Differences in wood properties among *Eucalyptus grandis* and *Eucalyptus grandis* x *Eucalyptus urophylla* with different degrees of ploidy

Diferenças nas propriedades da madeira entre *Eucalyptus grandis* e *Eucalyptus grandis* x *Eucalyptus urophylla* com diferentes graus de ploidia

Diferencias en las propiedades de la madera entre *Eucalyptus grandis* y *Eucalyptus grandis* x *Eucalyptus urophylla* con diferentes grados de ploidía

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Abstract
We compared the anatomy, density, chemical contents, and bioenergy values of *Eucalyptus grandis* and hybrids of *Eucalyptus grandis* x *Eucalyptus urophylla* wood originating from diploids, triploids and tetraploids. We hypothesize that *Eucalyptus grandis* and hybrids of *Eucalyptus grandis* x *Eucalyptus urophylla* with different degrees of ploidy have variations as a result of different sets of chromosomes producing different phenotypic expressions and chemical constituents, such as variation in cell size and frequency, which would directly influence wood quality. Twenty-year-old trees were cut, eight for each ploidy: diploids and tetraploids are *E. grandis*; triploids are *E. grandis* x *E. urophylla*. We use standardized techniques. Our hypothesis was confirmed. Triploid and tetraploid trees presented wider trunks, taller trees with longer stems and wider crowns compared to diploid trees. Wood density showed significant radial variation only in diploids, while triploid and tetraploid trees were more homogeneous. In polyploid trees, the anatomical features did not clearly present a radial pattern. Triploid and tetraploid trees presented higher density wood than diploid trees. The chemical constituents varied from pith to bark in the three ploidies, but no differences between ploidies were found. For energy generation purposes, diploid and triploid trees are more desirable than tetraploid trees.
Keywords: Chromosomes; Biomass; Wood quality; Forest breeding; Polyploidy.

Resumo
Comparamos a anatomia, densidade, conteúdo químico e valores de bioenergia de *Eucalyptus grandis* e híbridos de madeira de *Eucalyptus grandis* x *Eucalyptus urophylla* originários de diplóides, triploides e tetraploides. Nossa hipótese é que *Eucalyptus grandis* e híbridos de *Eucalyptus grandis* x *Eucalyptus urophylla* com diferentes graus de ploidia têm variações como resultado de diferentes conjuntos de cromossomos produzindo diferentes expressões fenotípicas e constituintes químicos, como variação no tamanho e frequência das células, o que influenciaria diretamente a madeira qualidade. Foram cortadas árvores com 20 anos, oito para cada ploidia: diplóides e tetraplóides são *E. grandis*; triplóides são *E. grandis* x *E. urophylla*. Usamos técnicas padronizadas. Nossa hipótese foi confirmada. Árvores triplóides e

tetraplóides apresentaram troncos mais largos, árvores mais altas com troncos mais longos e copas mais largas em comparação com árvores diplóides. A densidade da madeira apresentou variação radial significativa apenas nos diplóides, enquanto árvores triplóides e tetraplóides foram mais homogêneas. Nas árvores poliplóides, as características anatômicas não apresentaram claramente um padrão radial. Árvores triplóides e tetraplóides apresentaram maior densidade de madeira do que a das árvores diplóides. Os constituintes químicos variaram da medula a casca nas três ploidias, mas não foram encontradas diferenças entre as ploidias. Para fins de bioenergia, árvores diplóides e triplóides são mais adequadas do que árvores tetraplóides.

Palavras-chave: Cromossomos; Biomassa; Qualidade da madeira; Melhoramento florestal; Poliploidia.

Resumen

Comparamos la anatomía, densidad, contenido químico y valores de bioenergía de *Eucalyptus grandis* e híbridos de madera de *Eucalyptus grandis* x *Eucalyptus urophylla* provenientes de diploides, triploides y tetraploides. Planteamos la hipótesis de que *Eucalyptus grandis* e híbridos de *Eucalyptus grandis* x *Eucalyptus urophylla* con diferentes grados de ploidía tienen variaciones como resultado de diferentes conjuntos de cromosomas que producen diferentes expresiones fenotípicas y constituyentes químicos, como la variación en el tamaño y la frecuencia de las células, lo que influiría directamente en la calidad de la madera. Se cortaron árboles de veinte años, ocho por cada ploidía: diploides y tetraploides son *E. grandis*; los triploides son *E. grandis* x *E. urophylla*. Usamos técnicas estandarizadas. Nuestra hipótesis fue confirmada. Los árboles triploides y tetraploides presentaron troncos más anchos, árboles más altas con tallos más largas y copas más anchas en comparación con los árboles diploides. La densidad de la madera mostró una variación radial significativo solo en diploides, mientras que los árboles triploides y tetraploides fueron más homogéneos. En árboles poliploides, las características anatómicas no presentaban claramente un patrón radial. Los árboles triploides y tetraploides presentaron madera de mayor densidad que los árboles diploides. Los componentes químicos variaron de la médula a la corteza en las tres ploidías, pero no se encontraron diferencias entre las ploidías. Para la generación de energía, los árboles diploides y triploides son más deseables que los tetraploides.

Palabras clave: Cromosomas; Biomasa; Calidad de la madera; Mejoramiento forestal; Poliploidía.

1. Introduction

Polyploidy is a term used to describe more than two sets of homologous chromosomes in nucleus, cell, or organism. It has been recognized as a major force in angiosperm evolution (Soltis, 2009; Diallo, 2016). Polyploidy techniques are widely used in forest production to acquire a new variety which can increase yield and tree quality, as well as improve resistance and reproduction (Janick & Moore, 1996; Bao & Louis, 1984). Hoshino et al. (2011) reported triploid plants, which have economic benefits in several kinds of farming systems. According to Zobel and Jett (2012), morphology and physiology of plants respond to genetic control, and these, in turn, influence the properties of wood.

Studies have shown the successful application of polyploidy in *Eucalyptus* used worldwide for breeding, e.g., Roth (1984) with *Eucalyptus urophylla* clones; Praça et al. (2009) with *Eucalyptus* spp., Lin et al. (2010) with *Eucalyptus globulus* and Han et al. (2011) with *Eucalyptus grandis*. Studies with polyploids are certainly a necessary first step, however more field results are needed to be sure of stability of wood properties found in polyploids and that, in fact, these wood properties are superior compared to diploids.

The use of polyploids in other species is also studied and has consistent results, e.g., Kang (2020) in triploid clones of *Populus* spp. in China suggest that triploid breeding will play an important role in increasing yield of forest production, improving wood quality and stress resistance. Chi et al. (2018) in triploids of *Acacia auriculiformis* and its hybrid with *A. mangium* report the advantage of using triploid clones, because in addition to characteristics of interest of plants, these triploids are infertile, which is desirable when natural regeneration is not aimed at. Griffin et al. (2015) in study with *Acacia* species in Vietnam report that development of polyploids is a means of introducing diversity in populations, in addition to improvements in wood fibers and other properties.

We didn't find many studies with tetraploid planting, according to Griffin et al. (2014) the wood has interesting properties, because growth rates are not adequate. Thus, our study provides important information for potential use of tetraploids in practice.

Among tree species, many of the *Eucalyptus* and *Corymbia* genera have been widely used for their rapid growth, productivity, adaptive capacity, as well as their diversity of applications. Species of both aforementioned genera represent a large

portion in the Brazilian timber sector, both in the production of pulp and paper and for production of lumber, the demand of which has increased in recent years. According to IBÁ (2019), Brazil reported planting of 7.83 million hectares, and country stands out in world trade as the largest exporter of cellulose.

Because of the importance of *Eucalyptus* and *Corymbia* wood, studies on plant breeding are justified, particularly in the context of polyploidy.

Here, we compared wood anatomy, wood density, chemical contents, and bioenergy values of *Eucalyptus grandis* and hybrids of *Eucalyptus grandis* x *Eucalyptus urophylla* originating from different ploidy degrees (diploids, triploids and tetraploids). We hypothesized that *Eucalyptus grandis* and hybrids of *Eucalyptus grandis* x *Eucalyptus urophylla* with different ploidy degrees have variations in the aforementioned characteristics, as a result of different sets of chromosomes producing different phenotypic expressions and chemical constituents, such as variation in cell size and frequency, which would directly influence wood density. This cellular variation has already been noticed in the dimensions of stomata, leaf and fruit size (Oda et al., 2014); thus, variation is also likely to occur in wood cells, thus affecting their properties.

2. Methodology

In our study, we used qualitative methods according to Pereira et al. (2018). In our study, we performed field and laboratory activities to achieve the necessary results for discussion and conclusion. Additionally, in methodologies described below there are standardized references for each variable.

2.1 Sampling

Eucalyptus grandis trees were produced from half-sibling seeds of a clonal planting, and the 10,000 largest seeds were selected for this study in 1987. For polyploidy induction, aqueous solution containing 0.5% colchicine and 10% glycerol (both from Sigma) was used. The seeds were incubated at 25°C on an orbital shaker (100 rpm) for 24 hours using a solution of colchicine. In 200 seeds, water treatment was carried out as a control group. Seeds were germinated, and plants were cultured for 84 days in pots (55 mm diameter) containing autoclaved sand soaked with mineral salts.

At 84 days of age, the leaves of each plant were visually evaluated, and plants with thicker, wider leaves and darker colour were selected and planted in test in the field. At 1.5 years of age, polyploid plants were identified by number of leaf stomata and greater length of branch fibers compared to diploid control. The number of stomata was assessed according to Roth (1984). Epidermis strips were plucked from the abaxial surface of leaves and observed under a light microscope. From each plant evaluated, two leaves were chosen, and the frequency of stomata was microscopically measured, as a criterion used for selection.

The project continued in a population formation regime with open-pollinated seed harvested in polyploid *E. grandis*. These *E. grandis* matrices were next to the commercial plantation of *E. grandis* and experimental plot of *E. urophylla*.

Seed collection was carried out on *E. grandis* plants, probable tetraploids. With the seeds obtained through open pollination, an experimental planting was established.

In this experimental planting, at age of 1.5 years, the selection of superior plants was carried out, which were evaluated for their morphology, and those with the most leathery, darker leaves and wavy edges were selected to evaluate stomata number, and fiber length (stem). The polyploid candidate plants were evaluated according to evaluation methodology available at the time, being considered polyploids when stomata number per leaf area was around 50% less than diploid plant (control) and fiber length was 15% greater than diploid (control). The candidate plants for polyploids were cloned and the second generation population clonal bank was installed in the field, with 99 clones and 3 replicates per clone, planted in 1995, at a spacing of 5 x 5 m, without experimental design, but with random distribution of clones and repetitions. According to Harbard et al. (2012), size and distribution of stomata can be used, but flow cytometry is preferred method for ploidy determination. Then, the ploidy of all

plants was evaluated by flow cytometry in 2013. At the time, in addition to triploids and tetraploids, diploid plants were also identified (Figure 1 and Table 1), showing ploidy behavior and plant flow cytometry results (Oda et al., 2014).

Figure 1. DNA-histograms showing G1/G0 peaks from nuclear suspensions stained with DAPI of leaves tissue of *Eucalyptus*. Note (a) diploid (internal standard), tuned to channel 200 and (b) triploid (channel 300), (c) tetraploid (channel 400) and (d) peaks resulting from simultaneous processing of nuclei plant 2C (standard) on channel 200, 3C (channel 300) and 4C (channel 400).
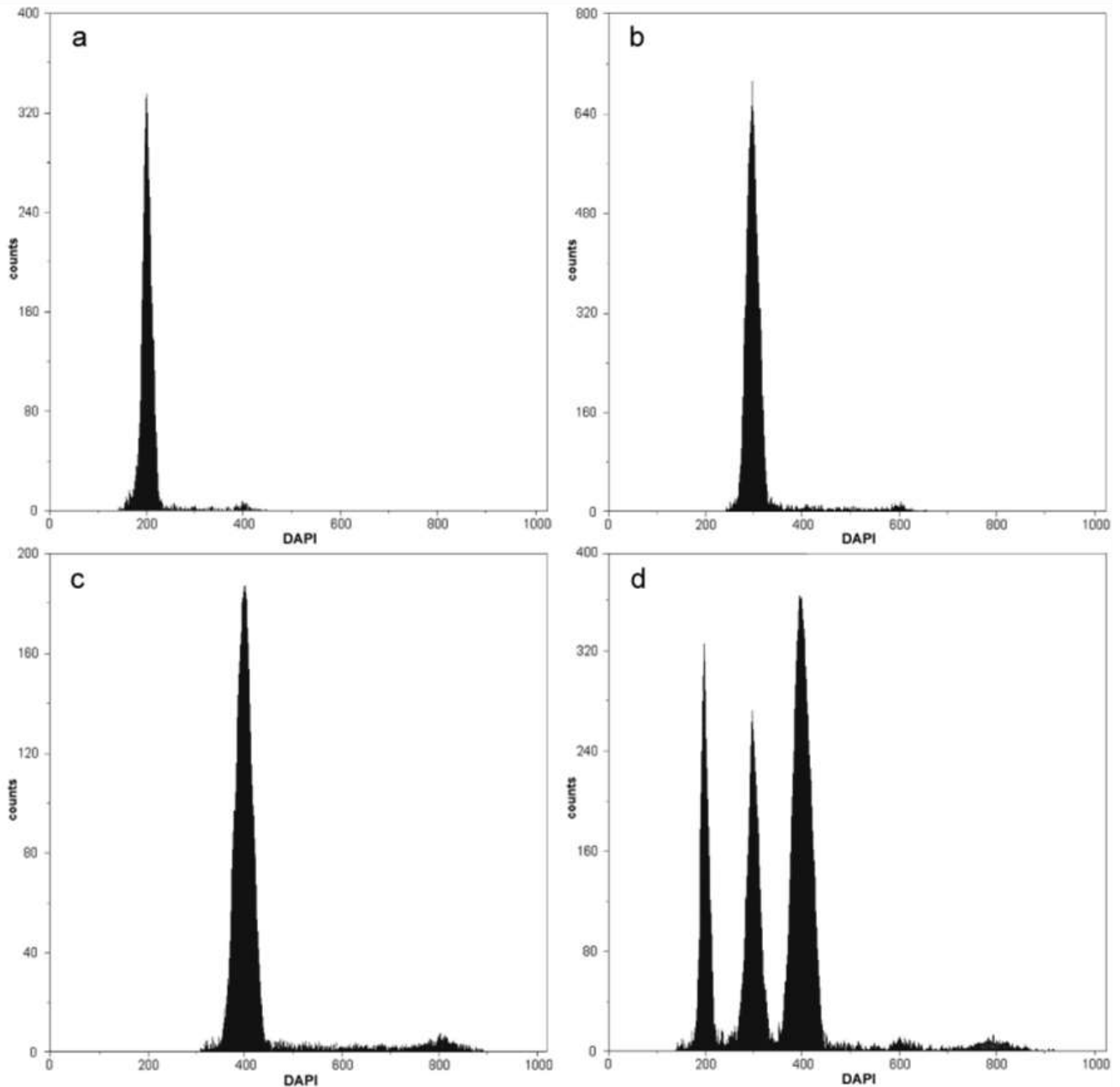
Source: Oda et al. (2014).

Table 1. Results of cytometry of *Eucalyptus* plants used in the present study.

Clone	Index	Ploidy
1	207	diploid
2	209	diploid
3	209	diploid
4	300	triploid
5	311	triploid
6	308	triploid
7	413	tetraploid
8	380	tetraploid
9	416	tetraploid
10	207	diploid
11	207	diploid
12	308	triploid
13	293	triploid
14	416	tetraploid
15	393	tetraploid

Source: Oda et al. (2014).

In 2015, 24 trees were collected from an exploratory forest inventory, eight from each ploidy (diploid, triploid and tetraploid); eight diploids and eight tetraploids from *E. grandis*, and eight triploids from *E. grandis* x *E. urophylla*.

Tree height was determined with a Forestor Vertex hypsometer. The diameter at breast height (DBH, 1.3 m from the ground) was measured with a caliper. A tape measure was used to determine mean diameter of crown (crown width) in the ground in two directions and dividing by two. A disc ≈ 10 cm thick in the stem base region was removed from each tree. In each disc, we cut out a strip, which was divided in half (transversely). The top was used for wood density and anatomical analysis, and the bottom was used for chemical assays. In each disc, strips were cut from pith to bark, and from each strip, six samples were taken for analysis. The samples were cut by ray percentage, i.e., in similar-sized parts from pith to bark. The pith region was considered 0% and the bark region 100%.

2.2 Wood density

Wood density (ρ12) was determined according to Glass and Zelinka (2010), evaluating the mass and volume at 12% moisture content (MC). Specimens of 5 x 2 x 2 cm were conditioned at constant temperature and MC (21ºC and 65% MC, respectively), and under these conditions, mass was determined with an analytical balance. The volume was estimated with a caliper rule used to measure their dimensions.

2.3 Anatomical features

The same samples used for wood density determination were reduced to blocks of 1.5 cm^3. The blocks were softened in boiling water and glycerin (4:1), and 18-20 μm thick sections were prepared on a sliding microtome (Zeiss Hyrax S50). Transverse and longitudinal sections were bleached with sodium hypochlorite (60%), washed in water, and stained with safranin (1%) (Johansen, 1940). Macerations were prepared according to the Franklin method (Berlyn & Miksche, 1976), stained with aqueous safranin, and mounted in a solution of water and glycerin (1:1). Terminology followed the IAWA list (IAWA Committee, 1989). All anatomical measurements were performed on a microscope (Olympus CX 31) equipped with a camera (Olympus Evolt E330) and a computer with image analyzer software (Image-Pro 6.3).

2.4 Chemical assays

To determine extractives (EX) and lignin (LI) contents, TAPPI standards T204 (2004a) and T222 (2004b) were used, respectively. The samples were fragmented into smaller pieces with a hammer and chisel and milled in a micro mill. The resulting powder was sieved through 40 and 60 mesh screens, and the material retained on the last sieve was used for analysis. The analyses were sequential such that the extractives were first removed, then lignin by acid treatment, followed by calculation of holocellulose content. For extractive contents, solutions of toluene: alcohol (2:1 v:v) and alcohol extractions were employed, at times exceeding 12 h in a Soxhlet extractor. For lignin, extractive-free powder was prepared in several stages with 72% sulfuric acid to obtain insoluble and soluble lignin (Cary 100 UV–visible spectrophotometer). Finally, the two values of lignin were added. Insoluble lignin (IL) content was determined (Equation 1). For soluble lignin (SL), we analyzed filtrates, and the blanks were read at two wavelengths (215nm and 280nm) using quartz cuvettes. Soluble lignin content was determined (Equation 2). Then, the holocellulose (HO) content was determined (Equation 3).

$$IL = \left(\frac{DWlig}{DW}\right) x\ 100$$

Equation 1

where DW = Dry sawdust weight, and DWlig = Dry weight of insoluble lignin.

$$SL = \left(\frac{4.53x(L.215 - blank) - (L.280 - blank)}{300x\ DW}\right) x\ 100$$

Equation 2

where DW = Dry sawdust weight. Ex and Li were expressed as a percentage (%) of oven-dry weight of unextracted wood.

$$HO = 100 - (Ex + Li)$$

Equation 3

2.5 Bioenergy values

In this step, samples from pith to bark (wood near the bark) were mixed. The moisture content was determined based on the methodology described by NBR 14929: 2003 (Wood - Determination of the Moisture Content of Chips - Method for Oven Drying). Wood chips were placed in a forced ventilation oven at $103°C ± 2°C$ until constant mass was obtained. Mass is considered constant when, after successive weighing, no change is observed in reading ($± 0.5$ g) within a time interval of one hour. The moisture content was determined (Equation 4).

We determined Higher Heating Value (HHV) using an adiabatic calorimeter pump, following NBR 8633: 1984 (Charcoal - Determination of Calorific Value - Test). The samples were fragmented into smaller pieces with a hammer and chisel and milled in a micro mill. The resulting powder was sieved through 40 and 60 mesh screens, and the material retained on the last sieve was used for analysis. A 1g sample was taken from each sample, homogenized and again separated into three fractions. We also determined Lower Heating Value (LHV) (Equation 5). To calculate Useful Heating Value (UHV), we used a moisture content of 20% in dry basis (average value at which firewood is burned for use in poultry), which represents 16.67% moisture in wet basis (Equation 6).

$$MC = \left(\frac{WM - DM}{DM}\right) x 100$$

Equation 4

where MC = moisture content (%), WM = Sample wet mass (g), and DM = Sample dry mass (g).

$$LHV = HHV - \left(600x8 - \left(\frac{H}{100}\right)\right)$$

<div align="right">Equation 5</div>

where LHV = Lower Heating Value (kJ.kg^{-1}), HHV = Higher Heating Value (kJ.kg^{-1}), and H = Hydrogen (%).

$$UHV = LHV \; x \; \left(100 - \frac{U}{100}\right) - 6 \; x \; U$$

<div align="right">Equation 6</div>

where UHV = Useful Heating Value (kJ.kg^{-1}), LHV = Lower Heating Value (kJ.kg^{-1}), and U = Wet basis moisture content (%).

2.6 Data analysis

We initially undertook descriptive statistical analysis and used Box Plot graphics to detect outliers. Thus, values 1.5 times higher than the 3rd quartile and values 1.5 times lower than the 1st quartile were excluded from the analysis. Normality tests were performed to check the distribution of data, and when a normal distribution was not observed, data were square root-transformed. Then, a parametric analysis of variance (one-way analysis of variance (ANOVA)) was performed. When a significant difference was observed, Tukey's test was used to identify pairs of significantly different means. We analyzed the radial variation from pith to bark and also six radial positions together to compare the different ploidy.

We also utilized a multivariate analysis via principal components analysis to verify the grouping of the different observed responses to different ploidies, taking into account the entire set of anatomical, physical, chemical and heating values. Because the measurement units differed among features, the data were log-transformed to reduce the effect of numeric scale (McGarigal et al., 2000).

3. Results

3.1 Tree size

Triploid and tetraploid trees had wider trunks, taller individuals with longer stems and wider crowns compared to diploid trees (Table 2).

Table 2. Dendrometric data of 20-year-old *Eucalyptus grandis* (diploid and tetraploid) and *Eucalyptus grandis* x *Eucalyptus urophylla* (triploid) trees. DBH = diameter at breast height. Average of eight trees in each ploidy.

Ploidy	DBH (cm)	Tree height (m)	Stem height (m)	Crown width (m)
Diploid	34.2b	40.1b	28.7b	3.1b
Triploid	41.1a	46.8a	32.8a	3.9a
Tetraploid	40.3a	45.1a	32.1a	3.7a

<div align="center">Source: Authors.</div>

3.2 Wood density

Wood density varied in each ploidy. In diploid plants, density was higher at the 60% position radially towards bark. In triploid plants, wood density did not vary much radially, and it was lower only in the 60% position. In tetraploid plants, wood density was higher near the pith and also at the 60% and 80% positions. Tetraploid plants presented denser wood (771 kg.m^{-3}) when compared to the other two ploidies (717 kg.m^{-3} and 727 kg.m^{-3}, diploid and triploid, respectively) (Table 3).

3.3 Anatomical features

Vessel element length variation was different among the three ploidies without an obvious common pattern. In the joint analysis, longer vessel elements occurred in triploids, while diploids and tetraploids did not differ from each other. Vessel diameter also showed variation in the three ploidies, in which wider vessels were observed close to the bark (100% position) in diploids, between 80% and 100% in triploids and between 40% and 80% in tetraploids. Tetraploids had wider vessels, and triploid vessels were narrower between the three ploidies. Vessel frequency in diploids decreased towards the bark. In triploid and tetraploid trees, vessel frequency did not show an evident pattern. Among ploidies, diploids showed higher and triploids lower vessel frequencies (Table 3).

Ray height and width varied radially in the three ploidies and oscillated without a pattern of increase or decrease towards the bark. When comparing the ploidies, higher rays were observed in tetraploid plants, and wider rays in triploid and tetraploid plants. Ray frequency was different among ploidies. In diploids, values oscillated; in triploids, no radial difference was noted, and in tetraploids, ray frequency increased towards to the bark. Among ploidies, diploids showed the highest and triploids the lowest RF values (Table 3).

Although fiber length did not present the same pattern among the three ploidies, longer fibers, in general, occurred closer to the bark. Fiber diameter oscillated radially without a clear pattern. Thicker wall fibers were observed near the bark in diploid and triploid plants, while in tetraploid plants, thicker wall fibers occurred from the 40% position. Among ploidies, we detected longer fibers in triploid plants, larger and thicker wall fibers in triploid and tetraploid plants, although wall thickness did not differ between tetraploids and diploids (Table 3).

3.4 Chemical assays

Extractive content varied similarly in the three ploidies, with increase toward the bark and higher contents in the 80% position in diploid plants and in positions 80% and 100% in triploid and tetraploid plants, respectively. Lignin content differed in radial direction among ploidies. In diploid plants, the highest value was observed in the 80% position. In triploids, the lowest extractive content occurred near the pith (0%). In tetraploid plants, higher values occurred in the 80% and 100% positions. Holocellulose content followed an inverse pattern of extractive content with decrease towards the bark in the three ploidies. The three chemical constituents studied did not show any variation among ploidies (Table 3).

3.5 Bioenergy values

Regarding bioenergy analysis, tetraploid wood showed lower values of HHV, LHV and UHV (Table 4). Table 5 and figure 2 show the correlations among the variables examined and the first and the second ordination axes responsible for 58.45% of the explanation of PCA. Axis 1 contributed 33.44% of the variability, and the variables most correlated to it were as follows: vessel diameter (r = 0.859), fiber length (r = 0.799), and holocellulose content (r = -0.787). Axis 2 contributed 25.01% of the variability, and the variables with higher correlation coefficients were as follows: higher heating value (r = 0.865), lower heating value (r = 0.865), and useful heating value (r = 0.811).

Table 3. Anatomical features, density and chemical contents of 20-year-old *Eucalyptus grandis* (diploid and tetraploid) and *Eucalyptus grandis* x *Eucalyptus urophylla* (triploid) trees.

Radial position	VEL (μm)	VD (μm)	VF ($n^o.mm^{-2}$)	RH (μm)	RW (μm)	RF ($n^o.mm^{-1}$)	FL (μm)	FD (μm)	FWT (μm)	$\rho12\%$ ($kg.cm^{-3}$)	EX (%)	LI (%)	HO (%)
Diploid													
0%(Pith)	502c	104c	30a	241b	23ab	8.2bc	973d	24a	4.2bc	628b	3.7c	32.4c	63.8a
20%	517c	118c	29a	236b	21b	7.8c	1092c	19c	3.8c	677b	3.5c	32.5c	63.9a
40%	511c	144b	25b	229b	22b	8.7ab	1136c	21bc	4.0c	637b	4.1c	33.9b	61.9a
60%	573ab	135bc	25b	282a	22b	8.4bc	1234b	20b	4.3bc	769a	5.0c	32.0c	62.9a
80%	526bc	145b	18c	271a	25a	9.2a	1288ab	21b	4.6b	809a	8.7a	35.5a	55.7c
100%(Bark)	595a	170a	23b	231b	21b	8.7ab	1313a	22b	52a	784a	6.9b	34.1b	58.9b
Mean	538B	135B	25A	248B	22B	8.5A	1181C	21B	4.3B	717B	5.3A	33.4A	61.1A
Triploid													
0%(Pith)	490b	123d	15c	249ab	22b	7.6a	970e	23ab	3.7d	738ab	3.8b	32.0b	64.1a
20%	509b	114d	12e	248ab	24ab	7.3a	1170d	20c	3.8d	715ab	3.7b	33.5a	62.7a
40%	605a	127cd	21a	248ab	24ab	7.7a	1286c	21bc	4.2cd	736ab	3.8b	34.4a	61.6a
60%	638a	148bc	17b	272a	26a	7.7a	1369ab	23b	4.4c	694b	4.6b	34.0a	61.3a
80%	631a	156ab	12e	251ab	25a	7.7a	1358bc	25a	5.4b	764a	7.0a	33.8a	59.1b
100%(Bark)	637a	173a	13d	232b	26a	7.4a	1440a	24ab	6.1a	729ab	6.2a	34.3a	59.4b
Mean	589A	127C	15C	250B	25A	7.6C	1278A	23A	4.7A	729B	4.9A	33.7A	61.2A
Tetraploid													
0%(Pith)	502c	148c	22b	274a	26a	7.2c	1051c	24ab	4.0b	830a	2.7c	32.1c	65.1a
20%	547abc	144c	24a	277a	21c	7.4c	1123b	23bc	4.0b	739b	3.0c	33.2b	63.7a
40%	575ab	188ab	20c	243b	25ab	8.1b	1223b	25a	4.4ab	711b	3.2c	33.2b	63.4ab
60%	534bc	203a	14e	252ab	23bc	8.4ab	1337a	25a	4.4ab	837a	4.4b	33.4b	62.0b
80%	584a	200a	18d	262ab	27a	8.6a	1283ab	21c	4.6ab	782a	7.2a	35.5a	57.1c
100%(Bark)	565ab	178b	14e	266ab	23bc	8.7a	1228b	24ab	5.0a	730b	6.4a	35.2a	58.3c
Mean	553B	178A	18B	262A	24A	8.1B	1214B	24A	4.5AB	771A	4.5A	33.8A	61.6A

VEL = vessel element length; VD = vessel diameter; VF = vessel frequency; RH = ray height; RW = ray width; RF = ray frequency; FL fiber length; FD = fiber diameter; FWT = fiber wall thickness; ρ12 = density 12% MC; EX = extractive content; LI = lignin content; HO = hollocellulose content. Difference between radial positions is represented by lowercase letters, while comparison between provenances is represented by uppercase letters. In the same column, distinct letters differ statistically (P<0.05) by Tukey's test. Source: Authors.

Table 4. Comparison among Higher Heating Value, Lower Heating Value and Useful Heating Value of 20-year-old *Eucalyptus grandis* (diploid and tetraploid) and *Eucalyptus grandis* x *Eucalyptus urophylla* (triploid) trees.

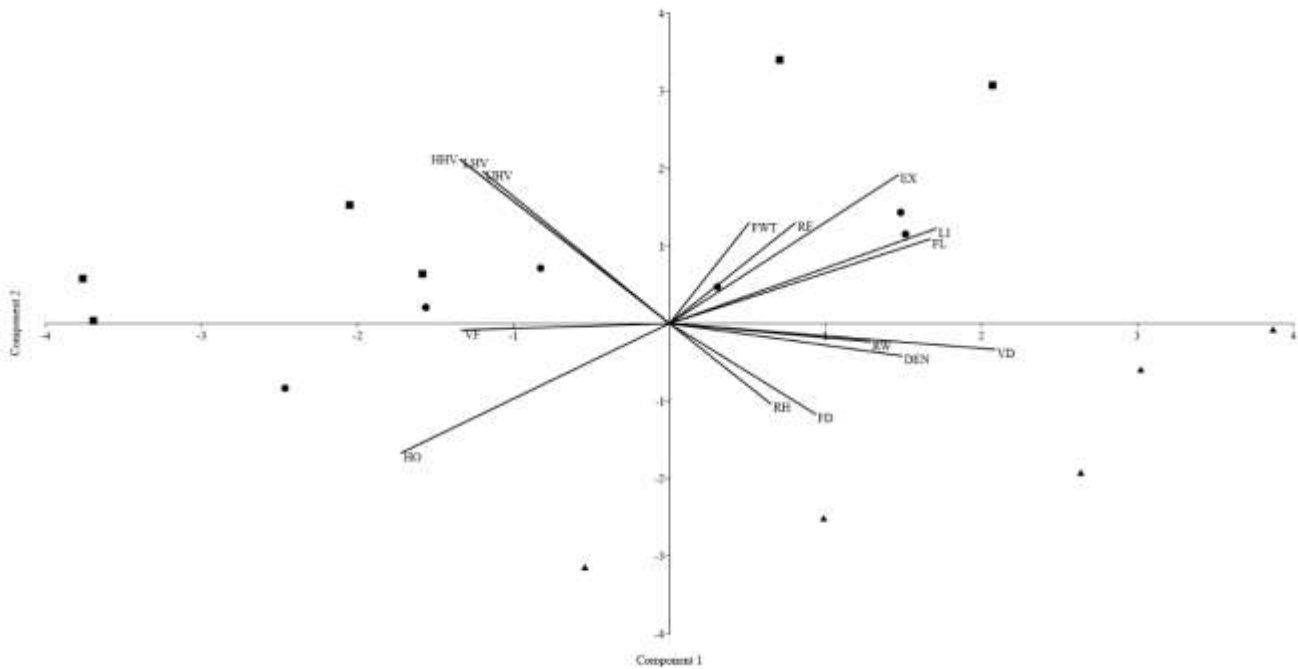|	Diploid	Triploid	Tetraploid
HHV (kJ.kg^{-1})	19867a	19766a	19446b
LHV (kJ.kg^{-1})	18594a	18494a	18173b
UHV (kJ.kg^{-1})	15769a	15834a	15319b

HHV = Higher Heating Value; LHV = Lower Heating Value; UHV = Useful Heating Value. Distinct letters in line differ statistically (P<0.001) by Tukey's test. Source: Authors.

Table 5. Principal component analysis of anatomical, physical, and chemical properties, as well as heating values of 20-year-old *Eucalyptus grandis* (diploid and tetraploid) and *Eucalyptus grandis* x *Eucalyptus urophylla* (triploid) trees.

	Principal Components	
Variables	PC 1	PC 2
VEL	0.628	0.287
VD	0.859	-0.245
VF	-0.568	0.040
RH	0.215	-0.424
RW	0.548	-0.147
RF	0.348	0.392
FL	0.799	0.331
FD	0.354	-0.472
FWT	0.286	0.451
$\rho 12$	0.582	-0.255
EX	0.699	0.611
LI	0.768	0.343
HO	-0.787	-0.502
HHV	-0.446	0.865
LHV	-0.446	0.865
UHV	-0.375	0.811
Percentage of explained variation	33.44%	25.01%

VEL = vessel element length; VD = vessel diameter; VF = vessel frequency; RH = ray height; RW = ray width; RF = ray frequency; FL fiber length; FD = fiber diameter; FWT = fiber wall thickness; $\rho 12$ = density 12% moisture content; EX = extractive content; LI = lignin content; HO = hollocellulose content; HHV = Higher Heating Value; LHV = Lower Heating Value; UHV = Useful Heating Value. Source: Authors.

Figure 2. Ordination generated via principal components analysis based on the entire dataset obtained from physical and chemical properties and heating values.

Ploidies are represented by (■) Diploid, (●) Triploid and (▲) Tetraploid. VEL = vessel element length; VD = vessel diameter; VF = vessel frequency; RH = ray height; RW = ray width; RF = ray frequency; FL fiber length; FD = fiber diameter; FWT = fiber wall thickness; ρ12 = density 12% moisture content; EX = extractive content; LI = lignin content; HO = holocellulose content; HHV = Higher Heating Value; LHV = Lower Heating Value; UHV = Useful Heating Value. Source: Authors.

4. Discussion

4.1 Tree size

In our study, all trees were the same age, planted at the same spacing and under the same soil conditions. However, as shown in results, triploid and tetraploid trees presented wider trunks, taller individuals with longer stems and wider crowns compared to diploid trees.

Dias (2016) studied polyploid induction in *Eucalyptus urophylla* and identified larger leaves with larger stomata at higher density in tetraploid plants when compared to diploids. On the other hand, Hao *et al*. (2013) reported larger stomata at a lower density in polyploid plants of *Atriplex canescens*. Vyas *et al*. (2007) studied the effects of polyploidy on photosynthetic properties and anatomy in leaves of *Phlox drummondii* and reported differences in the area of chloroplasts, Rubisco and chlorophyll contents, which could interfere with the photosynthetic capacity of polyploid plants, noting, however, that results may vary according to species. Similarly, Greer *et al*. (2018) reported that polyploidy affected photosynthesis by causing changes in morphology, anatomy and biochemistry in *Populus tremuloides*. The authors noted that triploid plants had higher leaf area, leaf mass, chlorophyll content and stomatal size than diploid plants. These differences corresponded to the higher potential of net carbon assimilation and stomatal conductance in triploid compared to diploid. According to Li *et al*. (1996), polyploid plants can maintain photosynthesis rate under negative water potentials at levels beyond which diploid plants have already stopped photosynthesis. Although we have not studied the stomata, it is possible that the changes described above may, at least in part, explain the higher growth of polyploid trees compared to growth in diploids. Beaulieu *et al*. (2008) reported polyploid plants as having, in general, larger stomata which can positively affect photosynthesis through efficient water use. Using the same trees as those of present study, Oda *et al*. (2014) reported that polyploid trees have larger leaves when compared to diploids and that this may be related to some change in stomata size or density. Griffin *et al*. (2014) in study comparing fibre and pulp properties

of diploid and tetraploid *Acacia mangium* grown in Vietnam, report that autotetraploid lines were shown to have superior wood properties, but with slower growth. In our study triploid and tetraploid trees had wider trunks, taller individuals with longer stems and wider crowns compared to diploid trees. Kang (2020) in triploid clones of *Populus* spp. found fast growth compared to diploid trees. In addition, tetraploids showed a higher wood density, which in general is associated with higher mechanical properties. Thus, it is suggested that there is a different behavior depending on the species and how planting was established, with need for further studies with tetraploids to confirm their properties and advantages over other ploidies.

4.2 Wood density

We emphasize that diploid tree wood followed a radial pattern of density variation, i.e., increase from pith to bark (Lachenbruch *et al*., 2011). In contrast, wood density oscillated radially in triploid and tetraploid trees. It is well known that wood density is influenced by anatomical features (Hoadley, 2000). In the diploid trees we studied, vessel element length, vessel diameter, fiber wall length and thickness increased toward the bark, corresponding to the typical radial pattern reported by Lachenbruch *et al*. (2011). Also, vessel density was inversely related to vessel diameter, which is commonly described in the literature (Baas *et al*., 2004). In polyploid trees, the anatomical features did not clearly present the radial pattern, which most certainly influenced wood density. In our study, triploid and tetraploid trees presented higher density wood than diploid trees.

Higher growth with higher wood density is not the most common result in the literature. In particular, Zobel and van Buijtenen (1989) claim that faster tree growth, in general, is related to lower wood density. However, Einspahr (1984) and Bedell (2006) proposed that polyploidy was successfully applied to *Populus tremula* to produce faster growth and higher wood density. Fantuzzi Neto (2012), investigating seven-year-old polyploid *Eucalyptus grandis* x *Eucalyptus urophylla* clones, found denser wood in polyploid trees compared with diploid trees. Zhai *et al*. (2012), studying wood physical properties of tetraploid *Paulownia fortunei*, reported higher basic density in tetraploid than that of diploid plants. Thus, by changing chromosome number, radial variation pattern can be altered, resulting in greater trunk growth with greater density. However, this does not normally occur in diploid trees; instead, it appears to be a pattern in polyploid wood trees. Additionally, Rossi *et al*. (2014) reports that relationships between growth rate and wood properties are related to species and environment in which trees grow and difficult to predict without previous investigations.

4.3 Anatomical features

Regarding anatomical features, as described above, diploid trees followed the typical radial pattern reported by Lachenbruch *et al*. (2011), which, in that report, did not occur in polyploid trees. These changes may have implications in wood strength, generally more associated with fiber features, as well as water transport. The latter case would be strongly associated with vessel variations, especially diameter. Zhang *et al*. (2017) studied four *Betula* species in China and found larger vessel diameters in polyploid plants compared to those of diploids. According to Tyree and Zimmermann (2002), an increase in vessel lumen diameter affects hydraulic efficiency with the fourth power of its diameter by the Hagen-Poiseuille equation. This could contribute to bigger tetraploid trees compared with diploids in the present study. Maherali *et al*. (2009) reported larger vessel diameter in polyploid *Chamerion angustifolium* compared with those of diploid plants, and this anatomical condition would increase stem hydraulic conductivity. Similar results were found by De Baerdemaeker *et al*. (2012) in *Malus x domestica*, indicating that tetraploid trees have larger vessel area and size, resulting in a significantly higher hydraulic capacitance and efficiency compared to diploid trees. Increase in vessel diameter may, however, carry a higher embolism risk (Tyree and Zimmermann, 2002). In association with larger vessels, Zhang *et al*. (2017) suggest that polyploid plants should develop anatomical and physiological changes that also improve water use efficiency. Thus, increase in vessel diameter would not imply a problem for hydraulic efficiency, even in stressful environments where water availability is scarce. In our study, vessel diameter

may have contributed, at least in part, to larger dimensions of polyploid trees when compared to diploids, and this may be related to water transport potential and vessel diameter. Although larger vessels occur in tetraploid trees and narrower vessels in triploid trees, it is assumed that 1) vessel diameter is a determinant of water conduction and its availability for photosynthesis and 2) other hydraulic system features, such as pits or stomata movements.

Regarding fiber dimensions, our results are similar to those of Zhai *et al*. (2012) in *Paulownia fortunei*, Fantuzzi Neto (2012) in polyploid clones of *Eucalyptus grandis* x *Eucalyptus urophylla*, and Griffin *et al*. (2014) in *Acacia mangium*, who found wood with longer, wider and thicker-walled fibers in polyploid (tetraploids) trees compared to diploid trees. Kang (2020) in triploid clones of *Populus* spp. found longer fiber compared to diploid trees. In our study, fiber length did not differ between tetraploids and diploids. In general, results show that polyploid plants produce fibers with larger dimensions compared to diploid plants. On the contrary, studying polyploidy in *Fraxinus americana*, Armstrong (1982) observed no relationship between ploidy level and fiber length and attributed fiber length both to an increase in the average cambial initial length and a proportionate ontogenetic elongation.

Increase in ray width was reported by Swamy and Govindarajalu (1957) with polyploidy in *Parthenium argentatum*. De Baerdemaeker *et al*. (2012) studied the effect of polyploidization on tree hydraulic function in *Malus x domestica* and found a higher amount of parenchyma, especially rays, in tetraploid plants compared to diploids. In our study, bulky rays (height x width) occurred in tetraploid trees, followed by triploid trees, which may have contributed to the larger size of triploid and tetraploid trees compared to diploid. This can be explained by bulky rays that can increase the radial transport of carbohydrates mobilized for cambial reactivation after a period of dormancy (Costa *et al*., 2006). Longui *et al*. (2011) reported bulky rays in *Gallesia integrifolia* provenance which, according to Sebbenn *et al*. (2009), presented faster growth and higher survival rate. In addition to storing sugar for cellular respiration, parenchyma cells (axial parenchyma or radial parenchyma) are essential to recover embolisms in vessels since parenchyma cells secrete solutes into the vessel, establishing an osmotic gradient, and water will gradually refill the air-filled vessels, either dissolving air micro-bubbles in the solution or forcing them into the surrounding hydrophobic microchannels in the vessel wall (Brodersen *et al*., 2010).

4.4 Chemical assays

In terms of radial variation, we highlight that lignin content differed in radial direction among ploidies. Additionally, homogeneity was observed in lignin content in triploid trees with variation only in the pith. It is well known that low lignin contents are desirable to improve the kraft pulping performance (Cardoso *et al*., 2011); however, a low value with homogeneity along the trunk is also expected.

In our study, the chemical constituents showed no differences among ploidies. Through polyploidy, Kang (2010) makes the argument that it is possible to reach characteristics of interest in *Populus* spp., among them low lignin content, which is also desirable for *Eucalyptus* species used for pulp and papermaking. Kang (2020) in triploid clones of *Populus* spp. found lower lignin and higher holocellulose content compared to diploid trees. Fantuzzi Neto (2012), investigating seven-year-old *Eucalyptus grandis* x *Eucalyptus urophylla* polyploid clones, found lower lignin content in polyploid trees compared with diploid trees. According to Corneillie *et al*. (2019), polyploidy affects plant growth and alters the cell wall composition, especially the basic somatic ploidy level is negatively correlated with lignin and cellulose content. Additionally, polyploidy conditions can influence the increase of biomass and consequently production of bioenergy and materials, resulting in an efficient strategy to further tailor crops for biomass production.

4.5 Bioenergy values

Some wood properties influence their energy potential. Extractives and lignin content (Telmo & Lousada, 2011; Günther

et al., 2012) and wood density (Silva *et al*., 2007) are positively related to wood energy values. Additionally, anatomical composition directly influences physical, mechanical and chemical wood properties (Hoadley, 2000). Thus, cell dimensions and frequency influence wood density, which, in turn, will influence energy values.

According to our experience, in the Brazilian market, HHV values between 16500 - 18000 kJ.kg^{-1} are suitable for use in bioenergy (Menucelli *et al*., 2019). In our study, all ploidies presented HHV values higher than 19000 kJ.kg^{-1}. This shows that all ploidies can be used as raw material for bioenergy. However, tetraploids had lower values than diploids and triploids, and this result became evident in the principal components analysis, in which axis 2 separated tetraploid from diploid and triploid trees. We suggest that lower calorific values in tetraploids may be related to bulkier rays and larger vessel diameter, which would increase the percentage of fines (fiber or other broken cell walls, vessel and parenchyma cells) in tetraploid wood. According to Foekel (2009), fines cause problems during pulping and refining, as well as further processing of the pulp. Although fines are highly related to wood quality for paper, we suggested that fines contribute negatively to energy value since ray or vessel cell walls are narrower than fiber walls, and such variations may influence the wood density that is directly related to energy values.

5. Conclusion

Results show that our hypothesis was confirmed by the differences noted in trees from different ploidies. More specifically, triploid and tetraploid trees presented wider trunks, taller trees with longer stems and wider crowns compared to diploid trees. Wood density showed significant radial variation only in diploids, while triploid and tetraploids trees were more homogeneous. The density variation was directly influenced by anatomical features. In polyploid trees, the anatomical features did not clearly present a radial pattern. In our study, triploid and tetraploid trees presented higher density wood than diploid trees. The chemical constituents varied from pith to bark in the three ploidies, but no differences were found among ploidies. For energy generation purposes, diploid and triploid trees are more desirable than tetraploid. In future studies, we suggest also studying the mechanical and workability properties of *Eucalyptus* spp. wood with different ploidies, so we have a broad view of how wood from plants with different chromosomal sets behaves.

Acknowledgments

References

Armstrong, J. E. (1982). Polyploidy and wood anatomy of mature white ash, *Fraxinus americana*. *Wood and Fiber*, *14*(3), 331-339.

Associação Brasileira de Normas Técnicas-ABNT. Norma NBR 8633.1984. Determinação do poder calorífico superior. 1, 13.

Associação Brasileira de Normas Técnicas-ABNT. Norma NBR 14929.2003. Determinação da umidade da madeira. 1,3.

Baas, P., Ewers, F. W., Davis, S. D., & Wheeler, E. A. (2004). Evolution of xylem physiology. In Poole, I., Hemsley, A. 273-295pp.

Beaulieu, J. M., Leitch I. J., Patel, S., Pendharkar, A. & Knight, C. A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol.*, *179*(4), 975-986. 10.1111/j.1469-8137.2008.02528.x.

Bedell, P. E. (2006). Tree Breeding for Genetic Improvement of Tropical Tree Specie*s*.

Berlyn, G. P. & Miksche, J. P. (1976). Botanical microtechnique and cytochemistry.

Brodersen, C. R., Mcelrone, A. J., Choat, B., Matthews, M. A. & Shackel, K. A. (2010). The dynamics of embolism repair in xylem: in vivo visualizations using high resolution computed tomography. *Plant Physiology*, *154*(3), 1088-1095. https://doi.org/10.1104/pp.110.162396

Cardoso, G. V., Foelkel, C. E. B., Frizzo, S. M. B., Rosa, C. A. B., Assis, T. F. & Oliveira, P. (2011). Effect of lignin content of *Eucalyptus globulus* labill. wood in kraft pulping performance. *Ciência Florestal*, *21*(1), 133-147. https://doi.org/10.5902/198050982756

Chi, N. Q., Griffin, R. A., Harbard, J. L., Harwood, C. E., Le, S., Nguyen, K. D. & Van Pham, B. (2018). Reduced fertility in triploids of *Acacia auriculiformis* and its hybrid with *A. mangium*. *Euphytica*, *214* (77), 1-14. https://doi.org/10.1007/s10681-018-2157-8

Corneillie, S., De Storme, N., Van Acker, R., Fangel, Ju., De Bruyne, M., De Rycke, R., Geelen, D., Willats, W. G. T., Vanholme B. & Boerjana, W. (2019). Polyploidy affects plant growth and alters cell wall composition. *Plant Physiology*, *179* (1), 74-87. https://doi.org/10.1104/pp.18.00967.

Costa, C. G., Callado, C. H., Coradin, V. T. R., Carmello-Guerreiro, S. M. (2006). Xilema. *In*: *Anatomia Vegetal*. 129-154pp.

De Baerdemaeker, N. J. F., Hias, N., Van Den Bulcke, J., Keulemans, W. & Steppe, K. (2012). The effect of polyploidization on tree hydraulic functioning. *American Journal of Botany*, *105*(2), 161-171. 10.1002/ajb2.1032

Diallo, A. M., Nielsen, L. R., Kjær, E. D., Petersen, K. K. & Ræbild, A. (2016). Polyploidy can Confer Superiority to West African *Acacia senegal* (L.) Willd. Trees. *Frontiers in Plant Science*, *14*(7), 1-10. 10.3389/fpls.2016.00821

Dias, R. Z. (2016). Poliploidização induzida in vitro, como estratégia biotecnológica para a otimização da cultura de eucalipto. (2016). *Dissertação de Mestrado*.

Einspahr, D. W. (1984). Production and utilization of triploid hybrid aspen. *Iowa State Journal of Research*, 58(4), 401-409.

Fantuzzi Neto, H. Qualidade da madeira de eucalipto para produção de celulose kraft. (2012). *Tese de doutorado.*

Glass S. V. & Zelinka, S. L. (2010). Moisture relations and physical properties of wood.

Greer, B. T., Still, C., Cullinan, G. L., Brooks, J. R. & Meinzer, F. C. (2018). Polyploidy influences plant-environment interactions in Quaking Aspen (*Populus tremuloides* Michx.). *Tree Physiology*, *38*(4), 630-640. 10.1093/treephys/tpx120

Griffin, A. R., Twayi, H., Braunstein, R., Downes, G. M., Son, D. H. & Harwood, C. E. (2014). A comparison of fibre and pulp properties of diploid and tetraploid *Acacia mangium* grown in Vietnam. *Appita Journal*, *67*(3), 43-49.

Griffin, A. R., Chi, N. Q., Harbard, J. L., Son, D. H., Harwood, C. E., Price, A., Vuong, T. D., Koutoulis, A. & Thinh, H. H. (2015). Breeding polyploid varieties of tropical acacias: progress and prospects. *Southern Forests*, *77*(1), 41-50. https://doi.org/10.2989/20702620.2014.999303

Günther, B., Gebauer, K., Barkowski, R., Rosenthal, M. & Bues, C.T. (2012). Calorific value of selected wood species and wood products. *European Journal of Wood and Wood Products*, *70*(5), 755-757. https://doi.org/10.1007/s00107-012-0613-z

Han, C., Xu, J. M., Du, Z. H., Li, G. Y., Zeng, B. S., Wu, S. J. & Wang, W. (2011). Polyploidy induction of clone of *Eucalyptus grandis* with colchicine. *African Journal of Biotechnology*, *10*(66), 14711-14717. https://doi.org/10.5897/AJB11.505

Harbard, J. L., Griffin, A. R., Foster, S., Brooker, C., Kha, L. D. & Koutoulis, A. (2012). Production of Colchicine-induced autotetraploids as a basic for sterility breeding in *Acacia mangium* Willd. *Forestry: An International Journal of Forest Research*, *85*(3), 427-436. https://doi.org/10.1093/forestry/cps041.

Hao, G. Y., Lucero, M. E., Sanderson, S. C., Zacharias, E. H. & Holbrook, N. M. (2013). Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytologist*, *197*(3), 970-978. https://doi.org/10.1111/nph.12051

Hoadley, B. (2000). Understanding wood: a craftsman's guide to wood technology.

Hoshino, Y., Miyashita, T. & Thomas, T. D. (2011). In vitro culture of endosperm and its application in plant breeding: approaches to polyploidy breeding. *Scientia Horticulture*, *130*(1), 1-8. https://doi.org/10.1016/j.scienta.2011.06.041

Indústria Brasileira de Árvores-IBÁ (2020). *The brazilian planted tree industry*. Disponível em: https://iba.org/datafiles/publicacoes/relatorios/iba-relatorioanual2019.pdf Acesso em: 26 nov. 2021

Janick, J. & Moore, J. N. (1996). Tree and Tropical Fruits, Fruit Breeding.

Johansen, D. A. (1940). Plant Microtechnique.

Kang, X. Y. (2010). Some understandings on polyploid breeding of poplars. *Journal of Beijing Forestry University*, *32*(5), 149-153.

Kang, X. Y. (2020). Research progress and prospect of triploid breeding of forest trees. *Scientia Sinica*, *50*(2), 136-143. https://doi.org/10.1360/SSV-2019-0126

Lachenbruch, B., Moore, J. R. & Evans, R. (2011). Radial variation in wood structure and function in woody plants, and hypotheses for its occurrence. In: Meinzer, F. C., Lanchenbruch, B. & Dawson, T.E. *Size-and age-related changes in tree structure and function* (pp. 121-164).

Li, W. L., Berlyn, G. P. & Ashton, P. M. S. (1996). Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). *American Journal of Botany*, *83*(1), 15-20. https://doi.org/10.1002/j.1537-2197.1996.tb13869.x

Lin, H., Jian, M., Liang, L. Y., Pei, W. J., Liu, X. Z. & Zhang, H. Y. (2010). Production of polyploids from cultured shoot tips of *Eucalyptus globulus* Labill by treatment with colchicine. *African Journal of Biotechnology*, *9*(15), 2252-2255. https://doi.org/10.5897/AJB2010.000-3028.

Longui, E. L., Lima, I. L., Andrade, I. M., Freitas, M. L. M., Florsheim, S. M. B., Zanatto, A. C. S. & Silva Júnior, F. G. (2011). Seed provenance influences the wood structure of *Gallesia integrifolia*. *IAWA Journal*, *32*(3), 361-374. 10.1163/22941932-90000064

Maherali, H., Walden, A. E. & Husband, B. C. (2009). Genome duplication and the evolution of physiological responses to water stress. *New Phytol.*, *184*(3), 721-731. https://doi.org/10.1111/j.1469-8137.2009.02997.x

Mc Garigal, K., Cushman, S. & Stafford, S. (2000). Multivariate Statistics for Wildlife Ecology Research.

Menucelli, J. R., Amorim, E. P., Freitas, M. L. M., Zanata, M., Cambuim, J., Moraes, M. L. T., Yamaji, F. M., Silva Júnior, F. G. & Longui, E. L. (2019). Potential of *Hevea brasiliensis* clones, *Eucalyptus pellita* and *Eucalyptus tereticornis* wood as raw materials for bioenergy based on higher heating value. *BioEnergy Research*, *12*(4), 992-999. 10.1007/s12155-019-10041-6

Oda, S., Mello, E. J., Menck, A. L. M., González, E. R., Souza, I. C. G., Siqueira, L. & Carvalho, C.R. (2014). Induction and identification of polyploidy *Eucalyptus grandis* and *Eucalyptus grandis* x *Eucalyptus urophylla* plants. Proceedings of the IUFRO Acacia 2014 Conference "Sustaining the Future of Acacia Plantation Forestry" *Anais.*.pp. 9, Hue, Vietnam.

Pereira, A. S., Shitsuka, D. M., Parreira, F. J., & Shitsuka, R. (2018). Metodologia da Pesquisa Científica. UFSM, https://repositorio.ufsm.br/bitstream /handle/1/ 15824/ Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1

Praça, M. M., Carvalho, C. R. & Boaventura-Novaes, C. R. D. (2009). Nuclear DNA content of three *Eucalyptus* species estimated by flow and image cytometry. *Australian Journal of Botany*, *57*(6), 524-531. 10.1071/BT09114

Rossi, S., Cairo, E., Krause, C. & Deslauriers, A. (2014). Growth and basic wood properties of black spruce along an alti-latitudinal gradient in Quebec, Canada. *Annals of Forest Science*, *72*(1), 77-87. 10.1007/s13595-014-0399-8

Roth, O. S. *Indução de Poliploidia em Clones de Eucalyptus urophylla S.T. Blake.* (1984). *Dissertação de mestrado.*

Sebbenn, A. M., Freitas, M. L. M., Zanatto, A. C. S., Morais, E. & Moraes, M. A. (2009). Comportamento da variação genética entre e dentro de procedências e progênies de *Gallesia integrifolia* Vell. Moq. para caracteres quantitativos. *Revista do Instituto Florestal*, *21*(2), 151-163.

Silva, M. G., Numazawa, S., Araujo, M. M., Nagaishi, T. Y. R. & Galvão, G. R. (2007). Carvão de resíduos de indústria madeireira de três espécies florestais exploradas no município de Paragominas, PA. *Acta Amazonica*, *37*(1), 61-70.

Soltis, D. E., Albert, V. A., Leebens-Mack, J., Bell, C. D., Paterson, A. H., Zheng, C., Sankoff, D., De Pamphilis, C. W., Wall, K. & Soltis, P. S. (2009). Polyploidy and Angiosperm Diversification. *American Journal of Botany*, *96*(1), 336-348. 10.3732/ajb.0800079

Swamy, B. G. L. & Govindarajalu, E. (1957). Anatomical studies in polyploid strains of *Parthenium argentatum. Journal of the Asiatic Society Letters & Science*, *23*(1-2), 43-54.

Technical Association of the Pulp and Paper Industry-TAPPI. (2011). Standard T 222 om-11: Acid insoluble lignin in wood and pulp.

Technical Association of the Pulp and Paper Industry-TAPPI. (1999). *Standard T 204 cm-97:* Solvent extractives of wood and pulp.

Telmo, C. & Lousada, J. (2011). Heating values of wood pellets from different species. *Biomass and Bioenergy*, *35*(7), 2634-2639. https://doi.org/10.1016/j.biombioe.2011.02.043

Tyree, M. T. & Zimmerman, M. H. (2002). Xylem structure and the ascent of sap.

Vyas, P., Bisht, M. S., Miyazawa, S. I., Yano, S., Noguchi, K., Terashima, I. & Funayama-Noguchi, S. (2007). Effects of polyploidy on photosynthetic properties and anatomy in leaves of *Phlox drummondii*. *Functional Plant Biology*, *34*(8), 673-682. https://doi.org/10.1071/FP07020

Zhai, X. Q., Zhang, X. S., Zhao, Z. L., Deng, M. J. & Fan, G. Q. (2012). Study on wood physical properties of tetraploid *Paulownia fortunei*. *Journal of Henan Agricultural University*, *46*(6), 651-654.

Zhang, W. W., Song, J., Wang, M., Liu, Y. Y., Lil, N., Zhang, Y. J., Holbrook, N. M. & Hao, G. Y. (2017). Divergences in hydraulic architecture form an important basis for niche differentiation between diploid and polyploid *Betula* species in NE China. *Tree Physiology*, *37*(5), 604-616. https://doi.org/10.1093/treephys/tpx004

Zobel, B. J. & Jett, J. B. (1995). Genetics of Wood Production.