ANATOMICAL, PHYSICAL, AND MECHANICAL PROPERTIES OF TRANSGENIC LOBLOLLY PINE (*PINUS TAEDA* L.) MODIFIED FOR INCREASED DENSITY

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Abstract. Traditional breeding methods are often constrained by the reproductive cycles of tree species and the difficulty in achieving significant improvements to complex traits; therefore, genetic manipulation of complex traits such as wood properties has the potential to resolve those issues. The objectives of this study were to analyze MOE, MOR, and the physical and anatomical properties of 2- to 3-yr-old field-grown transgenic *Pinus taeda* trees modified for increased density. This investigation consisted of a total of 55 sample trees in two separate experiments. Transgenic trees from sets OX41 and OX55, modified for increased density using two variants of the same HAP5 gene, exhibited higher mechanical properties with smaller stem diameter and tracheid lumen diameter than their set of control trees. In addition, set OX55 exhibited increased cell wall thickness. In the second experiment, the transgenic group WVK249, modified for higher density using an unrelated MYB gene, exhibited similar diameter growth and increased cell wall thickness and lower lumen/cell wall ratios but no change in mechanical properties compared with its control.

Keywords: Density, specific gravity, quantitative wood anatomy, tracheid, MOE, MOR, transgenic, *Pinus taeda* L.

INTRODUCTION

Loblolly pine (*Pinus taeda* L.), grown on more than 30 million acres (Smith et al 2009), is intensively managed for pulp and timber throughout the southeastern United States and, as such, is the most commercially important pine species in the southern United States (Fox et al 2007). Demand for valuable wood products has been increasing, whereas the timber base has been gradually declining (Prestemon and Abt 2002) and the land base is increasingly shifting from natural forests owned by private landowners to pine plantations (Allen et al 2005). The tree improvement industry hopes to meet these future demands using available tools such as good silvicultural methods, selection of elite trees through progeny testing, and, more recently, genetic manipulation (McKeand et al 2006; Aspinwall et al 2012).

The forest industry in many parts of the world is moving toward the usage of more fast-growing plantation trees, which must be harvested at a younger age, to keep up with their raw material needs and to reduce costs. This industrial practice will produce trees with a larger portion of juvenile wood with low specific gravity, more knots, and larger proportion of reaction wood (Larson et al 2001; Moore and Cown 2017). Juvenile wood has lower MOE and MOR (Pearson and Ross 1984; Bendtsen and Senft 1986; Pearson 1988; Biblis 2006), and stability issues associated with the shrinking and swelling differential between juvenile and mature wood (Beard et al 1993). The primary breeding objectives for tree improvement programs focus on volume growth (height/diameter) and adaptability (survival and disease resistance) with wood quality as a secondary emphasis (Byram et al 2005). Stressing the importance of wood quality specifically from genetically improved loblolly pine stock can have a significant positive impact on the industry (Li et al 1999).

Physical (eg specific gravity) and mechanical properties (eg MOE and MOR) of wood are important indicators for solid and composite wood applications and can be measured in progeny testing to evaluate elite trees. Wood density and specific gravity have been shown to be a moderate predictor of MOR within species; however, they were not as effective at determining MOE (Zhang 1997). Specific gravity is defined by the oven-dry mass divided by the

volume of wood and is a more useful measurement because of the hygroscopic nature of wood (Zobel and Van Buijtenen 1989). Although specific gravity is a useful wood property and corresponds well with cambial age, it alone does not account for the full magnitude of increase in mechanical properties in radiata pine (Bamber and Burley 1983). Primary and secondary cell walls of plants contain cellulose microfibrils embedded in a matrix containing lignin, hemicellulose, and pectin (Harris 2006). The stiffness of wood is derived from cellulose microfibrils and their distribution within the cell wall (Cave and Walker 1994).

For juvenile trees, it is advantageous to be able to bend to dissipate forces from the wind so they form a more flexible wood, allowing it to bend at large angles to dissipate loads without failure in the fibers (Meinzer et al 2011). As the tree grows, to contain the significant weight from the rest of the tree, the stiffness of the wood must increase to prevent buckling. The performance and corresponding commercial value of structural lumber is limited by the MOE of juvenile corewood, eg a 3-fold difference in MOE was observed in radiata pine pith–containing boards by Tsehaye et al (2000). In a study by Biblis (2006), 90% of lumber cut from juvenile (19-yr-old) loblolly pine trees failed to reach the required design MOE standards set by the Southern Pine Inspection Bureau. For young trees (less than 4 yr old), measurements of MOE are difficult because of the considerable influence of compression wood, stem eccentricity, and taper (Lindstrom et al 2002), and standard specimens are sometimes not able to be cut from such small trees.

Genetic modifications of loblolly pine trees primarily focused on resistance to fusiform rust disease (Wilcox et al 1996; Myburg et al 2006; Lu et al 2007), faster growth (Farnum et al 2007), and, more recently, wood quality traits (Edmunds et al 2017). The potential to orchestrate the expression of multiple genes affecting secondary cell wall thickening and formation makes transcription factors of interest to researchers. MYB transcription factors (named for their partial similarity to mammalian myeloblastosis genes) regulate expression of genes involved in a wide variety of cell processes, such as secondary cell wall formation and phenylpropanoid biosynthesis in tobacco (Goicoechea et al 2005), *Eucalyptus* (Soler et al 2015), and *Populus* (Wilkins et al 2009) as well as cold stress tolerance in *Arabidopsis* (Borevitz et al 2000; Agarwal et al 2006). Ko et al (2017) observed increased lignocellulosic biomass accumulation in poplar through overexpressing MYB46. An MYB isolated from pine xylem is thought to induce lignification and play a role in wood formation in pine (Patzlaff et al 2003). Similarly, HAP transcription factors (named for the heme activator proteins of yeast, and also known as CCAAT-box or NF-Y transcription factors) regulate multiple genes involved in different traits including photomorphogenesis and timing of flowering in *Arabidopsis* (Cai et al 2007; Myers et al 2016). Little is known about the functions of HAP genes in tree species.

The objective of this investigation was to analyze the mechanical, physical, and anatomical properties of plantation-grown loblolly pine trees genetically engineered for increased density using either HAP or MYB transcription factors. Characterization of these trees was carried out to elucidate whether the increase in density corresponded to increases in MOE and MOR, changes in physical properties, and, also, to what extent the anatomical properties influenced the mechanical properties. To the authors' knowledge, this is the first study on transgenic pine genetically modified for increased density.

MATERIALS AND METHODS

Wood Materials

This investigation studied 2- to 3-yr-old field-grown transgenic loblolly pine trees. Trees were modified for increased density using either HAP5 or MYB-like genes as outlined by Rottmann et al (2014) and Wood et al (2011), respectively. Two experiments were evaluated with each containing its own control group. Depending on the size of stems, one to nine specimens were sawn from each tree, with 244 specimens in total available for analysis. Specimens were cut into an 1 cm $\times$ 1 cm (cross section) by 16 cm (axially) in between knots. The trees were grown and harvested

in a field trial located in Bamberg County, SC, by ArborGen Inc.

HAP5 Experiment

In this first experiment, three groups of transgenic trees were analyzed. The control group consisted of transgenic trees using an empty vector control without the HAP5 gene (WVK147: five trees from three lines). Trees in the two modified groups incorporated an HAP5 gene derived from *Pinus radiata* under the control of a xylem-preferred promoter (OX41: 17 trees from 9 lines) or a constitutive promoter (OX55: 16 trees from 8 lines).

MYB-like Experiment

In this second experiment, the control without the MYB-like gene (WVR31: nine trees from five lines) was compared with transgenic trees with an MYB-like gene derived from *Eucalyptus grandis* with the xylem-preferred promoter (WVK249: nine trees from five lines).

Mechanical Properties

Specimens were kept in a chamber at a constant 20°C and 65% RH to equilibrate to air-dry MC (~12%). MOE and MOR were tested according to a modified ASTM D143 standard (ASTM 2009) using an MTS Alliance machine (MTS Systems Corporation, Eden Prairie, MN) with a wooden block attached to a 250-pound load cell to apply the load. Each specimen with a span of 14 cm was loaded in bending on the tangential face nearest the pith, at a speed of 0.125 in./min so that the specimen would break in about 3-5 min.

Following the tests, static MOE was calculated as follows:

$$\text{Static MOE} = \left(L^3/4bh^3\right) \times \left(\mathrm{d}P/\mathrm{d}\delta\right)\left[10^3 \times \text{psi},\ \text{ksi}\right],$$

where $\mathrm{d}P/\mathrm{d}\delta$ = slope of the linear portion of the load-deflection diagram (lb/in.), L = span distance between the two supports in inches, b = breadth in inches, and h = depth in inches.

MOR was calculated as follows:

$$\text{MOR} = \left(3(P_{\text{Max}} \times L)/2bh^2\right)[\text{psi}],$$

where P_{Max} = maximum load (lb force), L = span distance between the two supports in inches, b = breadth in inches, and h = depth in inches.

Physical Properties

Density and specific gravity were determined from sections that were cut from the end of each specimen. Mass and volume were measured at ~12% MC using a balance with 0.01-g accuracy and calipers with 0.01-mm accuracy. Specimens were then oven-dried at 103 ± 2°C and mass measurements were obtained. Density was calculated at ~12% MC from the mass of the specimens divided by the calculated volume at ~12% MC, whereas specific gravity was calculated from the oven-dry mass of the specimens divided by the calculated volume at ~12% MC multiplied by the density of water.

Quantitative Wood Anatomy

Based on a range of measured mechanical properties, specimens with the lowest average and highest MOE and MOR were selected from each control and modified group for quantitative wood anatomy. From each specimen, three 20-μm-thick transverse microtome sections were cut and stained with 1% aqueous safranin solution to enhance contrast, washed with deionized water, and placed on a glass slide. Anatomical properties were measured using an image analyzer system, which consisted of a light microscope (Nikon E200, Melville, NY), a 3CCD color video camera (Sony DXC-390, Tokyo, Japan), and Image-Pro Plus 9.1 software (Media Cybernetics, LP, Silver Spring, MD). From each slide, five digital images with 273 μm × 205 μm size were taken randomly at ×400 magnification for studying cell properties. All images had 1.3-megapixel and 1080 ×

970 resolution. Double cell wall thickness (μm), lumen diameter (μm), lumen area (μm^2), and lumen/cell wall area ratio (%) were measured using randomly selected areas that were between rays and had no reaction wood and other abnormalities (eg scar tissue, traumatic resin canals, etc.). Double cell wall thickness was measured manually. Specimens were then examined for the absence (0) or presence (1) of compression wood, the reaction wood of softwoods, characterized microscopically as a group of thicker walled, rounded tracheids with many intercellular spaces (Timell 1986).

Growth Rate

At the time of harvest, stem diameters were measured as an indication of growth rate, and both growth rates and density/specific gravity were used for a regression analysis with mechanical properties and anatomical properties.

Statistical Analysis

All measured properties were analyzed using analysis of variance (ANOVA) descriptive statistics in SAS® Enterprise Guide 6.1 (SAS Institute, Inc., Cary, NC). Dunnett's multiple range tests were used to determine the significant differences between the properties of the transgenic group and those of the control using an $\alpha = 0.05$.

RESULTS AND DISCUSSION

Mechanical and Physical Properties

In the HAP5 experiment, mean MOE values of the control and modified loblolly pine trees ranged from 298.5 to 410.8 ksi, mean MOR values ranged from 5510 to 7971 psi, and mean specific gravity values ranged from 0.354 to 0.485 (Table 1). The transgenic group OX55 consistently had the highest MOE, MOR, specific gravity, and density values, but also had the lowest stem diameter. On the other hand, the control (WVK147) consistently had the lowest MOE, MOR, specific gravity, and density values, and also had the largest stem diameter. The transgenic OX41 had mechanical and physical property values and stem diameters in between those of the control and transgenic OX55, but still significantly different from the control.

Table 1. Experiment 1—mechanical (MOE and MOR) and physical properties (specific gravity) of individual genetic lines in control (WVK147) and modified (OX41 and OX55) loblolly pine specimens.

Promoter	MOE (ksi)	COV	MOR (psi)	COV	Specific gravity	COV	Diameter (cm)	Line	No of Trees	*n*	MOE (ksi)	MOR (psi)	Specific gravity	Diameter (cm)
								WT-1	2	8	298.5	5510	0.354	4.50
WVK147 Control	310.9	13%	5819	15%	0.374	10%	5.20	WT-2	1	7	302.9	5838	0.377	6.00
								WT-3	2	14	321.9	6028	0.383	5.50
								OX41-1	2	9	320.0	6208	0.410	4.00
								OX41-2	2	11	344.2	6857*	0.398	4.75
								OX41-3	2	6	315.6	6163	0.411	4.50
								OX41-4	2	6	400.0*	7164*	0.428*	3.00
OX41	336.6*	14%	6473*	12%	0.396*	9%	4.03	OX41-5	2	10	307.8	5964	0.375	4.50
								OX41-6	2	8	305.7	5904	0.366	3.50
								OX41-7	1	5	332.2	6524	0.412	4.00
								OX41-8	2	10	352.6	6847*	0.388	4.00
								OX41-9	2	7	366.6*	6693	0.399	4.00
								OX55-1	2	10	335.0	6185	0.367	4.50
								OX55-2	2	10	392.7*	7100*	0.387	4.50
								OX55-3	2	8	326.0	6382	0.408	3.50
OX55	359.9*	15%	6721*	16%	0.409*	12%	3.89	OX55-4	2	6	368.4*	6839	0.402	3.50
								OX55-5	2	10	410.8*	7971*	0.485*	3.75
								OX55-6	2	10	346.7	6212	0.411	3.75
								OX55-7	2	10	336.4	6336	0.401	3.75

Note: *n*, number of specimens cut for each genetic line; COV, coefficient of variation; asterisk (*) indicates statistically significant result compared with the control.

In the MYB-like experiment, mean MOE values of the control and modified loblolly pine trees ranged from 267.2 to 344.2 ksi, mean MOR values ranged from 5858 to 6679 psi, and mean specific gravity values ranged from 0.349 to 0.429 (Table 2). There was no statistically significant difference for MOE, MOR, and stem diameter between the transgenic WVK249 and the control WVR31; however, the specific gravity was higher in the transgenic WVK249.

These values are in the range of those reported in the literature for loblolly pine. A study by Pearson and Ross (1984) from a 15-yr-old loblolly pine progeny test and a 25-yr-old commercial plantation analyzing 0-2 rings from the pith found higher MOE values (ranging from 640 to 880 ksi) and higher MOR values (ranging from 7260 to 9080 psi) than those found in our study; however they found a similar specific gravity (ranging from 0.38 to 0.40). Biblis (2006) tested lumber cut from 19-yr-old loblolly pine trees and found much greater MOE values (750-1305 ksi), smaller MOR values (2385-5405 psi), and higher specific gravity values (0.45-0.48). Bendtsen and Senft (1986) found a mean MOE value of 292 ksi, mean MOR value of 4080 psi, and mean specific gravity value around 0.399 for the first- through the third-year growth rings in loblolly pine.

Quantitative Wood Anatomy

In the HAP5 experiment, the control WVK147 had a mean double cell wall thickness of 5.1 μm (ranging from 5.0 μm to 5.1 μm), a mean lumen area of 452 μm^2 (ranging from 369 μm^2 to 535 μm^2), a mean lumen diameter of 23.4 μm (ranging from 21.1 μm to 25.6 μm), and a mean lumen/cell wall ratio of 60.7% (ranging from 56.5% to 64.8%). The transgenic group OX41 had a mean double cell wall thickness of 5.7 μm (ranging from 4.6 μm to 6.9 μm), mean lumen area of 376 μm^2 (ranging from 368 μm^2 to 513 μm^2), mean lumen diameter of 21.2 μm (ranging from 18.3 μm to 22.8 μm), and mean lumen/cell wall ratio of 59.0% (ranging from 55.2% to 63.9%). The transgenic group OX55 had a mean double cell wall thickness of 6.0 μm (ranging from 5.6 μm to 6.6 μm), mean lumen area of 368 μm^2 (ranging from 319 μm^2 to 459 μm^2), mean lumen diameter of 21.0 μm (ranging from 19.7 μm to 23.2 μm), and a mean lumen/cell wall ratio of 57.9% (ranging from 53.2% to 65.6%) (Table 3). Both transgenic groups, OX41 and OX55, had significantly smaller lumen diameters and lumen areas, whereas only OX55 had thicker double cell walls than the control and the thickest double cell walls in this study. The quantitative wood anatomy measurements showed a significantly smaller lumen area and thicker double cell walls for the transgenic group OX55.

In the MYB-like experiment, the control WVR31 had a mean double cell wall thickness of 4.7 μm (ranging from 4.3 μm to 5.0 μm), mean lumen area of 389 μm^2 (ranging from 376 μm^2 to 401 μm^2), mean lumen diameter of 21.6 μm

Table 2. Experiment 2—mechanical and physical properties of individual genetic lines in control (WVR31) and modified (WVK249) loblolly pine specimens.

Promoter	MOE (ksi)	COV	MOR (psi)	COV	Specific gravity	COV	Diameter (cm)	Line	No of trees	*n*	MOE (ksi)	MOR (psi)	Specific gravity	Diameter (cm)
								WT-1	2	10	344.2	6336	0.349	4.50
								WT-2	2	9	318.9	6022	0.383	3.50
WVR31	328.2	10%	6164	10%	0.367	9%	3.89	WT-3	2	10	336.0	6390	0.381	5.00
Control								WT-4	1	1	327.2	5938	0.429	2.00
								WT-5	2	10	309.1	5858	0.357	3.50
								WVK249-1	1*	5	329.4*	6498	0.395	4.00
								WVK249-2	2	5	339.1	6442	0.375	4.50
WVK249	325.9	10%	6422	9%	0.394*	8%	3.78	WVK249-3	2	8	334.7	6679	0.420*	4.00
								WVK249-4	2	7	319.7	6173	0.402	3.50
								WVK249-5	2	3	267.2*	6123	0.410	3.00

Note: *n*, number of specimens cut for each genetic line; COV, coefficient of variation; asterisk (*) indicates statistically significant result compared with the control.

Table 3. Mechanical, physical, and wood anatomical properties for individual specimens selected for quantitative wood anatomy measurements for WVK147 as the control, OX41, and OX55.

Specimen (plasmid-tree-specimen)	WVK147-3	OX41-1	OX41-2	OX41-11	OX41-12	OX55-1	OX55-2	OX55-9	OX55-10
Approx stem diam (cm)	6	4	4	3	4	4	5	3.5	4
MOE (ksi)	315.3	331.1	302.1	316.9	303.8	344.4	337.8	399.6	474.6
MOR (psi)	6050.2	6146.0	6095.9	6391.5	5885.6	6212.7	6304.2	7575.2	9410.5
Mass (g)	1.10	1.31	1.14	1.12	1.05	1.06	1.22	1.49	1.68
Oven-dry mass (g)	0.98	1.17	1.01	1.00	0.93	0.93	1.08	1.31	1.49
MC	13%	12%	13%	12%	12%	14%	13%	14%	13%
Length (mm)	25.70	25.95	25.54	25.77	26.05	26.10	25.51	26.24	26.24
Width1 (mm)	10.36	10.56	10.20	9.94	10.40	10.18	10.27	10.37	10.34
Width2 (mm)	10.41	10.39	10.18	10.38	10.46	10.17	10.60	10.50	10.37
Volume (mm^3)	2771	2845	2651	2659	2832	2700	2774	2858	2811
Density (kg/m^3)	397	460	430	421	369	391	438	520	598
Double cell wall (μm)	5.1	5.6	5.2	5.0	5.9	6.0	6.2	5.7	6.1
Cell lumen area (μm^2)	452	315	398	425	367	437	356	326	354
Lumen diameter (μm)	23.4	19.3	21.9	22.7	20.9	22.8	20.8	19.9	20.7
Lumen/cell wall ratio (%)	60.7	57.1	61.3	63.9	57.8	62.2	58.5	55.4	55.6
Compression wood	1	1	1	1	0	0	0	1	1

Note: Compression wood: 0-absent; 1-present.

(ranging from 21.2 μm to 21.9 μm), and mean lumen/cell wall ratio of 63.8% (ranging from 63.2% to 66.4%). The transgenic group WVK249 had a mean double cell wall thickness of 6.0 μm (ranging from 5.5 μm to 6.6 μm), mean lumen area of 385 μm^2 (ranging from 312 μm^2 to 440 μm^2), mean lumen diameter of 21.6 μm (ranging from 19.7 μm to 23.1 μm), and mean lumen/cell wall ratio of 57.8% (ranging from 55.3% to 59.0%) (Table 4). No significant difference was found between the transgenic WVK249 and the control WVR31 for lumen area and lumen diameter either. However, the control WVR31 had lower double cell wall thicknesses and larger lumen/cell wall ratios than the transgenic group WVK249.

Results were comparable with those reported for plantation-grown loblolly pine trees. McMillin (1968) found tracheid wall thicknesses ranging from 4.3 μm in earlywood to 9.4 μm in the latewood and tracheid diameters ranging from 31.9 μm in the latewood to 56.1 μm in the earlywood for 0-10 rings growth from the pith. Shupe et al (1996) found tracheid wall thicknesses ranging from 4.6 μm in the earlywood to 13.1 μm in the latewood for 9-yr-old loblolly pine trees at one site and 3.3 μm in the earlywood and 6.4 μm in the latewood at another site.

Quantitative wood anatomy measurements in the HAP5 experiment were analyzed and compared with other wood properties such as MOE, MOR, and density. The double cell wall thickness showed positive but weak correlations with density ($R^2 = 0.07$), MOE ($R^2 = 0.12$), and MOR ($R^2 = 0.01$). The lumen/cell wall ratio seems to negatively affect density ($R^2 = 0.30$), MOE ($R^2 = 0.28$), and MOR ($R^2 = 0.15$). The weak correlation can be due to the narrow range of measurements and the relatively small number of specimens, even though we selected the specimens with the broadest range in properties. In the MYB-like experiment, the double cell wall thickness showed a positive but weak correlation with density ($R^2 = 0.29$) and MOR ($R^2 = 0.24$), but a very weak negative correlation was found with MOE ($R^2 = 0.04$). The lumen/cell wall ratio negatively affected density ($R^2 = 0.19$) and MOR ($R^2 = 0.08$), but the correlation was low, whereas MOE showed a weak positive correlation of $R^2 = 0.15$. MOE showed an opposite trend with the double cell wall thickness and lumen/cell wall ratio in this study, but it must be pointed out that the range of the measured properties was narrow.

In summary, for the HAP5 experiment, both transgenic groups, OX41 and OX55, had significantly higher densities, specific gravities,

Table 4. Mechanical, physical, and wood anatomical properties for selected individual specimens for quantitative wood anatomy measurements for WVK249 and WVR31 as controls.

Specimen (plasmid-tree-specimen)	WVK249-2-4	WVK249-3-3	WVK249-4-2	WVK249-5-4	WVR31-4-3	WVR31-4-4
Approx stem diam (cm)	4	5	4	4	4	4
MOE (ksi)	348.4	341.2	320.8	387.9	359.9	355.1
MOR (psi)	6582	6147	7150	7617	6895	6181
Mass (g)	1.23	1.09	1.22	1.14	1.22	1.08
Oven-dry mass (g)	1.09	0.98	1.09	1.01	1.08	0.97
MC	13%	11%	12%	13%	13%	11%
Length (mm)	26.25	25.56	25.79	25.11	25.49	25.51
Width1 (mm)	10.34	10.04	9.58	10.37	10.41	10.31
Width2 (mm)	10.43	10.72	10.24	9.66	10.43	10.28
Volume (mm^3)	2831	2751	2530	2515	2768	2704
Density (kg/m^3)	434	396	482	453	441	399
Double cell wall (μm)	5.5	5.6	6.6	6.3	4.3	5
Cell lumen area (μm^2)	360	440	312	426	376	401
Lumen diameter (μm)	20.8	23.1	19.7	22.8	21.2	21.9
Lumen/wall ratio (%)	58.4	59	55.3	58.6	66.4	63.2

Note: Compression wood: 0-absent; 1-present.

MOE and MOR values, and thicker double cell walls than the control. The control had significantly larger stem diameter, indicating a faster growth. The control also had larger lumen diameters and lumen areas than both transgenic groups. For the MYB-like experiment, there were no significant differences in MOE, MOR, or stem diameter values between the transgenic WVK249 and the control WVR31; however, specific gravity was significantly greater for WVK249. No significant difference was found between WVK249 and the control WVR31 for lumen area and lumen diameter either; however, the control WVR31 had smaller double cell wall thickness and larger lumen/cell wall ratio than the transgenic group WVK249.

CONCLUSIONS

Specific gravity, density, MOE, and MOR in bending and anatomical properties were measured in plantation-grown 2- to 3-yr-old loblolly pine trees genetically modified to increase density. Compared with the control, all modified groups had significantly higher double cell wall thickness and density. For transgenic groups OX41 and OX55, both stem diameter growth and tracheid lumen diameter were reduced, leading to greater MOE and MOR. On the other hand, the transgenic group WVK249 had no change in stem diameter growth, MOE, or MOR. The increased density of these trees during the first three years of growth may be realized at the age of harvest; nonetheless, the trade-off has shown that growth may be negatively affected. These genetically engineered trees with increased density could present a significant opportunity to improve the quality of fast-growing trees and to increase the value of juvenile wood in fast-growing trees used for solid wood applications, so more research is needed.

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