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Clonal Propagation of *Detarium microcarpum* from Root Cuttings

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Detarium microcarpum is a valuable tree species for fuelwood, timber, food and medicine in sub-Saharan Africa. However, its population is dwindling due to overexploitation, its seedlings' low survival rate and slow growth. Vegetative propagation might enhance both survival and growth, but to date a successful clonal method does not exist for *D. microcarpum*. We conducted four experiments to examine the effects of propagation environment (high versus low humidity), cutting length and diameter, alignment of root segments (horizontal versus vertical) and distance from the root collar of donors on the regeneration ability of root segments collected from field-grown *D. microcarpum* trees in Burkina Faso. The size of root segments significantly affected their regeneration ability, while alignment had no effect. Sprouting was possible from 10 and 20-cm long segments of 15–60 mm diameter with 7–43% sprouting efficiency and multiple shoots while 5 cm long segments were unsuitable with 0–3% sprouting efficiency. Cuttings maintained at low humidity produced larger diameter sprouts than those in greenhouse. All cuttings showed strong polarity with most of the shoots developing at the proximal end. Rootlings from 20 cm root segments produced more new roots (0.62 ± 0.08 g) than those from 10 cm segments (0.34 ± 0.09 g), but they were similar for sprout and leaf growth. We conclude that lateral roots of field-grown mature trees can be used to produce rootlings in a nursery. Since this study is the first attempt to propagate *D. microcarpum* from root cuttings, further investigations are required to optimize the technique.

Keywords Burkina Faso, sprouting efficiency, rootling, vegetative propagation
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1 Introduction

Detarium microcarpum Guill. & Perr. is a deciduous tree of the family *Legumunoseae*, subfamily *Ceasalpinioideae* (Watson and Dallwitz 1993, Vautier et al. 2007). It is found in semi-arid sub-Saharan Africa from Senegal to Cameroon, extending east to the Sudan. It has an irregular distribution, but it can be locally very common. Typically, it is found in high rainfall savanna areas, dry forests and fallow lands on sandy or iron rich hard soils as well as scattered trees on farms. It also occurs in dry savanna as a more stunted tree with smaller fruits (Vautier et al. 2007) reaching ca. 10 m high and with a dense rounded crown; in wet areas it can grow up to 25 m tall.

The fruits that are drupe-like, circular and disc-shaped, containing fibers are edible and rich in vitamin C, potassium and calcium. The seeds, singly embedded within the hard fruits are used to thicken soups (Akpata and Miachi 2001). *D. microcarpum* is classified as a major African medicinal plant. The roots, stems, bark, leaves and fruits are all used to treat ailments such as tuberculosis, meningitis, itching, syphilis and diarrhea (Arbonnier 2000, Abreu and Relva 2002, Kouyaté and van Damme 2006, Vautier et al. 2007). Isolation of terpenoids and anti-HIV flavans from *D. microcarpum* extracts have been reported (Abreu and Relva 2002). In Burkina Faso, *D. microcarpum* is also known as the most important commercial fuelwood species harvested from the State forests (Kaboré 2005, Sawadogo 2007). Its hard dark brown wood provides very high quality fuelwood (19 684 kj/kg) and charcoal (Kaboré 2005) and good quality timber that is used in carpentry and construction (Vautier et al. 2007).

In Burkina Faso, current forest management regimes are based on coppicing and natural regeneration supplemented by direct seeding; these approaches are often unsuccessful. This may threaten the sustainability of the most commonly cut tree species, such as *D. microcarpum*, *Vitellaria paradoxa* C.F.Gaertn., *Terminalia* spp. and *Crossopteryx febrifuga* Benth. (Kaboré 2005, Sawadogo 2007). Savadogo et al. (2007) have, for example, classified *D. microcarpum* and *V. paradoxa* as being vulnerable species in Tiogo forest, one of the State forests of Burkina Faso, due to overexploitation and diminishing populations. Natural regeneration of *D. microcarpum* is often established as a mixture of seedlings and suckers (Bationo et al. 2001, Ky-Dembele et al. 2007), but seedlings have a low survival rate (Bationo et al. 2001, Kaboré 2005) and slow growth (Bationo et al. 2001, Ky-Dembele et al. 2008). Seedling shoot die back for several unknown number of years is one of the reasons for the slow progression of seedlings towards an advanced stage of regeneration (Bationo et al. 2001, Bastide and Ouédraogo 2008, Ky-Dembele et al. 2008). Selection and vegetative propagation of superior families could be a way to improve the regeneration of *D. microcarpum* and to increase the quality and quantity of forest products derived from this species. However, no successful clonal propagation method is currently available.

We, therefore, examined the potential of using root segments for the clonal propagation of *D. microcarpum*. Stem cuttings seem to be difficult to root irrespective of whether they are derived from the shoots of mature trees, coppices or 3-month-old seedlings (Ky-Dembele, personal observation), but the species' ability to sucker naturally suggests that there is potential for propagating it from root cuttings. Root cuttings has been defined as a propagation technique in which plant roots are severed into individual pieces, segments or cuttings, each of which is capable of developing adventitious buds and roots and, therefore, of regenerating into complete plants (Macdonald 1990). The plants thus formed are known as 'rootlings' (Hall et al. 1989, Snedden et al. 2010). The method has been used for propagating some forest trees species, such as poplars (Eliasson 1961, Hall et al. 1989, Stenvall et al. 2004, Snedden et al. 2010), with varying success. An understanding of the main factors affecting the regeneration vigor of root segments, such as cutting size (length, diameter), the original location of the segment in the root system and growing conditions are essential (Hartmann et al. 2002). Hence, the present study was intended to determine the effects of root segment length and diameter, propagation environment, alignment and insertion mode and the distance from the root collar on the regeneration ability of root segments collected from mature plants of *D. microcarpum*. As the method is inexpensive and does not require special equipment (Hall et al. 1989, Meunier et

al. 2008), root cuttings could be a cost-effective method of cloning *D. microcarpum*.

2 Materials and Methods

2.1 Stockplants and Cuttings Preparation

Naturally regenerated mature trees of *D. microcarpum* from the Nazinon forest were used; this is a tree and shrub savanna woodland located ca. 100 km south of Ouagadougou (11°30´–11°51´N and 1°27´–1°50´W) in Burkina Faso. The donor trees involved in the study were, on average, 6.3 ± 0.2 m tall, had an average diameter at breast height (DBH) of 12.3 ± 0.6 cm and an average canopy width of 4.1 ± 0.2 m. Lateral roots were excavated, and a 1–1.5 m long section was removed from each tree. Root fragments were cut to the desired size, placed in water to avoid dehydration and then taken to the laboratory of the Forest Productions Department of the Environment and Agricultural Research Institute (INERA/DPF). The distal end (towards the root tip) of each root segment was cut obliquely in order to differentiate it from the proximal end. In the laboratory, cuttings were soaked in a solution of Ivory 80 WP, a fungicide containing 80% Mancozeb for 10 min. The cuttings were planted in a sterilized mixture of soil, sand and cattle manure (1:1:1, v/v/v) in plastic containers. The mixture had a sandy-clay-silt texture (Bayala et al. 2009). The containers were covered with transparent plastic sheets and placed in a greenhouse at a humidity of 70–100% and a temperature of 22–37 °C, unless otherwise stated. Cuttings were watered manually every second day.

2.2 Experimental Designs

Four series of experiments were performed to identify factors that influence the sprouting ability of root segments. In the first experiment, the effects of root segment length (5 and 10 cm), diameter (11–20 mm and 21–40 mm) and propagation environment (inside a greenhouse with high humidity of 70–100% and a temperature in the range 22–37 °C or outdoors in the shadow of a tree, where the humidity was low. i.e. 25–70%, and the temperature in the range 22–40 °C) were tested in a completely randomized factorial design with 10 replicates and three cuttings per experimental unit. The tree which was used for shade was a mature *Sclerocarya birrea* Hochst., measuring 13.5 m tall, 55.4 cm DBH, 15.2 m across the canopy from east to west and 8.5 m from north to south. From March 26 to 27, 2009, a total of 240 root segments were prepared from eleven lateral roots (cut to 5 or 10 cm) collected from eleven mature trees. Root segments were grouped into four categories: 5 cm long, 11–20 mm diameter; 5 cm long, 21–40 mm diameter; 10 cm long, 11–20 mm diameter; and 10 cm long, 21–40 mm diameter. Three segments were taken randomly from each set and assigned to the corresponding experimental unit. They were then buried horizontally, 1 cm below the surface of the growing medium in a plastic box (75 × 15 × 12 cm). The experiment lasted for eight weeks, starting on March 26, 2009.

In the second experiment, the effects of root segment length was further tested, using 10 cm and 20 cm lengths, all with a diameter of 20–40 mm, in combination with vertical insertion modes (exposed versus buried) in a completely randomized factorial design involving four treatments and 10 replicates, with three root segments per replicate. In the exposed insertion mode, the proximal ends of the segments were kept 2 cm above the surface of the medium while in the buried insertion mode, the proximal ends were kept 1 cm below the surface of the growing medium (Fig. 1). A total of 120 root segments were collected from twenty mature trees, four trees at a time (to set up two replicates), from May 29 to June 2, 2009. Each day segments were grouped into two sets (10 and 20 cm). Three segments were taken randomly from each set and planted in a perforated black polythene bag (27 cm diameter × 40 cm height). The experiment ran for ten weeks starting on May 29, 2009.

In the third experiment, we examined whether regeneration from root segments is dependent on distance from the root collar of the mother tree; we used a completely randomized design with 10 replicates. One lateral root (1.5 m long) was excavated from each of 30 mature trees, six trees at a time (to compose two replicates) between October 29 and November 2, 2009. Twenty-centimeter

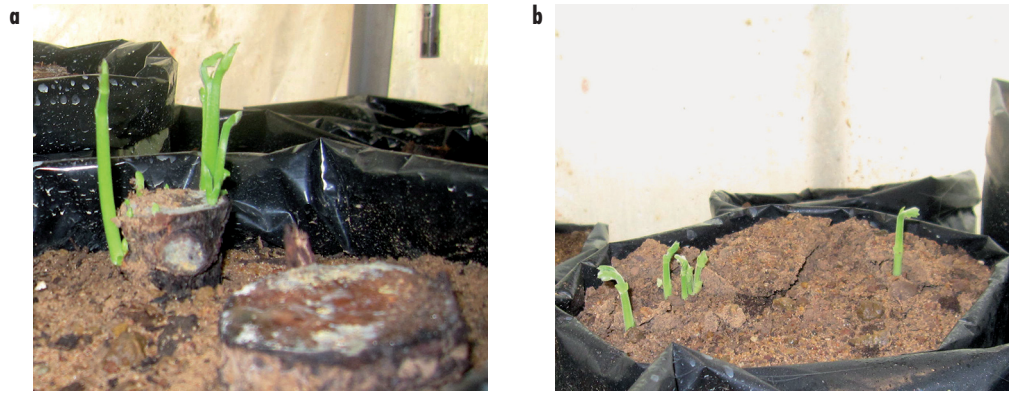

Fig. 1. Vertical insertion modes used for clonal propagation of *Detarium microcarpum* from root cuttings: proximal end exposed (a) or buried 1 cm bellow the surface of the medium (b) in Ouagadougou, Burkina Faso.

root segments were cut at three distances from the root collar, 0, 60 and 120 cm; these are referred to as treatments, and pooled in three sets. Three cuttings were taken randomly from each set and assigned to the corresponding experimental unit. The root segments were 15–60 mm in diameter. The root segments were buried vertically, with the proximal end 1 cm below the surface of the medium, which had been placed in perforated black polythene bags (27 cm diameter × 40 cm height). The experiment ran for eleven weeks.

Finally, we examined the effects of alignment of root segments (vertical and horizontal) in combination with cutting length (10 cm and 20 cm) in a completely randomized factorial experiment with 10 replicates and three cuttings per experimental unit. Root segments (10 cm and 20 cm) of 20–40 mm diameter were obtained from the same lateral roots collected for the third experiment. One set of root segments (10 cm and 20 cm) was taken 20–50 cm from the root collar and the other set 80–110 cm from the root collar. To avoid a potential confounding effect of distance from the root collar, root segments from both sets were equally distributed between treatments. The root segments were buried 1 cm below the surface of the growing medium in plastic boxes (75 × 15 × 12 cm) for the horizontal alignment and in perforated black polythene bags (27 cm diameter × 40 cm height) for the vertical alignment. The experiment ran for eleven weeks in a greenhouse from October 29, 2009.

In order to assess rootling establishment further, a total of 21 sprouted root segments, 15 from 20 cm root segments and six from 10 cm root segments were replanted in perforated black polythene bags (27 cm diameter × 40 cm height) filled with the same mixture as described previously, containing soil, sand and manure. On August 14, 2010, seven months after planting, 21 rootlings (the surviving sprouted root segments) were examined.

2.3 Data Recording and Analysis

At the end of each experiment, all root segments were removed from the growing medium, washed, the number of sprouts taller than 0.5 cm and the number of new roots were recorded per cutting and the length of the longest sprout was measured. The origins of the sprouts on each segment (whether in the proximal, central or distal region of the segment) were also recorded. The sprouting efficiency was calculated as the percentage of sprouted cuttings to the total number of root segments planted in each experimental unit. For rootling establishment, the number of sprouts, the length and the basal diameter of the longest sprouts were recorded. Because the new roots were fine, embedded in the soil and therefore difficult to count, the root systems were gently washed manually over a 0.5 mm sieve to separate roots, excluding the initial root segments. The dry

biomass of the stems, leaves and roots was determined after oven drying at 70 °C for 48 hours. The total biomass of the rootling was calculated by summing the stem, root and leaf biomass.

Data were checked for normality and analyzed using the GLM procedure of Statistical Analysis System (SAS Institute Inc. 2002–2008). The two-sample T-Test procedure in Minitab 15 (Minitab Inc., State College, PA, USA) was used for data relating to rootling establishment. The dependent variables were mean sprouting percentage (sprouting efficiency), mean number of sprouts per sprouted segment, mean length and basal diameter of the longest sprout per sprouted segment, number of sprouts per rootling, length of the rootling's longest sprout, new root biomass, and total rootling biomass. Significant differences at $P<0.05$ were further tested using Tukey's HSD multiple comparison test.

3 Results

3.1 Effects of Size of Root Segments and Propagation Environment

New shoots started to appear above the surface of the growing medium from the fifth week after the root segments were planted; this was the case in both the high-humidity environment of the greenhouse and the dry outdoor environment. Propagation environment only significantly influenced the diameter of the longest sprout ($P=0.0056$). The length of the root segments significantly affected sprouting efficiency ($P<0.0001$), while the diameter of the root segments influenced both sprouting efficiency ($P=0.0318$) and diameter of the longest sprout ($P=0.0271$). Cuttings grown outdoors had a larger collar diameter than those grown in the greenhouse, while longer root segments (10 cm) exhibited a higher sprouting efficiency than shorter ones (5 cm). Root segments with a larger diameter (21–40 mm) produced the largest, most vigorous sprouts (Table 1). There were no interaction effects on any of the parameters assessed. Shoot formation occurred most frequently at the proximal ends of cuttings (62%) compared to the distal end (20%) and the middle section (18%). No segment produced new roots from either the proximal or distal end in eight weeks of observation.

3.2 Effects of Root Segment Length and Vertical Insertion Mode

The length of root segment affected sprouting efficiency ($P=0.0002$) and the length of the longest sprout produced per sprouted segment ($P=0.0306$), while the mode of insertion had a significant effect on the diameter of sprouts ($P=0.0088$). No interaction effect was observed for any of the parameters assessed. On average, 20 cm long root segments sprouted five times more efficiently than 10 cm long segments. Buried root segments produced larger sprouts than unburied root segments. Neither the length of the root segments nor their insertion mode significantly influenced the number of sprouts per sprouted root segment, but multiple shoots were produced in many cases (Table 2). There was a pronounced polarity along the root segments, with the major-

Table 1. Effects of environment, root segment length and diameter on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
Environment	Greenhouse	16 ± 4a	1.8 ± 0.2a	2.5 ± 0.2a	7.44 ± 1.56a
	Outdoor	11 ± 4a	1.6 ± 0.4a	3.6 ± 0.3b	10.56 ± 2.13a
Cutting length	5 cm	1 ± 1a	1.0 ± 0.0a	2.1 ± 0.0a	7.00 ± -a
	10 cm	26 ± 5b	1.8 ± 0.2a	2.9 ± 0.2a	8.50 ± 1.34a
Cutting diameter	11–20 mm	8 ± 3a	1.3 ± 0.2a	2.5 ± 0.3a	7.29 ± 1.59a
	21–40 mm	19 ± 5b	2.0 ± 0.2a	3.0 ± 0.2b	8.94 ± 1.73a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

Table 2. Effects of root segment length and vertical insertion mode on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
Length	10 cm	7 ± 4a	2.5 ± 0.5a	3.0 ± 0.9a	10.70 ± 3.22b
	20 cm	33 ± 5b	4.7 ± 1.0b	4.0 ± 0.4b	5.24 ± 0.80a
Insertion	Buried	22 ± 6a	4.4 ± 1.1a	5.1 ± 0.4b	7.42 ± 0.92a
	Exposed	18 ± 5a	4.3 ± 1.5a	2.7 ± 0.4a	4.92 ± 1.49a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

Table 3. Effects of distance from the root collar of the donor tree on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Distance	Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
0 cm	40 ± 12a	3.3 ± 0.6a	3.6 ± 0.4a	10.60 ± 2.23a
60 cm	27 ± 11a	3.4 ± 0.5a	4.2 ± 0.7a	11.84 ± 3.81a
120 cm	20 ± 0.9a	3.5 ± 0.9a	3.4 ± 0.5a	6.99 ± 1.56a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

Table 4. Effects of root segment length and alignment on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
Length	10 cm	12 ± 4a	1.8 ± 0.4a	3.6 ± 0.6a	7.10 ± 2.17a
	20 cm	25 ± 5a	3.0 ± 0.6a	3.8 ± 0.2a	8.88 ± 1.90a
Alignment	Horizontal	15 ± 4a	1.9 ± 0.4a	3.7 ± 0.4a	6.33 ± 1.48a
	Vertical	22 ± 6a	3.3 ± 0.8a	3.7 ± 0.2a	10.24 ± 2.38a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

ity of shoots arising from the proximal end (88%) compared to the distal end (4%) and the middle part of the root segments (8%). No more new roots were produced within ten weeks.

3.3 Effects of Distance From Tree Root Collar, Root Segment Length and Alignment

There was no clear effect of distance from the root collar of the mother tree on the regeneration of root segments, but sprouting efficiency varied from 20% to 40% and there was multiple shoot production (Table 3). Most of the shoots were produced from the proximal end (85%), followed by the middle part of the root segments (15%), but no shoots originated from the distal end of the cuttings. None of the cuttings produced new roots.

The alignment of root segments and their length did not significantly influence the sprouting efficiency, the number of sprouts per sprouted segment or the diameter and length of the longest sprout per sprouted segment, even though the

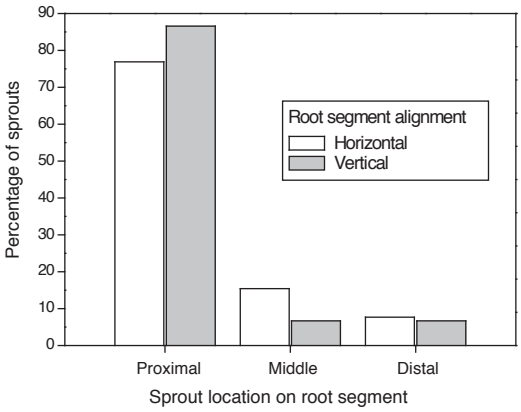

Fig. 2. Percentage of sprouts produced at different locations, the proximal ends (towards the mother tree root collar), the middle and the distal end (towards the root tip), on root segments of *Detarium microcarpum* aligned horizontally and vertically (experiment 4) in Ouagadougou, Burkina Faso.

overall mean tended to be higher for root segments that were 20 cm long (Table 4). However, the polarity was greater for the vertical alignment than the horizontal (Fig. 2). Root segments aligned vertically produced 87% of their sprouts along the proximal third part compared to 77% for the horizontally aligned cuttings.

All sprouted root segments, when replanted, produced new roots (0.05 g–1.08 g) originating from the original root segments (Fig. 3). There was a significant difference in mean new root biomass ($p=0.044$) between the two root segment lengths, 10 and 20 cm. Rootlings derived from 20 cm root segments produced a greater biomass of new roots (0.62 ± 0.08 g) than 10 cm root segments (0.34 ± 0.09 g). Root segment length did not influence the other parameters used to assess rootling establishment even though the mean values tended to be higher for those derived from 20 cm root segments. The mean values regarding these parameters for rootlings derived from 10 cm and 20 cm long segments, respectively, were: 1.5 ± 0.3 and 1.7 ± 0.2 for sprout number; 16.5 ± 3.8 cm and 19.0 ± 2.1 cm for sprout length; and 0.98 ± 0.20 g and 1.7 ± 0.30 g for the total rootling biomass.

4 Discussion

The results from the present study clearly demonstrate that *D. microcarpum* can be regenerated from root segments collected from mature, field-grown trees. The segments exhibited a relatively good capacity to produce new shoots and roots. The average sprouting efficiency obtained in our study appears to be lower than that reported for *Ficus* spp. (Danthu et al. 2002), *Maerua crassifolia* Forssk. (Houmey et al. 2007) and *Spathodea campanulata* P.Beauv. (Meunier et al. 2008), but

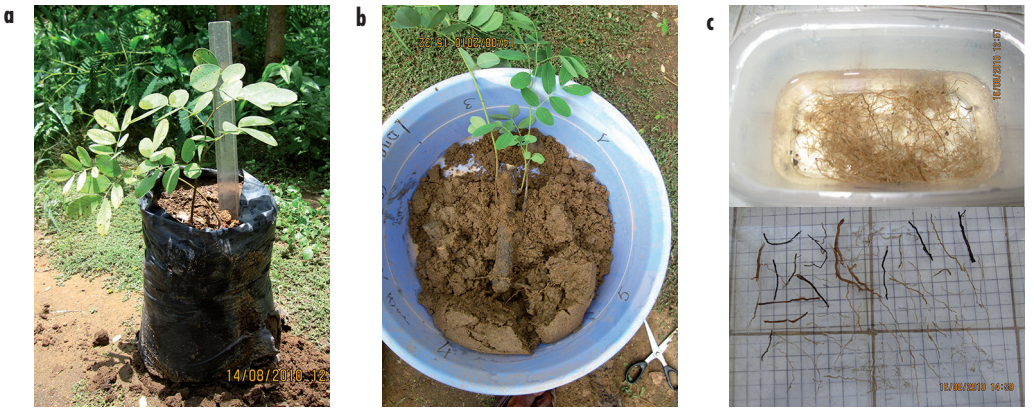

Fig. 3. *Detarium microcarpum* rootling (a) obtained from a vertically planted root segment (b) and regenerated new roots (c) in Ouagadougou, Burkina Faso.

comparable to that of field-grown mature *Faidherbia albida* (Delile) A.Chev. (Danthu 1991, Harivel et al. 2006) and higher than that of *Sclerocarya birrea* Hochst and *Diospyros mespiliformis* Hochst. ex A.DC. (Zida 2009). Generally, the discrepancy can be related to species or donor plant genotypes (Schier 1974, Yu et al. 2001, Stenvall et al. 2004), age of donor plants (Stenvall et al. 2004), growing conditions or application of hormones (Ede et al. 1997, Tsipouridis and Schwabe 2006) and phenological stage or seasonal effects (Tsipouridis and Schwabe 2006, Stenvall et al. 2009, Snedden et al. 2010).

In our study, however, the regeneration capacity was affected mainly by root segment size, diameter and length. Sprouting in *D. microcarpum* was possible for 10 and 20 cm long root segments of 15–60 mm diameter, while cuttings 5 cm long were unsuitable due to poor sprouting ability. This is in agreement with a number of previous studies, in which a similar range of lengths or diameters has resulted in successful sprouting. For *F. albida* and *Spathodea campanulata*, Harivel et al. (2006) and Meunier et al. (2008) successfully used 15–20 cm long of 10–20 mm or 20–40 mm diameter root segments while for *M. crassifolia*, 10 cm long root segments exhibited the best sprouting efficiency (Houmey et al. 2007). Root segments 10 and 15 cm long were the most efficient in *Prunus avium* (L.) L. propagation (Ghani and Cahalan 1991) while Robinson and Schwabe (1977a) found that root segments 16 cm long were more productive than several shorter roots amounting to the same total length for clonal propagation of *Malus domestica* Borkh.

Moreover, throughout the experiments, the sprouting efficiency of 10 cm root segments varied considerably. The highest sprouting efficiency was obtained from root segments collected in March (26%) followed by October (12%) and the end of May (7%). This variation might be related to the effect of phenological stage, since sprouting efficiency is known to be correlated to concentrations of carbohydrates in roots, which fluctuate under field conditions throughout the year; they are highest in the dormant period and the lowest during the vegetative growth period of donor plants (Schier and Zasada 1973, Stenvall et al. 2009, Snedden et al. 2010). Likewise, March corresponds to the end of leaf senescence that starts in November and, thus, coincides with the end of the dormant period during the dry season and the beginning of leaf production, while May corresponds to the vegetative growth period, before flowering which occurs from June to September. In October and through to January, *D. microcarpum* trees produce fruits (Kouyaté and van Damme 2006, Bastide and Ouédraogo 2009). Thus, phenological stage could be an important criterion affecting sprouting efficiency of root segments. However, more experiments examining the season and length of segments as factors are needed before drawing any conclusions.

Root thickness has a clear effect on survival, shoot production and vigor when propagating woody species from root segments. Work on the propagation of apple and kiwifruit from root segments has demonstrated better performance of thicker roots; this may be related to greater assimilate reserves available for regeneration (Robinson and Schwabe 1977a, Lawes and Sim 1980). In particular, carbohydrates have been considered to be key determinants of good shoot regeneration from root segments (Lawes and Sim 1980). Very thin root segments may lack sufficient nutritional reserves for bud and shoot growth (Eliasson 1971c, Robinson and Schwabe 1977b, Stenvall et al. 2009). On the other hand, thick roots may regenerate slowly because the tissue may be too mature and inactive (Stenvall et al. 2006). Thus, there is an optimum size that results in successful regeneration of root segments; in our case 21–60 mm seems promising.

Of the external factors considered, humidity (higher in the greenhouse) and light (lower in the greenhouse) did not seem to be important for shoot initiation on root segments of *D. microcarpum* but probably would have an effect on subsequent growth and survival, as revealed by studies of apple and aspen (Eliasson 1971a, Robinson and Schwabe 1977a, Stenvall et al. 2005). Stenvall et al. (2005) found that light did not affect the sprouting of hybrid aspen root segments but that it had a negative effect on their subsequent rooting, while 30 °C was the best soil temperature for sprouting.

Sprouting efficiency, the number of sprouts and the length and diameter of the longest sprout per sprouted root segment did not vary significantly between distances from the root collar, even

though cuttings taken from near the root collar of the mother tree exhibited the best sprouting efficiency (40%) compared to the middle part, 60 cm (27%) and the distal part, 120 cm (20%) away from the root collar. These results are in accordance with previous studies of aspen (Starr 1971) and *Paulownia tomentosa* (Thunb.) Steud. (Ede et al. 1997), where no clear differences were found between the number of shoots and roots produced near the main tree and those produced at the extremity. Our data, however, contrast with results presented by Houmey et al. (2007) who found the middle part of the root better at producing shoots than the proximal or distal parts. As thickness and location are related to each other, the location of the root segment is often significantly correlated with cutting regeneration ability (Ede et al. 1997); thicker root segments originating near the root collar usually produce more shoots, faster and more efficiently than thinner cuttings from the distal parts of the root system.

Assuming that the type of containers, the boxes and perforated polythene bags, did not affect the sprouting capacity of the root segments, they should not have confounded the comparison between horizontal and vertical alignments. Therefore, we consider that sprouting efficiency did not differ between horizontally and vertically aligned roots or between the exposed and the buried cuttings. This is in agreement with Brouard et al. (2005) but not with Ghani and Cahalan (1991), who found that exposing the proximal end of *P. avium* roots increased the number of successfully regenerated segments. However, vertical insertion, where the proximal end was buried 1 cm below the surface of the medium, was preferable, because root segments in this alignment are better anchored than the horizontally aligned ones, which are unstable and prone to lodging. In addition, the buried cuttings produced sprouts with a larger basal diameter than the exposed ones, thus providing support for a better shoot growth and a higher survival rate.

Root segments of *D. microcarpum* showed strong polarity, with most of the shoots developing towards the proximal end. This was expected because of hormonal control, a mechanism which interacts with carbohydrate supply for bud initiation and subsequent growth from root segments of woody plant species (Eliasson 1971b, Schier and Campbell 1976, Robinson and Schwabe 1977a, Ede et al. 1997). According to these authors, the polarity is due to the transport of auxin, a shoot suppression hormone that is acropetal in roots, away from the proximal end towards the root tip. In attached roots, auxin from the aerial part of the tree would normally prevent bud initiation, but when this supply ceases upon detachment of the root, depletion of auxin will allow preferential bud initiation to occur at the proximal end, a phenomenon regarded as an extension of apical dominance.

Rootling assessment showed that both sprouted root segments of 10 cm and 20 cm were able to produce new roots from the initial root segments. However, the regeneration of new roots was a slower process compared to shoot regeneration. This is in accordance with all previous studies consulted, in which rooting time is often longer than sprouting time (Hartmann et al. 2002, Stenvall et al. 2005). As suggested by these authors, this feature may indicate that the sprouting process promotes initiation of adventitious rooting because the carbohydrate supply from the leaves may support root elongation (Eliasson 1968). However compared to aspen, which has a rooting time of less than a month (Stenvall et al. 2005), *D. microcarpum* roots slowly, requiring more than two months. The optimal time needed for root formation ought to be determined in relation to other factors, such as root length, hormone application and season of collection. The development of new roots directly from the original root segments instead of the base of the new shoot has been revealed as a feature common in poplars but not aspen (Schier and Campbell 1976), and may suggest that such new roots originate from latent lateral root initials on the original root segments (Hartmann et al. 2002). Moreover, because the longest root segments that we investigated (20 cm) produced more new roots than the 10 cm root segments, these may be better for *D. microcarpum* propagation from root cuttings.

In conclusion, the findings from our study indicate that lateral roots from field-grown mature trees can be used for clonal propagation of *D. microcarpum* in a nursery. Cutting length and diameter are both important factors that affect the sprouting and rooting ability of root segments. Root segments, measuring 20 cm long and 15–60

mm in diameter, were the most successful in terms of sprouting efficiency and new roots production, whether planted horizontally or vertically and with the proximal end exposed or buried. Because this study is the first attempt to clonally propagate *D. microcarpum* from root cuttings, further work is required to optimize the technique. In particular, the effects of age of donor plants, application of shoot and root inducing hormones, and season of collection of root segments on shoot as well as new root formation need to be studied.

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