Casuarina Research and Development in China

Chonglu Zhong[1*], Yong Zhang[1], Yu Chen[1], Qingbin Jiang[1], Zhen Chen[1], Chong Wu[1], Khongsak Pinyopusarerk[2], Claudine Franche[3] and Didier Bogusz[3]

1. Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou 510520, China
2. CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia
3. Equipe Rhizogenèse, UMR DIAPC, Institut de Recherche pour le Développement (IRD), 911 avenue Agropolis, BP 64601, 34394 Montpellier Cedex 5, France

* Email: zclritf@gmail.com; zclritf@21cn.com

Abstract Casuarina trees are planted along the coastal area of southern China as windbreaks, in agroforestry systems and for wood and fuelwood production. At present, casuarina plantations cover about 300,000 ha. *Casuarina equisetifolia*, *C. cunninghamiana*, *C. glauca* and *C. junghuhniana* are the most commonly planted species. Series of field trials have been carried out using different *Casuarina* species, provenances and clones to screen for adaptability to biotic and abiotic stress in different areas. A simple technique for the mass propagation of casuarina seedlings is rooting of cuttings in water culture. Experiments conducted in the nursery, glasshouse and field showed that ectomycorrhizal (ECTM) or arbuscular mycorrhizal (AM) fungi or *Frankia*-tree genotype symbiotic associations played an important role in improved management of casuarina growth.

1 Introduction

The family Casuarinaceae includes tree and shrub species that have the capacity to enter different endosymbiotic interactions with soil microbes including the nitrogen-fixing actinomycete *Frankia*, ectomycorrhizal and endomycorrhizal fungi (Zhong *et al*., 1995). Many casuarinas are economically and ecologically important as they provide a wide range of goods and services. Their wood is a main source of fuelwood and charcoal, and is also used for general construction and other wood-based industries, e. g. woodchips for paper pulp and veneer for plyboard. Along the southern coast of China, casuarinas are commonly planted as windbreaks to stabilize the moving sand, in agroforestry, and for general rehabilitation programmes, e. g. pioneer trees for degraded sites and soil improvement. Very few species can replace casuarinas at the foreshores.

Casuarinas were introduced to the tropical and sub-tropical zones of China in the early 1960s, and currently cover about 300,000 ha mostly in the coastal areas. Among the large number of species introduced, *Casuarina equisetifolia* L. Johnson, *C. cunninghamiana* Miq., *C. glauca* Sieber ex Sprengel and *C. junghuhniana* Miq., are the most successful, and are the focus of ongoing research and development. This paper summarizes research and development on casuarinas conducted in China over the past 25 years.

2 Improvement Programmes for Key *Casuarina* Species

2.1 Provenance/family/clone selection

Since 1984, with the support from projects funded by ACIAR, CSIRO-coordinated international trials, FAO and the Chinese government, 23 *Casuarina* and *Allocasuarina* species comprising 204 bulked seedlots, 230 individual families and more than 100 clones have been field tested in China. The results showed that *C. cunninghamiana*, *C. equisetifolia* and *C. junghuhniana* were most suitable for the tropical region. *C. equisetifolia*, *C. glauca* and some provenances of *C. cunninghamiana* were suitable for the subtropical region along the coast in the southern part of Zhejiang province and the north-eastern part of Fujian province. The summarized results are as follows:

C. equisetifolia: By ACIAR projects and CSIRO-IPTC projects, more than 10 international provenance trials have been established in Guangdong, Hainan, Fujian and Guangxi in southern China since 1985. Based on more than ten quantitative and qualitative traits (Pinyopusarerk *et al.*, 1995), better-than-average provenances were 14233, 18008, 18013, 18015, 18086, 18118, 18119, 18127, 18128, 18134, 18143, 18153, 18154, 18244, 18288, 18348 and 18355, with possible suitable areas predicted by means of Booth's model (Zhong, 1990; Zhong and Bai, 1996; Zhong, 2000; Zhong *et al.*, 2001).

C. cunninghamiana: Around 35 seedlots have been introduced into China since 1980s. Based on results from ACIAR projects 8457 and 8848, and FAO project GCP/CRP/005/FRA, better-than-average seedlots were 13513, 13514, 13515, 13516, 13518, 13519, 13520 and 15574. In southern China, the provenances from northern Queensland performed better than those from southern Queensland and New South Wales (Zhong and Bai, 1996).

C. glauca: Around 28 seedlots have been introduced to China since 1984. Based on the results from ACIAR project 8457 and 8848, FAO project GCP/CRP/005/FRA and Chinese projects, the better-than-average seedlots were 13141, 14146, 15217, 15218 and 15579. In southern China, northern provenances from its native distribution performed better than those from southern provenances (Zhong and Bai, 1996).

C. junghuhniana: Since 1985, over 36 provenances have been introduced and tested in southern China. In 1986, two provenances were tested in Qionghai of Hainan and Zhangzhou of Fujian. In 1991, seven provenances were planted in Yangxi of Guangdong. In 1996, two international provenance trials involving 28 seedlots were established in Zhangzhou, Fujian and Dianbai, Guangdong. The results showed that better-than-average provenances were 17877, 18844, 18847, 18852, 18853, 18949, 18950, 19238, 19239, 19240, 19489, 19490 and 19491 (Zhong and Bai, 1996; Zhong, 2000; Zhong *et al.*, 2003).

In 2001, a progeny test of *C. equisetifolia* with 230 individual families was set up. In 2008, more than 400 genetic resources of the four key *Casuarina* species were used to establish seed orchards in Hainan Island. Since 2002, tests including more than 50 clones have been carried out at six sites with a total area of over 10 ha in Hainan and Guangdong provinces.

2.2 Hybridization

Based on introduction, conservation, provenance trials and progeny and clone tests, Chinese researchers have been working on the selection of new *Casuarina* varieties to enhance productivity and adaptability. Chinese researchers created new varieties by producing *Casuarina* hybrids during the 1950s. The following hybrids were reportedly produced: *C. equisetifolia* × *C. glauca*, *C. glauca* × *C. equisetifolia*, *C. cunninghamiana* × *C. equisetifolia* and *C. cunninghamiana* × *C. glauca* (Xu and Lao, 1984). Unfortunately, these hybrid resources have been lost. At present, *Casuarina* hybrid clones in China are natural hybrids selected from clonal plantations of mainly *C. cunninghamiana*, *C. equisetifolia* and *C. glauca*. Current hybridization work has been conducted in India (Nicodemus *et al.*, 2010). El-lakany (1983) reported a hybrid of *C. cunninghamiana* × *C. glauca* in Egypt. Clonal plantations of a male hybrid of *C. junghuhniana* × *C. equisetifolia* have been established in Thailand and India (Kondas, 1983).

From 2007 to 2010, 136 crosses were performed by controlled pollination, and seeds were harvested from 64 successful crosses. Progeny tests of these seeds were conducted in 2008 in Hainan and Fujian provinces.

2.3 Molecular biotechnology

The genetic structure and diversity of *C. equisetifolia* provenances were revealed by RAPD and AFLP markers (Guo *et al.*, 2003; Huang *et al.*, 2009). The information has provided baseline data for selection and breeding of this species in China. Meanwhile, *C. equisetifolia* and *C. cunninghamiana* have been used for genetic transformation in China (Zhong, unpublished data).

3 Propagation Techniques

3.1 Propagation by cuttings

Branch cuttings are used to propagate planting material. The vegetative material is the young needle-like branchlet, preferably less than three months old taken from stock plants in hedge orchards. Water culture is now a common method for propagation of casuarina cuttings in China (Liang and Chen, 1982). The procedure consists of soaking the bottom part of 8-10 cm long branchlets in 50-100 mg kg^{-1} of naphthalene-acetic acid (NAA) or indole-butryic acid (IBA) solution for 24 hours. The bottom 3-4 cm of the plant material is then soaked in tap water and placed near sunlight. The tap water is changed every day. At 25-32°C water temperature, cuttings will root after 7-10 days, and the rooting percentage reaches over 80% after 15 days. If the water temperature is lower than 25°C, it may take 15-30 days to root. The rooted branchlets are then transplanted in growing containers filled with standard potting mix. This technique has been extended to county foresters and farmers.

Rooting in moist, fine sand, and applying the same hormone treatment as described above for water culture, is an alternative method. Generally, a sand bed 15-18 cm in depth, 80-100 cm in width and 5-10 m in length, is built with bricks. A plastic sheet is placed inside the bed to keep the sand clear of soil, and water pipes are laid at one end to supply water. The sand bed is always kept moist and is covered with a clear plastic sheet to increase the temperature in winter. This method is also suitable for mass production of planting material.

Both water culture and sand culture are used successfully for *C. equisetifolia*, *C. cunninghamiana*, *C. glauca* and *C. junghuhniana*. Other species such as *C. cristata*, *Allocasuarina littoralis* and *A. torulosa* are more difficult to root.

3.2 *In vitro* tissue culture

In vitro tissue culture propagation of casuarina is also an important technique to produce seedlings, particularly species that are difficult to root or when propagating old trees. Since 1980s, tissue culture methods for propagating casuarinas have been reported (Abo El-Nil, 1987; Cao *et al.*, 1990;

Duhoux *et al.*, 1990). Suitable tissue materials include slender branches, young buds, immature male inflorescence and female flower buds. The success of the approach depends on appropriate disinfection of the plant material, the composition of the nutrient medium, rooting hormone (NAA, 6-BA or IBA), temperature, light, and the biological characteristics of the species. Generally, slender tissue-cultured material is easier to root than material from cuttings. In 2000, Chinese researchers started to work on *in vitro* casuarina trees. Up to now, *in vitro* plants of *C. cunninghamiana*, *C. equisetifolia* and *C. glauca* have been obtained, but there are considerable differences in organogenesis among species (Liu *et al.*, 2003).

4 Selection for *Frankia*/Mycorrhizal Fungus-Association of Tree Genotypes and Application of Inoculum

Over 20 strains of *Frankia*, eight genera of ectomycorrhizal fungi and 15 AM fungi have been collected from casuarina plantations in China (Kang and Zhong, 1999; Zhong *et al.*, 2010). Mycorrhizal fungi and *Frankia* can improve the growth and biomass production of casuarina seedlings or saplings. Chinese researchers have been working on the selection of casuarina symbiotic genotypes, and inoculation with ectomycorrhizal fungus significantly improved the diameter and height of seedlings of *C. equisetifolia* and *C. junghuhniana* (Zhong, 1993; Zhong, 2000). There was also variation among seedlots in response to the inoculation (Zhong *et al.*, 2003). A *Frankia* inoculation experiment on *C. cunninghamiana* was carried out in the hot, dry river valley in Yuanmou, Yunnan province, which is located far from the coastal area and there is no natural *Frankia* presence in the soil. Survival of inoculated seedlings was 10-20% higher than that of uninoculated seedlings. Tree height after two years differed significantly among *Frankia* treatments, and not all *Frankia* strains improved tree growth (Yang *et al.*, 2007). It is important to screen suitable *Frankia* strains for different species and different sites. Since 1989, many field inoculation trials have been conducted in Hainan, Guangdong and Yunnan provinces. More than 18 years of experience in applying casuarina inocula in China have shown that application of a symbiotic microorganism (*Frankia* and mycorrhizal fungi) can effectively improve survival and biomass productivity of casuarina plants (Zhong *et al.*, 2010), and is recommended when casuarina trees are planted at sites where casuarinas have not previously been planted. Pure culture strains or isolate mycorrhizal fungus inoculants in liquid form can be used to inoculate seedlings in the nursery.

5 Casuarina Trees for Degraded Lands

Based on previous experiments, we studied pest and disease resistance at seedlot and clone levels; wind resistance at seedlot and clone levels; salt tolerance at species and clone levels; and cold tolerance at species and clone level. Tissue-cultured seedlings of three *C. equisetifolia* clones were subjected to salt-tolerant tests, and the best clone was able to grow with 0.3% NaCl in the growing medium (Liu *et al.*, 2003). Tolerance to salt, drought and disease of cuttings material using some physiological traits and growth indexes have been reported (Yang *et al.*, 2003; Zhang *et al.*, 2008). Since 2001, we have introduced *C. equisetifolia*, *C. glauca*, *C. cunninghamiana*, *C. junghuhniana*, *C. obesa*, *C. cristata* and *A. littoralis* to the hot dry river valley in Yuanmou, Yunnan province, which is characterized by degraded soil, low annual rainfall (600 mm) and high evaporation (3,800 mm). The first five species have potential but further observations are needed (Yang *et al.*, 2007).

6 Wilt Disease

Dieback of casuarina trees caused by bacterial wilt disease *Ralstonia solanacearum* (formerly

Pseudomonas solanacearum) is a very serious problem in many plantations in southern China. Selection of wilt-resistant genotypes started in the 1980s. Some clones have shown resistance but their growth is slow. In addition, new strains of *R. solanacearum* tend to develop in new environments. A precautionary measure is to broaden the genetic diversity by planting many clones in bacterial-wilt prone areas. A minimum of 10 clones may be required in large-scale planting by forest farms. However, in China, plantation stakeholders often only consider the short-term financial gain and plant only very few highly productive clones.

7 Conclusions

Four *Casuarina* species, *C. equisetifolia*, *C. cunninghamiana*, *C. glauca* and *C. junghuhniana* are the most promising for plantation establishment in southern China. These species have shown good adaptability to degraded sites, e. g. salt alkaline land in coastal areas and stone mountains and hot dry river valleys in inland areas. Casuarina trees display marked variation in quantity and quality traits. The extent of these variations needs to be studied for the selection of superior materials. Among propagation methods, root cuttings of young branchlets yields very good results and is the most commonly used method for mass propagation. Inoculation experiments in China have also shown that symbiotic microorganisms can improve survival and growth, especially on degraded lands. It is thus important to screen the best *Frankia* and mycorrhizal strains for different species and different sites.

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