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# Vegetative propagation of *Argania spinosa* (L.) Skeels cuttings: Effects of auxins and genotype

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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**Abstract: *Argania spinosa* (L.) is an endemic tree species of south-western Morocco; it plays a very important socio-economic and environmental role. However, the vegetative propagation of the argan tree by traditional cuttings is limited by the difficulty of rooting and survival during transplantation in the field. Considering these facts, this study intended to investigate the rooting ability and growth performance of argan tree cuttings, collected from four élite trees rated ASOC1, ASOC2, ASOC3 and ASOC4, and treated with four concentrations (0, 1000, 3000 and 5000 mgL$^{-1}$) of IBA, NAA and IAA. The results revealed cuttings of ASOC2 and ASOC3 genotypes were relatively less responsive than ASOC1 and ASOC4, this genotype effect was more pronounced in auxin treated cuttings. Treatment of cuttings by IBA was more effective than treatments by either NAA or IAA. Among all the media tested, 3000 mgL$^{-1}$ of IBA with ASOC1 resulted in higher sprouting (81.75%), rooting (60.75%) and survival rates (96.25%). However, with the increase of IBA concentration levels (>3000 mgL$^{-1}$), adventitious roots and sprouts performances decreased in all the genotypes. *Argania spinosa* could be successfully propagated by cuttings from selected élite trees.**

## 1. Introduction

The argan tree - *Argania spinosa* (L.) Skeels - is a monoecious tree species, evolving in arid and semiarid areas and belonging to the tropical *Sapotaceae* family (M'Hirit *et al.*, 1998). Conventionally, argan plants are propagated by seeds. However, this method is not adequate for argan trees domestication. This species is allogamous and shows extreme variability and heterogeneity (Nouaim *et al.*, 2002; Alouani and Bani-Aameur,

2004; Msanda *et al.*, 2005). In addition, seed propagation does not ensure preserving selected genetic characters for the next generation and could also result in a loss or dilution of favorable mother trees genes (Hartmann *et al.,* 2002). However, vegetative propagation techniques (cuttings, layering, division or separation, budding, grafting and tissue culture) are not used for clonal forestry purposes because the argan tree is a hard-to-root species (Nouaim *et al.*, 2002). Thus, the development of an efficient vegetative propagation technique will provide an opportunity to facilitate domestication, improvement and mass multiplication of élite trees of this species (Leakey *et al.,* 1990; Benbya *et al*., 2018). The most common vegetative propagation methods that have the highest short-term potential for large scale production of woody plants are micropropagation (tissue culture) and macropropagation (rooted cuttings) (Duryea and Dougherty, 1991). The success of cutting techniques depends on several factors that influence rooting efficiency (Hartmann *et al.,* 2002). Adventitious root formation (ARF) is a synchronized developmental process involving various biochemical, physiological and histological events in the induction, initiation, expression and elongation phases of adventitious roots (Nemeth, 1986; Soundy *et al.*, 2008). The large variation in adventitious root formation in *A. spinosa* species is attributed to genotype (Nouaim *et al.*, 2002). In fact, the loss of ability to regenerate roots and shoots by cuttings can be described in terms of ontogenetic stage, physiological and chronological ages, which may eliminate the possibility of successful propagation of selected trees (Rasmussen *et al.,* 2014). The physiological age may also depend on environmental growth conditions and plant responses to stress (Greenwood *et al.,* 2001; Rasmussen *et al.*, 2014). Among external factors, the most important role for adventitious root formation is ascribed to growth regulators, which have been successfully employed in many plant species to improve the rooting ability of cuttings (Singh *et al.*, 2011; Sağlam *et al.*, 2014). The balance of plant hormones in the cutting could affect the development of root primordia, initial root development, root elongation, hardening and further development of the rooted cutting (Jaenicke and Beniest, 2003). In fact, adventitious root induction in cuttings is promoted by high auxin levels and low cytokinin levels in the rooting zone (De Klerk *et al.,* 2001). Auxin, the first identified plant hormone, is involved in various plant growth and development processes, including embryogenesis, organogenesis, vascular tissue patterning (xylem and phloem), flower development, fruit setting, ripening and senescence (Vanneste and Friml, 2009; Davies, 2010). Auxin homeostasis within plant tissues is regulated by the interplay of biosynthesis, conjugation, transport, and signaling pathways (Zhao, 2010). The endogenous auxin, indole-3-acetic acid (IAA), plays a central role in adventitious rooting of cuttings (De Klerk *et al.*, 1999). The difference in rooting ability between easy and hard-to-root cuttings can be attributed to IAA transport and accumulation (Ford *et al.*, 2002). The endogenous auxin increased up to fifteen days to decline thereafter, and exogenous auxin application increased the indole/auxin content (Kochhar *et al*., 2008). Many studies have shown that application of exogenous auxin results in an increased rooting initiation and development. The most common root-promoting compound in the nursery industry is indole-3-butyric acid (IBA) (Hartmann *et al.,* 2002). In addition to enhancing the rate of adventitious root development, exogenous auxin application has been found to reduce rooting development time, to increase the number of roots per plant and root system uniformity (Leakey *et al.,* 1990; Overvoorde *et al.*, 2010). The present study was undertaken to investigate the influence of genotype, auxin type and its concentration, and their combined interaction effect on adventitious rooting capacity and vegetative growth attributes of mature semi-hardwood cuttings of *Argania spinosa.* A low-cost technology of non-mist propagation system was used for all the experiments.

## 2. Materials and Methods

*Experimental site and selection of plant material*

This study was conducted under an experimental greenhouse at the biotechnology unit of the Regional Center of Agricultural Research of Rabat, Morocco. Cuttings grew from May 2014 to September 2015. *Argania spinosa* cutting materials were collected from trees of Oued Cherrat forest Arboretum, Benslimane province, Morocco (33°81'96" N ; 7°11'03" W; 45 m altitude), which is located within 2000 m of the Moroccan Atlantic coast and with an average annual rainfall of 460 mm.yr$^{-1}$. The selection of a healthy mature Candidate Plus Tree (CPT) was based on general growth, phenology (leafing, initiation of flowering, initiation of fruiting, fruiting period, maturation of fruiting, fruit shape and caliber), high biomass (crown diameter) and regeneration ability. The four genotypes (stock plants) were tagged for

identification and were pruned regularly (thrice a year) to encourage production of good shoots and maintain juvenility of the trees. After one-year, semi-hardwood cuttings were collected sequentially from shoots located in the middle-part of the tree crown in the months of April to June. The cuttings were taken early in the morning by using sterile pruning scissors and the collected shoots were kept under shade. After harvesting, these shoots were kept in perforated plastic bags inserted in a cool box to minimize a possible desiccation effect during collection and transportation. At the laboratory, the cuttings were kept in a cold room (4°C) for 48 hours.

*Preparation of cuttings and application of auxins*

Cuttings were screened by using a calibrated electronic digital Vernier caliper for desired and uniform size of (5±1) mm width, (10±0.5) cm length, with 4-6 leaves and 7-10 nodes per cutting, after removal of the apices. Each cutting was granted by two vertical cuts below the node on the basal end and a slanting cut above the node on the apical portion. The lower thorns and leaves (50% of total leaves) of each cutting were removed, keeping intact leaf buds in each cutting. The base of each cutting was freshly trimmed by 0.5 mm, then it was immersed in a 0.2% fungicide solution (Dithane with active ingredient mancozeb 750 g/kg) for 10 min and washed thoroughly with distilled water. The apical cut ends of the treated cuttings were sealed with tree wound dressings to reduce water loss, prevent diseases and decay. The lower 5 cm portion of the cuttings was dipped for 5 min in a concentrated auxin solution prepared by dissolving the hormone powder into 10 ml of ethanol (95%), and then sterile distilled water was added to a final volume of 1L. The concentrations of auxin solutions were 1000, 3000 and 5000 $mgL^{-1}$ which corresponds to (5.71, 17.12, and 28.54) mM IAA, (5.37, 16.11 and 26.85) mM NAA and (4.92, 14.76 and 24.60) mM IBA respectively. Untreated cuttings (cuttings were dipped in distilled water) were considered as a control set.

*Experimental growth conditions*

Cuttings were initially raised in a non-mist greenhouse to allow root initiation to take place. The temperature of the greenhouse was 32±2°C, with a 16h/8h photoperiod and 80% humidity. In the greenhouse, the basal cut portion was inserted vertically according to the positive polarity in 1000 cc polystyrene pots (two cuttings per pot). The pots perforated at the bottom were filled with a rooting medium of sterilized and sieved fine river sand. Holes were punched into the rooting medium to allow the insertion of the cuttings without damaging the cambium or removing the rooting hormone. After sticking of the cuttings, the rooting medium was pressed slightly around them. Cuttings were irrigated regularly to field capacity by tap water every two days. The rooting experiment was conducted for several weeks until the cuttings initiated roots. Then, rooted cuttings were removed from pots and substrate was carefully washed away from the root system. Cuttings with roots (≥ 1 mm) were considered as rooted and were included for calculating the rooting ratio. Root shoots (≥ 1 cm) were considered for calculating mean number of roots. The root systems were handled carefully so that no visible damage occurred during transplantation. After data recording, these plantlets were transferred to larger black polyethylene pots (20 cm diameter, 20 cm depth) containing a mixture of sterilized forest soil, peat moss (pH of 6, water retention of 800 ml/l and organic matter content of 20%) and sieved fine river sand rooting medium (1:1:1 v/v). These pots were placed in the greenhouse with full sunlight, at a spacing of 20 cm × 20 cm. A Hoagland nutrient solution (Hoagland and Arnon, 1950) was also used once a week to provide the nutritional needs of plantlets. The cuttings were recorded after 48 weeks, a period that was considered sufficient to measure the survival of rooted cuttings, following a preliminary study in the laboratory.

*Experimental design and treatments*

The experiment was conducted in greenhouse using a Randomized Complete Block Design (RCBD) with four replications to study the effect of four genotypes (ASOC1, ASOC2, ASOC3 and ASOC4), three auxin types (IBA, IAA and NAA), four concentrations (control, 1000, 3000 and 5000 $mgL^{-1}$) and their interactions.

*Measured parameters*

Eight morphological characteristics per cutting, including the number of leaves (LN), leaf size in $cm^2$ (LS), number of sprouts (SN), sprout length in cm (SL), sprouting rate (SP), number of roots (RN), longest root length in cm (RL), and rooting rate (RP) were measured 12 weeks after planting. The survival rate (SR) was recorded 48 weeks after rooting induction.

*Statistical analysis*

The data was submitted to tests of analysis of variance (ANOVA) for treatment effects using the general linear model (GLM) procedure of SAS program version 9.1 (SAS Institute, Cary, NC, USA), for all

the evaluated parameters. Comparisons between treatments were performed by using Duncan's Multiple Range Test (DMRT) at P<0.05 level of significance. Values were means ± standard deviation (SE). Data given in percentages was subjected to arcsine $\sqrt{X}$ transformation before statistical analysis.

## 3. Results

The results have shown that sprouting preceded rooting initiation on the cuttings. Indeed, cuttings developed leaflets after about ten days, sprouts within six weeks and there was no rooting till twelve weeks after planting (Fig. 1), although there were significant differences between genotypes treated with different auxin types and concentrations in the process of adventitious root development and shoot growth of *Argania spinosa* cuttings (Table 1).

*Effect of auxin type, concentration and genotype on the number of leaves and leaf size per cutting*

The number and size of leaves followed the same pattern; they have a significant response (P<0.05) to auxin type, concentration and for the different genotypes studied. The interaction between auxin type, concentration and genotype was also significant on the mean number of leaves and leaf size (Table 1) The results show that genotypes responded to all treatments including low concentrations (1000 mg $L^{-1}$). The highest numbers of leaves values (35.75 and


Fig. 1 - Vegetative propagation of *Argania spinosa* through mature semi-hardwood cuttings. (A) Selected plus tree in natural conditions (month of May). (B) Cuttings in polyethylene pots according to a Randomized Complete Block Design (RCBD). (C) Leaf initiation (10 days) and sprouts elongation (6 weeks). (D) Root primordia initiation and adventitious root development (12 weeks). (E) High rate of adventitious roots from semi-hardwood cuttings treated with 3000 mgL-1 IBA (48 weeks). (F) Plant produced from cuttings transplanted in black polyethylene pots under non-mist greenhouse conditions (3 years).

Table 1 - Three-way analysis of variance (ANOVA) for effects of genotype (ASOC1, ASOC2, ASOC3 and ASOC4), auxin type (IAA, NAA and IBA), concentration (0; 1000; 3000; 5000) and their interactions on measured parameters of *A. spinosa* cuttings

| Source of variance | Dependent variable | df | F-value | P-value |
|---|---|---|---|---|
| Genotype | | | | |
| | No. of leaves | 3 | 99.127 | 0.000 |
| | Leaf size ($cm^2$) | 3 | 66.694 | 0.000 |
| | No. of sprouts | 3 | 56.110 | 0.001 |
| | Sprout length (cm) | 3 | 10.321 | 0.000 |
| | No. of roots | 3 | 11.500 | 0.000 |
| | Root length (cm) | 3 | 12.100 | 0.000 |
| | Sprouting rate (%) | 3 | 343.16 | 0.000 |
| | Rooting rate (%) | 3 | 109.75 | 0.000 |
| | Survival rate (%) | 3 | 57.643 | 0.000 |
| Auxin | | | | |
| | No. of leaves | 2 | 15.180 | 0.000 |
| | Leaf size ($cm^2$) | 2 | 9.750 | 0.000 |
| | No. of sprouts | 2 | 1.295 | 0.274 |
| | Sprout length (cm) | 2 | 3.084 | 0.046 |
| | No. of roots | 2 | 33.270 | 0.000 |
| | Root length (cm) | 2 | 38.420 | 0.000 |
| | Sprouting rate (%) | 2 | 65.200 | 0.000 |
| | Rooting rate (%) | 2 | 861.00 | 0.000 |
| | Survival rate (%) | 2 | 1125.5 | 0.000 |
| Concentration | | | | |
| | No. of leaves | 3 | 148.10 | 0.000 |
| | Leaf size ($cm^2$) | 3 | 165.34 | 0.000 |
| | No. of sprouts | 3 | 512.55 | 0.000 |
| | Sprout length (cm) | 3 | 480.47 | 0.000 |
| | No. of roots | 3 | 281.49 | 0.000 |
| | Root length (cm) | 3 | 252.28 | 0.000 |
| | Sprouting rate (%) | 3 | 4638.9 | 0.000 |
| | Rooting rate (%) | 3 | 2830.6 | 0.000 |
| | Survival rate (%) | 3 | 8245.3 | 0.000 |
| Genotype x auxin x concentration | | | | |
| | No. of leaves | 18 | 6.832 | 0.000 |
| | Leaf size ($cm^2$) | 18 | 8.511 | 0.000 |
| | No. of sprouts | 18 | 00.164 | 1.000 |
| | Sprout length (cm) | 18 | 8.425 | 0.000 |
| | No. of roots | 18 | 57.646 | 0.000 |
| | Root length (cm) | 18 | 14.281 | 0.000 |
| | Sprouting rate (%) | 18 | 3.681 | 0.000 |
| | Rooting rate (%) | 18 | 4.715 | 0.000 |
| | Survival rate (%) | 18 | 1.692 | 0.047 |

Df= degrees of freedom; level of significance P<0.05; sprouting rate, rooting rate and survival rate were subjected to arcsine $\sqrt{X}$ transformation before statistical analysis.

34.56) were recorded from ASOC1 treated respectively with 3000 and 5000 $mgL^{-1}$ IBA, while the lowest number of leaves (13.31) was observed for ASOC2 cuttings treated with 1000 $mgL^{-1}$ IAA (Fig. 2). It was noticed that the number of leaves of ASOC1 cuttings, exposed to 3000 $mgL^{-1}$ IBA, is 77% higher compared to the control group.

The number of leaves for ASOC1 cuttings - that received 1000 $mgL^{-1}$ NAA is 73% greater than the control group, while it is 52% higher for the 5000 $mgL^{-1}$ IAA application. The mean number of leaves produced in the control group of ASOC1 is 19.29 leaves per cutting (Fig. 2). According to the results, the highest leaf size mean (32.42 $cm^2$) was observed in ASOC1 treated by 3000 $mgL^{-1}$ IBA and it is 3 times greater than the control group. Similarly, leaf size (27.29 $cm^2$) of ASOC1 cuttings receiving 1000 $mgL^{-1}$ NAA is 2.87 times higher than leaf size in the control set. While, the cuttings of ASCO1 that received 5000 $mgL^{-1}$ IAA developed a leaf size 1.52 times bigger than control. Whereas the minimum leaf size (6.82 $cm^2$) was recorded for the ASOC2 cuttings treated with 1000 $mgL^{-1}$ IAA (Fig. 2).

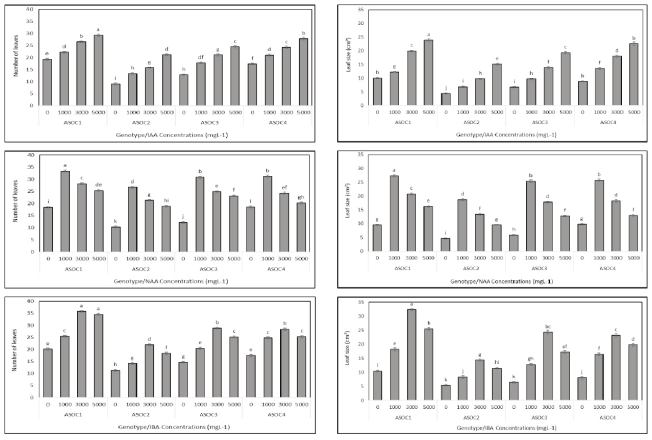

Fig. 2 - Effects of auxin type, concentration and genotype on leaves development (Mean values of leaves number and leaf size cm2) of *A. spinosa* cuttings. Within each treatment, the mean values followed by the same letter are not significantly different (Duncan's Multiple Range Test, P<0.05, mean± SE, n = 32).

*Effect of auxin type, concentration and genotype on the number of sprouts and sprout length per cutting*

The comparison between various treatments revealed that genotype, auxin type, concentration and their interaction had a significant influence (P < 0.05) on sprout length per cutting. However, the mean number of sprouts is influenced only by genotype and auxin concentration (Table 1). The number of sprouts and sprout length were induced by all the treatments except control (Fig. 3). The pretreatment of ASOC1 with 3000 $mgL^{-1}$ IBA enhanced the number of sprouts (1.81 times) and sprout length by 15.94 cm, in comparison with the control. Thereby, NAA and IAA applications also increased the number and length of sprouted cuttings but were less effective than IBA. Indeed, by using 1000 $mgL^{-1}$ NAA treatment on ASOC1 cuttings, the number of sprouts was 1.63 times greater and sprouts were longer by 12.38 cm than the control set, while by using 5000 $mgL^{-1}$ IAA treatment, the greatest number of sprouts was 1.69 times greater for ASOC4 and the highest sprouts length was 11.19 cm longer for ASOC1 (Fig. 3).

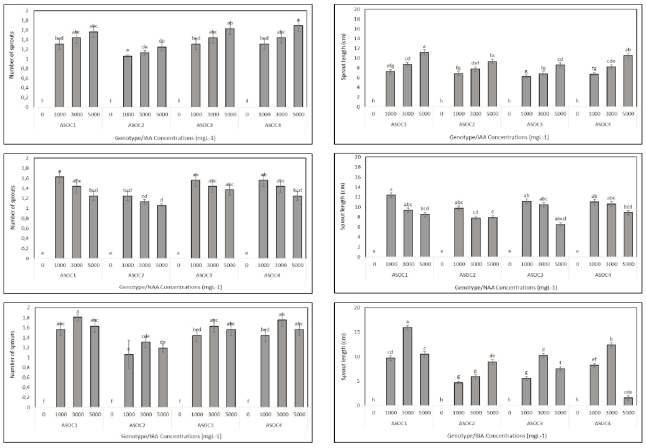

Fig. 3 - Effects of auxin type, concentration and genotype on sprouts growth (Mean values of sprouts number and sprout length) of A. spinosa cuttings. Within each treatment, the mean values followed by the same letter are not significantly different (Duncan's Multiple Range Test, P<0.05, mean± SE, n = 32).

*Effect of auxin type, concentration and genotype on the number of roots and longest root length per cutting*

The analysis of variance indicated that there were significant differences between auxin type and concentration as well as genotype on the number of roots produced and root length formed on *A. Spinosa* cuttings. The interactions between auxin type and its concentration with genotype influenced (P<0.05) root number and root length (Table 1). It may be inferred from above that there is a synergism between the number of roots and length of roots in all genotypes studied. Indeed, the more there are roots per cuttings and the more there will be longer roots. The adventitious root production increased at lower concentrations of IBA and began to level off between 1000 and 3000 $mgL^{-1}$, whereas with bigger hormone concentration levels (>3000$mgL^{-1}$), the number and length of roots were smaller. Similar

rooting response was observed with NAA hormonal treatments (Fig. 4). According to the results of the study, IBA significantly promoted the number and length of roots in comparison with NAA and IAA. Genotypes ASOC2 and ASOC3 were relatively less responsive than genotypes ASOC1 and ASOC4 in term of root number and root length per cutting. The cuttings in the control group and cuttings treated with 1000 $mgL^{-1}$ IAA failed to produce roots, while the cuttings belonging to ASOC1 receiving a 3000 $mgL^{-1}$ IBA application developed a maximum number of roots (41) with an average length of 22.94 cm.

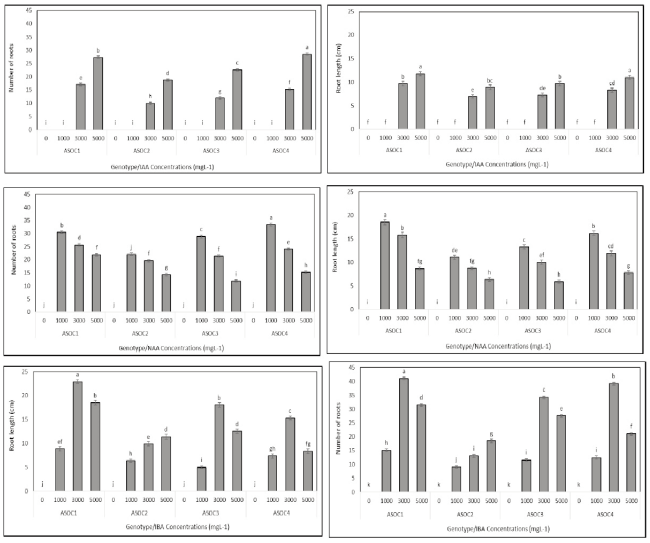

Fig. 4 - Effects of auxin type, concentration and genotype on growing roots (Mean values of roots number and mean longest root length) of *A. spinosa* cuttings. Within each treatment, the mean values followed by the same letter are not significantly different (Duncan's Multiple Range Test, P<0.05, mean ± SE, n = 32).

*Effect of auxin type, concentration and genotype on sprouting, rooting and cuttings survival rates*

Sprouting, rooting and survival ratios of *A. spinosa* cuttings were significantly influenced (P<0.05) by the genotype from which cuttings were collected, as well as by the type and amount of auxin applied (Table 1). Most cuttings that rooted in the non-mist greenhouse survived and ultimately produced sprouts as a sign of their successful regeneration. The results indicate that an application of IBA, NAA and IAA increased sprouting, rooting and survival rates, and IBA was the most effective auxin. When treated with concentrations >3000 $mgL^{-1}$ sprouting, rooting and survival rates began to decrease rapidly in all cuttings treated with NAA and IBA (Fig. 5). The results indicate that cuttings collected from ASOC1 and treated with 3000 $mgL^{-1}$ IBA had the highest sprouting percentage (81.75%), rooting percentage (60.75%) and survival percentage (96.25 %) per rooted cutting, followed by ASOC4 which had a sprouting ratio of 74%, a rooting ratio of 55.5% and a survival ratio of 94.5%. Among the different concentrations of NAA used, the maximum sprouting percentage (71.5%), rooting percentage (46.5%) and survival percentage (94.5%), were obtained in ASOC1 cuttings treated with 1000 $mgL^{-1}$. Besides, compared with the control, all IAA treatments enhanced the rooting rate of *A. spinosa* cuttings but were less effective than IBA and NAA. Indeed, the highest sprouting ratio (63.25%), rooting ratio (27.75%) and survival ratio (94.0%) with IAA treatment was obtained with ASOC1 by using 5000 $mgL^{-1}$ IAA.

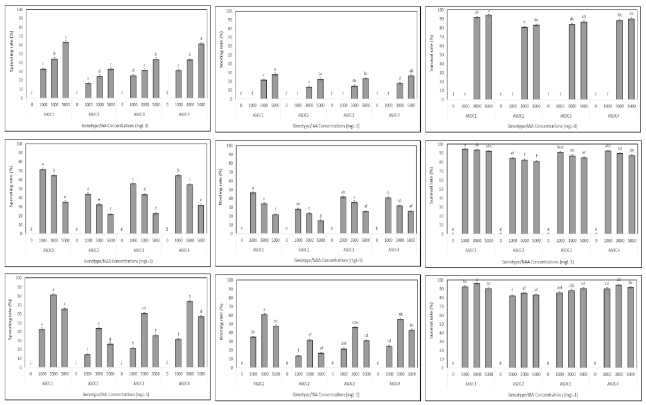

Fig. 5 - Effects of auxin type, concentration and genotype on sprouting, rooting and survival rate of *A. spinosa* cuttings. Within each treatment, values marked by the same letter are not significantly different (Duncan's Multiple Range Test, P<0.05, mean ± SE, n = 32). Mean values of sprouting percentage, rooting percentage, and survival rate were subjected to arcsine √X transformation before statistical analysis.

## 4. Discussion and Conclusions

The results of the present study indicate that there is a large amount of variability between the genotypes in their sprouting, rooting and survival measurements. These results fit well with those obtained by Nouaim *et al*. (2002), which reported that the rooting capacity of *Argania spinosa* cuttings has proven to be difficult and strongly genotype dependent. The significant variation among genotypes could be due to considerable genetic variation (Prat *et al*., 1998). It could be possible that some of the variation for root response results from the differences in age or in physiological states of initial cutting sources. It has also been reported that cuttings time of collection in different seasons could also

affect the rooting potential in clonal propagation. In fact, the varied effectiveness of auxin concentrations among genotypes and species is likely to be related with differences in the amount of endogenous hormone and associated to root co-promoters in the plant tissues at the time of severance (Hartmann *et al*., 2002). Otherwise, the decreasing in rooting ability could be assigned to low sensitivity of the tissues to auxin or by secondary metabolites accumulation, causing oxidation and inactivation of enzymes and phytohormones which inhibit regenerative potentialities of tissues (Wilson, 1994; Husen and Pal, 2007). Similar observations have been reported by Mabizela *et al*. (2017), who concluded that four studied genotypes of honey bush (*Cyclopia subternata*) had significant differences in rooting potential. Considering the above results, sprouting, rooting and survival ability of *Argania spinosa* cuttings were significantly higher (P> 0.05) in cuttings treated with exogenous auxins. The fact that exogenous auxin treatment exhibited a greater potential for adventitious rooting as well as a rapid growth of cuttings is widely recognized on different plant species (Hunt *et al*., 2011; Gehlot *et al*., 2014). The effect of auxin on adventitious roots growth and development can be explained by its role in wound healing through the inhibition of IAA-oxidase activity and the activation of the enzymatic antioxidant defense system, which helps to restore the redox balance and protect tissues from oxidative damage, particularly during the different steps of adventitious root development (Rout, 2006). While in contact with the basal cell, the auxin pool may have an indirect influence by promoting activity of starch hydrolytic enzymes and enhancing the translocation speed of carbohydrates to the cuttings base (Haissig, 1974; Aminah *et al*., 1995). Consequently, it supplies the cutting with the required energy for hastening cell differentiation of root primordia, growth and development via cell divisions and elongation (Husen, 2008). Auxin could also act through selective proteolysis and cell-wall loosening (Schopfer, 2001). Hence, it regulates the organ growth and development, promotes emergence of shoot buds, sprout length and consequently resulting in a better overall growth of the cuttings (Schroeder and Walker, 1990). In the present study, cuttings treated with distilled water without growth regulators did not show any response on rooting and sprouting. Thus, it has been proved that the IBA application is more effective than NAA and IAA. In most cases, 3000 $mgL^{-1}$ of IBA was the most effective concentration for promoting sprouting, rooting and survival rates of all studied genotypes. Higher efficiency of IBA at inducing adventitious rooting may be explained by higher chemical stability against catabolism and inactivation by conjugation, nontoxic over a wide concentration range, low mobility and available over a longer period of time in the plant tissue (Barrel *et al*., 2001; Ludwig-Muller, 2000; Hartmann *et al*., 2002). Whereas, the higher concentration of IAA stimulates ethylene production in plant cells, which is known to inhibit root induction and elongation (Mulkey *et al*., 1982). Similar to our results, Kesari *et al*. (2009) found a relatively poor rooting yield with IAA treated stem cuttings of *Pongamia pinnata* in comparison to IBA. The result shows that IBA had a stronger effect on sprouting and rooting than NAA, the reason may be that NAA is very stable and more persistent than other auxins and remains present in the tissue in its free form (Dunlap *et al*., 1986).

Thus, NAA decreases the level of nutrients mobilization and translocation to the root primordia and blocks the roots outgrowth (Husen and Pal, 2006). For most genotypes, auxin responses are concentration dependent and tissues react in a distinct manner to varying amounts of exogenous auxins. Thereby, adventitious root production decreased at very low concentrations of IBA (<1000 $mgL^{-1}$) and increased to levels between 1000 and 3000 $mgL^{-1}$. However, with the increase in hormone concentration levels (>3000 $mgL^{-1}$), the number and length of roots decreased. In accord to our results, Akakpo *et al*. (2014) showed a decline in rooting rates of *Vitellaria paradoxa* stem cuttings for IBA concentrations exceeding 3000 $mgL^{-1}$. This result confirms the findings of Hartmann *et al*. (2002), who demonstrated that too little IBA can decrease rooting and concentrations substantially higher than those normally found in plant tissues may be inhibitory, phytotoxic or even cause cell death. The results of our study revealed that IBA concentrations and genotype strongly influence the sprouting and rooting ability of *A. spinosa*, which confirms previous studies indicating that the optimal IBA concentrations for suitable adventitious root responses vary according to different species. Indeed, Singh and Rawat (2017) achieved a maximum increase in sprouting and rooting on semi-hardwood cuttings of *Zanthoxylum armatum* treated with 3000 $mgL^{-1}$ IBA. Besides, Tsipouridis *et al*. (2003) observed a maximum rooting rate in *Prunus persica* treated with 2000 $mgL^{-1}$ IBA. Husen (2008) reported that cuttings of *Dalbergia sissoo* treated with 2000 $mgL^{-1}$ IBA induced a stronger rooting system. Also, in *Aesculus indica*, the highest rooting rate was recorded in stem

cuttings treated with 4000 $mgL^{-1}$ IBA (Majeed *et al*., 2009). The maximum rooting rate for *Tectona grandis* was obtained with 4000 $mgL^{-1}$ IBA as compared to the other treatments (Husen and Pal, 2007). However, in *Pongamia pinnata*, IBA at 1000 $mgL^{-1}$ was found to be the most effective concentration for rooting ratio and root number (Kesari *et al*., 2009).

The results of this study revealed that adventitious root development and shoot growth of *Argania spinosa* semi-hardwood cuttings were significantly influenced by auxin type, concentration, genotype and their interactions. There was a variation among the different genotypes studied, indeed cuttings taken from ASOC1 and ASOC4 were rooted and sprouted better than those from ASOC2 and ASOC3. It was observed that almost all the treatments, except the control set, were able to induce sprouting and rooting in cuttings, and the IBA application is more effective than NAA and IAA. IBA application at a concentration of 3000 $mgL^{-1}$ to ASOC1 cuttings seemed to be the best treatment in terms of sprouting, rooting and survival rates, and also for the number of leaves, leaf size, number of sprouts, sprout length, number of roots, and root length per cuttings. The present study indicated that *A. spinosa* can be propagated through semi-hardwood cuttings of élite trees and can be usefully applied to promote the role of vegetative propagation of this species through forest restoration and for breeding programs. Thus, further experiments should be conducted to extend the techniques to induce rooting of cuttings from selected élite genotypes used as a source material for argan tree orchards.

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