

Below- and aboveground architecture of *Androstachys johnsonii* prairies: topological analysis of the root and shoot systems

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Abstract

Aim This study aimed to analyse the topological branching pattern, area-preserving branching, and fractal branching pattern (self-similarity) of the root and shoot systems of 93 *Androstachys johnsonii* trees with diameter-at-breast heights of 5–32 cm.

Methods Topological parameters were calculated.

Results Visual analysis indicated herringbone-like branching pattern for both the root and shoot systems. However, the topological index (TI) and topological trend (TT) suggested otherwise. This discrepancy was attributed to the fact that *A. johnsonii* has multiple laterals per stem/taproot node, suggesting that the topological indexes (TI and TT) might yield biased conclusions regarding the branching pattern when the main axis has multiple laterals per node. Hence, modified topological index (TIM) that could be applied in the cases of multiple laterals per node while conserving the values of TI for cases with one lateral per node

was developed; the modified index was more efficient and realistic than TI.

Conclusion The area preserving branching was confirmed for each stem node confirming thus, the self-similar branching. For the root system, the area-preserving branching was only confirmed for the first node; therefore, self-similarity was not confirmed.

Keywords Topology · Leonardo da Vinci rule · Branching pattern · Self-similarity · Mécresse

Introduction

Architecture of biological objects refers to the spatial configuration of the assemblage of subunits such that the overall configuration has some functional significance (Lynch 1995). Root architecture refers to the spatial configuration of the root system (Lynch 1995) and, analogously, shoot architecture refers to the spatial configuration of the shoot system.

Root architecture determines the ability of plants to exploit soil resources (Lynch 1995), thereby affecting water and nutrient acquisition, carbon metabolism, and environmental stress resistance (Trubat 2012). Shoot architecture, on the other hand, affects the allocation of light to leaf area and the manner in which leaves are arranged and displayed (Valladares 1999), thereby playing an important role in plant growth and survival (Valladares and Percy 2000).

Plants are known to respond to nutrient limitation by modifying branching and root system architecture (Trubat

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et al. 2012; Lynch and Ho 2005) towards herringbone-like root system (Fitter 1987; Fitter et al. 1991). In a similar way the response to water limitation is also by inhibiting lateral branching (Malamy 2005) and thus promoting herringbone-like root systems (Fitter 1987). Therefore, during the life of a tree, from seedling to adult stage, the root system can undergo transformations according to the availability of nutrients and water resources.

The majority of the existing studies on root system architecture focus on seedlings (Martínez-Sánchez et al. 2003; Trubat et al. 2012; Chiatante et al. 2004; Berntson 1997; Fitter and Stickland 1991; Larkin et al. 1995; Nicotra et al. 2002; Tworkoski and Scorza 2001; Cortina et al. 2008; Riccardo 2007) and saplings (Coll et al. 2008; Spanos et al. 2008; Salas et al. 2004; van Noordwijk and Purnomosidhi 1995; Oppelt et al. 2001); this is probably because of the difficulty in excavating the root system of adult trees. Further, if the architecture of the root system of an adult tree is studied, often the root system is not totally removed and is therefore only partially analysed (Kalliokoski 2011; Kalliokoski et al. 2008; Soethe et al. 2007).

Field studies of below- and aboveground architecture are relatively scarce in Africa (Oppelt et al. 2000, 2001). To our knowledge, similar studies in Mozambique and, especially, on *Androstachys johnsonii*, an commercially important woodland tree species restricted only to Mozambique (Cardoso 1963), are lacking. Hence, we aimed to investigate branching behaviour and determine the application of the Leonardo da Vinci rule and fractal branching pattern (self-similarity) of the root and shoot systems of *A. johnsonii*.

Material and methods

Study area

Mecrusse is a forest type where the main species, and occasionally, the only one in the upper canopy, is *A. johnsonii*. It is the dominant and co-dominant species with a relative cover varying from 80 to 100 % (Matilla and Timane 2005).

In Mozambique (18°15'S and 35°00'E), Mecrusse-dominated woodlands are mainly found in Inhambane and Gaza Provinces and in Massangena, Chicualacuala, Mabalane, Chigubo, Guijá, Mabote, Funhalouro, Panda, Mandlakaze, and Chibuto Districts. The east-most Mecrusse forest patches, covering the last five districts,

were defined as the study area. The study area has an extension of 4,502,828 ha (DINAGECA 1997), of which 226,013 ha (5 %) are covered by Mecrusse woodlands.

In the study area, the climate is dry tropical except in the west part of Panda district and south-west part of Mandlakaze district where the climate is humid tropical (Dinageca 1997; Mae 2005a; b, c, d, e). The climate is divided into two seasons: warm or rainy season from October to March and cool or dry season from March to September (Mae 2005a; b, c, d, e).

The mean annual temperature is generally greater than 24 °C, and the mean annual precipitation varies from 400 to 950 mm (Dinageca 1997; Mae 2005a; b, c, d, e). According to the FAO classification (FAO 2003), the soils in the study area are mainly Ferralic Arenosols covering more than 70 % of the study area (Dinageca 1997). Arenosols, Umbric Fluvisols, and Stagnic soils are also predominant in the north-most part of the study area (Dinageca 1997).

The study area is characterised by shortage of water resources as well as precipitation; of the five districts comprising the study area, only Chibuto and Mandlakaze districts have water resources (Dinageca 1997; Mae 2005a; b, c, d, e).

Data collection

Ninety-three trees with diameter-at-breast heights varying from 5 to 32 cm and heights varying from 5.69 to 16 m were randomly selected within 23 circular plots of 20-m radius and divided into root and shoot systems. The entire root system was completely excavated, and the distal diameters before branching and the proximal diameters after branching were measured at each node by using a calliper or calliper rule. Similarly, the shoot system was measured. Only the primary laterals (lateral roots or branches), those originating from the main axis (taproot or stem), were considered. The link length (internode distance: internal link; distance from the last node to the apex (meristem): external link; Fig. 1) was measured using a tape.

Additionally, the dry weight of the taproot, primary lateral roots, higher-order lateral roots (mainly secondary roots), primary branches, and higher-order branches were determined by multiplying the ratio of fresh- to oven-dry weight of samples taken from those components by the total fresh weight of the relevant component. Dry weight of the stem was obtained by

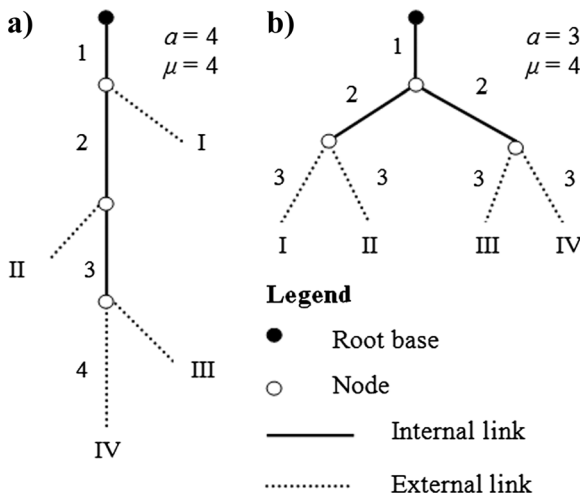


Fig. 1 Altitude and magnitude for (a) herringbone and (b) dichotomous root systems. The altitude is numbered in Arabic numerals and the magnitude is numbered in Roman numerals

multiplying the estimated density of each sample by the stem volume. Dry weights of the root and shoot systems were obtained by summing the dry weights of their constituent components. This information was used to determine how much biomass is allocated in higher-order axes (branches and roots).

Data processing and analysis

The altitude (*a*) and magnitude (*μ*) of topological parameters were determined for each root and shoot system. Altitude refers to the number of links in the longest individual path in the system (Fitter 1987; Fitter and Stickland 1991; Echeverria 2008), from the root or shoot base to an external link (Coll et al. 2008; Echeverria 2008), and magnitude refers to the total number of external links (those ending with a meristem; Fitter et al. 1991; Fitter and Stickland 1991; Riccardo 2007; Coll et al. 2008) (Fig. 1).

The branching tendency of the root and shoot systems to one of the two extremes of branching patterns, herringbone and dichotomous, was estimated by calculating two distinct indexes, the topological index (TI) and the topological trend (TT). TI was computed as the slope of the linear regression between $\log_{10}(a)$ and $\log_{10}(\mu)$ as proposed by Fitter et al. (1991); it can also be computed as follows: $\log_{10}(a) : \log_{10}(\mu)$ ratio (Riccardo 2007; Glimskar 2000), in the cases of single root or shoot systems. TI values close to 1 are associated with herringbone branching pattern and those close to 0.53 are associated with random growth of the roots or

branches (Fitter et al. 1991). TT was computed using Equation 1 (Trencia 1995). TT values close to 1 are associated with herringbone branching pattern, and those close to 0 are associated with dichotomous branching pattern (Trencia 1995). Therefore, TI values were tested under the null hypothesis of being equal to 1 and 0.53, and average TT values were tested under the null hypothesis of being equal to 1 and 0 by using Student’s *t*-test.

$$TT = [Pe_0 - Pe_{(min)}] / [Pe_{(max)} - Pe_{(min)}] \tag{1}$$

where Pe_0 is the number of observed Pe , and Pe (external path length) is the sum of the number of links in all paths from all external links to the base link (Fitter 1987). $Pe_{(max)}$ and $Pe_{(min)}$ are the possible maximum and minimum Pe values, respectively, and are computed as $Pe_{(max)} = 1/2 (\mu^2 + 3\mu - 2)$ and $Pe_{(min)} = \mu (a_{min} + 1) - 2a_{min} - 1$, where $a_{min} = \log_2(\mu - 1) + 2$.

The proportionality factor (*p*), a parameter that describes the changes in cross-sectional area (CSA) from parent root or branch to the total daughter roots or branches (van Noordwijk and Mulia 2002), i.e., the changes of CSA during branching (Soethe et al. 2007) was computed using Equation 2. The allocation factor (*q*), a parameter that describes the equity in CSA among daughter roots or branches (van Noordwijk and Mulia 2002) was computed using Equation 3. Since only the primary roots or branches were considered, the parent root or branch was always the taproot or stem and the daughter roots or branches included the taproot or the stem after branching and the daughter roots or branches after branching.

$$p = \frac{D^2 \text{ before branching}}{\sum D^2 \text{ after branching}} \tag{2}$$

$$q = \frac{\max D^2 \text{ after branching}}{\sum D^2 \text{ after branching}} \tag{3}$$

where *D* is the root or branch diameter.

The Leonardo da Vinci rule or the area-preserving branching was tested for the root and shoot systems by using four different methods. First, the average parameters *p* and *q* calculated for each *ith* node of the 93 trees and for the entire population of nodes were tested under the null hypothesis of being equal to 1 and 0.5, respectively, by using Student’s *t*-test. Second, these

parameters (p and q) were tested for independence to the link diameter by running a linear regression of p and q against the link diameter and testing the significance of the regression slope. Third, the diameter exponent Δ from Equation 4 of each stem or taproot node in each tree was estimated separately by nonlinear optimisation using Newton coordinate search, and the average was obtained per *ith* node and per total number of nodes. The diameter exponent is the value of exponent Δ that is used to solve Equation 4.

$$d_b^\Delta = \sum_{a=1}^n d_a^\Delta \quad (4)$$

where d_b is the distal diameter before branching, and d_a is the proximal diameter after branching. Finally, assuming $\Delta=2$, the regression through the origin (RTO) of the CSA before branching against total CSA after branching was run by using Equation 5 reported by Spek and van Noordwijk (1994).

$$d_b^2 = \alpha \sum_{a=1}^n d_a^2 \quad (5)$$

The average parameter Δ and the regression slope α were tested under the null hypothesis of being equal to 2 and 1, respectively, by using Student's *t*-test. The regression slope α is also referred to as the proportionality factor (p) by some authors such as Oppelt et al. (2001), although their values estimated from equations 2 and 5 are distinct. In this study, these parameters were treated distinctly.

In the cases where the average parameter p and slope α were statistically different from 1 and the average parameter Δ was not statistically different from 2, the area-preserving branching was confirmed. This was because if $p=1$ (i.e., $\Delta=2$ or $\alpha=1$), the CSA does not change across a branching point (node), whereas if $p>1$ (i.e., $\Delta>2$ or $\alpha>1$), the CSA decreases from the parent to daughter and, if $p<1$ (i.e., $\Delta<2$ or $\alpha<1$), it increases (Kalliokoski 2011; Oppelt et al. 2001; Richardson and zu Dohna 2003). Further, in the cases where the average parameter q was not found to be statistically different from 0.5, equity in CSA was implied among daughter roots or branches.

Furthermore, in the cases in which the area-preserving branching was observed for all nodes across the stem or taproot, the self-similar branching pattern was confirmed (van Noordwijk and Mulia 2002; Soethe

et al. 2007 and Richardson and zu Dohna 2003). In other words, if the branching parameters (p and q) for all nodes across the stem or taproot were not found to be dependent on the link diameter, the self-similar branching pattern was confirmed (Salas et al. 2004; van Noordwijk and Purnomosidhi 1995). All the statistical analyses were performed at the 5 % significance level using Microsoft Excel Data Analysis Tools.

Results

Topology

The branching topology of the root and shoot systems of *A. johnsonii* can be considered as perfectly to nearly herringbone, respectively, i.e., the stem or taproot is the main axis (Kalliokoski 2011) and the longest and thickest branch (Richardson and zu Dohna 2003; Fig. 2) as opposed to dichotomous branching topology where the parent branch divides into two daughter branches of the same size (Richardson and zu Dohna 2003), i.e., branching occurs with equal probability on all links (Kalliokoski 2011). However, while the shoot system occasionally violated the definition of herringbone branching by having branching laterals, the root system conformed to the definition by Riccardo (2007) and Kalliokoski (2011) since it mostly had non-branching laterals.

The shoot system consisted mostly of 7 nodes with an average of 2 laterals per node, a minimum of 1 and maximum of 4. The root system, on the other hand, consisted mostly of 4 nodes with 75 % of the laterals

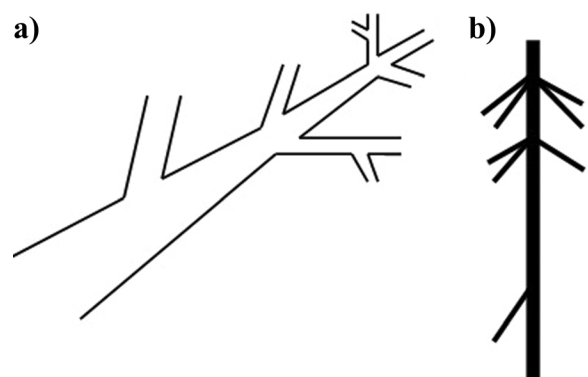


Fig. 2 Topological representation of the (a) shoot system and (b) root system. All the laterals of the root system emerge from the main axis (taproot); however, in the shoot system, branching of some primary laterals is noted

located in the first and second nodes. The average number of laterals per node in the root system was 4 and 3 for the first and second nodes, respectively, and 2 per remaining nodes; the minimum and maximum numbers of laterals per node were 1 and 11, respectively. Table 1 provides details on the number of nodes and laterals per node for the root and shoot systems.

In all, 81 % of the stem nodes had only 1 lateral; 15 %, had 2 and 3; and 1 %, had 3 and 4 laterals. Further, 32 % of the taproot nodes had only 1 lateral, and 17, 13, and 6 % had 2, 3, and 4 laterals, respectively; in 32 % of the nodes, the number of laterals varied from 5 to 11. In the shoot system, the laterals per node increased with height; on the other hand, in the root system, the laterals decreased with depth. The diameters of the laterals at the insertion point decreased with height (aboveground) and depth (belowground).

In the shoot system, the average link length per tree varied from 115 to 719 cm, with an overall weighted average of 193 cm; on the other hand, in the root system, it varied from 35 to 235 cm, with an overall weighted average of 90 cm. The average link diameter in the shoot system was 9.73 cm, varying from a minimum of 2.5 to a maximum of 32 cm; in the root system, the average link diameter was 12.59 cm, ranging from 3.5 to 47 cm. The biomass allocated in each tree component is given in the Table 2; where it can be seen that higher-order roots account, on average, with 2.922 % (0.704 kg) of

the biomass of all lateral roots (24.083 kg); and higher-order branches account with 5.631 % (3.130 kg) of the biomass of all branches (55.586 kg).

The lower amount of biomass allocated to higher-order axes (roots and branches) support the visual analysis results that the branching topology of the root and shoot systems is herringbone; with the root system showing a tendency to a perfect herringbone branching, judging by the amount of biomass allocated to higher-order roots when compared to the shoot system.

The altitude of the shoot system varied from 2 to 8, with an average of 4.6, and the magnitude varied from 2 to 13, with an average of 5.40. The TI for the shoot system was 0.70, consequently the hypotheses of being equal to 1 ($P < 0.0001$) or 0.53 ($P < 0.0001$) were rejected. The altitude of the root system varied from 3 to 5, with an average of 2.82, and the magnitude varied from 2 to 15, with an average of 7.29. The TI for the root system was 0.30, and the hypotheses of being equal to 1 ($P < 0.0001$) or 0.53 ($P = 0.0002$) were rejected.

The external path length (P_e) of the shoot system varied from 4 to 68, with an average of 21.40; the average TT value was 0.90; however, the hypotheses of being equal to 1 ($P < 0.0001$) or 0 ($P < 0.0001$) were rejected. P_e for the root system varied from 4 to 46, with an average of 17.80; the average TT was 0.37, and the hypotheses of being equal to 1 ($P < 0.0001$) or 0 ($P < 0.0001$) were rejected.

Table 1 Summary description of the number of nodes and laterals in the sampled trees

		First node	Second node	Third node	Fourth node	Fifth node	Sixth node	Seventh node	Total
Shoot system	Number of nodes in the sampled trees	86.00	80.00	69.00	44.00	23.00	6.00	1.00	309.00
	Number of branches after bifurcation	175.00	168.00	158.00	110.00	57.00	20.00	3.00	691.00
	Average number of branches per node per tree	2.03	2.10	2.29	2.50	2.48	3.33	3.00	17.74
	Number of lateral branches after bifurcation (excluding the stem)	172.00	160.00	141.00	93.00	48.00	17.00	2.00	633.00
	Average number of lateral branches per node per tree (excluding the stem)	2.00	2.00	2.04	2.11	2.09	2.83	2.00	15.08
Root system	Number of nodes in the sampled trees	73.00	40.00	19.00	1.00				133.00
	Number of lateral roots after bifurcation	386.00	160.00	43.00	3.00				592.00
	Average number of lateral roots after bifurcation per node per tree	5.29	4.00	2.26	3.00				14.55
	Number of lateral roots after bifurcation (excluding the taproot)	327.00	134.00	39.00	2.00				502.00
	Average number of lateral roots after bifurcation per node per tree (excluding the taproot)	4.48	3.35	2.05	2.00				11.88

Table 2 Summary basic statistics of the biomass per tree (kg) allocated in different tree components

#	Tree component	Minimum (kg)	Mean (kg)	Maximum (kg)	SD (kg)	CV (%)
1	Taproot	1.474	23.651	71.926	18.926	80.019
2	Primary lateral roots	0.000	23.379	95.737	22.551	112.085
3	Higher-order lateral roots	0.000	0.704	5.600	2.267	57.200
4	Lateral roots (2+3)	0.746	24.083	100.815	23.945	99.428
5	Root system (1+4)	2.545	47.735	162.105	41.210	86.331
6	Stem	5.636	138.267	413.153	110.577	79.974
7	Primary branches	2.310	52.456	209.586	57.492	109.600
8	Higher-order branches	0.000	3.130	12.858	1.954	62.437
9	Branches (7+8)	2.583	55.586	211.320	57.355	103.183
10	Shoot system (6+9)	9.823	196.659	590.863	163.713	83.247

SD is the standard deviation and CV is the coefficient of variation. The major components and their values are indicated in bold font

Branching parameters (p and q)

The average p values per node for the shoot system varied from 0.99 to 1.05, with an overall average of 1.03 (Table 3); none of the values were statistically different from 1 ($P \geq 0.05$). For the root system, the average p values per node varied from 1.02 to 2.65, with an overall average of 1.41, and only the average p value of the first node was not found to be statistically different from 1 ($P = 0.7185$). Therefore, the p values suggested that the area-preserving branching was confirmed for all stem nodes, thereby ensuring the self-similar branching pattern. For the root system, the area-preserving branching was only confirmed for the first node; for the second and third nodes, the CSA was found to decrease with branching, since the average p values were larger than 1.

The average q values per node for the shoot system ranged from 0.46 to 0.60, with an overall average of 0.56; however, these values were not statistically

different from 0.5 only for the last three nodes (Table 4). For the root system, where the average q values per node ranged from 0.45 to 0.53 with an overall average of 0.46, only the second and third nodes had average q values not different from 0.5.

Since the diameters of the stems and taproots after branching were mostly larger than those of the laterals, the average q values were not statistically different from 0.5, suggesting that there is equity in terms of CSA between the stem or taproot after branching and the laterals. The average q values markedly larger than 0.5 for the first, second, and third and for all stem nodes suggested that the largest share of the CSA was for the stem after branching. Similarly, average q values markedly smaller than 0.5 for the first and all taproot nodes suggested that the largest share of CSA was in the laterals.

The linear regressions of p against the link diameter for each stem node and for all stem nodes together were not significant (Adjusted $R^2 < 0.015$ and $P > 0.05$), suggesting

Table 3 Summary of the basic statistics for the branching parameter p

	Shoot system							Root system			
	p1	p2	p3	p4	p5	p6	p for all nodes	p1	p2	p3	p for all nodes
Minimum	0.5025	0.692	0.2549	0.4612	0.5104	0.5900	0.2549	0.4073	1.0544	0.7987	0.3352
Mean	0.9968	1.0541	1.0518	1.0245	1.0334	0.9925	1.0306	1.0169	2.6541	1.3887	1.4182
Maximum	1.5444	2.2669	1.7622	2.3607	2.3059	2.1202	2.3607	2.8412	7.4903	2.2368	7.4903
SD	0.1749	0.2481	0.3021	0.4621	0.3666	0.5786	0.3100	0.3994	1.6807	0.3448	1.2750
CV%	17.5461	23.5386	28.7237	37.1072	35.4722	58.2980	29.1482	39.2799	63.3242	24.8304	89.9028
Probability	0.8659	0.0546	0.1587	0.1035	0.6668	0.9759	0.0584	0.7185	0.0000	0.0001	0.0003

p_i is the p statistic for the i th node

Table 4 Summary of the basic statistics for the branching parameter q

	Shoot system							Root system			
	p1	p2	p3	p4	p5	p6	p for all nodes	p1	p2	p3	p for all nodes
Minimum	0.3846	0.4773	0.2941	0.2667	0.2759	0.2375	0.2375	0.1714	0.2000	0.4091	0.1714
Mean	0.6096	0.5902	0.5473	0.5062	0.5058	0.4554	0.5645	0.4483	0.4518	0.5334	0.4607
Maximum	0.8367	0.8158	0.7647	0.8387	0.7083	0.6000	0.8387	0.8478	0.6970	0.6316	0.8478
SD	0.0926	0.0781	0.1097	0.1297	0.1131	0.1455	0.1099	0.1870	0.1508	0.0735	0.1659
CV%	15.1835	13.2265	20.0488	25.6324	22.3619	31.9532	19.4659	41.7199	33.3873	13.7893	36.0118
Probability	0.0000	0.0000	0.0006	0.7538	0.8077	0.4867	0.0000	0.0208	0.0500	0.0634	0.0000

qi is the q statistic for the i th node

the independence of p values from link diameter (Table 5, Fig. 3). The independence of q values from link diameter for each stem node and for all stem nodes together was also confirmed (Adjusted $R^2 < 0.018$ and $P > 0.05$) except for the fourth node (Adjusted $R^2 = 0.27$ and $P = 0.0002$). The independence of branching parameters (p and q) for all nodes to link diameter reconfirms the self-similar branching pattern of the shoot system (Salas et al. 2004; van Noordwijk and Purnomosidhi 1995).

For the root system, independence of p values to link diameters was verified only for the first and third nodes (Adjusted $R^2 < 0.039$ and $P > 0.05$), and independence of q values to link diameters was only confirmed for the first and second nodes (Adjusted $R^2 = 0.028$ and $P > 0.1$). The dependence of p and q to link diameter for all nodes suggested that there is no self-similar branching pattern for the root system. For the second taproot node, p values increased with the link diameter (Fig. 3c).

Diameter exponent (Δ)

For 25 of the 309 stem nodes and 34 of the 133 taproot nodes, there was no solution for Δ , i.e., the nonlinear optimisation of Equation 4 did not converge. The non-convergence was mainly because the diameters of these stem or taproot nodes after branching were larger than were those before branching.

For the shoot system, the average Δ per node ranged between 1.79 and 2.06, with an overall average of 1.99 (Table 6). The null hypothesis of average Δ to be equal to 2 was not rejected for any node or population of nodes ($P > 0.09$). This confirms the area-preserving branching for each stem node and the self-similar branching pattern.

For the root system, the average Δ per node ranged between 1.22 and 1.86, with an overall average of 1.54

(Table 6). The null hypothesis of average Δ to be equal to 2 was not rejected only for the first node ($P = 0.06$). Therefore, the area-preserving branching was only reconfirmed for the first taproot node. The average Δ values for the second, third, and all nodes were markedly smaller than 2, suggesting that the CSA increased with branching; contradicting the conclusion obtained by the proportionality factor (p).

RTO of CSA before branching against CSA after branching

The slope α of RTO of CSA before branching against CSA after branching (Table 7) was not statistically different from 1 ($P > 0.07$) for any stem node or for the entire population of nodes. This result was expected since the average branching parameter p was not significantly different from 1. These results also suggest the area-preserving branching for each stem node and for the entire population of nodes and thus the self-similarity.

For the root system, the slope α was not found significantly different from 1 ($P > 0.05$) only for the first node (as expected) and for the entire population of nodes. Therefore, the area-preserving branching was not observed for any subsequent branching points (nodes), and thus, self-similarity was not confirmed for the root system.

Discussion

Topology

The TI values of the shoot and root systems were statistically different from 1 and 0.53, respectively, implying

Table 5 Regression of the branching parameters against the link diameter for the shoot and root systems

Shoot system		$p=b_0+b_1d$		Adjusted R^2		Probability		$q=b_0+b_1d$		Adjusted R^2		Probability	
Node sequence	b_0	b_1	Adjusted R^2	Probability	b_0	b_1	Adjusted R^2	Probability	b_0	b_1	Adjusted R^2	Probability	
First	0.9066	0.0056	0.0141	0.0530	0.6114	-0.0001	-0.0118	0.9380	0.6114	-0.0001	-0.0118	0.9380	
Second	1.1189	-0.0048	-0.0018	0.3576	0.5607	0.0022	0.0102	0.1823	0.5607	0.0022	0.0102	0.1823	
Third	1.0843	-0.0028	-0.0131	0.7275	0.4719	0.0064	0.0161	0.0523	0.4719	0.0064	0.0161	0.0523	
Fourth	1.0287	0.0206	0.0108	0.2319	0.3303	0.0167	0.2662	0.0002	0.3303	0.0167	0.2662	0.0002	
Fifth	1.0759	-0.0047	-0.0458	0.8493	0.5082	-0.0003	-0.0476	0.9716	0.5082	-0.0003	-0.0476	0.9716	
Sixth	0.2681	0.0852	-0.1605	0.6082	0.5219	-0.0078	-0.2381	0.8540	0.5219	-0.0078	-0.2381	0.8540	
All nodes	1.0770	-0.0010	-0.0029	0.7297	0.4971	0.0052	0.0176	0.0593	0.4971	0.0052	0.0176	0.0593	
Root system		$p=b_0+b_1d$		Adjusted R^2		Probability		$q=b_0+b_1d$		Adjusted R^2		Probability	
Node sequence	b_0	b_1	Adjusted R^2	Probability	b_0	b_1	Adjusted R^2	Probability	b_0	b_1	Adjusted R^2	Probability	
First	1.4050	-0.0147	0.0389	0.0580	0.5056	-0.0022	0.0284	0.3954	0.5056	-0.0022	0.0284	0.3954	
Second	-0.3843	0.1096	0.3514	0.0000	0.5555	-0.0037	0.0284	0.1519	0.5555	-0.0037	0.0284	0.1519	
Third	1.1501	0.0174	-0.0140	0.3980	0.6538	-0.0088	0.1920	0.0346	0.6538	-0.0088	0.1920	0.0346	
All nodes	0.7324	0.0333	0.0625	0.0022	0.5487	-0.0035	0.0348	0.0179	0.5487	-0.0035	0.0348	0.0179	

d is the link diameter; probability refers to the significance of the regression slope

that their branching patterns could not be considered to be herringbone or to have a random growth, thereby contradicting the results of visual analysis. Nonetheless, the TI values were closer to 0.53 than to 1, suggesting a tendency to grow randomly. On the other hand, the TT values, although not statistically different from 1 and 0, were considerably closer to 1 for the shoot system (a tendency to assume the herringbone branching pattern) and 0 for the root system (a tendency to assume the dichotomous branching pattern).

The TI might indicate a non-herringbone branching pattern since *A. johnsonii* has multiple laterals per stem or taproot node. However, the herringbone mathematical tree based on which TI is calculated, as described by Fitter (1987), Fitter and Stickland (1991), Spek and van Noordwijk (1994), Larkin (1995), van Noordwijk and Purnomosidhi (1995), Richardson and zu Dohna (2003), and Riccardo (2007), has only one lateral per node, and thus, a is equal to μ , as revealed by Fitter (1991), and TI is equal to 1 (Martínez-Sánchez et al. 2003). However, the maximum number of laterals per node of *A. johnsonii* was 4 for the shoot system and 11 for the root system, making μ considerably larger than a , and thus, TI was lesser than 1 and closer to 0.53. This

was confirmed by our results, where the average μ of the root system was 3 times the average a .

The same holds true for TT values, at least for the root system, since $P_{(max)}$ and $P_{(min)}$ are functions of μ , making the denominator of Equation 1 larger than the numerator and causing TT to be much lesser than 1 and closer to 0. The TT value for the shoot system was closer to 1 because the average a was closer to average μ , which in turn was due to the smaller number of laterals per node associated with the larger number of links, unlike in the root system.

This suggests that the TI and TT defined by Fitter et al. (1991) and Trencia (1995), respectively, might lead to biased conclusions with regards to the branching pattern when the main axis has multiple laterals per node, i.e., in these cases, a herringbone branching pattern might be regarded as a dichotomous one or as having random branching according to the topological indexes (TI and TT), even if the branching pattern is clearly herringbone as is the case of the root system of *A. johnsonii*.

This situation can be overcome by using the modified TI (TIM) that addresses the situation of multiple laterals per node while conserving the value of TI for the cases of one lateral per node. TIM is computed as the slope of

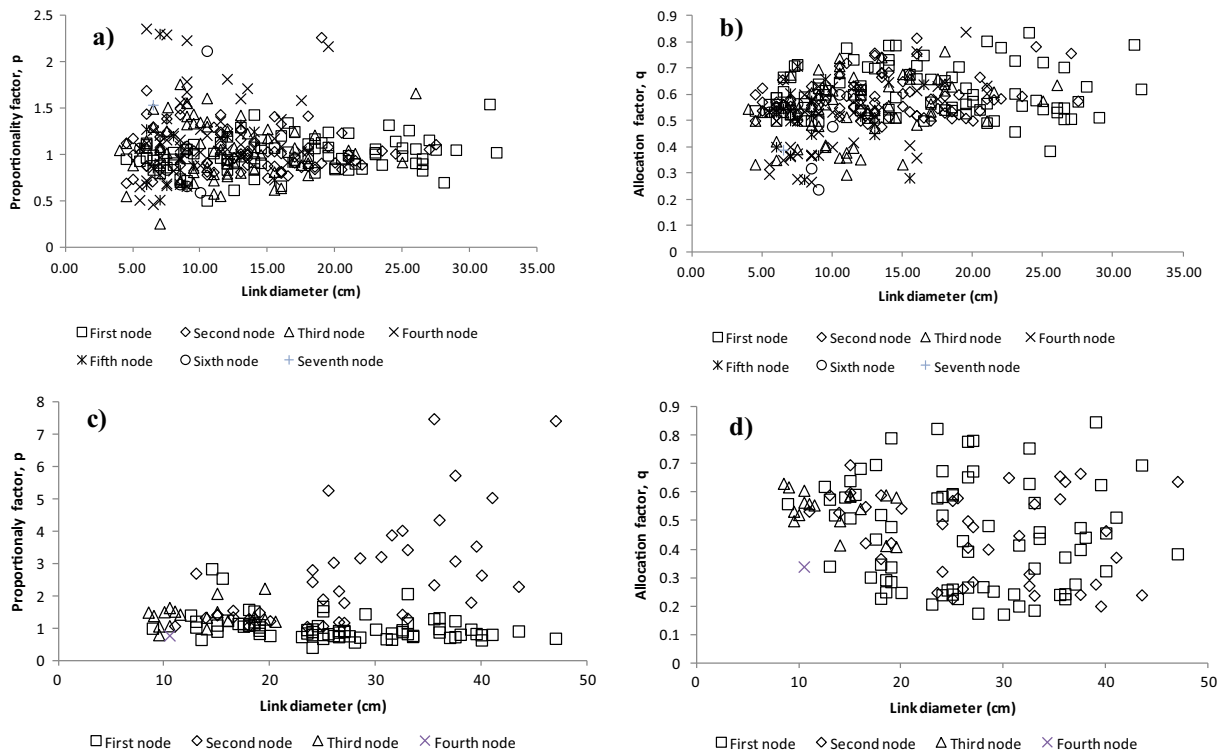


Fig. 3 Regression of branching parameters (p and q) against link diameter for (a, b) shoot system and (c, d) root system

the regression between $\log_{10}(a)$ and $\log_{10}(N+1)$, where N is the number of nodes in the root or shoot system. In the cases of single observation, TIM is computed as the ratio between $\log_{10}(a)$ and $\log_{10}(N+1)$.

The index TI is computed based on μ and a ; and the modified one (TIM) is computed based on $N+1$ and a . Therefore, whenever a mathematical tree has only one lateral per node, the equation for TIM yields the same value as that obtained using the equations for TI described by Fitter et al. (1991), since, in this case, $N+1 = \mu$, regardless of exhibiting a herringbone or dichotomous branching, no distinguished main axis, higher-order branching, or lateral axes with more than one link. However, the TIM value is different from the TI value when there are multiple laterals per node ($N+1 \neq \mu$), but, nonetheless, it is consistent with the visual analyses results, and thus more realistic.

Multiple branching per node is common in the shoot system; for example, multiple laterals per stem node are found in *Ceiba pentandra* (L.) Gaertn, *Araucaria columnaris* (G. Forst.) Hook, *Araucaria heterophylla* (Salisb.) Franco, *Pinus ponderosa* Douglas, and *Pinus cembra* L. (visual observation).

In Fig. 4, the topological index TI described by Fitter et al. (1991) is compared with the modified one (TIM). In the herringbone branching with multiple laterals per node (Fig. 4a), although the mathematical tree has perfectly herringbone branching pattern, the TI value is much closer to 0.53, suggesting that the tree tends to have a random growth of roots. However, the TIM indicates a perfect herringbone branching pattern consistent with the visual analysis results. For the herringbone branching with a single primary lateral per node (Fig. 4b), dichotomous branching with no laterals (Fig. 4c), when there is no distinguished main axis (Fig. 4c), and when there is

Table 7 RTO of CSA before branching (CSA_b) against CSA after branching (CSA_a)

Shoot system			
Node sequence	$CSA_b = b_1 CSA_a$		
	b_1	Adjusted R^2	Probability
First	0.9785	0.9552	0.2750
Second	0.9791	0.9637	0.2259
Third	0.9443	0.9164	0.0781
Fourth	1.0120	0.9050	0.0830
Fifth	0.8962	0.9034	0.0688
Sixth	0.7116	0.6417	0.0909
All nodes	0.9775	0.9580	0.1002
Root system			
Node sequence	$CSA_b = b_1 CSA_a$		
	b_1	Adjusted R^2	Probability
First	0.8070	0.9355	0.0516
Second	2.1123	0.7322	0.0000
Third	1.3099	0.9009	0.0002
All nodes	0.9033	0.7591	0.3879

Probability refers to the significance of the regression slope

one primary lateral per node with secondary branching (or higher-order branching) (Fig. 4d), the TIM yields the same results as TI, since $N+1 = \mu$.

Further research is needed to develop a modified topological trend (TTM) that can cope with the situation of multiple laterals per node while conserving the TT values by Trencia (1995) in cases of one lateral per node.

The average value of TIM computed for *A. johnsonii* (for both the root and the shoot system) was 1, and thus consistent with the visual analysis results. The TIM was not expected to be exactly 1 for the shoot system not because the shoot system does not have a perfectly herringbone pattern since some laterals still branch, but

Table 6 Summary of the basic statistics for the diameter exponent Δ

	Shoot system							Root system			
	Δ_1	Δ_2	Δ_3	Δ_4	Δ_5	Δ_6	Δ for all nodes	Δ_1	Δ_2	Δ_3	Δ for all nodes
Minimum	0.9700	0.8914	1.0811	0.6879	0.9031	0.9325	0.6879	0.7828	0.4858	0.9524	0.4858
Mean	2.0588	1.9885	2.0319	1.7932	2.0807	2.2172	1.9968	1.8593	1.2177	1.5112	1.5407
Maximum	3.8018	4.3501	4.8206	4.7337	3.3546	2.8178	4.8206	2.6272	1.9390	2.9877	2.9877
SD	0.5360	0.5937	0.8064	0.7902	0.6564	0.8302	0.6723	0.4331	0.4169	0.4569	0.5242
CV%	26.0337	29.8564	39.6877	44.0660	31.5457	37.4408	33.6711	23.2927	34.2323	30.2350	34.0215
Probability	0.3328	0.8686	0.7567	0.0935	0.5796	0.5899	0.9363	0.0596	0.0000	0.0002	0.0000

Δ_i is the Δ statistic for the i th node

because the secondary branches (those originating from the primary lateral branches) were not considered in this study and regarded as non-existing by the modified index.

A. johnsonii is a tree species found in regions with scanty rainfall and limited water resources (Mae 2005a; b; c; d; e; Dinageca 1997) and soil resources. This might be the reason why this tree species has a herringbone branching pattern of the root system (Fitter 1987; Fitter et al. 1991; Fitter and Stickland 1991; Malamy 2005; Echeverria et al. 2008).

Leonardo da Vinci rule

Van Noordwijk and Mulia (2002) suggested that a minimum of 50 but preferably 100 branching points (nodes) should be used for deriving the branching parameters. In this study, 309 and 133 branching points were used for deriving the branching parameters of the root and shoot systems, respectively.

The da Vinci rule was confirmed for each stem node and for the entire population of nodes by using four different methods: proportionality factor p , independence of p to link diameter, diameter exponent Δ , and the regression of CSA before branching against CSA after branching. The assumption of self-similarity was confirmed by the repetition of the area preserving branching (by all methods) in all stem nodes and by the independence of p and q to the link diameter for the entire population of nodes (Noordwijk and Purnomosidhi 1995; Richardson and zu Dohna 2003).

For the root system, the area-preserving branching was only confirmed for the first node. However, the area-preserving branching was confirmed for the entire population of nodes by using the regression between CSA before branching and that after branching. Since the area-preserving branching was not confirmed for every node and there was a significant dependence of p and q to link diameter for the entire population of nodes, the self-similarity was not confirmed.

The area preservation that was analysed statistically here can be explained based on eco-physiologic principles. Variations might be attributed to the effect of different heartwood proportions of the root diameter. Nikolova et al. (2009) have reported different proportions of physiologically inactive heartwood in European species, which influences the remaining capacity of a root to conduct water. Area preservation in roots and stems is better interpreted as a derivate from the physiologically

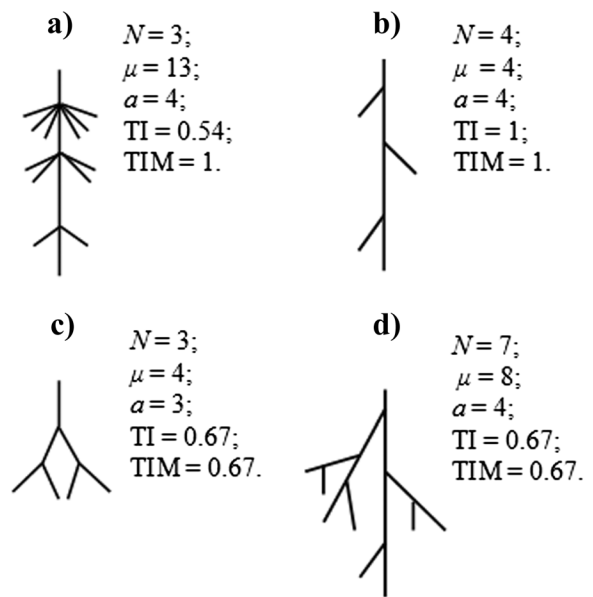


Fig. 4 Comparison of the topological index by Fitter et al. (1991) and the one modified by us for (a) herringbone branching with multiple laterals per node (b) herringbone branching with a single lateral per node, (c) dichotomous branching, and (d) dichotomous with higher-order branching. TI and TIM are computed as $\log(a) : \log(\mu)$ and $\log(a) : \log(N+1)$ ratios, respectively

founded pipe model theory (Shinozaki et al. 1964a, b) that links the conductive sapwood area in the conductive elements of a tree to the foliage attached. Stem diameters are highly correlated with heartwood area (Seifert et al. 2006), even if Shinozaki's pipe model concept has to be extended to the real water flow in conductive elements (Nikolova et al. 2009) since doubling the size of conduits in the actively transporting sapwood increases water transport by the power of four (Schiller 1933). It has been observed for several tree species that conduits taper with increasing height in the tree, which seems to be a reason for a deviation of sapwood areas from a strict pipe-model based principle (Anfodillo et al. 2006) and might be affecting area preservation as a correlated variable as well. Here, further research would be needed to reveal the relationships between root dimension, sapwood and conduit anatomical traits to determine water conducting capacity in *A. johnsonii*.

The average proportionality factor p for each stem node and the overall average p for the entire population of stem nodes were in the range of those of the shoot system (Noordwijk and Purnomosidhi 1995). Similar results were obtained by using the average p of the root system to those reported by Noordwijk and Purnomosidhi (1995), except for the second node.

The diameter exponent Δ in this study for the root and shoot systems was in the range of those reported by Oppelt et al. (2001) and Richardson and zu Dohna (2003), although, in this case, the self-similarity was only confirmed for the shoot system. Further, the lack of dependence of p and q to link diameter of the shoot system and for the first taproot node was also in accordance with the finding by Noordwijk and Purnomosidhi (1995), Soethe et al. (2007), and Salas et al. (2004).

The average p for the second and third taproot nodes suggested that the CSA decreased with branching since the average p values were larger than 1. However, this contradicts the conclusion obtained using average Δ , since, for the second and third nodes, the average Δ values were markedly smaller than 2, suggesting that the CSA increased with branching. These contradictions are attributed to the fact that, for 34 of the 133 observed taproot nodes, there was no solution for Δ , which affected each node's average Δ and the overall average Δ .

Conclusion

The newly developed topological index TIM is an unbiased estimator of the branching tendency, since this index considers both the situations of single and multiple branching per node as opposed to the traditionally used topological index TI. The branching topology of the root and shoot systems of *A. johnsonii* trees was found to be perfectly to nearly herringbone, respectively. The area preserving branching was confirmed for each stem node, thereby confirming the self-similar branching. For the root system, the area preserving branching was only confirmed for the first node; therefore, the self-similarity was not confirmed.

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Conflicts of interest The author(s) declare that they have no competing interests

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