Selection for Cold Tolerance of Casuarinas in China

Qingbin Jiang[1], Fen Liu[1], Yong Zhang[1], Yu Chen[1], Zhen Chen[1], Ni Ma[1], Pan Hu[1], Guiping He[2], Zhihui Gao[3], K. Pinyopusarerk[4], C. Franche[5], Chonglu Zhong[1]*

[1] *Research Institute of Tropical Forestry, Chinese Academy of Forestry, Longdong, Guangzhou 510520, China*

[2] *Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, Hangzhou 311400, China*

[3] *Zhejiang Academy of Forestry, Hangzhou 310023, China*

[4] *CSIRO Plant Industry, GPO Box 1600, ACT 2601, Australia*

[5] *Equipe Rhizogenèse, Institut de Recherche pour le Développement, 911 avenue Agropolis, 34394 Montpellier cedex 5, France*

**Email : zcl@ritf.ac.cn*

Abstract

Selection and breeding of *Casuarina cunninghamiana, C. equisetifolia* and *C. glauca* for cold tolerance by inoculation of mycorrhizal fungi (MF) and molecular biotechnology were conducted under glasshouses and field conditions. Physiological and biochemical response traits and gene selection and expression under cold treatments could explain behind molecular mechanism cold tolerance. Key results are as follows:

1) Field introduction trials carried out around Hangzhou Bay of Zhejiang identified strongly cold-tolerant clones of *C. glauca* which could survive low temperature stress at -5 °C. For propagation of the selected *C. glauca* clones, young branchlet characteristics, substrate ratio, moisture and temperature were determined. Percentage of rooted cuttings reached 90%.

2) Branchlets of *C. cunninghamiana*, *C. equisetifolia* and *C. glauca,* were tested for electrical conductivity and percentage of electrolyte leakage traits under cold treatments. The relative conductivity, the soluble protein content, MDA content and SOD activity can be used as basic indices for the identification and selection of cold tolerance.

3) *C. equisetifolia* seedlings inoculated with different MF in glasshouse and cut branchlets under low temperature stress were studied. Results showed that some

treatments inoculated with MF in combination with appropriate low temperature stress could significantly improve the protection of enzyme activity, reduce cell membrane permeability and MDA content to increase their cold resistance. No mycorrhizal fungal treatments could promote cold tolerance.

4) To understand the gene expression and molecular mechanism of cold tolerance, selected *C. cunninghamiana* clones from Hanzhong, Shanxi (33° 20'N) were exposed to extreme cold temperature (-5 to -10 °C) to study transcriptome which would include relative cold genes. Illumina Solexa sequencing technology was used to obtain the cold genes which were designed for their primers for RT-PCR and real-time quantitative PCR tests to obtain the differential expression of the cold gene sequences.