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Research Article

Selection and Multiplication of Argan (*Argania spinosa* L.) Superior Clones for Conservation Purposes

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Abstract

An effective rejuvenation approach to improve *Argania spinosa* L. rhizogenesis was developed through the clonal propagation from selected field-grown trees. After transitional cutting, shoots, from the forced greenhouse trees, were transferred on Murashige and Skoog medium with various hormonal combinations. The maximum frequency of *in vitro* shoot development (93%) was induced on MS medium containing 2.5 mg/L BA combined with 1 mg/L indole-3-acetic acid (IAA). Repeated sub-culturing of the nodal segments was also undertaken on medium supplemented with lower BA concentrations (1.5 mg/L). The ability to induce in vitro rooting of *A. spinosa*, was achieved on MS basal medium containing 5 mg/L Indole-3-butyric acid (IBA) and 1mg/L α-naphthaleneacetic acid (NAA). The well-rooted shoots were successfully acclimatized and transferred to field conditions with 100% survival rates. The work described here is the first successful attempt toward generating true to type copies of superior argan forest trees to facilitate there in situ conservation and re-introduction.

Keywords: Conservation; Cutting; Argan Tree; Shoot Proliferation; Rejuvenation; Rhizogenesis

Introduction

Argania spinosa L. (family-Sapotaceae) is an endemic tree of Morocco where it covers more than 800 000 Km [1] alleviating significantly the rural poverty [2]. The demand for this multipurpose tree is increasing due to its ecological interest, socio-economic value [3] and more particularly to its fruit that gives a much-appreciated oil with relatively high and increasing market prices. Moreover, due to its tolerance to drought [4], argan tree is currently considered the best approach toward an environmentally friendly agriculture. It is a sustainable and highly recommended species to replace the crops used in the traditional system susceptible to biodiversity degradation and climate variations [5].

However, despite the oil growing need, argan native populations have been reported to decline by the half over the past years, due to human activity and ecological imbalances [2,6,7] with more conceivable contractions under future climate change scenarios [8]. To alleviate the pressure exerted on the natural forest, it becomes a necessity to conserve and to successfully set-up and manage orchards geared to argan-oil production in particular under arid and semi-arid conditions.

Currently, a higher percentage of argan trees regenerated in Morocco by seed [9]. Propagation by seed can generate genetic diversity [10], but the seedling-derived progenies of outcrossed heterozygous individuals lead to heterogeneous material. The

seed based orchards are subjected to severe impairment in oil production, which doesn't meet farmer's expectation [11]. The lack of standard varieties and the extension of the period required to achieve the reproductive stage are also major factors that restrict the use of seedlings for argan oil production [10].

The possibility to produce genetically identical argan plants and capture interesting traits from selected superior genotypes become a necessity for the successful conservation and to switch from an exclusively wild plantation to an oil crop. However, when trees age, this approach is confronted by the difficult rooting [11]. The biotechnological approach represents a valuable tool to meet the increasing requirements for the in situ reproduction and conservation of the agroforestry systems in degradation [11,12]. Our approach is based on the use of the existing diversity to build superior orchards with a selected clonal mix with similar phenotype but different genotypes, to respond to various environmental changes and ensure sustainability.

The clonal propagation, via meristem culture of selected field grown argan trees, have been challenging to establish with success due to many problems, especially the age related maturation problems and the not-operational or under functioning roots under *ex vitro* conditions [10,13].

The aim of the study is to get an efficient procedure that make possible the production of homogeneous material, ensuring the capture of the forest elite genetic diversity, to set up orchards with elite argan genotypes for high yield oil production and in fine to develop an agroforestry system based on argan tree.

Materials and Methods

Explants preparation and surface sterilization

Superior trees of at least 100-year-old were selected in the Agadir argan forest based on high oil yield. The trees were clonally propagated in the nursery through cutting for their subsequent use as mother trees. This work has been conducted by the Regional Center for Water and Forests of Marrakech [14,15].

Shoots of about 10 to 20 cm length with dormant axillary buds were collected from the well-established clones of 6 to 7-year-old. To remove the genotype effect on multiplication, the shoots were collected from more than 30 genotypes and mixed in the different experiments. The shoots were washed with tap water supplemented by few drops of commercial detergent and treated by 4% (w/v) of the fungicide methyl-thiophanate (Pelt 44) for about 10 min.

The surface sterilization was followed under aseptic conditions. The explants were then surface sterilized by 20 min soaking in 3% (v/v) Sodium hypochlorite containing few drops of Tween-20 followed by several washes with sterile distilled water. 1-2 cm of nodal segments with one to two axillar buds were then excised and used as explants.

Culture establishment

The surface sterilized explants were cultured on shoot-inducing medium, which consists of Murashige and Skoog (MS) basal salts and vitamins (0.1%) mixture [16]. The latter was supplemented with plant growth regulators (PGRs), 100 mg/1 myo-inositol and 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 and solidified with 0.8% (w/v) agar prior to sterilization at 121°C for 20 minutes.

This first experiment aimed to adjust hormonal requirements for bud sprouting. We have evaluated separately the influence of three cytokinins, N6-benzyladenine (BA), Kinetin (Kn) and 2-isopentenyladenine (2-iP) on bud formation using the concentrations of 0 (control), 1, 2, 2.5 and 3 mg/L.

Subsequently, a combined BA effect, the most performing cytokinin with low concentrations of different auxins, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA), at 0, 0.25, 0.5, 1 mg/L, was also studied in order to determine the required auxin/cytokinin ratio for the maximum shoot development.

All cultures were maintained in a growth chamber under 16 h photoperiod and cool-white light (36 µmol.m-2.s^{-1}) at a temperature of 25 ± 2°C. Cultures were maintained by a constant subculturing in three-week intervals to the same fresh medium. The percentage of explants producing shoots and shoot length were recorded after four weeks.

Shoot multiplication

The optimized combinations for bud breaking were investigated for multiplication. MS medium of the same composition was used for the multiplication of further shoot buds. Furthermore, lower concentrations of BA (1, 1.5 and 2 mg/L) were also tested for shoot multiplication.

The primary proliferated shoots (> 3 cm) were isolated from the original explants after axillary shoot proliferation and dissected into uninodal microcuttings (> 1cm) containing 1 to 2 axillary

buds without leaves. Subsequent subcultures were carried out at four-week intervals. The new developed shoots (higher than 2 cm) were either separated and transferred to the rooting medium or sub-cultured onto a fresh multiplication medium for further multiplication.

Rooting of regenerated shoots

To induce roots, the *in vitro* produced shoots (2 to 4 cm in length) were excised. All the genotypes regenerated were used in order to eliminate any effect of clonal differences. The shoots were aseptically cut at the base and individually transferred to root-inducing media consisting of one-quarter MS basal salts medium, fortified with different types and concentrations of auxins IAA, IBA and NAA (0, 1 and 5 mg/L) alone or in combinations. Cultures were maintained for one week in darkness and subsequently moved to light conditions with a 16 h photoperiod. The number of rooted explants was recorded after two months.

After the root induction, the shoots with roots were transferred to a growth-regulator-free and half strength MS basal salts medium to induce the root elongation. The plantlets were transferred to a solidified MS medium fractured with a sterilized scalpel to improve the aeration of the solidified media and simulate soil texture.

Acclimatization

The plantlets with well-developed roots were washed to remove adhered agar, treated with methyl-thiophanate (Pelt 44) and transferred to sterilized jars containing peat and sand (1:1). Jars were covered with polyethylene bags and maintained inside a growth chamber under normal conditions (25 ± 2°C with a 16 h photoperiod). The plantlets were irrigated every 4 days with a half-strength MS basal salt solution devoid of sucrose and vitamins. The relative humidity was gradually decreased (how?) and the plantlets were finally transferred to the glasshouse. The survival of acclimatized plants was recorded.

Statistical analysis

Data were analyzed by the analysis of variance ANOVA in SPSS software (Version 10.0; Norusis 1986). Treatment means were separated using Tukey's Post hoc test at $P \leq 0.05$ to compare significant differences. All experiments were repeated at least three times.

Results

Influence of cytokinins

The results show that shoot induction was achieved with all cytokinin types regardless of the genotype. However, it was well elicited from the explants cultured on MS medium supplemented with BA at the optimal concentration of 2.5 mg/L without vitrification (Table 1) and without significant shoot elongation. The MS medium containing 2.5 mg/L BA induced bud break in 70% of nodes. In addition, 2-iP showed the poorest shoot production rate (39%) and was the least effective cytokinin followed by Kn.

PGR (mg/L)				
BA	**Kn**	**2-iP**	**Shoot number/ explant (%)**	**Shoot length (cm)**
-	-	-	30.55 ± 0.27^{e}	2.10 ± 0.08^{a}
1	-	-	65.17 ± 0.29^{abc}	2.40 ± 0.04^{a}
2	-	-	66.67 ± 0.23^{ab}	2.56 ± 0.08^{a}
2.5	-	-	70.30 ± 0.11^{a}	2.61 ± 0.05^{a}
3	-	-	64.96 ± 0.46^{abc}	2.38 ± 0.07^{a}
-	1	-	47.22 ± 0.35^{cde}	2.27 ± 0.05^{a}
-	2	-	54.27 ± 0.20^{abcd}	2.33 ± 0.04^{a}
-	2.5	-	54.59 ± 0.23^{abcd}	2.39 ± 0.06^{a}
-	3	-	54.27 ± 0.29^{abcd}	2.38 ± 0.03^{a}
-	-	1	38.89 ± 0.35^{de}	2.17 ± 0.06^{a}
-	-	2	46.15 ± 0.29^{de}	2.12 ± 0.09^{a}
-	-	2.5	47.22 ± 0.13c^{de}	2.14 ± 0.08^{a}
-	-	3	50.00 ± 0.46^{bc}d	2.28 ± 0.06^{a}

Table 1: Effect of different concentrations of diverse cytokinins on shoot induction and elongation from nodal bud explants of *A. spinosa* after four weeks of culture.

Each experiment consisted of at least 13 explants and was repeated thrice. Values represent means ± SE of three replicates. Data were recorded at the end of the fourth week. Values in each column with the same lower-case letters are not significantly different according to the one-way ANOVA, Least Significant Difference multiple range test at $P \leq 0.05$.

Effect of cytokinin-auxin combination

In this study, it has been shown that an optimized quantity of cytokinin is required for shoot proliferation. However, the addition of low auxin concentrations has improved significantly the shoot formation from nodal explants. Our results show also that the shoots, developed after auxin application, were significantly longer relatively to those grown on auxin-free medium. The combination of 2.5 mg/L BA and 1 mg/L IAA has induced the formation of additional shoots (88%) (Table 2).

Multiplication of shoots

Cytokinin application was tested starting from the third subculture. Among the various concentrations of BA tested, 1.5 mg/L

PGR (mg/L)				
NAA	IBA	IAA	Shoot number/ explant (%)	Shoot length (cm)
-	-	-	70.30 ± 0.11 ^{bc}	2.61 ± 0.05 ^{a}
0.25	-	-	71.68 ± 0.11 ^{bc}	3.25 ± 0.08 ^{b}
0.5	-	-	72.55 ± 0.18 ^{bc}	3.22 ± 0.08 ^{b}
1	-	-	77.01 ± 0.10 ^{b}	3.25 ± 0.08 ^{b}
-	0.25	-	65.47 ± 0.18 ^{c}	3.17 ± 0.08 ^{b}
-	0.5	-	67.43 ± 0.10 ^{bc}	3.40 ± 0.05 ^{b}
-	1	-	78.43 ± 0.07 ^{b}	3.30 ± 0.07 ^{b}
-	-	0.25	71.46 ± 0.27 ^{bc}	3.41 ± 0.06 ^{b}
-	-	0.5	82.35 ± 0.20 ^{ab}	3.40 ± 0.09 ^{b}
-	-	1	88.23 ± 0.11 ^{a}	3.54 ± 0.08 ^{b}

Table 2: Effect of various concentrations of auxins in MS medium supplemented by 2.5 mg/L BA on shoot regeneration and elongation through nodal explants of *A. spinosa* after four weeks of culture.

Each experiment consisted of at least 16 explants and was repeated thrice. Values represent means ± SE of three replicates. Data were recorded at the end of the fourth week. Values in each column with the same lower-case letters are not significantly different according to the one way ANOVA, Least Significant Difference multiple range test at P ≤ 0.05.

was selected for shoot multiplication (Table 3). The decrease in BA concentration from high amounts, for establishment, to a reduced amount, for multiplication, has improved shoot proliferation.

BA (mg/L)	Shoot multiplication (%)	Shoot length (cm)
2.5	57.94 ± 2.79 ^{b}	1.44 ± 0.30 ^{b}
2.0	70.17 ± 3.50 ^{b}	1.62 ± 0.46 ^{b}
1.5	86.98 ± 2.57 ^{a}	2.20 ± 0.50 ^{a}
1.0	73.65 ± 2.69 ^{ab}	2.07 ± 0.58 ^{a}

Table 3: Influence of BA on the *in vitro* shoot multiplication of *A. spinosa*.

Each experiment consisted of at least 20 replicates and repeated three times. Values represent means ± SE of three replicates. Data were recorded at the end of the fourth week. Values in each column with the same lower-case letters are not significantly different according to the one-way ANOVA, Least Significant Difference multiple range test at P ≤ 0.05.

Rooting and acclimatization

The effect of different auxins on the rooting process of the *in vitro* regenerated shoots of *A. spinosa* was tested (Table 4). The results show that the shoots cultivated on MS auxin-free medium did not induce roots. Among the auxins tested, IAA failed in inducing roots. The best results were observed with IBA combined with NAA. Shoots cultured on root induction medium containing 5 mg/L IBA and 1 mg/L NAA provided the best result with 55% rooting rate (two to three roots/shoot). The well-rooted shoots were transferred to culture bottles containing sterilized peat and sand (1:1) fortified with full strength MS nutrients and maintained in culture room for 3 weeks with relative humidity gradual decrease.

IBA (mg/L)	NAA (mg/L)	IAA (mg/L)	Rooting (%)
-	-	-	-
5	-	-	41.82 ± 0.94b
-	5	-	37.27 ± 2.31^{bcd}
1	5	-	39.39 ± 1.56^{bc}
5	1	-	54.85 ± 1.49^{a}
5	5	-	46.66 ± 1.72^{ab}
-	-	5	-
1	0	5	18.18 ± 2.7^{e}
5	-	1	27.27 ± 2.71^{de}
5	-	5	30.30 ± 1.56^{cd}

Table 4. Effect of auxins on the rooting of the *in vitro* induced shoots of *A. spinosa* after two months of culture.

Each experiment consisted of at least 30 explants and repeated thrice. Values represent means ± SE of three replicates. Data were recorded at the end of the fourth week. Values in each column with the same lower-case letters are not significantly different according to the one-way ANOVA, Least Significant Difference multiple range test at P ≤ 0.05.

Discussion

The major problem faced when culturing tissues derived from field grown trees are contaminations, recalcitrance and medium and/or explants browning due to phenolic discharges [17]. In our experiment, the contaminations were the major obstacle for the establishment of the aseptic cultures derived from the glasshouse-forced cuttings. Among the different sterilizing agent tested, sodium hypochlorite was acceptably efficient preventing tissues death and browning relatively to the other agents.

In *in vitro* conditions, the nodes are reported to be responsive independently of the season of culture [18]. In this experiment, the period of culture lasted from 2010 to September 2014, with acceptable rates. Argan shoot proliferation was possible year-round, with the maximum effectiveness achieved between March and May similarly to other plant systems [19,20].

To optimize explants response, different morphogenic processes were evaluated. It was clear that shoot development and multiplication were directly correlated ($P < 0.05$) to the PGR's type and concentration. Bud break and shoot proliferation is a cytokinin function (Table 1) similarly to other woody plants [21], pointing to the important BA stimulating effect over the other tested cytokinins. BA at 2.5 mg/L was used as optimal concentration for further experiments. The majority of researches have also reported BA effectiveness for shoot induction [22-24]. These results may be attributed to the ability of tissues to metabolize BA more than other PGR's and its ability to induce natural hormones production such as zeatin [25]. 2-iP was found to be ineffective followed by Kn at the contrarily of many other woody plants [26].

The development of multiple shoots was not achieved by the single cytokinin addition from each axillar node. Auxin addition promoted additional shoot growth counteracting the BA inhibitory effect on shoot elongation. The combination of cytokinin and auxin stimulated additional shoot multiplication. Several studies reported so far did also show the cytokinin-auxin synergetic effect on the proliferation rates [27]. In our study, shoots were induced preferably combining 2.5 mg/L BA and 1 mg/L IAA (88%) (Table 2). The synergetic effect of IAA, in particular, was previously confirmed [27-29]. In other studies, a single BA addition was suitable for shoot induction from meristem cultures [19,30]. In this study, the auxin addition did not allow the multiple shoot development.

After successive subculturing, the shoots manifested shoot-tip necrosis and leaf yellowing as it was reported with many other woody species [31]. $AgNO_3$ was used positively to enhance culture's quality (Data not shown). The cultures remained green and healthy exhibiting also an indirect effect on the quantity of the regenerated shoots as it was previously reported [32]. The development of new axillar shoots after two weeks of apical bud excision was observed in many other plants as a consequence of the apical dominance suppression and the induction of other meristematic dominances [33]. In this study, it was also observed that the apical bud decapitation incites the formation of a basal crop of shoots (Figure 1). When the newly formed shoots are cut into segments, they gave rise to new axillary shoots. This multiplication process was successfully repeated every 4 weeks intervals. However, after the second subculture, a gradual decline was observed with the development of dwarf shoots. This could be explained by the extended cytokinin exposure, which led to an endogenous accumulation inhibiting the shoot growth [25,27]. Thus, BA requirements for shoot multiplication were decreased compared to those required for shoot initiation. 1.5 mg/L BA was preferably used for shoot multiplication. Low cytokinin amounts promoted shoot proliferation for other plants [24]. With the BA restriction, it was possible to develop additional vigorous shoots. This shoot multiplication process was maintained more than 1 year without vigor loss.

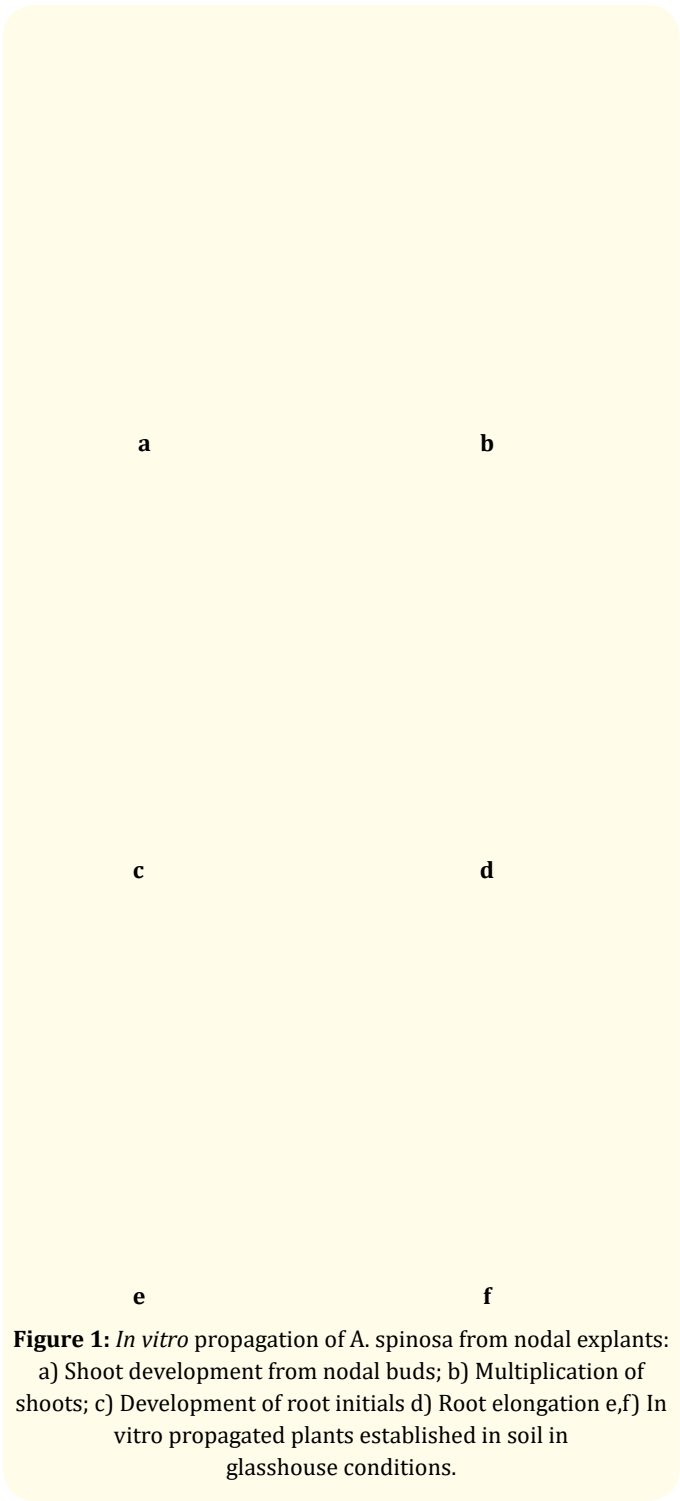

Figure 1: *In vitro* propagation of A. spinosa from nodal explants: a) Shoot development from nodal buds; b) Multiplication of shoots; c) Development of root initials d) Root elongation e,f) In vitro propagated plants established in soil in glasshouse conditions.

In this study, we were able to develop shoots year-round. This reveals an important shoot development relatively to the previously reported argan trials conducted on mature trees [10,13]. This result could be linked to the rejuvenised tissues used in this study. Rejuvenation process through traditional vegetative propagation is well known to facilitate the *in vitro* propagation of mature woody trees [34].

The auxin nature and concentration are specific to each species. In our study, *in vitro* root induction was possible only in an auxin-rich medium. Other results indicate that IBA is the most adequate for root induction [35,36]. It is considered as the most effective auxin involved in rooting process [37].

In woody trees, low mineral salt concentrations are better for rooting [38]. In our experiment, shoots were cultured on various strengths, full-, half- and quarter-strength MS medium in order to improve the overall root growth and reduce the time requested for root induction. The obtained results revealed that the one-fourth strength improves root induction (data not shown). However, in this experiment, the rooting process was relatively slow. This process begins with two to three root emergences with a linear constant growth without manifestation of a limited vegetative growth due to the poor mineral composition of the medium (Figure 1).

Development of well-formed and functional root system is one of the essential steps for water and mineral uptake and for successful *ex vitro* transfer [39]. Thus, root ramification is a critical step to overcome the poor rooting system development. In this study, we used conditions to facilitate the contact between roots, air and the medium substrates. This system was indeed successful for improving roots ramification.

However, despite the shoot regeneration efficiency exhibited, the rooting rates achieved were still not sufficient. Flowering was observed among the collection of mother tree used, which indicates the beginning of the mother trees maturity stage.

Furthermore, the acclimatization of the micro-propagated plantlets is a particular challenge. According to this study, *A. spinosa* microcutting using nodal explants requires 5 to 6 months to produce plantlets ready for field transfer. Acclimatization of the micro-propagated plants was accomplished in glasshouse. The plantlets with a well-developed root system of 5 to 6 months showed good survival percentage (100%) with lack of vegetative growth.

Conclusion

We investigated the possibility of selected argan tree's clonal propagation. The results confirmed the practical applicability of the procedure despite the tree reputation for its recalcitrance to micro-culture. To our knowledge, this is the first time that roots were induced and elongated *in vitro* from axillary nodal explants of selected superior genotypes. From a young material collected between March and May, it was possible to obtain good rates in term of establishment, multiplication, rooting and transplantation. The strength of the protocol described here is in the ability to clonally propagate and guarantee the production of true-to-type offspring and elite plantations for both agriculture and forestation programs from very old trees due to a transitional rejuvenation step of horticultural cuttings. The procedure was adapted efficiently to heterogeneous material, eliminating the genotype effect on the variability in plant reactivity, insuring the robustness and reproducibility of the procedure. A domestication program of the *A. spinosa* has been considered based on those results, repeated cuttings and grafting are considered to get the starting stock of material from superior trees. With the high commercial value of argan oil, the cost-effectiveness of the micro-propagation is justified.

We expect that our results will contribute to the development of plantations of argan trees that would lead to improving revenue of small farmers, addressing climate change by using a more drought tolerant crop than those currently used in semi-arid regions and improving soil conditions by increasing organic matter.

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Bibliography

1. Mateille T., *et al*. "Aridity or low temperatures: What affects the diversity of plant-parasitic nematode communities in the Moroccan argan relic forest?". *Applied Soil Ecology* 101 (2016): 64-71.

2. Lybbert TJ., *et al*. "Booming markets for Moroccan argan oil appear to benefit some rural households while threatening the endemic argan forest". *Proceedings of the National Academy of Sciences* 108 (2011): 13963-13968.

3. Le Polain Y., *et al*. "Dégradation environnementale et développement économique dans l'arganeraie d'Aoulouz (Maroc)". *Sécheresse* 24 (2013): 29-38.

4. Chakhchar A., *et al*. "Physiological and Biochemical Mechanisms of Drought Stress Tolerance in the Argan Tree". In Plant Metabolites and Regulation Under Environmental Stress, (2018): 311-322.

5. Mizrahi Y., *et al*. "New fruits for arid climates. Trends in new crops and new uses". *ASHS Press* (2002).

6. Charrouf Z and Guillaume D. "Argan oil: Occurrence, composition and impact on human health". *European Journal of Lipid Science Technology* 110.7 (2008): 632-636.

7. Defaa C. "Analyse de l'itinéraire technique d'un périmètre réussi de régénération d'arganier". In Actes du Premier Congrès International de l'Arganier Agadir (2013).

8. Alba Sánchez F., *et al*. "Long term climate forcings to assess vulnerability in North Africa dry argan woodlands". *Applied Vegetation Science Journal* 18.2 (2015): 283-296.

9. Ferradous A and Hafidi M. "Production de plants d'arganier (Argania spinosa) au Maroc: choix du conteneur et du substrat". *Bois et Forêts Des Tropiques Journal* 334.4 (2018): 37-47.

10. Nouaim R., *et al*. "The argan tree (Argania spinosa) in Morocco: Propagation by seeds, cuttings and in-vitro techniques". *Agricultural Systems* 54.1 (2002): 71-81.

11. Lindgren D. "Advantages of clonal propagation". Draft for proceedings from a meeting at Ronne by Department of Forest Genetics and Plant Physiology, Sweden (2001).

12. Justamante MS., *et al*. "Vegetative propagation of argan tree (Argania spinosa (L.) Skeels) using *in vitro* germinated seeds and stem cuttings". *Scientia Horticulture* 225 (2017): 81-87.

13. Bousselmame F., *et al*. "Optimisation des conditions de culture pour l'enracinement *in vitro* de l'arganier (Argania spinosa L.)". Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie 324.11 (2001): 995-1000.

14. Bellefontaine R., *et al*. "Multiplication végétative de l'arganier, Argania spinosa, au Maroc: le projet John Goelet". Bois et *Forêts Tropiques Journal* 304.304 (2010): 47-59.

15. Ferradous A., *et al*. "Optimisation des techniques de bouturage sous mist chez l'arganier". In Actes du Premier Congrès International de l'Arganier, Agadir (2011).

16. Murashige T and Skoog F. "A medium for rapid growth and bioassay with tobacco tissue culture". *Physiologia Plantarum* 15.3 (1962): 473-497.

17. Hiti-Bandaralage JC., *et al*. "Micropropagation of Avocado (Persea americana Mill.) ". *American Journal of Plant Science*s 8.11 (2017): 2898-2921.

18. Shahzad A., *et al*. "Historical Perspective and Basic Principles of Plant Tissue Culture". In Plant Biotechnology: Principles and Applications (2017) Edited by M. Z. Abdin and U. Kiran, M. Kamaluddin, Ali A. Springer, Singapore.

19. Phulwaria M., *et al*. "Micropropagation of Salvadora persica - a tree of arid horticulture and forestry". New Forests 42.3 (2011): 317-327.

20. Cardoso JC and Teixeira da Silva JA. "Gerbera micropropagation". *Biotechnology Advances* 31.8 (2013): 1344-1357.

21. Shyamkumar B., *et al*. "Multiple shoot induction from cotyledonary node explants of Terminalia chebula". *Biologia Plantarum* 47.4 (2003): 585-588.

22. Germana MA., *et al*. "Organogenesis and encapsulation of *in vitro*-derived propagules of Carrizo citrange [Citrus sinensis (L.) Osb. × Poncirius trifoliate (L.) Raf]". *Plant Cell Tissue Organ Culture* 106.2 (2011): 299-307.

23. Sivanandhan G., *et al*. "Effect of nitrogen and carbon sources on *in vitro* shoot multiplication, root induction and withanolides content in Withania somnifera (L.) Dunal". *Acta Physiologiae Plantarum* 37 (2015): 12.

24. Moharana A., *et al*. "High frequency shoot proliferation from cotyledonary node of Lawsonia inermis L. and validation of their molecular finger printing". *Journal of Crop Science and Biotechnology* 20.5 (2017): 405-416.

25. Malik SK., *et al*. "Rapid *in vitro* multiplication and conservation of Garcinia indica: A tropical medicinal tree species". *Scientia Horticulturae* 106.4 (2005): 539-553.

26. Mohammed A., *et al*. An efficient *in vitro* shoot regeneration from leaf petiolar explants and ex vitro rooting of Bixa orellana L.- A dye yielding plant". *Physiology and Molecular Biology of Plants* 21.3 (2015): 417-424.

27. Shekhawat MS., *et al*. "High Frequency Plantlet Regeneration from Nodal Segment Culture of Female Momordica dioica (Roxb.)". *Journal of Crop Science and Biotechnology* 14.2 (2011): 133-137.

28. Orlińska M and Nowaczyk P. "*In vitro* plant regeneration of 4 Capsicum spp. genotypes using different explant types". *Turkish Journal of Biology* 39.1 (2015): 60-68.

29. Haque SKM and Ghosh B. "An improved micropropagation protocol for the recalcitrant plant Capsicum – a study with ten cultivars of Capsicum spp. (C. annuum, C. chinense, and C. frutescens) collected from diverse geographical regions of India and Mexico". *Journal of Crop Science and Biotechnology* 93.1 (2017): 91-99.

30. Shahzad A., *et al*. "Development of a regeneration system via nodal segment culture in Veronicaanagallis - aquatica L. – an amphibious medicinal plant". *Journal of Plant Interactions* 6.1 (2011): 61-68.

31. Kermani SA., *et al*. "The evaluation of the effect of multiwall carbon nano tube (mwcnt) on *in vitro* proliferation and shoot tip necrosis of pistachio rootstock UCB-1 (Pistacia integrima × P. atlantica)". *Journal of Nuts* 8.1 (2017): 49-59.

32. Yildirim AB and Turker AU. "Effects of regeneration enhancers on micropropagation of Fragaria vesca L. and phenolic content comparison of field-grown and *in vitro*-grown plant materials by liquid chromatography–electrospray tandem mass spectrometry (LC–ESI–MS/MS)". *Scientia Horticulturae* 69 (2014): 169-178.

33. Karalija E., *et al*. "Media composition affects seed dormancy, apical dominance and phenolic profile of Knautia sarajevensis (Dipsacaceae), Bosnian endemic". *Acta Botanica Croatica* (2017).

34. Oliveira MLP., *et al*. "Growth regulators, culture media and antibiotics in the *in vitro* shoot regeneration from mature tissue of citrus cultivars". *Pesquisa Agropecuaria Brasileira* 45.7 (2010): 654-660.

35. Panwar D., *et al*. "An improvised shoot amplification and ex vitro rooting method for offsite propagation of Tinospora cordifolia (Willd.) Miers: a multi-valued medicinal climber". *Indian Journal Plant Physiology* (2018): 1-10.

36. da Silva JAT., *et al*. "Smoke tree (Cotinus coggygria Scop.) propagation and biotechnology". *South African Journal of Botany* 114 (2018): 232-240.

37. Thomas TD and Maseena EA. "Callus induction and plant regeneration in Cardiospermum halicacabum L. –a medicinally important plant". *Scientia Horticulturae* 108.3 (2006): 332-336.

38. Patil P., *et al*. "A revised protocol for *in vitro* propagation of Carica papaya using lateral buds from field-grown trees". *Journal of Horticultural Sciences* 2.2 (2016): 99-103.

39. Nowak J and Shulaev V. "Priming for transplant stress resistance in *in vitro* propagation". *In vitro* Cellular and Developmental Biology - Plant 39.2 (2003): 107-124.

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