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VARIATION IN ANATOMICAL AND CHEMICAL PROPERTIES IN DIFFERENT CLONES OF *Casuarina junghuhniana* AND ITS HYBRIDS

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ABSTRACT

Casuarinas are extensively cultivated across tropical, subtropical, and Mediterranean regions due to their rapid growth, adaptability to diverse environmental conditions, multifaceted utility, and suitability for short-rotation agroforestry systems. This study investigated clonal variation in elite Casuarina germplasm obtained from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, and planted under subtropical conditions in Punjab, India. The focus was on examining wood properties at different tree heights (base, 25%, 50%, and 75%) in a six-year-old plantation. Standard methods recommended by the Technical Association of Pulp and Paper Industry (TAPPI) were employed to assess the wood characteristics. The results revealed significant variability in wood properties among clones. Clone IFGTB CH 6 exhibited the highest mean fibre length (1068.6 µm), while clone IFGTB CH 2 demonstrated the largest fibre diameter (19.16 µm). Clone IFGTB CH 4 had the longest vessel length (512.78 µm), while IFGTB CH 6 exhibited the widest vessel diameter (189.90 µm). In terms of longitudinal distribution, the maximum average fibre length was recorded at the tree base, while the largest fibre diameter was observed at 75% of the tree height. With regard to chemical composition, clone IFGTB CH 2 recorded the highest holocellulose content (64.40%), while IFGTB CJ 108 had the lowest lignin content (31.24%), making it particularly suitable for pulp and paper production. The distribution of holocellulose and lignin content along the height of the tree showed that the maximum holocellulose was found at 75% of the tree height, while the lowest lignin content was observed at the tree base. These findings highlight the considerable variation in wood properties across clones and vertical tree height, with clone IFGTB CH 6 standing out as a promising candidate for wood-based industries due to its superior fibre dimensions, high holocellulose content, and relatively low lignin content. The observed clonal variability in wood properties offers significant potential for advancing tree improvement programs aimed at enhancing the performance of Casuarina in industrial applications.

Keywords: Anatomical, *Casuarina*, Chemical, Clonal variability, Wood properties

Wood has been a fundamental resource for human civilization for millennia, playing a crucial role in construction, furniture making, pulp and paper production, and various other industries. With population growth and the depletion of natural forests, the global demand for wood and wood-based products has surged (ISFR, 2021). In the context of modern technology and industrialization, developing countries face significant challenges in meeting the growing demand for high-quality paper, particularly due to the scarcity of long fibres and woody species with low lignin content. Furthermore, the capacity for expansion is limited, as both natural and artificial forests are insufficient to meet the raw material needs of the pulp and plywood industries (Gregory *et al.*, 2002). In this scenario, several fast-growing exotic tree species have been explored for short-rotation forestry in India, including in the Punjab region, where certain species and clones have demonstrated potential for thriving under the subtropical and semi-arid conditions. Casuarina is one such species, with elite germplasm introduced to Punjab in 2016 from the Institute of Forest Genetics and Tree Breeding (IFGTB) in Coimbatore, Tamil Nadu (Garg *et al.*, 2022).

Casuarina is a genus of 17 tree species, originally native to regions such as Australia, Southeast Asia, Malaysia, and New Caledonia (Brewbaker *et al.*, 1990). The wood of Casuarina, classified as hardwood, is dense, heavy, and dark red, commonly referred to as she-oak, river oak, or Australian pine (Potgieter *et al.*, 2013). Most Casuarina species are found in nutrient-poor environments, and their remarkable adaptability is one of the reasons they have been widely planted across the globe. After being introduced to Australia in the mid-19th century, Casuarina was subsequently planted in various regions, including China, India, the Middle East, East Africa, and the southwestern United States (Zhang *et al.*, 2006). In India, the most commonly planted species are *C. junghuhniana* and *C. equisetifolia*. One of the distinctive characteristics of Casuarina is

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its ability to thrive in poor soil conditions, such as dry, sandy soils, nutrient-depleted soils, and those with free drainage. This is largely due to its nitrogen-fixing capacity, which enables it to grow in environments with limited fertility (Parrotta, 1993). Casuarina trees can also adapt to high salinity, waterlogged areas, and harsh climatic conditions, further enhancing their potential for widespread planting in challenging environments (Zhong *et al.*, 2013).

Due to its durability, Casuarina wood has been historically used for a variety of purposes, including firewood, boat building, house construction, and furniture making (Nicodemus *et al.*, 1996). Additionally, the bark of the Casuarina tree, rich in tannins, has been utilized for dyeing leather and producing fishing nets. Moreover, Casuarina serves as a valuable raw material for the production of chemical and semi-chemical pulps, making it an important species in the forestry and paper industries (Guha *et al.*, 1970). In India, the pulp and paper industry is a significant sector, yet it faces challenges in meeting the growing demand for paper products. Despite having 813 pulp and paper mills, India produces only 14.9 million metric tons (MMT) of paper, accounting for just 3.7% of global paper production. This relatively low output is primarily due to the acute shortage of fibrous raw materials available to the industry. As a result, there has been a concerted effort to explore and develop new, fast-growing Casuarina germplasm that can be effectively utilized by the pulp and paper industry. With approximately 800,000 hectares of Casuarina plantations, India is the world's largest producer of Casuarina (Pinyopusarerk and Williams, 2000). The states of Orissa, Tamil Nadu, Andhra Pradesh, and the Union Territory of Puducherry alone account for around 500,000 hectares of these plantations (Nicodemus, 2009). Previous studies have demonstrated the suitability of Casuarina for use in the pulp and paper industries. Kandeel *et al.* (1982) found that Casuarina yields more pulp with higher tear strength compared to *Eucalyptus camaldulensis*. Guha and Karira (1981) reported that *Casuarina equisetifolia* produced kraft pulp with strength properties comparable to those of *E. grandis* and *E. citriodora*, confirming its suitability for the paper industry. Maheswari *et al.* (1979) suggested that long-fiber pulps, such as those from bamboo, could be blended with Casuarina to mitigate the inadequate availability of bamboo. Additionally, Kumar *et al.* (1996) achieved more than 50% pulp yield from Casuarina, further highlighting its potential for pulp production.

Short-rotation tree species such as *Populus*, *Eucalyptus*, and *Melia* are commonly used by farmers for block and boundary plantations on agricultural lands (Kaur *et al.*, 2017). In recent years, the southwestern districts of Punjab have been grappling with waterlogging and salinity issues, which have disrupted the traditional cropping patterns. As a result, farmers are increasingly seeking alternative tree plantations that are better suited to these challenging soil conditions. Given Casuarina's proven adaptability to saline and waterlogged environments, and the relatively low adoption of *Eucalyptus* plantations in Punjab, there is an urgent need to expand Casuarina cultivation, particularly with elite germplasm, to meet the growing demand for raw materials in the pulp and paper industries.

Understanding the variation among Casuarina clones is crucial for optimizing their use across various wood-based industries, from timber production to environmental management. Casuarina's ability to thrive in saline and waterlogged soils makes it particularly valuable for reclamation and sustainable land use in such areas. This study aims to explore the factors contributing to the variation in wood anatomical and chemical properties among different Casuarina clones. By examining the role of genetic diversity and environmental factors in shaping these traits, the research seeks to identify superior clones that are most suitable for paper and pulp production, thereby contributing to the efficient use of Casuarina in the pulp and paper industry.

MATERIALS AND METHODS

Location and climate

The experimental site is located at 30°59'28"N latitude and 75°52'38"E longitude, at an elevation of 240 meters above mean sea level, representing the subtropical climatic conditions of Punjab. The soil at the site is loamy in texture, with high organic carbon content, and the pH ranges from 8.0 to 8.5. The electrical conductivity of the soil varies between 0.18 and 0.28 mS/cm. The experiment was conducted under optimal irrigation conditions. The climate of the study site is characterized as subtropical to tropical, with three well-defined seasons: a hot and dry summer from April to June, a hot and humid monsoon from July to September, and a cold winter from December to February. Frost occurrences are not common in this region.

Experimental material and design

The clonal evaluation experiment involved nine clones of *Casuarina spp.*, including five hybrids of *C. equisetifolia* × *C. junghuhniana* and four clones of *C. junghuhniana*. The experiment was established at the University Seed Farm, Ladhowal (Ludhiana), Punjab Agricultural University, Ludhiana. The experimental design followed a Completely Randomized Block Design (CRBD), with four trees per clone and three replications, spaced at 4 x 2 meters. This resulted in a total of twelve plants per treatment across the three replications. The total area used for the experiment was 1584 square meters, which included border rows and the space between replications. The *Casuarina* clones used in this study were developed by the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu. Each replication consisted of four trees per clone, with three replications in total. The trees, aged six years and three months, were harvested in 2022 for wood sample collection to assess their wood properties.

Methodology and data collection

Trees were selected based on the higher growth along with straight stem (data not presented here). Samples for the estimating the wood properties were taken in four replications following the simple random sampling method from four different tree heights i.e. Base (0%), 25%, 50 % and 75% of total tree height. Fibre and vessel characteristics, holocellulose, and lignin content were estimated by following the procedure (Jane, 1956) by collecting the wood samples. Vessel length and diameter were estimated by taking the wood samples at diameter at breast height of the tree only, while remaining traits were estimated at 4 different tree heights. The analysis of fibre and vessel parameters were carried out in Electron Microscopy and Nanoscience laboratory and analysis of chemical properties were done in Department of Biochemistry, Punjab Agricultural University, Ludhiana as per procedure given by Technical Association of the pulp and paper industry.

Statistical analysis

Statistical analysis was done as per the procedure laid down for Completely Randomised Design for the interpretation of results as per the method suggested by Gomez and Gomez (1984). The statistical analysis was carried out using IBM SPSS statistics software's *version* 21. Critical difference was calculated to compare the treatment means for all the characters. Standard error (S.E.) was used to determine the CD using the values of the treatments and the difference between the two means at the 5% level of significance (t).

RESULTS AND DISCUSSION

Significant differences among clones, longitudinal directions and their interactions were observed for the fibre dimensions at the age of six years and three months (Table 1 and 2). The maximum fibre length was observed in clone IFGTB CH 6 (1068.6μm), whereas, the minimum was observed in clone IFGTB CH 4 (881.2μm). The fiber length gradually decreased from base to top of the tree (Table 1). The maximum fibre length was observed at the base level (1047.3 μm) and the minimum was observed at 75% of tree height (900.8 μm). Interaction among clone × longitudinal directions revealed significant effects for fibre length. The maximum fibre length (1221.6 μm) was observed from the interaction between the clones and IFGTB CH6 recorded at the base of the tree. Our result for fibre length was similar to those obtained by other researchers. Fibre length varied among five clones of *Eucalyptus tereticornis* at different ages (Rao *et al.,* 2005). In the present study, fibre diameter showed a significant variation for clones and longitudinal

Table 1. Variation in fibre length (μm) among *Casuarina* clones with respect to longitudinal tree height

Clone	Longitudinal directions				
	Base	25%	50%	75%	Mean
IFGTB CH 1	1014.1	1036.3	953.5	972.0	994.0
IFGTB CH 2	1022.2	1012.2	1002.7	826.1	965.8
IFGTB CH 4	986.3	943.3	902.1	693.1	881.2
IFGTB CH 5	978.0	936.2	921.3	1042.5	969.5
IFGTB CH 6	1221.6	1137.6	1055.8	859.2	1068.6
IFGTB CJ 7	1153.2	1125.3	1075.5	861.5	1053.9
IFGTB CJ 9	1009.0	996.1	907.5	898.8	952.8
IFGTB CJ 10	987.8	983.4	879.4	983.3	958.5
IFGTB CJ 108	1053.2	880.3	856.2	970.8	940.1
Mean	1047.3	1005.6	950.5	900.8	976.04

SEm(±) for clone: 5.51
SEm(±) for longitudinal directions: 3.67
SEm(±) for clone × longitudinal directions:11.03
CD ($p \leq 0.05$): For clones: 15.59; Longitudinal directions: 10.40; Clone×longitudinal directions: 31.19

Table 2. Variation in fibre diameter (μm) among *Casuarina* clones with respect to longitudinal height

Clone	Longitudinal Directions				
	Base	25%	50%	75%	Mean
IFGTB CH 1	15.21	14.42	17.11	19.45	16.55
IFGTB CH 2	19.20	19.40	19.41	18.63	19.16
IFGTB CH 4	16.31	16.89	15.08	15.19	15.87
IFGTB CH 5	10.99	12.31	17.38	18.71	14.85
IFGTB CH 6	20.80	15.09	16.73	21.45	18.52
IFGTB CJ 7	14.61	16.28	11.80	16.45	14.79
IFGTB CJ 9	16.10	16.21	19.57	17.04	17.23
IFGTB CJ 10	14.33	15.21	15.18	13.03	14.44
IFGTB CJ 108	14.94	15.78	16.21	15.95	15.72
Mean	15.83	15.73	16.50	17.32	16.34

SEm(±) for clones: 0.115
SEm(±) for longitudinal directions: 0.077
SEm(±) for clones × longitudinal directions: 0.230
CD ($p \leq 0.05$): For clones: 0.33; Longitudinal directions: 0.22; Clone × Longitudinal directions: 0.65

directions of the trees (Table 2). The maximum value was observed in clone IFGTB CH 2 (19.16 μm) followed by clone IFGTB CH6 (18.52 μm) and the minimum was observed in clone IFGTB CJ 10 (14.44 μm). Among the longitudinal directions of tree, the maximum value was observed at 75% of tree height (17.32 μm) and the minimum was observed at 25% of tree height (15.73 μm). Interaction among clone × longitudinal directions revealed significant effects for fibre diameter. In general, the maximum value was observed for clone IFGTB CH 6 (21.45μm) at 75% of tree height.

Fibre dimensions are critical quality parameters in pulp and paper production. In the present study, the fibre dimensions of *Casuarina* clones were found to be larger than those reported in previous studies. For instance, Varghese and Sivaramakrishna (1996) observed that 10-year-old *C. equisetifolia* trees grown in India had fibre diameters ranging from 4.0 to 10.8 μm. In contrast, *C. equisetifolia* grown in Egypt exhibited a larger fibre diameter of 13.8 μm (El-Osta *et al.*, 1981). Mahmood (1993) reported an average fibre diameter of 14.81 ± 4.9 μm for *C. equisetifolia* trees in Pakistan, while Aree *et al.* (2021) found an overall fibre diameter value of 15.542 μm. Among the different clones in this study, *IFGTB CH 4* recorded the highest vessel length (512.78 μm), significantly surpassing the other clones, followed by *IFGTB CH 2* (501.47 μm) (Table 3). The lowest vessel length was observed in *IFGTB CJ 10* (404.30 μm). Clones *IFGTB CH 1*, *IFGTB CH 2*, and *IFGTB CH 4* exhibited average vessel lengths greater than the overall mean of all clones, indicating their above-average performance. Vessels, a characteristic

Table 3. Variation in vessel length (μm) and diameter (μm) among *Casuarina* clones

Clones	Vessel length (μm)	Vessel diameter (μm)
IFGTB CH 1	494.82	145.35
IFGTB CH 2	501.47	181.34
IFGTB CH 4	512.78	138.74
IFGTB CH 5	428.27	178.33
IFGTB CH 6	405.50	189.90
IFGTB CJ 7	420.32	136.32
IFGTB CJ 9	446.24	132.22
IFGTB CJ 10	404.30	147.35
IFGTB CJ 108	448.77	146.86
Mean	451.38	155.15
SEm(±)	5.768	2.549
CD ($p \leq 0.05$)	17.26	7.63

feature of hardwoods, are essential for the conduction of water and mineral nutrients in living trees (Panshin and Zeeuw, 1980). Vessel size directly influences the properties of paper, with smaller vessels generally being preferred for better paper quality (Sreevani and Rao, 2015). Vessel length is one of the major anatomical factors used to evaluate the quality of pulpwood. The maximum vessel diameter was recorded in *IFGTB CH 6* (189.90 µm), followed by *IFGTB CH 2* (181.34 µm), while the minimum was observed in *IFGTB CJ-9* (132.22 µm). Clones *IFGTB CH 2*, *IFGTB CH 5*, and *IFGTB CH 6* demonstrated vessel diameters greater than the overall average for all clones, suggesting their superior performance. These findings align with previous studies, such as Sreevani and Rao (2015), who reported similar results for *Eucalyptus tereticornis*, and Pande and Singh (2009), who observed vessel diameter variation across 17 clones of *E. tereticornis*. Aree *et al.* (2021) also noted similar values for vessel length (0.545 mm) and vessel diameter (179.806 µm).

Cellulose and lignin are the primary structural and chemical components of wood, with species exhibiting high cellulose content and low lignin levels being particularly suitable for pulp and paper production. In this study, while all nine *Casuarina* clones exhibited relatively similar chemical compositions, there were significant differences in their holocellulose content (Table 4). The highest holocellulose content was recorded in *IFGTB CH 2* (64.40%), which was significantly superior to all other clones. However, the holocellulose content in the *Casuarina* clones was generally lower than that observed in *Eucalyptus* species. The lowest holocellulose content was found in *IFGTB CJ 108* (57.92%). Regarding longitudinal variation, the highest holocellulose content was observed at 75% of tree height (64.83%), followed by 50% of tree height (63.02%). The lowest content was recorded at the base of the tree (58.24%). The interaction between clone and longitudinal direction showed significant effects, with an increase in holocellulose content at higher tree heights. Specifically, the maximum holocellulose content was observed in *IFGTB CH 2* (68.40%) at 75% of tree height, followed by *IFGTB CH 4* (67.40%) at the same height. Holocellulose is a crucial factor in determining the quality of wood for pulp and paper production, as it directly influences the yield and strength of the pulp.

Earlier studies have reported that holocellulose content in *Eucalyptus* clones ranged from 68.5% to 74.6% (Vennila *et al.*, 2011), which is consistent with the findings in the present study, where significant variation in holocellulose content was observed among *Casuarina* clones. The differences in holocellulose content across longitudinal directions, as reported in this study, align with similar findings in 8-year-old *Leucaena leucocephala* (Nazri *et al.*, 2009). Plant materials with a cellulose content of 34% or higher are typically considered promising for pulp and paper production from a chemical composition perspective (Nieschlag *et al.*, 1960). While cellulose content was not measured separately in this study, the high holocellulose content (above 64%) serves as a clear indication of high cellulose content (likely exceeding 34%). According to Madakadze *et al.* (1999), cellulose content is directly proportional to the mechanical strength of pulp, particularly its tensile strength. The higher the cellulose content in the wood, the greater the pulp yield it will produce (Sykes *et al.*, 2003). Increasing cellulose content in wood not only enhances pulp yield

Table 4. Variation in holocellulose content (%) among *Casuarina* clones with respect to longitudinal height

Clones	Longitudinal directions				
	Base	25%	50%	75%	Mean
IFGTB CH 1	59.80	60.50	61.50	62.40	61.05
IFGTB CH 2	61.40	61.80	66.00	68.40	64.40
IFGTB CH 4	58.40	59.50	65.90	67.40	62.80
IFGTB CH 5	56.90	60.80	62.10	64.30	61.02
IFGTB CH 6	58.60	61.30	62.10	64.10	61.52
IFGTB CJ 7	58.60	61.10	63.40	66.40	62.37
IFGTB CJ 9	58.00	60.30	63.30	64.40	61.50
IFGTB CJ 10	59.10	60.10	63.30	62.80	61.32
IFGTB CJ 108	53.40	55.40	59.60	63.30	57.92
Mean	58.24	60.08	63.02	64.83	61.54

SEm (±) for clone: 0.468
SEm (±) for longitudinal directions: 0.312
SEm (±) for clone × Longitudinal position: 0.936
CD ($p \leq 0.05$): For clones: 1.32; Longitudinal directions: 0.88 ; Clone × Longitudinal directions: 2.64

Table 5. Performance of various clones of *Casuarina* for lignin content (%) at different heights

Clones	Longitudinal Directions				
	Base	25%	50%	75%	Mean
IFGTB CH 1	31.40	33.79	33.80	33.80	33.20
IFGTB CH 2	30.00	32.68	32.68	32.69	32.01
IFGTB CH 4	35.00	31.79	31.80	31.80	32.60
IFGTB CH 5	31.40	32.00	32.00	32.00	31.85
IFGTB CH 6	30.20	31.92	31.93	31.93	31.49
IFGTB CJ 7	31.80	31.29	31.29	31.30	31.42
IFGTB CJ 9	33.60	34.19	34.20	34.20	34.05
IFGTB CJ 10	30.00	33.83	33.83	33.84	32.88
IFGTB CJ 108	30.60	31.45	31.46	31.46	31.24
Mean	31.55	32.55	32.55	32.56	32.30

SEm (±) for clones: 0.240
SEm (±) for Longitudinal directions: 0.160
SEm (±) for clones × Longitudinal directions: 0.481
CD ($p \leq 0.05$): For clones: 0.68, Longitudinal directions: 0.45'
For clone × Longitudinal directions: 1.36

but also reduces pulping costs and improves the overall efficiency of pulp and paper mills.

Lignin content exhibited significant variation among the *Casuarina* clones studied (Table 5). The lowest lignin content was observed in *IFGTB CJ 108* (31.24%), which was significantly lower than that of *IFGTB CH 1*, *IFGTB CH 2*, *IFGTB CH 4*, *IFGTB CJ 9*, and *IFGTB CJ 10*. On the other hand, the highest lignin content was recorded in *IFGTB CJ 9* (34.05%). Regarding longitudinal variation, the minimum lignin content was observed at the base of the tree (31.55%), with the maximum lignin content found at 75% of the tree height (32.56%). The interaction between clones and longitudinal directions showed significant effects, with *IFGTB CH 2* and *IFGTB CJ 10* exhibiting the lowest lignin content (30.00%) at the base of the tree. Lignin distribution in wood significantly influences all methods of turning wood into paper (Westermark *et al.*, 1988). In the pulp and paper industry, lignin is generally considered undesirable because its presence leads to the yellowing of paper products over time if not adequately removed. Additionally, higher lignin content increases pulping costs, as it requires more intensive chemical breakdown, a process that can be expensive. Therefore, reducing lignin content in wood can reduce processing costs and improve pulp quality. Parthiban *et al.* (2011) reported that the lignin content in 25 *Casuarina* hybrid clones (*C. junghuhniana* × *C. equisetifolia*) ranged from 24.2% to 27.8%. In contrast, Topalogu and Erisir (2018) observed an increase in lignin concentration from the base to the top of *Fagus orientalis* and *Abies nordmanniana* stems. In the present study, the lignin content in the *Casuarina* clones was found to be at satisfactory levels (~30%), which suggests that these clones are suitable for pulp and paper production. Kojimal *et al.* (2007) also found that Klason lignin content in wood was inversely correlated with pulp sheet density, a key characteristic influencing the physical properties of pulp.

CONCLUSION

A significant variation in fibre and vessel dimensions, as well as chemical constituents such as holocellulose and lignin, was observed among the nine clones of *Casuarina* and its hybrids grown under the subtropical conditions of Punjab. These parameters exhibited varying levels along the tree height, from the base to 75% of the total tree height. Among the clones, the highest holocellulose content was recorded in *IFGTB CH 2* (64.40%), while the highest holocellulose content among the longitudinal directions of tree height was observed at 75% of the tree height (64.83%). The minimum lignin content was found in *IFGTB CJ 108* (31.24%). For anatomical properties, the maximum fibre length was observed in *IFGTB CH 6* (1068.6 µm), while the maximum fibre diameter was found in *IFGTB CH 2* (19.16 µm). The longest vessel length was recorded in *IFGTB CH 4* (512.78 µm), and the largest vessel diameter was observed in *IFGTB CH 6* (189.90 µm). Based on the high holocellulose content and the desirable values for lignin, fibre, and vessel traits, *IFGTB CH 2* emerged as the superior clone for use in the pulp and paper industry. These findings serve as a valuable baseline for the industry, highlighting *IFGTB CH 2* as a prime candidate for future cultivation and processing. The results of the present study hold significant practical implications. Identifying superior clones with high wood quality is crucial for advancing *Casuarina* cultivation in subtropical climates such as Punjab. These findings pave the way for the wider adoption of *Casuarina* in the region, promoting its potential as a sustainable and valuable resource for the pulp and paper industry.

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