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Clonal propagation of *Argania spinosa* (L.) skeels: effects of leaf retention, substrate and cutting diameter

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Abbreviations: IBA= Indole-3-Butyric Acid, FS= Fine sand substrate, PM= Peat moss substrate, FS/PM= mixture of fine sand and peat moss.

Abstract: To evaluate the rooting ability and growth performance in semi-hardwood cuttings of *Argania spinosa* under non-mist greenhouse conditions, our experimentation was conducted with three cutting diameters (0.1-0.3, 0.3-0.6 and 0.6-0.9 cm), four leaf retention treatments (leafless, 2, 4 and 8 leaves) and three different rooting substrates (fine sand, peat moss, a 1:1 mixture of fine sand/peat moss). Significant effects of cuttings diameter, leaf retention and rooting substrate on sprouting, rooting and survival ability from *A. spinosa* semi-hardwood cuttings were observed. Among all diameters tested, a diameter of (0.3-0.6 cm) showed maximum rooting and survival capacity, while cuttings with a diameter of 0.6-0.9 cm resulted in the greatest sprouting ability. Successful vegetative propagation was restricted to leafy stem cuttings. Moreover, it was observed that the highest rooting ability was reached in cuttings planted in fine-sand substrate. However, the highest sprouting ratio (85.0%) and survival rate (92.5%) were achieved in a mixture of sand and peat moss. Thus, argan trees vegetative propagation could be most effectively achieved using semi-hardwood cuttings with a 0.4 cm diameter and 4 leaves, planted in a fine sand substrate during the root initiation period and grown in a mixture of fine sand and peat moss for hardening.

1. Introduction

The Argan tree [*Argania spinosa* (L.) Skeels] is the only species representing the tropical Sapotaceae family in Morocco (Emberger, 1925). It is a prodigious perennial thermophilic and xerophilic thorny species (Ehrig, 1974; Morton and Voss, 1987). The argan tree can be found in a wide array of environments where soils are ranging from heavy clay to poor and rocky desert soils, it grows in altitudinal ranges extending from sea level (along the Atlantic coast) up to 1500 m (Msanda, 1993). Moreover, it has the ability to survive in very dry conditions at very hot temperatures up to 50°C and low rainfall levels between 150 and 400 mm/year, shedding foliage and remaining in a state of dormancy for several years during prolonged drought (M'Hirit *et al.*, 1998). Recently, *A. spinosa* has attracted worldwide attention as a source of highly valued oil (Charrouf and Guillaume, 2008). It's becoming the most expensive edible oil in the world, as the present supplies does not meet demand (Lybbert *et al.*, 2011). Despite the ecological value and local economic importance of this species, its forest density has been divided by two during the twentieth century and the natural regeneration rate is very low and is worsened by climate changes and the negative impact of over exploitation (Kenny and De Zborowski, 2007). Besides, its cultivation is not sufficiently developed. In part, this is due to excessive utilization of kernels for oil production which hindered the conventional propagation through seed germination (Nouaim *et al.*, 2002); on the other hand, it is due to the slow progress in propagation methods, mainly because of the generally low capacity of this species to form adventitious roots (Nouaim *et al.*, 2002; Justamante *et al.*, 2017). Therefore, research is required to develop efficient vegetative propagation techniques for the production of more planting material and conservation of this species. The rooting efficiency and growth performance of cuttings in many species have been found to be dependent of the cutting thickness (Foster *et al.*, 2000). The rooting effectiveness in function of cuttings diameter could be explained by different factors (Foster *et al.*, 2000). The variation observed among different cutting diameters could be influenced by physiological conditions (nutrients, carbohydrates, auxin, phenolics, lignification etc.), their position within the branch or their ontogenetic aging (Kaul, 2008; Wendling *et al.*, 2014). Moreover, leaf retention was also reported to influence rooting behaviour of cuttings (Leakey and Coutts, 1989). The proportion of leaves retained by the cuttings during propagation is related to the percentage of cuttings that form adventitious roots (Tchoundjeu and Leakey, 1996; Mesén *et al.*, 1997a). Another factor which creates a suitable environment for rooting is substrate (Tchinda *et al.*, 2013). The importance of substrate type on the rooting capacity of cuttings is widely recognized as the starting point to be addressed for a successful vegetative propagation using stem cuttings (Hartmann *et al.*, 2002; Leakey, 2004). Cuttings of many species root successfully in a variety of 50 propagation substrates, but the rooting performance may be greatly influenced by the kind of substrate which is linked to the hydromorphic or xeromorphic status of the species (Hartmann *et al.*, 2002; Leakey, 2004). The present study aims to evaluate the effect of three independent factors (cuttings diameter, leaf retention and substrate) on sprouting, adventitious rooting and survival ability of *Argania spinosa* semi-hardwood cuttings to develop an efficient technique for achieving large-scale production of superior clonal stock plants and conservation of genetic resources. It is a continuation of our previous research on Argan vegetative propagation, which concerned the nutrient solution influence (Benbya *et al.*, 2018), and the effects of auxin type and genotype (Benbya *et al.*, 2019) on the multiplication process.

2. Materials and Methods

Experimental site and plant materials

Vegetative propagation experiment was carried out during the vegetative period of the tree (May to September) at non-mist greenhouse of the biotechnology unit of the Regional Center of Agricultural Research of Rabat (INRA-Morocco), under natural photoperiod and a mean temperature of 32±2°C. The semi-hardwood cuttings of *A. spinosa* were collected from selected adult mature trees, naturally growing at the Arboretum of Oued Cherat in the province of Bouznika, Morocco (33° 81' 96" N; 7° 11' 03" W; 45 m Altitude), which is located within 2 km of the Moroccan Atlantic coast and with an average annual rainfall of 460 mm/yr.

Preparation of cuttings and experimental establishment

Plant material was placed in cold dark storage at 4°C for 48h before planting. Then, semi-hardwood cuttings were screened for uniform length (10±0.5 cm) and were divided into three groups by diameter: 0.1-0.3 cm with 10 to12 nodes; 0.3-0.6 cm with 8 to 10 nodes and 0.6-0.9 cm with 6 to 8 nodes using calibrated electronic digital Vernier caliper. Given cuttings were prepared as multiple nodes, without leaves (leafless); or with two leaves, four leaves or eight leaves. The tip of shoot was removed and the lower leaves of each cutting were removed. Before planting, cuttings were treated with aqueous solution of 0.2% (w/v) fungicide (Dithane 750 g/kg Mancozeb) for 10 minutes and subsequently washed with distilled water to remove excess fungicide. Then, the basal end (5.0 cm) of the cuttings was soaked for 5 min in a freshly prepared aqueous solution of auxins (17.12 mM of IBA). Prepared cuttings were immediately inserted in different polyethylene (PE) pots (1000 cc) containing three different substrates: sterilized sieved fine sand (FS) (pH of 7.3, pH-KCl method; water retention of 160 ml/l; organic matter content of 0.2%, Walkley & Black method); peat moss (PM) pH of 6; water retention of 800 ml/l; organic matter content of 20%); mixture of fine sand and peat moss (1:1 v/v) (FS/PM). The rooting period for the experiment was run for 12 weeks, and then cuttings were uprooted carefully without harming root with running water. Successfully rooted cuttings (at least one root with a length of at least 1 cm long) were transplanted into polyethylene pots (4500 cc) and were maintained for hardening at a spacing of 20 cm × 20 cm for 48 weeks. Given cuttings were regularly watered twice a week with tap water during the first twelve weeks; after a Hoagland & Arnon nutrient solution (Hoagland and Arnon, 1950) was used as irrigation source.

Data collection

The morphological characteristics assessments were realized after 12 weeks for the number of sprouts (NS), length of the longest sprout (SL, cm), sprouting percentage (SP, %), number of roots (NR), length of the longest root (RL, cm), and rooting percentage (RP, %), whereas the survival rate success (SR, %) was recorded at week 48 of the hardening phase.

Experimental design and treatments

The experiment was conducted in Randomized Complete Block Design (RCBD) with four blocks and three independent variables (cutting size, leaf retention and substrate) (Fig. 1A). For each treatment there were 32 cuttings (eight per block), randomly allocated in groups of two cuttings (forming four

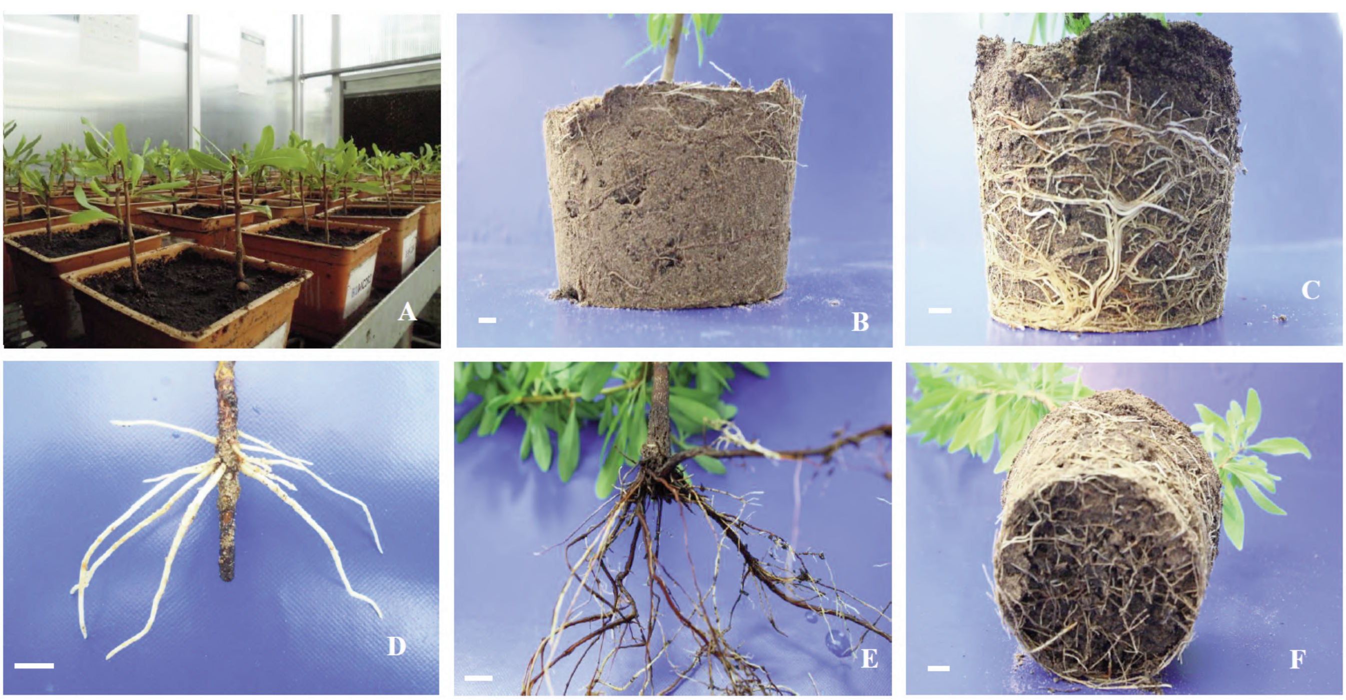

Fig. 1 - Clonal propagation of *Argania spinosa* semi hardwood cuttings. (A) Cuttings planted in peat moss substrate according to a randomized complete block design (RCBD) under non mist greenhouse conditions (24 weeks). (B) Rooted plant cutting set in sand substrate (2 years). (C) Well rooted plant cutting set in a mixture of sand and peat moss (1:1 v/v) substrate (3 years). (D) Cutting with developed root primordia from fine sand substrate (12 weeks). (E) Vigorous adventitious roots of semi hardwood cuttings grown in a mixture of sand and peat moss substrate (2 years). (F) Argan plantlet in a mixture of fine sand and peat moss substrate under non-mist greenhouse conditions. (3 years). Scale bars: 10 mm.

experimental units for thirty-six treatment combinations). The experiment was established with three cutting diameters (0.1-0.3 cm; 0.3-0.6 cm and 0.6-0.9 cm), four leaf number treatments (leafless, 2, 4, and 8 leaves) and three different substrates (FS, PM and FS/PM).

Statistical analysis

The main effects and interactions of cutting diameter, leaf retention and substrate were determined using a general linear model (GLM) procedure in SAS program version 9.1 (SAS Institute, Cary, NC) for all the evaluated parameters. The differences between the treatments were tested using Duncan's Multiple Range Test (DMRT) with at least 95% level of statistical reliance, and as a result, homogenous groups were acquired and interpreted. Collected data were subjected to simple linear regression analysis. All data were reported as means ± standard error (SE). Sprouting, rooting and survival percentage data were arcsine transformed to ensure normal distribution and homogeneity of variances.

3. Results

Effect of the cutting diameter, leaf retention and substrate on number of sprouts and sprouts length of the Argania spinosa cuttings

Cuttings with 0.6-0.9 cm diameter and four leaves set in FS/PM substrate showed the greater mean number of sprouts and the longest sprout of cuttings (1.81±0.10 cm and 17.25±0.96 cm respectively), while the lowest number of sprouts and the shortest sprout length occurred for cuttings with 0.3-0.6 cm diameter and eight leaves planted in FS substrate (1.06±0.09 cm and 8.19±1.11 cm, respectively). However, thinner cuttings (0.1-0.3 cm) and leafless cuttings for all diameters failed to produce any sprouts and are therefore not shown on the graphs (Fig. 2).

The mean number of sprouts per rooted cutting showed a progressive increase with increasing cutting diameter (r= 0.56***). A similar trend was also observed in sprouts length per rooted cutting (Table 1). Results showed that PM substrate as well as

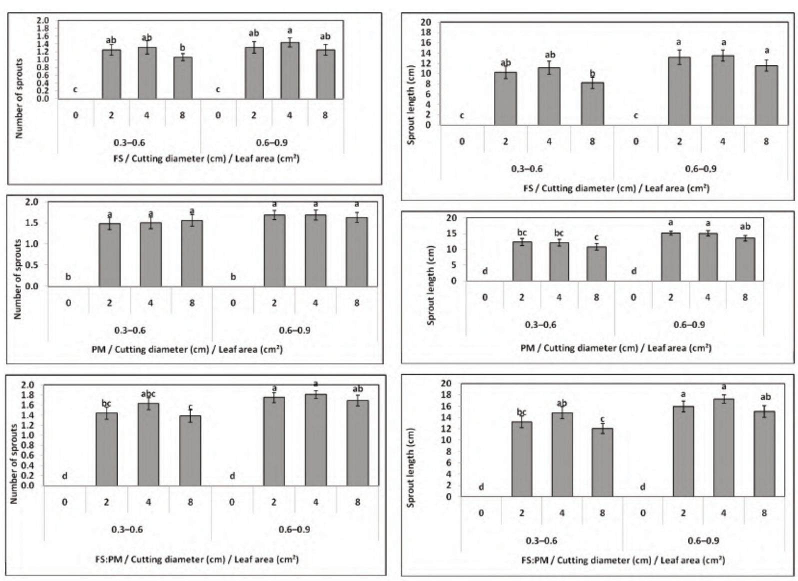

Fig. 2 - Effects of cutting diameters, four leaf number treatments (leafless, 2, 4, and 8 leaves) and three different substrates (FS, PM and FS/PM) on mean values of number of sprouts (NS) and length of the longest sprout (SL) per rooted cutting of *Argania spinosa*. Values followed by a common letter within each column are not significantly different at P<0.05 using the Duncan's Multiple Range Test (DMRT). A value represents mean ± standard error of means (n = 32).

Table 1 - Simple linear regression for cutting diameter and number of sprouts or sprout length (cm) of *Argania spinosa* cuttings

Dependent variable (Y)	Independent variable (X)	Correlation coefficient *(r)*	Regression equation	P-Value
Number of sprouts	Cutting diameter (cm)	0.56	Y= 0.59 X - 0.44	0.000 ***
Sprout length (cm)	Cutting diameter (cm)	0.58	Y= 5.42 X - 4.32	0.000 ***

***= Highly significant (P<0.001).
n = 32.

FS/PM substrate exhibited higher means number of sprouts compared to FS substrate. However, the FS/PM substrate showed the longest sprouts, followed with PM substrate, while FS exhibited the shortest sprout per cutting (Table 1). Over all the combined data for leaf retention treatments, it showed that with cutting diameter increase, there was an increase in the number of sprouts and sprout length (r=0.58***) (Table 1). Indeed, cuttings with 0.6-0.9 cm diameter showed the greater mean number of sprouts and sprout length of cuttings, followed by cuttings with 0.3-0.6 cm. Among leaf retention tested, cuttings with 4 leaves produced the longest sprouts compared with 2 and 8 leaves (Table 2).

Based on the analysis of variance (ANOVA), cutting diameter and substrate had a significant effect (P<0.05) on the mean number of sprouts, while leaf retention was not found significant on the number of sprouts per cuttings (Table 3). Moreover, cutting diameter, leaf retention and substrate had a highly significant effect (P<0.001) on the sprout length, whereas leaf retention had only significant effect on sprout length (Table 3). Neither mean number of the sprouts per cuttings nor the sprout length was significantly (P > 0.05) affected by the two-way and three-way interaction between the three factors tested (Table 3).

Table 2 - Main effects of cutting diameter, leaf retention and substrate on the number of sprouts and length of the longest sprout (cm) of *Argania spinosa* cuttings

Treatments	Sprouts number	Sprout length
Cutting diameter (cm)		
0.1-0.3	-	-
0.3-0.6	1.40 ± 0.05 b	11.65 ± 0.46 b
0.6-0.9	1.58 ± 0.05 a	14.46 ± 0.44 a
Leaf retention		
Leafless	-	-
2	1.48 ± 0.06 a	13.34 ± 0.58 ab
4	1.56 ± 0.05 a	13.98 ± 0.55 a
8	1.43 ± 0.06 a	11.85 ± 0.56 b
Substrate		
Fine sand (FS)	1.27 ± 0.07 b	11.30 ± 0.63 c
Peat moss (PM)	1.58 ± 0.06 a	13.13 ± 0.52 b
Mixture of FS and PM	1.62 ± 0.05 a	14.74 ± 0.51 a

Values followed by the same letter(s) are not significantly different at P<0.05 using the Duncan's Multiple Range Test (DMRT). A value represents mean ± standard error of means (n = 32).

Effect of the cutting diameter, leaf retention and substrate on number of roots and length of the longest root of the A. spinosa cuttings

The highest number of roots and root length were observed in cuttings with 4 leaves, followed by 2 leaves though not significantly, while lowest number of roots and root length were mostly recorded in cuttings with 8 leaves. Best response in term of number of roots was obtained when cuttings were set in FS substrate with four leaves and diameters of 0.3-0.6 cm for which the mean number of adventitious roots was 45.06±1.14 (Fig. 1E), followed by cuttings with four leaves set in FS substrate with diameters of 0.6-0.9 cm (44.130±1.21). The greatest value of root length was recorded when cuttings were grown in FS substrate with 0.3-0.6 cm thickness and with four leaves (33.63±2.12), while cuttings set in a PM sub-

Table 3 - Analysis of variance (ANOVA) for the effects of cutting diameter, leaf retention and substrate and their interactions on number of sprouts and length of the longest sprout cm per *Argania spinosa* cuttings

Source of variance	Dependent variable					
	No. of sprouts			Sprout length		
	df	F-value	P-value	df	F-value	P-value
Cutting diameter	2	7.36	0.0071 *	2	20.36	<0.0001 ***
Leaf retention	3	1.30	0.2736 NS	3	4.08	0.0179 *
Substrate	2	10.08	<0.0001 ***	2	10.15	<0.0001 ***
Cutting diameter × Leaf retention	6	0.03	0.9702 NS	6	0.9559	0.9559 NS
Cutting diameter × Substrate	4	0.39	0.6750 NS	4	0.01	0.9896 NS
Leaf retention × Substrate	6	0.35	0.8451 NS	6	0.23	0.9232 NS
Cutting diameter × Leaf retention × Substrate	12	0.20	0.9399 NS	12	0.03	0.9983 NS
Block	1	0.10	0.7534 NS	1	2.43	0.1200 NS

NS= Not significant (P>0.05); *= Significant (P<0.05); ***= Highly significant (P<0.001).
df= degrees of freedom.
×= Interaction between treatments.

strate didn't exceed 23 cm (Fig. 3; Fig. 1E). However, the incidence of necrosis was the greatest among thinner cuttings (0.1-0.3 cm) and leafless cuttings, indeed these cuttings failed to produce any adventitious roots and are therefore not on the graphs (Fig. 3).

The mean number of roots was dependent on type of substrate; cuttings grown in FS and FS/PM substrates rooted significantly better, whereas the lowest mean number of adventitious roots per cuttings was recorded in cuttings propagated in PM substrate (Table 4). Over the entire experimental period, the three substrates showed pronounced differences in the root length: cuttings planted in FS substrate were found to produce significantly longest adventitious roots, followed by FS/PM substrate then cuttings set in PM substrate (Table 4). Among the cutting diameters studied, (0.3-0.6) cm showed the highest number of roots and the longest root, followed by the cutting diameter (0.6-0.9) cm, while cuttings with (0.1-0.3) cm were the least performing and failed to produce roots (Table 4).

Regarding the ANOVA test values, the effects of cutting diameter, leaf retention and substrate were found highly significant (P<0.001) on the number of adventitious roots formed per cutting (Table 5). Moreover, cutting diameter and substrate had also a highly significant effect (P<0.001) on root length of cutting, while leaf retention had only a significant effect (P<0.05). Root length was significantly (P<0.05) affected by the interaction between cutting diameter and substrate, while the other interactions between the three factors studied were not significant on the number of roots and root length of cuttings (Table 5).

Table 4 - Main effects of cutting diameter, leaf retention and substrate on the number of roots and the length of the longest root (cm) of *Argania spinosa* cuttings

Treatments	Roots number	Root length
Cutting diameter (cm)		
0.1-0.3	-	-
0.3-0.6	38.38 ± 0.74 a	27.24 ± 0.73 a
0.6-0.9	35.61 ± 0.90 b	24.42 ±0.69 b
Leaf retention		
Leafless	-	-
2	36.95 ± 0.94 b	25.65 ± 0.93 ab
4	38.97 ± 1.04 a	27.47 ± 0.83 a
8	35.07 ± 1.03 b	24.36 ± 0.85 b
Substrate		
Fine sand (FS)	42.07 ± 0.66 a	30.76 ± 0.76 a
Peat moss (PM)	27.44 ± 0.82 b	18.25 ± 0.63 c
Mixture of FS and PM	41.48 ± 0.75 a	28.47 ± 0.65 b

Values followed by the same letter(s) are not significantly different at P<0.05 using the Duncan's Multiple Range Test (DMRT). A value represents mean ± standard error of means (n = 32).

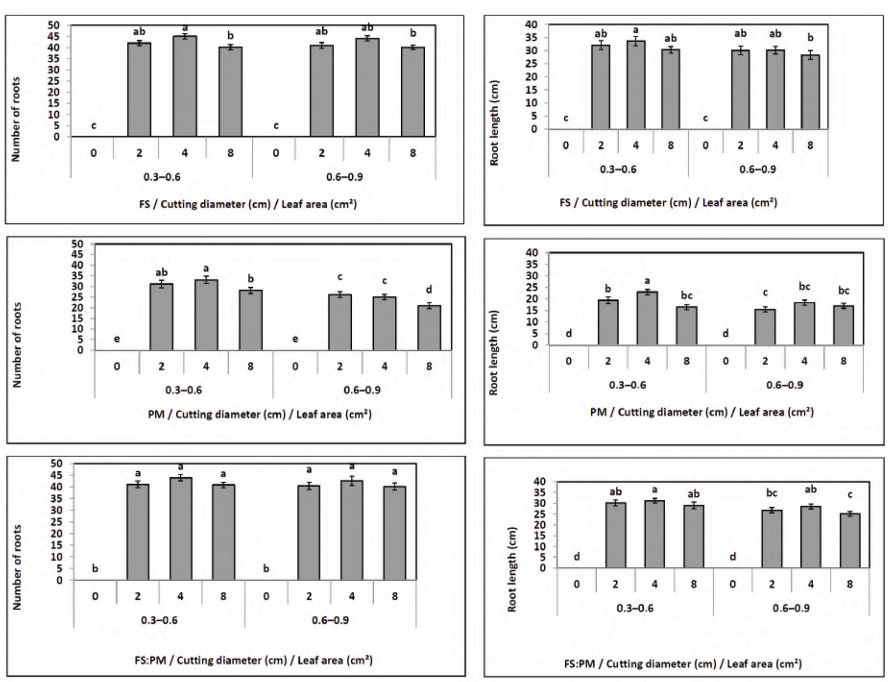

Fig. 3 - Effects of cutting diameters, four leaf number (leafless, 2, 4, and 8 leaves) and three different substrates (FS, PM and FS/ PM) on mean values of number of adventitious roots (NR) and length of the longest root cm (RL) per rooted cutting of Argania spinosa. Values followed by a common letter within each column are not significantly different at P < 0.05 using the Duncan's Multiple Range Test (DMRT). A value represents mean ± standard error of mean (n = 32).

Table 5 - Analysis of variance (ANOVA) for the effects of cutting diameter, leaf retention and substrate and their interactions on the number of adventitious roots and the length of the longest root (cm) of *Argania spinosa* cuttings

Source of variance	Dependent variable					
	No. of roots			Root length		
	df	F-value	P-value	df	F-value	P-value
Cutting diameter	2	11.27	0.0009 ***	2	13.29	0.0003 ***
Leaf retention	3	7.43	0.0007 ***	3	5.42	0.0049 *
Substrate	2	134.30	<0.0001 ***	2	98.88	<0.0001 ***
Cutting diameter × Leaf retention	6	0.18	0.8318 NS	6	0.46	0.6345 NS
Cutting diameter × Substrate	4	5.81	0.0034 *	4	0.11	0.8990 NS
Leaf retention × Substrate	6	0.72	0.5809 NS	6	0.30	0.8766 NS
Cutting diameter × Leaf retention × Substrate	12	0.14	0.9657 NS	12	0.58	0.6775 NS
Block	1	2.46	0.1178 NS	1	0.26	0.6133 NS

NS= Not significant (P>0.05); *= Significant (P<0.05); ***= Highly significant (P<0.001).
df= degrees of freedom.
×= Interaction between treatments.

Effect of the cutting diameter, leaf retention and substrate on sprouting percentage, rooting percentage and survival rate of the Argania spinosa cuttings

Although the thinner cuttings gave no sprouts, among the other different treatments, the highest sprouting percentage was 85.00 ± 3.76% when cuttings with 4 leaves and 0.6-0.9 cm diameter were planted in FS/PM substrate, while cuttings with 8 leaves and 0.3-0.6 cm diameter propagated in FS substrate gave the poorest sprouting percentage (61.50± 5.68%). However, leafless cuttings performed least and failed to produce any sprouts (Fig. 4). The proportion of cuttings forming new shoots during growth varied between the three substrates. Cuttings planted in PM and FS/PM substrates were the most successful in sprouting, while cuttings propagated in FS

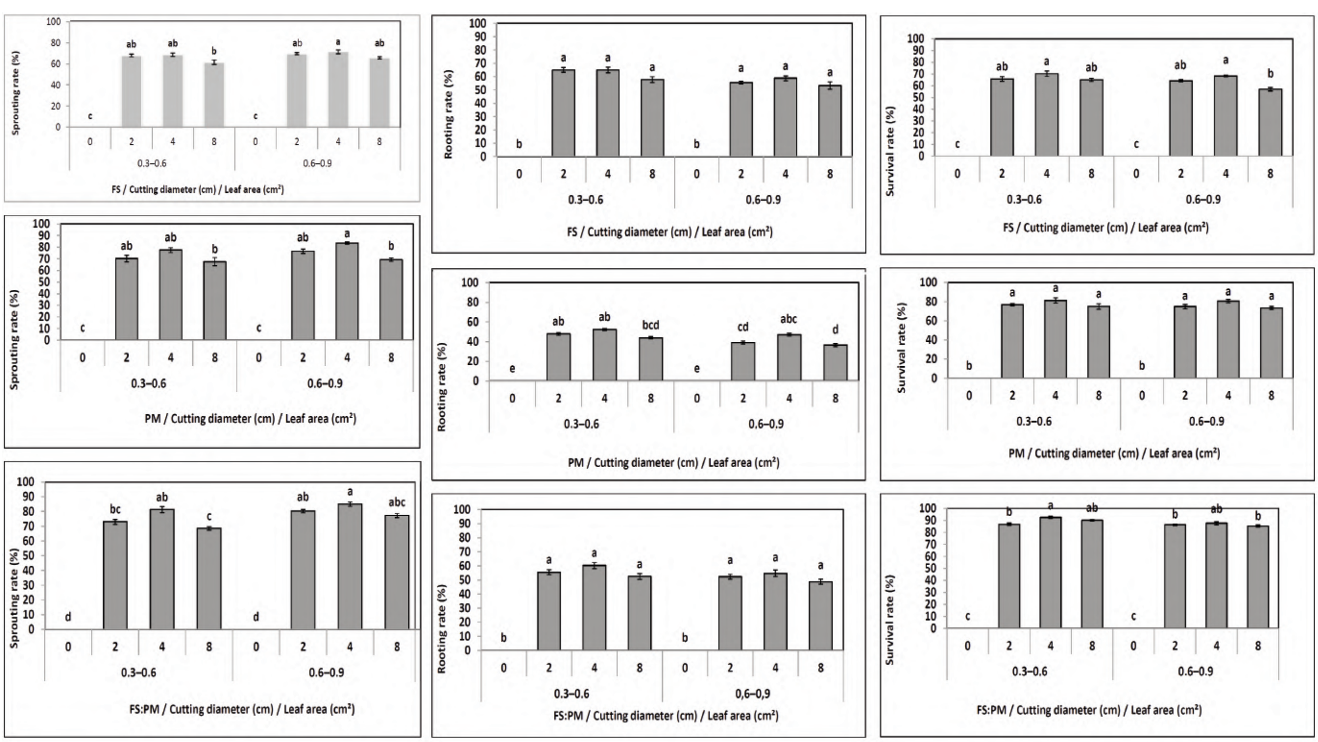

Fig. 4 - Effects of cutting diameters, four leaf number treatments (leafless, 2, 4, and 8 leaves) and three different substrates (FS, PM and FS/PM) on mean values of sprouting percentage (SP), rooting percentage (RP), and survival rate (SR) of *Argania spinosa* cuttings. Values followed by a common letter within each column are not significantly different at P<0.05 using the Duncan's Multiple Range Test (DMRT). A value represents mean ± standard error of mean (n = 32).

substrate showed the lowest sprouting ability (Table 6).

Results showed that cuttings with 0.3-0.6 cm diameter with two and four leaves set in a FS substrate showed maximum rooting: (65.00 ± 5.15% and 65.00 ± 5.87% respectively). Compared with the cuttings of 0.1-0.3 cm thickness and leafless cuttings which failed to root, all cutting diameters and leaf retention treatments enhanced the rooting percentage. Moreover, percentage of cuttings developing adventitious roots decreased when the leaf number exceeds 4 leaves (Fig. 4). The maximum rooting rates were obtained for cuttings grown in FS substrate, followed by FS/PM substrate, while the cuttings grown on PM substrate showed the lowest rooting capacity (Table 6). The survival rate ranged from 57.00 ± 4.53% for plantlets with 0.6-0.9 cm diameter and 8 leaves grown in FS substrate, to 92.50 ± 1.94% for plantlets derived from cuttings with 0.3-0.6 cm diameter and 4 leaves potted on substrate containing a mixture of fine sand and peat moss. However, cutting mortality was the greatest in thinner cuttings (0.1-0.3) cm and leafless cuttings which showed 100% mortality rate within forty-eight weeks (Fig. 4). The rooted plantlets were successfully hardened and acclimatized under non-mist greenhouse conditions (Table 6). These plants showed the best survival rate and performed better growth and development when they were grown in a FS/PM substrate compared with cuttings raised in PM and FS substrates (Fig. 1F).

The data revealed that the substrate caused significant ($P < 0.05$) variations in sprouting, rooting and survival percentage of the rooted cuttings, while cutting diameter and leaf retention were not significant on the survival rate (Table 7). However, there was no significant effect ($P>0.05$) of treatment interactions between the three independent factors on the three parameters studied.

4. Discussion and Conclusions

The present investigation revealed that cutting diameter (0.1-0.3, 0.3-0.6 or 0.6-0.9 cm), leaf retention (0, 2, 4 or 8 leaves) and substrate (FS, PM or FS/PM) were found to be important factors for successful adventitious rooting, sprouting and survival ability of *Argania spinosa* semi-hardwood cuttings.

This experiment on *A. spinosa* cuttings concerning cutting diameter indicated a significant effect on the sprouting, rooting and survival performances. Among the different cutting diameters tested, cuttings with 0.3-0.6 cm diameter tended to root better and develop more rooting capacity compared with thinner diameter (0.1-0.3 cm), which failed to produce any sprouts or roots. Moreover, larger cuttings (0.6-0.9 cm) showed the best sprouting ability but did not result in any significant improvement in root growth and development. The greatest rooting potential of cuttings with 0.3-0.6 cm diameter could be due to their good storage capacity of carbohydrates and

Table 6 - Main effects of cutting diameter, leaf retention and substrate on sprouting percentage, rooting percentage, and survival rate of *Argania spinosa* cuttings

Treatments	Sprouting (%)	Rooting (%)	Survival (%)
Cutting diameter (cm)			
0.1-0.3	-	-	-
0.3-0.6	70.69 ± 1.58 b	55.56 ± 2.22 a	78.14 ± 1.72 a
0.6-0.9	75.42 ± 2.52 a	49.55 ± 1.75 b	75.25 ± 2.20 a
Leaf retention			
Leafless	-	-	-
2	72.96 ± 1.87 ab	52.54 ± 2.03 ab	75.75 ± 1.04 ab
4	77.92 ± 1.93 a	56.33 ± 1.82 a	80.04 ± 2.16 a
8	68.29 ± 1.52 b	48.79 ± 1.90 b	74.29 ± 2.03 b
Substrate			
Fine sand (FS)	67.54 ± 1.94 b	59.21 ± 2.27 a	65.08 ± 2.37 c
Peat moss (PM)	74.08 ± 2.01 a	44.42 ± 2.19 b	76.96 ± 2.44 b
Mixture of FS and PM	77.54 ± 2.17 a	54.04 ± 2.41 a	88.04 ± 2.83 a

Values followed by the same letter(s) are not significantly different at $P<0.05$ using the Duncan's Multiple Range Test (DMRT). A value represents mean ± standard error of means (n = 32).

Table 7 - Analysis of variance (ANOVA) for the effects of cutting diameter, leaf retention and substrate and their interactions on sprouting percentage, rooting percentage and survival rate of *Argania spinosa* cuttings

Source of variance	Dependent variable								
	Sprouting (%)			Rooting (%)			Survival (%)		
	df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
Cutting diameter	2	4.28	0.0434 *	2	6.70	0.0124 *	2	1.96	0.1668 NS
Leaf retention	3	5.93	0.0047 *	3	3.53	0.0363 *	3	2.80	0.0694 NS
Substrate	2	6.60	0.0027 *	2	13.98	<0.0001	2	41.37	<0.0001
Cutting diameter × Leaf retention	6	0.02	0.9849 NS	6	0.06	0.9444 NS	6	0.23	0.7918 NS
Cutting diameter × Substrate	4	0.22	0.8072 NS	4	0.17	0.8411 NS	4	0.13	0.8775 NS
Leaf retention × Substrate	6	0.27	0.8937 NS	6	0.12	0.9745 NS	6	0.29	0.8855 NS
Cutting diameter × Leaf retention × Substrate	12	0.15	0.9633 NS	12	0.09	0.9860 NS	12	0.14	0.9655 NS

NS= Not significant (P>0.05); *= Significant (P<0.05); ***= Highly significant (P<0.001).
df= degrees of freedom.
×= Interaction between treatments.

other reserves for adventitious root formation (Leakey and Storeton-West, 1992; Tchoundjeu and Leakey, 1996). The good sprouting response may be due to the presence of adequate sugar reserves such as fructose, glucose and sucrose (Gehlot *et al.*, 2015). Moreover, the level of endogenous auxins and other rooting cofactors might be lower in cuttings with a small diameter, which leads to reduced rooting percentage or even the absence of rooting (Wilson and Van Staden, 1999). The rooting efficiency of these cuttings could be also due to the lower content of mineral elements, especially nitrogen which is positively correlated with rooting (Budiarto *et al.*, 2006). In addition, the effect of cutting diameter on rooting capacity could also be attributed to the origin of a cutting within shoots, and to its position of the stock-plant (Leakey, 2004). Indeed, thin cuttings are produced from shoots which arise in sub-dominant positions of the mother plants which contain a low amount of auxin, grow slowly and stop growth early (Howard and Ridout, 1991). The lower rooting capacity of cuttings with larger diameters (0.6-0.9 cm) may probably be due to changes in the extent of lignification and the degree of secondary thickening along their stems (Girouard, 1969; Hartmann *et al.*, 1990). These results are consistent with other studies that found a positive effect of cutting diameter on sprouting, rooting and survival success of rooted cuttings. Palanisamy and Kumar (1997) showed that the higher rooting efficiency of *Picea abies* was obtained by cuttings of 0.3-0.4 cm diameter. OuYang *et al.* (2015) confirmed the highest rooting efficiency of *Picea abies* obtained by cuttings of 0.3-0.4 cm diameter. Moreover, Foster *et al.* (2000) observed that the cuttings of *Pinus taeda* with an average diameter of 0.2-0.3 cm tended to root better and develop more roots.

Concerning leaf retention, this study showed that successful vegetative propagation of *Argania spinosa* was restricted to leafy stem cuttings. Indeed, defoliated cuttings failed to produce any sprouts / roots and showed a mortality rate of 100% within forty-eight weeks after planting. This result could be explained by the direct influence of the presence of leaves on the primary shoots, because initial growth of shoots depends on assimilates supplied by leaves and also through their influence on the cutting's water status (Newton *et al.*, 1992; Van Labeke *et al.*, 2001). Moreover, the leaf retention could also exert a strong influence on root initiation and development because it allows post-severance carbon assimilation (Leakey and Coutts 1989; Hartmann *et al.*, 1990; Thomas and Schiefelbein, 2004). Leafy cuttings provide a continuous supply of photosynthates besides their reserves after implementing them into the substrate (Tchoundjeu *et al.*, 2002; Leakey, 2004). On the other hand, the inability of leafless cuttings to root has been associated with the rapid depletion of stored carbohydrates in stem tissues after excision from the stock plants (Hoad and Leakey, 1996; Druege *et al.*, 2000). In addition, the leaf retention may also influence the endogenous auxins of the cutting. Since rooting is stimulated by the level of auxins available, it is expected that adventitious rooting response of a cutting will be proportional to the number of leaves retained (Tchoundjeu and Leakey, 1996). In fact, our results showed that the ability of cuttings to develop adventitious roots decreased when the leaf number exceeds 4 leaves. This could be explained by the balance between the positive effects of assimilate production through allowing sufficient photosynthesis and the negative effects of water loss via transpiration (Leakey and Coutts, 1989; Mesén *et al.*, 1997a). However, cuttings with eight leaves exhibited early root growth and delayed sprouting and subsequently a decreased rooting efficiency. Indeed, cuttings with a high number of leaves appear to suffer from water deficit and a consequent reduction in photosynthetic activity as reflected by higher transpiration rates and leaf shedding compared to cuttings with an optimal number of leaves (Leakey and Coutts, 1989; Newton *et al.*, 1992; Aminah *et al.*, 1997). Thus, the balance between photosynthesis, transpiration and nutrient transport is an important factor influencing rooting (Leakey and Coutts, 1989). The positive effect of leaf retention on cutting success has been reported across a wide range of species including *Triplochiton scleroxylon* (Leakey and Coutts, 1989), *Cordia alliodora* (Mesén *et al.*, 1997a), *Vitis vinifera* (Thomas and Schiefelbein, 2004), *Eucalyptus hybrids* (Trueman and Adkins, 2013), *Santalum austrocaledonicum* (Tate and Page, 2018).

Finally, the study of the substrate effect showed that a significant difference in sprouting and rooting as well as in survival ability was found between the three substrates tested. It was observed that the number of roots, root length and rooting percentage reached significantly higher values in cuttings set in the FS substrate compared to the PM substrate and to the mixture of fine sand and peat moss. This effectiveness of adventitious rooting by rooted cuttings set in FS substrate could be related to its optimal volume of gas-filled pore-space and oxygen diffusion rate which create an adequate aerated environment for increased transpiration and respiration and enhanced adventitious root formation (Andersen, 1986). Moreover, sand is a porous substrate that limits the development of microbial pathogens and where roots can be settled well without damage (Tchinda *et al.*, 2013). Though, the poor aeration and high water holding capacity of peat moss substrate is a fundamental problem for adventitious root formation, leading to enhance the rate of fungal infection and decay of cuttings before root initiation (Schmitz *et al.*, 2013). Furthermore, the higher water content in the peat moss substrate induces closing of the stomata and inhibits oxygen diffusion. This anoxia caused by oxygen deficiency or by accumulation of toxic substances, including bicarbonate and carbon dioxide, is suspected of being linked to the rooting problems due to tissue death, wilting, severe defoliation, and reduced water absorption and leaf water potential (Mesén *et al.*, 1997b; Drew, 1983; Veen, 1988). The results also showed that cuttings set in a mixture of sand and peat moss performed better than those set in sand or peat moss substrate alone, in terms of sprouting and survival rate. It has been suggested that the addition of peat moss to fine sand improved the water holding capacity and promoted mineral nutrients absorption (Leakey *et al.*, 1990). The uptake of water by cuttings is positively related to the water content of the substrate and this may enhance survival rate by reducing water deficits, leaf abscission and cutting necrosis (Newton *et al.*, 1992). A number of comparative studies between different substrates have indicated that cuttings set in a sand substrate performed better in term of rooting and survival capacity. Atangana *et al.* (2006) reported that the higher rooting percentages were observed in sand for *Allanblackia floribunda*. Gehlot *et al.* (2014) also recommended that higher rooting percentages of *Azadirachta indica* were recorded for cuttings planted in sand. Moreover, Adugna *et al.* (2015) stated that in *Vanilla planifolia*, the cuttings set in sand performed the highest rooting percentage.

In conclusion, the results of the present study clearly indicate that cuttings with thinner diameters (0.1-0.3 cm) and defoliated cuttings have failed to produce any sprouts or roots and showed a 100% mortality rate within forty-eight weeks after planting. The greatest mean number of sprouts, sprout length and sprouting capacity as well as survival success was performed by cuttings with diameter of 0.3-0.6 cm and 4 leaves set in a mixture of sand and peat moss. The best mean number of roots, root length and rooting ability was achieved by cuttings with diameter of 0.3-0.6 cm and 4 leaves in a fine sandy substrate. This study also reveals that *Argania spinosa* is amenable to clonal propagation using sand-based rooting substrate which could provide a promising prospect for the conservation of this endemic species. In addition, it is suggested that this low-cost propagation techniques could greatly facilitate the domestication and the development of superior tree crops as a commercial agroforestry species with genetically homogenous plant material. The cuttings used in the experiments were taken during the spring season. It is therefore recommended that further works should be done to determine the optimal period for cutting collections for successful clonal propagation of *A. spinosa* trees.

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