and Management. November 22-25, Arid Forest Research Institute (Indian Council of Forestry Research and Education), Jodhpur, India, pp 266.
- Warrier, K.C.S., Kumar, A.K.G., Warrier, R.R. and Gurumurthi, K. 2000. Studies on *Casuarina equisetifolia* Forst with reference to salt stress, experimental clonal seed orchard and sex expression. In: *Proceedings of the 4th Annual Workshop on Casuarina*

Twenty-Five Years of Research on Casuarinas at IFGTB

- (Compiled by K. Gurumurthi). September 18, 2000. Institute of Wood Science and Technology, Bangalore. pp.53-58.
- Warrier, K.C.S., Kumar, A.K.G., Warrier, R.R. and Gurumurthi, K. 2001. Studies on sexual variation in clones of *Casuarina equisetifolia* Forst. *Indian Forester* 127 (8): 865-870.
- Warrier, K.C.S., Suganthi, A. and Gurudevsingh, B. 2012. Cloning techniques for enhancing growth rate in *Casuarina equisetifolia*. In Jayaraj, R.S.C., Warrier, R.R., Nicodemus, A. and Kumar, N.K. (Eds) *Advances in Casuarina Research in India*, Proceedings of the 2nd National Seminar on Casuarinas, Institute of Forest Genetics and Tree Breeding Coimbatore, pp 61-64.
- Warrier, K.C.S., Suganthi, A. and Singh, B.G. 2013. A new record of abnormal phylloclad modification in *Casuarina equisetifolia*. *International Journal of Agricultural Science Research* 2 (1): 8-11.
- Warrier, K.C.S., Suganthi, A. and Subramani, S.P. 2012. Genetic variability in clones of *Casuarina equisetifolia* L. In: Jayaraj, R.S.C., Warrier, R.R., Nicodemus, A. and Kumar, N.K. (Eds), *Advances in Casuarina Research in India*, Proceedings of the 2nd National Seminar on Casuarinas, Institute of Forest Genetics and Tree Breeding Coimbatore, pp 231-237.
- Warrier, K.C.S., Venkataramanan, K.S., Kumar, A. and Gurumurthi, K. 2001. Clonal seed orchards for quality seed production in *Casuarina equisetifolia*. In: Gurumurthi, K., Nicodemus, A., and Siddappa (Eds.), *Casuarina: Improvement and Utilization*. Institute of Forest Genetics and Tree Breeding, Coimbatore, India pp 21-28.
- Warrier, K.C.S., Warrier, R.R., Singh, N.G. and Kumar, N.K. 2012. Special purpose seed orchards – A viable option to enhance productivity of wastelands. In: Balaji, S., Sarcar, M.K., Upreti, A., Kumaran, K., Pradmod, P. and Chandrasekaran, G. (Eds.), TNFA Centenary National Seminar on Forestry Vision 2023, Abstracts. 5-6 July 2012, Tamil Nadu Forest Academy, Coimbatore, pp 57.
- Warrier, R.R., Warrier, K.C.S. and Gurumurthi, K. 1999. Standardisation of protocol for isolation of protoplasts in *Casuarina equisetifolia* Forst. National Conference on Biotechnology: Hitherto and Henceforth. December 28-30, 1999, The American College, Madurai.

- Yasodha R, Kathirvel,M., Sumathi, R., Gurumurthi,K. and Nagaraju, J. 2004. Clonal identification of *Casuarina equisetifolia* using DNA polymorphisms generated by PCR with arbitrary primers. IUFRO NFT News, Working party 2.08.02, 7: 5-6.
- Yasodha R, Kathirvel,M., Sumathi, R., Gurumurthi,K. and Nagaraju, J. 2004. Genetic analyses of Casuarinas using ISSR-PCR and FISSR-PCR markers. *Genetica*, 122 : 161-172.
- Yasodha,R., Sumathi, R., Ravi, N. and Gurumurthi, K. 1999. Optimisation of RAPD assay in *Casuarina equisetifolia*. *Indian Journal of Plant Physiology*, 4: 58-60.

CASUARINA RESEARCH TEAM

Anandalakshmi, R	Jesubalan, D	Natarajan, P
Ashok Kumar	John Prasanth Jacob	Naveenkumar, A
Bachpai, V.K.W	Johnson Prabhu, S	Nepolean, P
Balasaravanan, T	Kamalakaran, R	Nicodemus, A
Balasubramanian, A	Kannan C.S. Warriar	Pauldasan, A.
Balu, A	Kannan, K	Ponraj, G
Beula, R	Karpaga Raja Sundari, B	Radha Veluthakkal
Buvaneswaran, C	Karthikeyan, A	Rajagopal, K
Chezhian, P	Krishna Kumar, N	Rajeshkannan, C
Christi Sagaria, Y	Lalitha, S	Ramasubramanian, N
David Raja	Madhavan Pillai, S.R	Rangarajan, R.
Deeparaj, B	Mahadevan, N.P	Ravi, R
Dhanya N. Nair	Manivachakam, P	Rekha R. Warriar
Durai, A	Manokaran, P	Roopesh, M
Edwin Raj, E	Maria Dominic Savio, M	Sangeetha, M
Ganesan, M	Masilamani, P	Santan Barthwal
George, M	Mathish Nambiar-Veetil	Sanu M. Abraham
Gopi, K.C	Modhumita Dasgupta	Saravanan, S
Gurudev Singh, B	Mohan Varghese	Selvakesavan, R.K
Gurumurthi, K	Murugesan, S	Senthilkumar, N
Hemalatha, V	Nagarajan, B	Shanthi, A
Jayaraj, R.S.C	Narayanan, C	Shashi Bhushan Tripathi

Twenty-Five Years of Research on Casuarinas at IFGTB

Shelke, G.R	Suresh Babu, D	Venkataramanan, K.S
Sivakumar, V	Sushamani, K	Vijayam, C.V
Sowmiya Rani, K.S	Suvega, T	Vinothkumar, K
Subramani, S.P	Thangamani, D	Viswanath, S
Suganthi, A	Thushara, P	Yasodha, R
Suguna, P	Velumani, R	
Sumathi, R	Venkatachalam, R	

ANNEXURES

Annexure - I

Yield Table for stem wood of *Casuarina equisetifolia* on green weight basis (kg tree⁻¹)

Best-fit equation used for construction of table: $y = 3.187 X^{1.013}$

DBH (cm)	Stem wood biomass (kg tree ⁻¹)										
	Height (m)										
	5	6	7	8	9	10	11	12	13	14	15
3	1.42	1.71	2.00	2.29	2.57	2.86	3.16	3.45	3.74	4.03	4.32
4	2.54	3.06	3.58	4.09	4.61	5.13	5.65	6.17	6.69	7.21	7.74
5	4.00	4.81	5.62	6.43	7.25	8.06	8.88	9.70	10.52	11.34	12.16
6	5.78	6.95	8.13	9.31	10.49	11.67	12.85	14.03	15.22	16.4	17.59
7	7.90	9.50	11.11	12.72	14.33	15.94	17.59	19.18	20.88	22.42	24.04
8	10.35	12.45	14.56	16.67	17.78	20.89	23.01	25.13	27.26	29.38	31.51
9	13.14	15.81	18.48	21.16	23.84	26.53	29.22	31.91	34.60	37.30	40.00
10	16.27	19.57	22.88	26.20	29.51	32.84	36.17	39.50	42.84	46.18	49.52

The values outside the thick lines are beyond the range of observed data

Annexure - II

Yield Table for stem wood of *Casuarina equisetifolia* on dry weight basis (kg tree⁻¹)

Best-fit equation used for construction of table: $y = 1.458 X^{1.034}$

DBH (cm)	Stem wood biomass (kg tree ⁻¹)										
	Height (m)										
	5	6	7	8	9	10	11	12	13	14	15
3	0.64	0.77	0.90	1.04	1.17	1.31	1.44	1.58	1.72	1.85	1.99
4	1.16	1.40	1.64	1.88	2.13	2.37	2.62	2.86	3.11	3.36	3.61
5	1.84	2.22	2.60	2.99	3.37	3.76	4.15	4.54	4.93	5.33	5.72
6	2.68	3.23	3.79	4.35	4.92	5.48	6.05	6.62	7.19	7.76	8.34
7	3.68	4.45	5.22	5.99	6.76	7.54	8.32	9.11	9.89	10.68	11.47
8	4.85	5.86	6.87	7.89	8.91	9.94	10.97	12.00	13.04	14.08	15.12
9	6.19	7.48	8.77	10.07	11.37	12.68	13.99	15.31	16.63	17.96	19.29
10	7.70	9.30	10.90	12.52	14.14	15.77	17.40	19.04	20.68	22.33	23.98

The values outside the thick lines are beyond the range of observed data

Annexure - III

Carbon Table for Above Ground Biomass (AGB) of *Casuarina equisetifolia*
(kg C tree⁻¹) in Tamil Nadu

DBH (cm)	Carbon for above ground biomass (kg C tree ⁻¹)										
	Height (m)										
	5	6	7	8	9	10	11	12	13	14	15
3	0.793	0.884	0.976	1.067	1.157	1.247	1.338	1.429	1.519	1.610	1.701
4	1.148	1.308	1.470	1.630	1.790	1.949	2.109	2.268	2.427	2.584	2.742
5	1.600	1.850	2.098	2.346	2.593	2.840	3.087	3.331	3.577	3.820	4.064
6	2.149	2.506	2.861	3.202	3.569	3.920	4.271	4.620	4.968	5.314	5.659
7	2.791	3.273	3.752	4.230	4.706	5.176	5.649	6.115	6.583	7.054	7.505
8	3.527	4.154	4.773	5.390	6.004	6.609	7.217	7.818	8.416	9.005	9.596
9	4.358	5.143	5.919	6.691	7.457	8.212	8.967	9.712	10.455	11.183	11.910
10	5.271	6.229	7.186	8.126	9.043	9.974	10.889	11.788	12.683	13.558	14.432

Annexure – IV

Carbon table for below ground biomass of *Casuarina equisetifolia* (kg C tree⁻¹)
in Tamil Nadu

Best-fit equation used for construction of table: $y = 0.616 X^{0.832}$

DBH (cm)	Carbon for below ground biomass (kg C tree ⁻¹)										
	Height (m)										
	5	6	7	8	9	10	11	12	13	14	15
3	0.139	0.161	0.184	0.205	0.225	0.247	0.267	0.287	0.306	0.326	0.346
4	0.224	0.260	0.296	0.331	0.364	0.398	0.431	0.463	0.495	0.527	0.558
5	0.324	0.377	0.429	0.479	0.529	0.577	0.624	0.672	0.717	0.763	0.808
6	0.439	0.511	0.581	0.649	0.715	0.780	0.846	0.909	0.971	1.034	1.095
7	0.567	0.660	0.751	0.839	0.926	1.010	1.093	1.176	1.257	1.335	1.414
8	0.709	0.825	0.936	1.048	1.155	1.260	1.366	1.467	1.568	1.669	1.768
9	0.862	1.003	1.141	1.275	1.405	1.533	1.661	1.784	1.908	2.030	2.150
10	1.027	1.195	1.360	1.519	1.677	1.830	1.979	2.126	2.272	2.418	2.561

REFERENCES

- Amanulla, B.K.M., Srinivasamurthy, K.V., Chandra, K.M.S., Pathak, S.D. and Chandrasekaramurthy, N. 2001. Variation in growth among *Casuarina cunninghamiana* provenances in Karnataka, India, 195-197: In: Gurumurthi, K., Nicodemus, A. and Siddappa (Eds.) *Casuarina Improvement and Utilization*, Institute of Forest Genetics and Tree Breeding, Coimbatore, India.
- Gurumurthi, K. and Subramanian, K. 1998. Casuarina – Favourite tree of farmers in peninsular, India. In: Joshi, S.C., Raturi, A.S., Singh, R.P. and Pant, S.C. (Eds), *Proc. National Seminar on Popularising Theme Trees among People including Rural and Urban Forest Industry*. Indian Council of Forestry Research and Education, Dehra Dun, India, pp. 83-88.
- Jain, J.K. and Mohan, N.M. 2001. The Andhra Pradesh Paper Mills and sustainable pulp wood production from *Casuarina equisetifolia*, 193-194: In: Gurumurthi, K., Nicodemus, A. and Siddappa (Eds.) *Casuarina Improvement and Utilization*, Institute of Forest Genetics and Tree Breeding, Coimbatore, India.
- Kaikini, D.S. 1937. Note on *Casuarina equisetifolia* plantation in Karwar. *Indian For.* 63(10): 661-668.
- Kondas, S, 1983. *Casuarina equisetifolia*, a multipurpose tree cash crop in India. In: Midgley, S.J., Turnbull, J.W. and Johnston, R.D. (Eds), *Casuarina Ecology, Management and Utilization* CSIRO, Canberra, Australia. pp. 66-76.
- Nicodemus, A. 2009. *Casuarina – A Guide for Cultivation*. Institute of Forest Genetics and Tree Breeding (Indian Council of Forestry Research and Education) Coimbatore, India, 16 p.
- Pinyopusarerk, K. 1996. *Casuarina equisetifolia*: a breeding program for India. Forestry Dept., FAO, Rome (Italy), Report No: FAO-FO—RAS/91/001, 44p.

- Pinyopusarker, K. and Williams, E.R. 2000. Range-wide provenance variation in growth and morphological characteristics of *Casuarina equisetifolia* grown in Northern Australia. *For. Ecol. Manage.* 143: 219-232.
- Prasad, N.S. and Dieters, M.J. 1998. Genetic control of growth and form in early-age tests of *Casuarina equisetifolia* in Andhra Pradesh, India. *For. Ecol. Manage.* 110(1-3): 49-58.
- Rawat, G.S., Kumar, N.K. and Nicodemus, A. 2011. Research and development of Casuarinas in India. In: Zhong, C., Pinyopusarker, K., Kalinganire, A. and Franche, C. (Eds), *Improving Smallholder Livelihoods through Improved Casuarina Productivity*, Proceedings of the 4th International Casuarina Workshop, Haikou, China, 21-25 March 2010. China Forestry Publishing House, pp. 11-16.
- Saravanan, S., Buvaneswaran, C., Veeramani, T. and Jayaraj, R.S.C. 2012. *Casuarina equisetifolia* – cotton based agroforestry system in Cauvery delta zone of Tamil Nadu for higher economic returns. In: In Jayaraj, R.S.C., Warriar, R.R., Nicodemus, A. and Kumar, N.K. (Eds) *Advances in Casuarina Research in India*, Proceedings of the 2nd National Seminar on Casuarinas, Institute of Forest Genetics and Tree Breeding Coimbatore, pp 385-390.
- Turnbull, J.W. 1990. Taxonomy and genetic variation in casuarinas. In: El-Lakany, M.H., Turnbull, J.W. and Brewbaker, J.L. (Eds), *Advances in Casuarina Research and Utilization*. (Desert Development Centre AUC, Cairo, pp. 1-11.
- Viswanath, S., Manivachakam, P. and George, M. 2001. *Casuarina equisetifolia* in agroforestry practices, 187-192: In: Gurumurthi, K., Nicodemus, A. and Siddappa (Eds.), *Casuarina Improvement and Utilization*, Institute of Forest Genetics and Tree Breeding, Coimbatore, India.
- Warrier, K.C.S. and Venkataramanan, K.S. 2011. Suitable Clones of *Casuarina equisetifolia* for Sodic Soils in India. In: Zhong, C., Pinyopusarker, K., Kalinganire, A. and Franche, C. (Eds), *Improving Smallholder Livelihoods through Improved Casuarina Productivity*, Proceedings of the 4th International Casuarina Workshop, Haikou, China, 21-25 March 2010. China Forestry Publishing House, pp. 194-198.