ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 02, pp.64866-64873, February, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANATOMICAL DIFFERENCES IN TISSUE CHARACTERISTICS BETWEEN JUVENILE AND ADULT MATERIALS AS WELL AS MALE, FEMALE AND MONOECIOUS TREES IN *CASUARINA EQUISETIFOLIA*

***Kannan C.S. Warrier, John Prasanth Jacob Rekha R Warrier and V. Sivakumar**

Senior Principal Scientist Institute of Forest Genetics and Tree Breeding, (Indian Council of Forestry Research and Education), Forest Campus, PB 1061, R.S. Puram, Coimbatore, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 18th November, 2017
Received in revised form
06th December, 2017
Accepted 20th January, 2018
Published online 18th February, 2018

Key words:
Casuarina,
Anatomical Studies,
Juvenility,
Maturity,
Sex expression

ABSTRACT

An attempt was made at the Institute of Forest Genetics and Tree Breeding, Coimbatore to understand the anatomical differences in tissue characteristics between juvenile and adult materials as well as male, female and monoecious trees in *Casuarina equisetifolia*. Traditionally, the transition from juvenile to adult phase has been explained as a change in the reproductive competence but it also is marked by species specific changes in a variety of vegetative traits including leaf shape, leaf anatomy, adventitious root production, disease resistance and a number of secondary compounds. However, attempts to identify sex of the plants based on morphological and physiological parameters have not been consistent. In recent years, there have been serious efforts to understand the genetic basis of sex determination in plants and to develop methods to identify sex at an early stage by using molecular / biochemical markers. Phylloclad cuttings collected from four different positions from lower (juvenile) to upper (mature) parts of 9 years old male, female and monoecious trees were subjected to the anatomical investigations.Various anatomical parameters including pith diameter, thickness of the phloem tissue, number of xylem vessels per unit area, diameter and area of the xylem vessels, roundness, aspect ratio and fullness ratio of the xylem vessels were measured using an Image Analyser.Anatomical studies carried out in the present study revealed significant differences between the juvenile and adult tissues. Pith diameter and thickness of phloem tissue varied among the stem cuttings obtained from the four different positions. A decreasing trend was observed from position 1 to position 4 for pith diameter. A reverse trend was observed for the thickness of phloem tissue. Similar was the trend for diameter of the remaining area (excluding pith and phloem), which was observed to decrease from position 4 to position 1. None of the other parameters examined in this study could be used to distinguish juvenile and adult materials at the anatomical level. Pith diameter, diameter of the area excluding pith and phloem, diameter, area, roundness and aspect ratio of the xylem vessels varied significantly among the male, female and monoecious trees. Pith diameter was found to be maximum for monoecious trees followed by female and male trees. A reverse trend was noticed for diameter of the remaining area. Average diameter and area of the xylem vessels were higher for the male trees than the female and monoecious individuals. With reference to the shape of the xylem vessels, male trees exhibited better roundness than the female and monoecious trees.

Citation: Kannan C.S. Warrier, John Prasanth Jacob Rekha R Warrier and V. Sivakumar2018. "Anatomical Differences in Tissue Characteristics between Juvenile and Adult Materials as well as Male, Female and Monoecious Trees in *Casuarina equisetifolia*", *International Journal of Current Research*, 10, (02), 64866-64873.

INTRODUCTION

Growth of the plant shoot can be divided into several discrete phases based on the character of the organs produced during these phases and the capacity of the shoots for reproductive development (Zimmerman *et al*., 1985). Traditionally, the transition from juvenile to adult phase has been explained as a change in the reproductive competence but it also is marked by species specific changes in a variety of vegetative traits including leaf shape, leaf anatomy, adventitious root production, disease resistance and a number of secondary compounds (Hacket, 1985; Kerstetter and Poethig, 1998). Rooting potential has been used in several woody plants as a marker for juvenility (Huang *et al*., 1992). Several biochemical markers are also being employed to distinguish the juvenile from the adult developmental phases of certain plants. However, attempts to identify sex of the plants based on morphological and physiological parameters have not been consistent (Zachariah *et al*., 1986; Packiyasothy *et al*., 1991). In recent years, there have been serious efforts to understand

****Corresponding author:*** **Kannan C.S. Warrier,**
Senior Principal ScientistInstitute of Forest Genetics and Tree Breeding, (Indian Council of Forestry Research and Education), Forest Campus, PB 1061, R.S. Puram, Coimbatore, Tamil Nadu, India.

the genetic basis of sex determination in plants and to develop methods to identify sex at an early stage by using molecular / biochemical markers (Hormaza *et al*., 1994; Bendiab, 1999; Mori *et al*., 2000). In the present study an attempt was made to understand the anatomical differences in tissue characteristics between juvenile and adult materials as well as male, female and monoecious trees of *Casuarina equisetifolia*.

MATERIALS AND METHODS

Three trees, all 9 years old, were selected for the study. They included a female, a male and a monoecious individual grown in the Forest Campus, Coimbatore. Four positions were marked on these trees namely,

Positions	Description	Remarks
Position 1.	Upto 60 cm from the ground level	No flowering in any branch
Position 2.	Between 60 cm and 95 cm from the ground level	Occasional flowering in some branches
Position 3.	Between 95 cm and 145 cm from the ground level	Many branches showing flowering
Position 4.	Between 145 cm and 165 cm from the ground level	All branches showing flowering

Sprigs obtained from these different positions (position 1 to 4) were used for the anatomical studies. Transverse sections were prepared using a microtome cryostat (Kryostat 1720 Digital, Lauda, Leitz Germany). Manual sections were also used for the study. Various anatomical parameters including pith diameter, thickness of the phloem tissue, number of xylem vessels per unit area, diameter and area of the xylem vessels, roundness, aspect ratio and fullness ratio of the xylem vessels were measured using an Image Analyser (Leica-Quantimet - QWin 500, Leica, UK).

Roundness is a shape factor and is calculated using the formula given below (Sivakumar *et al*., 2002).

$$\text{Roundness} = \frac{(\text{Perimeter})^2}{4 \times \pi \times \text{2D surface area} \times 1.064}$$

where 1.064 is a correction factor due to digitization. (The roundness of a circle is unity). Aspect ratio is the ratio of length to breadth. Fullness ratio is also a shape factor measured as the square root of the ratio of area to circumscribed area as given below (Sivakumar *et al*., 2002).

$$\text{Fullness ratio} = \left(\frac{\text{2D surface area}}{\text{Convex area}}\right)^{1/2}$$

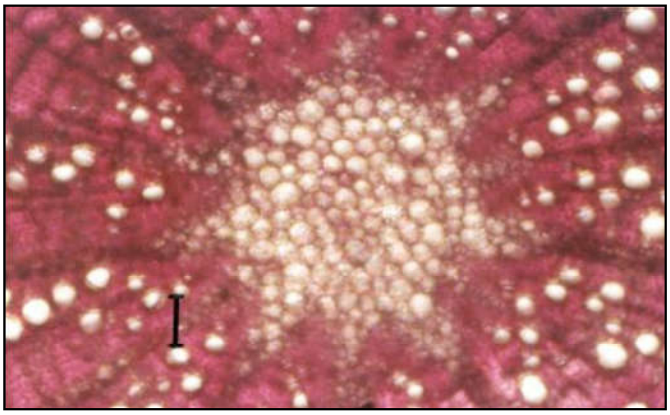
RESULTS AND DISCUSSION

The results obtained for the various anatomical parameters including ratios of (a) pith diameter, (b) phloem thickness and (c) diameter of the remaining area (excluding pith and phloem) to the total diameter of the cross section (PD/TD, PT/TD and RD/TD respectively), (d) number of xylem vessels per square mm of the section, (e) percentage of area occupied by the xylem vessels, (f) average diameter and area of the individual xylem vessels and (g) roundness, aspect ratio and fullness ratio of the xylem vessels are presented in Table 1. Position 1 recorded a value of 0.293 for the ratio of pith diameter to the total diameter of the section and was found superior to all the other positions. However, positions 2 (0.180), 3 (0.166) and 4 (0.168) were found on par with each other. The ratio of phloem thickness to the total diameter was maximum for position 4 (0.089) and was found on par with position 3 (0.086) while position 1 recorded the minimum value (0.062). Position 4, which recorded the maximum value (0.344) for RD/TD was observed to be on par with position 3 (0.331) and position 2 (0.321). Position 1 recorded the minimum value (0.291) for this ratio and was found on par with position 2. No significant variation was registered among the four positions with reference to any of the other parameters. The ratios, PD/TD and RD/TD and the characteristics of xylem vessels including average diameter, area, roundness and aspect ratio varied significantly among the sexes (Table 2). Monoecious trees recorded the maximum value for the ratio of pith diameter to total diameter (0.276) followed by female (0.190) and male (0.140) trees. Male and female trees were found on par for this parameter. Male trees registered the maximum value for RD/TD (0.347) and were on par with females (0.328). Monoecious individuals recorded a value of 0.290 for this ratio. Average diameter and area of the xylem vessels were higher for the male trees (2.675 µm and 6.989 µm^2 respectively). Female trees which recorded values of 2.443 µm and 6.014 µm2 respectively for these parameters were on par with the males. Monoecious individuals registered the minimum values (2.265 µm and 5.206 µm^2 respectively). With reference to roundness and aspect ratio, monoecious trees recorded the maximum values (1.376µm and 1.615 µm^{2}respectively). Female (1.312 µm and 1.571 µm^2 respectively) and male (1.298 µm and 1.551 µm^2 respectively) trees were on par when roundness and aspect ratios were studied.

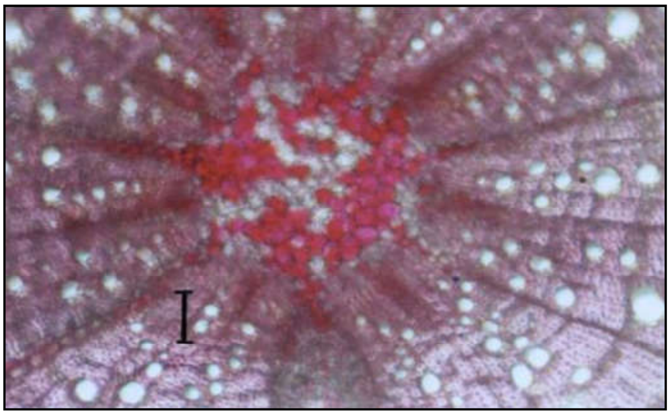
Ontogenetical ageing is accompanied by anatomical, cytological and physiological changes and these could be used for determining the phase change (Fortanier and Jonkers, 1976). Ewers and Aloni (1987) studied the variation in anatomical parameters including development of conducting tissues and thickness of cambial zones between juvenile and adult needles of *Pinus strobus* and *P. brutia*. Quantitative anatomical investigations in *Populus deltoides* revealed that the number of vessels per unit area decreased with maturity (Ifju, 1991). Significant variation was also observed for radial and tangential diameter of vessels and fibres. In *Pinus taeda*, Hamann (1998) studied the anatomical changes during adventitious root initiation for cuttings obtained from donors of different types and ages. The results showed that the anatomical changes during root formation and the origin of adventitious roots were the same in materials from different sources. Studies conducted in *Castanea sativa* revealed that the main anatomical difference between the juvenile (shoots collected from base) and adult (shoots collected from the crown) stems was in their secondary tissues. Secondary phloem was more developed in shoots from the crown than in shoots from the base (Ballester *et al*., 1999).

Anatomical studies carried out in the present study revealed significant differences between the juvenile and adult tissues. Pith diameter and thickness of phloem tissue varied among the stem cuttings obtained from the four different positions. A decreasing trend was observed from position 1 to position 4 for pith diameter. In other words, juvenile cuttings exhibited higher pith area than the mature ones (Plates 1 to 3). A reverse trend was observed for the thickness of phloem tissue (Table

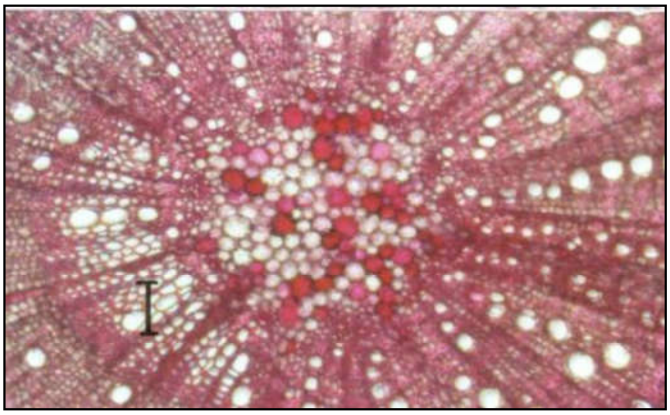
1). Similar was the trend for diameter of the remaining area (excluding pith and phloem), which was observed to decrease from position 4 to position 1. None of the other parameters examined in this study could be used to distinguish juvenile and adult materials at the anatomical level. Pith diameter, diameter of the area excluding pith and phloem, diameter, area, roundness and aspect ratio of the xylem vessels varied significantly among the male, female and monoecious trees (Table 2).



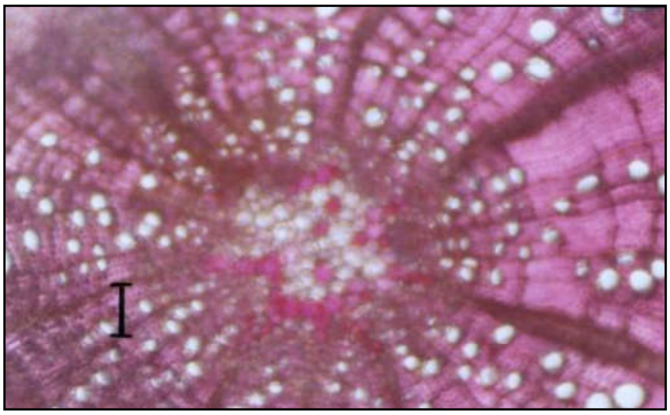
M1:
Upto 60 cm
from ground level



M2:
Between 60 & 95 cm
from ground level



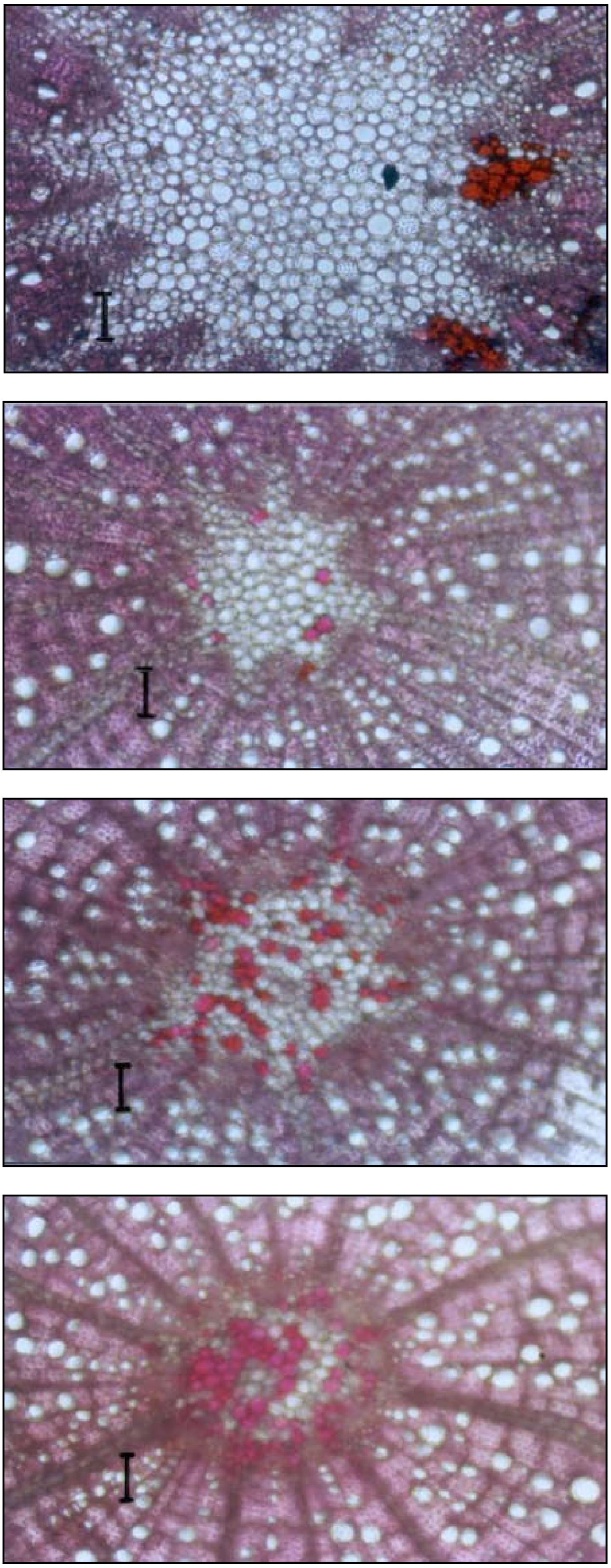
M3:
Between 95 & 145 cm
from ground level



M4:
Between 145 & 165 cm
from ground level

Bar represents 8μ

Plate 1. Variation in pith with reference to degree of juvenility in a male Casuarina tree



F1:
Upto 60 cm
from ground level

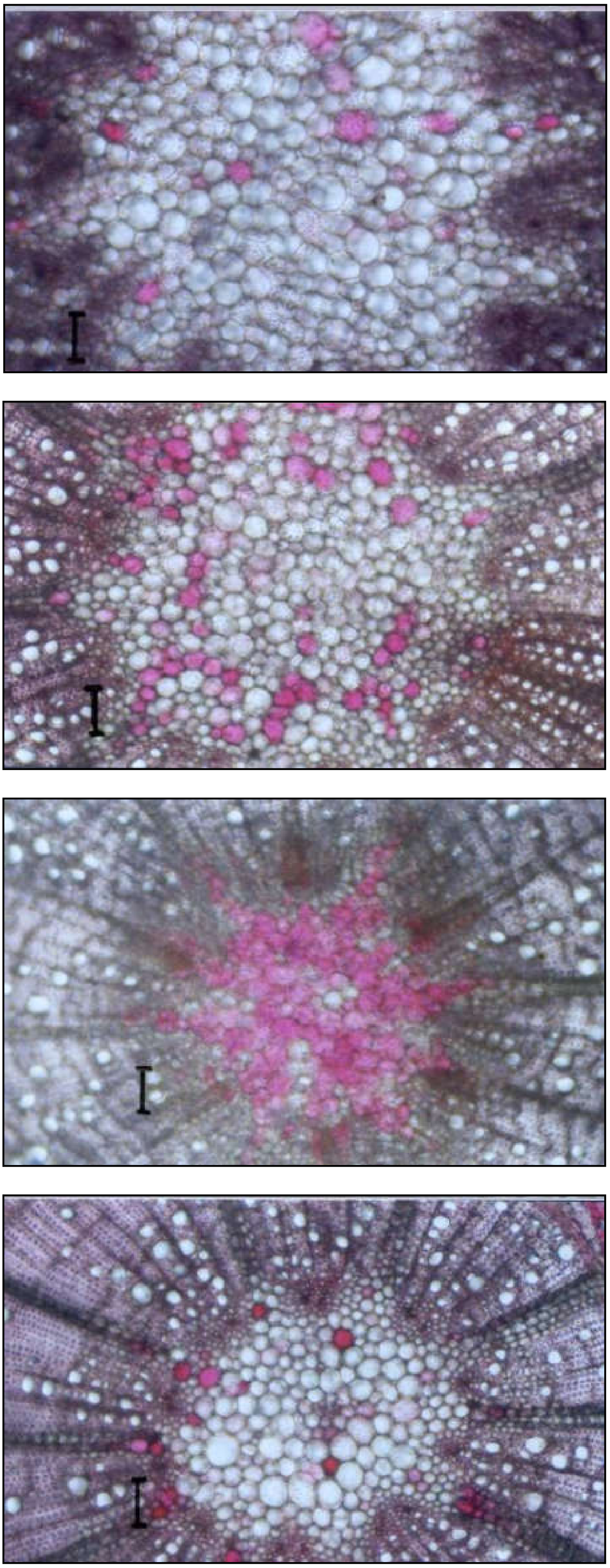
F2:
Between 60 & 95 cm
from ground level

F3:
Between 95 & 145 cm
from ground level

F4:
Between 145 & 165 cm
from ground level

Bar represents 8μ

Plate 2. Variation in pith with reference to degree of juvenility in a female Casuarina tree



B1:
Upto 60 cm
from ground level

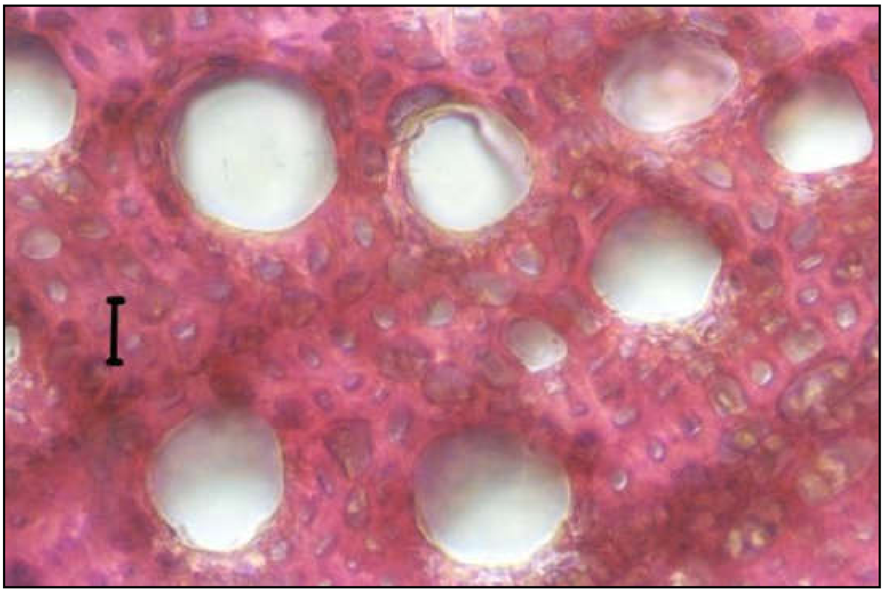
B2:
Between 60 & 95 cm
from ground level

B3:
Between 95 & 145 cm
from ground level

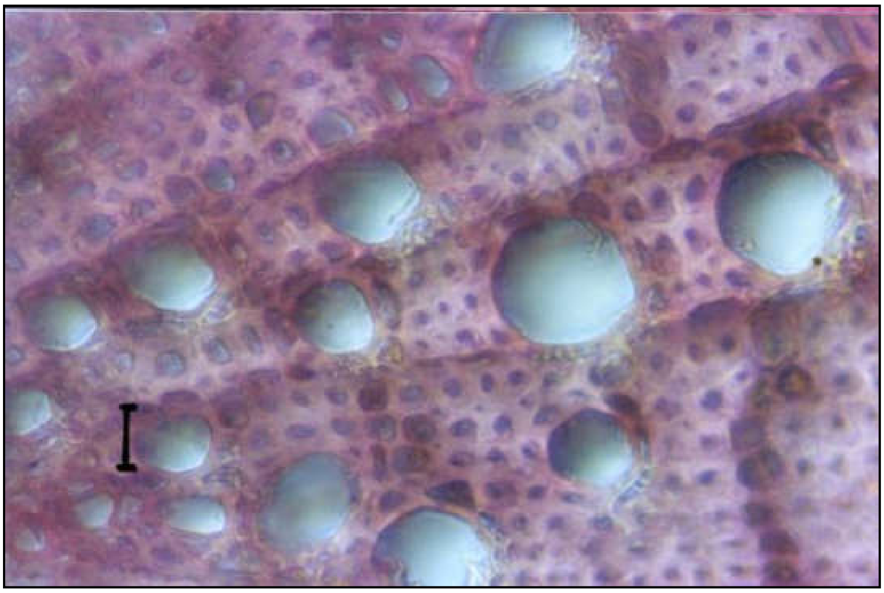
B4:
Between 145 & 165 cm
from ground level

Bar represents 8μ

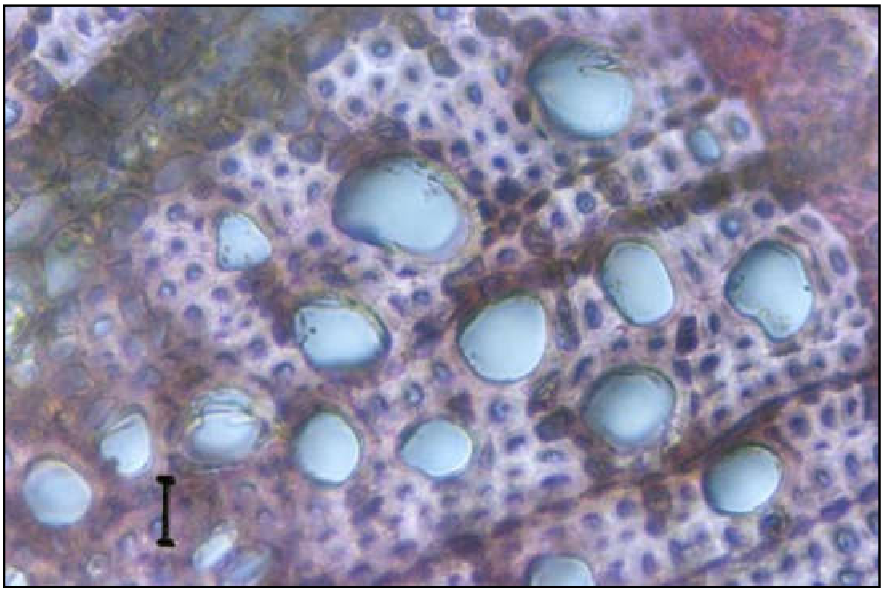
Plate 3. Variation in pith with reference to degree of juvenility in a monoecious Casuarina tree



Male



Female



Monoeciou

Bar represents 2μ

Plate 4. Variation in roundness of xylem vessels among male, female and *Monoecious casuarina* trees

Table 1. Difference in anatomy with reference to degree of juvenility in *Casuarina equisetifolia*

Position	Anatomical Measurements				
	PD/TD	PT/TD	RD/TD	NOVES ^{NS}	AREAVES ^{NS}
Position 1	0.293 ^{a}	0.062 ^{c}	0.291 ^{b}	53.765	3.420
Position 2	0.180 ^{b}	0.072 ^{b}	0.321 ^{ab}	64.135	4.026
Position 3	0.166 ^{b}	0.086 ^{a}	0.331 ^{a}	55.618	3.349
Position 4	0.168 ^{b}	0.089 ^{a}	0.344 ^{a}	66.408	3.794
SEM	0.023	0.004	0.011	5.142	0.433

Position	Anatomical Measurements				
	AVDIA ^{NS}	AVAREA ^{NS}	ROUND ^{NS}	ASPECT ^{NS}	FLRATIO ^{NS}
Position 1	2.472	6.089	1.340	1.598	1.078
Position 2	2.499	6.223	1.340	1.589	1.075
Position 3	2.489	6.121	1.314	1.552	1.074
Position 4	2.384	5.846	1.320	1.577	1.085
SEM	0.108	0.459	0.015	0.017	0.006

PD/TD Ratio of pith diameter to total diameter of the section
PT/TD Ratio of phloem thickness to total diameter of the section
RD/TD Ratio of diameter of remaining area (excluding pith and phloem) to total diameter of the section
NOVES Number of xylem vessels per mm^2 of the section
AREAVES Percentage of area occupied by the xylem vessels in the section
AVDIA Average diameter of the individual xylem vessels (µm)
AVAREA Average area of the individual xylem vessels (μm^2)
ROUND Roundness of the xylem vessels
ASPECT Aspect ratio of the xylem vessels
FLRATIO Fullness ratio of the xylem vessels

Means with the same letter in a column do not differ significantly as per Duncan's Multiple Range Test at 5 per cent level of significance

NS: Non-significant

Table 2. Difference in anatomy with reference to sex in *Casuarina equisetifolia*

Sex	Anatomical Measurements				
	PD/TD	PT/TD ^{NS}	RD/TD	NOVES ^{NS}	AREAVES ^{NS}
Male	0.140 ^{b}	0.083	0.347 ^{a}	53.233	3.757
Female	0.190 ^{b}	0.077	0.328 ^{a}	68.258	4.208
Monoecious	0.276 ^{a}	0.072	0.290 ^{b}	58.453	2.976
SEM	0.018	0.004	0.009	4.182	0.348

Sex	Anatomical Measurements				
	AVDIA	AVAREA	ROUND	ASPECT	FLRATIO ^{NS}
Male	2.675 ^{a}	6.989 ^{a}	1.298 ^{b}	1.551 ^{b}	1.069
Female	2.443 ^{ab}	6.014 ^{ab}	1.312 ^{b}	1.571 ^{b}	1.079
Monoecious	2.265 ^{b}	5.206 ^{b}	1.376 ^{a}	1.615 ^{a}	1.085
SEM	0.081	0.340	0.008	0.013	0.005

PD/TD Ratio of pith diameter to total diameter of the section
PT/TD Ratio of phloem thickness to total diameter of the section
RD/TD Ratio of diameter of remaining area (excluding pith and phloem) to total diameter of the section
NOVES Number of xylem vessels per mm^2 of the section
AREAVES Percentage of area occupied by the xylem vessels in the section
AVDIA Average diameter of the individual xylem vessels (µm)
AVAREA Average area of the individual xylem vessels (μm^2)
ROUND Roundness of the xylem vessels
ASPECT Aspect ratio of the xylem vessels
FLRATIO Fullness ratio of the xylem vessels

Means with the same letter in a column do not differ significantly as per Duncan's Multiple Range Test at 5 per cent level of significance

NS: Non-significant

Pith diameter was found to be maximum for monoecious trees followed by female and male trees (Plates 1 to 3). A reverse trend was noticed for diameter of the remaining area. Average diameter and area of the xylem vessels were higher for the male trees than the female and monoecious individuals. With reference to the shape of the xylem vessels, male trees exhibited better roundness (circular objects will have a roundness of 1) than the female and monoecious trees (Plate 4). Anatomical studies to distinguish between male and female trees have been carried out in *Bischofia javanica* (Zheng and Cheng, 1998) and the results showed that the ratio of the wood fibre wall thickness to lumen diameter in the female woods was larger than in the male woods and the numbers of septate fibres and the fibre tracheids were smaller.

Acknowledgement

Authors are grateful for the funding support from the Indian Council for Forestry Research and Education. Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors /

publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

Ballester, A., Fernandez-Lorenzo, J.L., Vidal, N., San-Jose, M.C. and Vieitez, A.M. 1999. Anatomical and biochemical events related to differential rooting competence in chestnut shoots cultured *in vitro*. In: *Proceedings of Application of Biotechnology to Forest Genetics* (Eds. Espinel, S. and Ritter, E.). Biofor 99, 22-25 September 1999, Victoria-Gasteiz, Spain, pp. 475-478.

Bendiab, K. 1999. Recherche de marqueurs biochimiques et moléculaire des sexes mâle et femelle du palmier dattier. Apport dans l'amélioration génétique pour la productivité. Atelier 'Constitution et organisation d'équipes de recherche scientifique dans les domaines de foresterie et des arbres fruitiers', Marrakech, 13-15 Avril.

Ewers, F.W. and Aloni, R. 1987. Seasonal secondary growth in needle leaves of *Pinus strobus* and *Pinus brutia*. *Amer. J. Bot*. 74(7): 980-987.

Fortanier, E.J. and Jonkers, H. 1976. Juvenility and maturity of plants as influenced by their ontogenetical and physiological aging. *Acta Hort*. 56: 183-194.

Hamann, A. 1998. Adventitious root formation in cuttings of loblolly pine (*Pinus taeda* L.):developmental sequence and effects of maturation. *Trees* 12: 175-180.

Hormaza, J.I., Dollo, L. and Polito, V.S.1994. Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. *Theor. Appl. Genet*. 89:9-13.

Hackett, W.P. 1985. Juvenility, maturation and rejuvenation in woody plants. *Hortic. Rev*. 7, 109–155.

Huang, L.C., Hsiao, C., Lee, S., Huang, B. and Murashige, T. 1992. Restoration of vigor and rooting competence in stem tissue of mature citrus by repeated grafting of their shoot apices onto freshly germinated seedlings *in vitro*. *In Vitro Cell Dev. Biol*. 28:30-32.

Ifju, G. 1991. Quantitative wood anatomy. Characterization of plantation grown cottonwood (*Populus deltoides* Bart. ex. Marsh.). *Acta Facultatis Ligniensis* 1: 7-40.

Kerstetter, R.A., and Poethig, R.S. 1998. The specification of leaf identity during shoot development. *A. Rev. Cell Dev. Biol*. 14: 373–398.

Mori, M., Suzuki, K. and Kohzaki, R. 2000. Variations in chlorophyll and carotenoid content in the growth process of the ginkgo leaf. *J.Japanese Soc.Food Sci.Technol*. 47(6): 448-451.

Packiyasothy, E.V., Jansz, E.R. and Dharmadasa, H.M. 1991. Studies on some chemical components of nutmeg (*Myristica fragrans* Houtt.) leaf directed at determination of sex of seedlings. *J. Natn. Sci. Coun. Sri Lanka* 19:91-98.

Sivakumar, V., Parthiban, K.T., Singh, B.G., Gnanambal, V.S., Anandalakshmi, R. and Geetha, S. 2002. Variability in drupe characters and their relationship on seed germination in teak (*Tectona grandis* L.f.). *Silvae Genetica* 51(5-6): 232-237.

Zachariah, J., Gopalan, A., Krishnamurthy, B. and Ravindran, P.N. 1986. Steroid degradation compound associated with sex expression in nutmeg (*Myristica fragrans* Houtt.) Proc. Indian Natn. Sci. Acad. 52:685-688.

Zheng, X.F. and Cheng, H.P. 1998. Structural comparison of dioecious wood among three species of broadleaved trees. *Acta Botanica Yunnanica* 20(4): 424-428.

Zimmerman, R.H., Hackett, W.P., and Pharis, R.P. 1985. Hormonal aspects of phase change and precocious flowering. *Encycl.Plant Physiol*. 11: 79-15.

****