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Gender identification in *Casuarina equisetifolia*

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ABSTRACT

Understanding the molecular factor behind sex expression has immense importance both in basic and applied research. Determination of sex in *Casuarina equisetifolia* is of utmost important from the commercial forestry. Sex of plant is very important as number of female plants must be maximum. Plantation of seedlings of unknown sex leads to long term effects on yield. Hence correct identification of male and female genotypes at juvenile phase is important to maintain proper densities of female and male plants and dioecious nature also poses problem in tree breeding programmes. In many dioecious plants, gender influences economic value, breeding schemes, and/or opportunities for commercial use of genetically transformed materials.

Introduction

Casuarina equisetifolia is out- crossed species, selection of appropriate gender type of the progeny would be beneficial at the nursery stage for establishing seedling seed orchards for the supply of seeds and also male clones are preferable than the female clones in turns of wood biomass especially in the pulp industry. Specific marker information about gender detection in *Casuarina equisetifolia* is not available in the literature. Hence the present study has been attempted to detect gender identification in *Casuarina equisetifolia* through biochemical marker (isozymes). Use of isozymes for gender identification at nursery level was reported on *Actinidia delciosa* (Skirkot,2001), *Hippophae rhammnoides* (Sharma,2010), *Hippophae salicifolia* (Shirkot,2009). *Bursera penicillita* (Parthasarathi *et al* 1982 ; Parthasarathi and Angadi 1984) *Simmondsia chinensis* (Suganuma Hirotoshi, 1999).

Casuarina equisetifolia is a predominantly dioecious, wind pollinated and economically important multipurpose species. Three types of gender are exists in nature (ie) male, female and bisexual plants (Dioecious & Monoecious) in *Casuarina equisetifolia* Here the investigation was observed when the poly acrylamide gel for peroxidase patterns were stained in both Electrophoretic ways through Iso Electric Focusing (IEF) using acrylamide ready gels (Ins.Fasta Pharmacia system, USA) and also conventional methodology using 12% Acrylamide gel electrophoresis (Genei, PAGE system). The present study investigated a specific allele which was designated as male specific and help to identify gender discrimination in *Casuarina equisetifolia*.

Fresh and young needle like leaves were collected from twenty selected male and female clones of *Casuarina equisetifolia*. one gram of leaves was weighed and the tissues were grounded fine powder using liquid nitrogen. Using of liquid nitrogen helps to grind the tissues very well since the tissue has more fibrous in nature. The grounded tissue was added with 5 ml of slightly modified extraction buffer consists of Tris-Hcl Buffer with additives (1% PEG,1% Sucrose, 1% PVPP, 0.8% Ascorbic acid, 0.5% Triton x-100 0.5% Mgcl2, 0.001% mercaptoethanol.). Then these extracts were centrifuged at 11,000 rpm for 10 minutes at 4^0C in the refrigerated centrifuge.. The clear supernatant was taken and stored at -20 ^{0}C till use.

12% Poly acrylamide separating gel and stacking gels (4%) were casted. The sample extract of 40 ul was loaded. The whole system was kept in the refrigerated condition (4 ^{0}C) while running gel. The ambient current (150 volts with 50mA) was applied for 3 hours for running the gel . The gels were stained with twelve different enzyme systems (Table-1) using slight modification with standard staining procedure (Robert W. Murphy *et al*., 1996). The images were documented and photographed under white light transilluminator. The banding patterns were observed as number of alleles and locus present /absent in each enzyme systems.

Results of this present study (Fig-1&2) showed that five enzymes (Alcohol dehydrogenase (Adh), Malate dehydrogenase (Mdh), Lactate dehydrogenase (Ldh), Peroxidase (Pod), Isocitrate dehydrogenase (Idh,) were more stable expressions towards gender specific among the twelve different enzymes which were optimized in this species. The enzymes Aspartate Amino transferase (AAT), Aconitase (ACO), Alcohol dehydrogenase (ADH), Lactate dehydrogenase (LDH), Esterase (EST), Peroxidase (POD), Glucose 6-phosphate dehydrogenase (G6PDH) were monomeric structure (The quaternary structure of the polypeptide) in *Casuarina equisetifolia*, whereas enzymes Malic Enzyme (ME), Superoxide (SOD), Malate dehydrogenas (MDH), Isocitrate Dehydrogenase (IDH), Polyphenol Oxidase (PPO) were dimeric structure. The maximum number of locus was two in six different enzymes. The maximum number of alleles in LDH was eight followed by five in POD, PPO, G_6 PDH. The present study investigated a specific allele which was designated as male specific and help to identify gender discrimination in *Casuarina equisetifolia*. Here we have reported about the gender specific isozyme markers in *Casuarina equisetifolia* which was the first report of this species.

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Table-1. List of Enzymes and its details

Name of the enzyme	structure	No of alleles	Locus
Aspartate AminoTransferase (AAT)	monomer	1	1
Malic enzyme (ME)	dimer	2	1
Aconitase (ACO)	monomer	1	1
Alcohol dehydrogenase (ADH)	monomer	3	2
Superoxide dismutase (SOD)	dimer	3	1
Malate dehydrogenase (MDH)	dimer	2	1
Lactate dehydrogenase (LDH)	monomer	8	2
Isocitrate dehydrogenase (IDH)	dimer	4	2
Esterase (EST)	monomer	2	1
Peroxidase (POD)	monomer	5	2
Polyphenol oxidase (PPO)	dimer	5	2
Glucose6phosphate dehydrogenase (G6PDH)	monomer	5	2