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The argan tree (*Argania spinosa*) in Morocco: Propagation by seeds, cuttings and *in-vitro* techniques

R. Nouaim[1], G. Mangin[2], M. C. Breuil[3] and R. Chaussod[3,*]
[1] *Université Ibnou Zohr, Faculté des Sciences, B.P. 28/S Agadir (Maroc);* [2]*Agri Obtention UP-Vitro, BV 1540 – 21034 Dijon Cedex (France);* [3] *I.N.R.A., Microbiologie des Sols, B.V. 1540, 21034 Dijon Cedex (France) (*Author for correspondence: E-mail: Remi.Chaussod@dijon.inra.fr)*

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Abstract

In South-western Morocco, the argan tree (*Argania spinosa*) is basis of a traditional agroforestry system. However, this system is no longer at equilibrium and there is an urgent need to renovate it. The natural reproduction of the tree has become difficult, and most attempts of artificial regeneration have failed. We have assessed three different propagation methods: seedlings, cuttings, and *in-vitro* micro-propagation. Our experiments, involving several seed collections, showed that germination occurred easily when using young (< 12 months) and large seeds (nut weight > 3 g). Nuts with thin walls germinated better than nuts with thicker walls. Sterilization prevented microbial contamination and improved germination. Vegetative propagation of the argan tree by cuttings was tried using lignified cuttings collected from adult trees or young stems from managed stockplants (three years old). Rooting rate seems to be dependent on genotype, and the best results were obtained from young stems sterilized to avoid fungal contamination. The success of *in-vitro* micro-propagation is also highly genotype-dependant. We developed a modified medium enabling up to 80–95% rooting rate for some clones. However, other clones were still recalcitrant. Whatever the propagation method used, special attention must be paid to the architecture and growth of the root system.

Introduction

In south-western Morocco, traditional agroforestry systems based on the endemic argan tree (*Argania spinosa* L. Skeels, sapotaceae) are still surviving. In spite of its 'forest' status, the argan tree is indeed a multipurpose tree, mainly used for fodder and as an oil-yielding tree (Nouaïm et al., 1991). The fruit of the argan tree is (as for walnut tree or almond tree) a stone-fruit, with pulp covering a lignified endocarp (the nut) containing one to three kernels (the seeds) from which an highly appreciated edible oil is extracted by traditional, manual techniques. Moreover, crops such as barley are often cultivated in association with scattered argan trees, taking advantage of the higher fertility of the soil under the tree (Nouaïm, 1994). However, the sustainability of this agroforestry system is now threatened by overgrazing and over-exploitation in the mountains, and intensive agriculture in the plains. This results in serious degradation of the soils and a decrease in agricultural production, both directly (production of fodder and oil from the tree) and indirectly (crops and animal husbandry). This degradation leads to substantial rural depopulation. It is therefore urgent not only to preserve remaining argan trees, but also to rehabilitate the degraded areas.

Perfectly suited to its environment, this endemic tree can grow on poor, shallow soils, and owing to its deep rooting system, it is considered as having a strong effect against erosion and desertification, which are the main environmental problems in southern Morocco. The argan tree is monoecious and allogamous and exhibits high genetic diversity (Msanda, 1993). This diversity can both be preserved for ecological purposes and used through domestication.

Recent initiatives have promoted the domestication of multi-purpose agroforestry species for their ability to alleviate poverty and mitigate environmental degradation (Leakey and Simons, 1998). This approach is relevant to *A. spinosa* which could be domesticated for oil and fodder production. What is required is a package of technical procedures from the selection of the best genotypes to the production of cultivars in nurseries for successful integration into agroforestry systems.

Currently due to the failure of natural regeneration and reforestation, the only possibility of rejuvenating mature argan forest is through coppicing. Seedling production and use for plant production in nurseries could allow for the conservation of the genetic diversity. However, according to the literature, seed germination is difficult. Through selection and mass production of trees with desirable characteristics, biotechnology could improve argan tree productivity, as well as overall production (Sasson, 1993). Vegetative propagation offers the opportunity to multiply selected genotypes and to provide a significant step towards 'domestication'.

The purpose of the present study was:

- to examine the factors affecting variation in the germination of argan seeds,
- to check the feasibility of both vegetative propagation by cuttings and *in-vitro* propagation, the mass production of argan tree and the capture of genetic variation,
- to study the root growth of plantlets produced by the above-mentioned techniques, and to improve the success rates of nursery production and transplantation.

Material and methods

1. *Propagation by seeds*

The seeds used in this study came from ripe fruits collected from randomly chosen trees located in the Ademine forest. The fruits were dried and their pulp was removed by hand to obtain nuts. These nuts were classified according to weight. Just before germination, they were disinfected with hydrogen peroxide for 30 mn, rinsed with sterile water and stored in water in the dark for four days. Water was renewed daily.

Fresh nuts were compared with nuts that had been stored for two years in dark at room temperature. The seeds were sown in opened jars containing disinfected calcinated clay ('Terragreen'). For each weight class germination percentage and plantlet numbers were recorded.

During our studies, we noticed that people in the local communities were able to distinguish two different types of argan tree, according to the difficulty by which the nut was broken. We named the trees whose nut is easily broken 'f' type (f = facile) and trees whose nut is more difficult to break 'd' type (d = difficile).

To compare the germination response of nuts and seeds from the two above-mentioned types of trees ('f' and 'd'), germination tests were performed on the nuts as in the first experiment or on seeds extracted from the nuts. The seeds were sown in Petri dishes on sterile vermiculite. Dishes were watered as required for maintaining substrate moisture. The number of seeds germinating was recorded daily, the emergence of the radicle being used as germination criterion, and percentage germination was calculated.

2. *Propagation by cuttings*

Experiments were conducted on hardwood or softwood cuttings. Hardwood cuttings were collected from adult trees in two populations of occidental Anti-Atlas argan forest. Ten trees from each population and 20 cuttings from each tree were used. Softwood cuttings were taken from three year old stock plants grown in a greenhouse and obtained from seeds. Shoots were cut into cuttings 5–6 cm long, with 6–8 nodes. They were soaked 15 mn in a disinfectant of 1/10 diluted 'Domestos'

(10% sodium hypochlorite and 4% non-ionic surfactants and soap; Lever Brothers, Kingston upon Thames, Surrey, UK) and sterile distilled water. Then, they were thoroughly rinsed with sterile water. Leaves and thorns were eliminated from the bottom of the cuttings and the bases were soaked 5 mn in hormonal solution containing 0.5% indole-3-butyric acid (IBA).

Hardwood cuttings were placed for rooting in non-mist propagators containing a mixture of 50% calcinated clay (Terragreen) and 50% peat, disinfected by autoclaving and saturated with Long Ashton solution (Hewitt, 1966). Softwood cuttings (with leaves) were rooted on three different substrates: calcinated clay (Terragreen), vermiculite and an industrial siliceous substrate (named 'oil dri', US-special, Lobbe, Iserlohn, Germany). 50 cuttings were used for each substrate. Substrates were put in glass flasks plugged with cotton. The water content was close to water holding capacity and the substrate was disinfected for 20 mn at 120 °C. An additional treatment was carried out with cuttings from a selected tree, using Terragreen as the rooting substrate.

All treatments were made in axenic conditions. Experiments were performed under controlled conditions with day/night temperatures of 23/21 °C, a day length of 16 h and a photon flux density of 400 $\mu M.m^{-2}\ s^{-1}$. Throughout the experiments, water content was maintained close to the water holding capacity by axenic addition of sterile water.

3. In-vitro *microcuttings propagation*

Young non lignified shoots, 8–10 cm long, were collected from 28 *A. spinosa* mother plants, 1–3 years old, grown in glasshouse at INRA-Dijon (France).

3.1. *Disinfection*

Shoots were disinfected by immersion in disinfecting solution (as described for cuttings) for 15 mn and thoroughly rinsed with sterile distilled water. Then, they were cut into pieces of 2–3 nodes and all leaves were removed.

3.2. *Proliferation step*

10 pieces of 2–3 nodes were planted in 700 ml flasks (dia. 10 cm) containing 120 ml of agar (6 g/l) solidified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), with 0.7 g/l of indole-3-butyric acid (IBA) and with or without addition of 1 mg/l of vitamin E. Cultures were kept at 25 °C day and 21 °C night with 12 or 16 h photoperiod. After three weeks of growth, the microcuttings were cut into 2–3 nodal segments and transferred onto fresh medium. This step was repeated twice before transferring then onto rooting medium, when they reached 3–4 cm.

3.3. *Rooting step*

First experiment. After a proliferation period, micro-cuttings were transferred onto MS or Lepoivre (LP) media, containing only 0.7 mg/l IBA or with a mixture of 0.7 mg/l IBA, 0.1 mg/l of benzylaminopurine (BAP) or 13.4 mg/l of phloroglucinol or 1 ml of vitamin E solution. The micro-cuttings were then placed into a climatic chamber either directly or after being stored for seven days in the dark at 21 °C.

Second experiment. After the proliferation period, microcuttings of two clones, 1 and 17, were transferred on different rooting media (Table 1).

Agar-based media I, II, III, IV, V and VI were distributed into 24 × 160 mm tubes and sterilized for 20 mn at 115 °C. Terragreen-based media (T) were made of sterilized terragreen (20.5 g/tube) and nutrient solution (18.5 ml/tube). For II and T media, the hormonal and vitamin solution was sterilized by filtration and then added into sterilized tubes. One litre of this solution was prepared with 100 ml of Walkey vitamin, 50 ml of gibberilic acid, 70 ml of IAB (0.7 g/l), 10 ml of vitamin E (1 mg/l) and 1 g of inositol.

3.4. *Acclimatization step*

After the rooting period, plantlets were harvested from tubes, and put into pots containing Terragreen saturated with 'Long Ashton' nutrient solution. Pots were placed in a climatic chamber where the relative moisture was progressively reduced from saturation to 80% on a period of four weeks.

4. *Root growth measurements*

Seedlings or *in-vitro* produced plantlets (clones 1 and 17) were placed in minirhizotrons (Riedacker,

Table 1. Composition of rooting media for *in-vitro* propagation of *A. spinosa*.

Media	I	II	III	IV	V	VI	T
ML macroelements	50 ml	50 ml	50 ml	50 ml	–	–	50 ml
MA macroelements	–	–	–	–	50	50	–
MS microelements	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
FeEDTA	1 ml	1 ml	5 ml	1 ml	1 ml	1 ml	5 ml
Saccharose	30 g	30 g	30 g	30 g	30 g	30 g	–
Agar T	6 g	6 g	6 g	6 g	6 g	6 g	–
Walkey vitamin*	10 ml	–	10 ml	10 ml	10 ml	10 ml	–
Giberillic acid	5 ml	–	5 ml	5 ml	5 ml	5 ml	–
IBA	7 ml	–	7 ml	7 ml	7 ml	7 ml	–
Vitamin E	1 ml	–	1 ml	1 ml	1 ml	1 ml	–
Inositol	100 mg	–	100 mg	100 mg	100 mg	100 mg	–
Naphtalene-acetic-acid (NAA)	–	–	–	–	–	7 ml	–
Hormonal and Vitamin solution	–	390 µl/tube	–	–	–	–	390 µl/tube
Terragreen	–	–	–	–	–	–	20.5 g/tube
pH	6.2	6.2	5.8	6.7	6.7	6.7	6.7

* Walkey vitamin solution contains 0.08 g of thiamin, 0.1 g of nicotinic acid, 0.1 g of pyridoxine in distilled water q.s.p. 1 liter.

1974) tilted at 15° from vertical position and containing Terragreen saturated with 'Long Ashton' solution. The minirhizotrons were placed in a climatic chamber (23/21 °C, day length of 16 h, photon flux density of 400 $\mu M.m^{-2}$ s^{-1}, 80% relative humidity). Plantlets were watered daily with sterile distilled water and received 'Long Ashton' nutritive solution every two weeks. Root growth was recorded by marking their position on a transparent sheet every day. An analytical image system (Samba 7, TITN society, Grenoble) was used to measure taproot and total root system length. After two months, plantlets were harvested and the fresh and dry weight of shoots and roots measured.

5. *Statistical analysis*

Both parametric (*t* test) and non-parametric tests (Mann-Whitney-Wilcoxon test, based on sum of ranks) were performed on experimental data for comparison of treatments (Conover, 1980).

Results and discussion

1. *Seedling propagation*

Our measurements showed that the weight of the nuts differed more between trees than within a tree, with a range from 0.5 to 8.6 g, the majority being between 1.5 and 4.5 g. Weight distribution for six randomly chosen trees in the Ademine forest varied between 0.75 and 6.25 g, with a large part of the nuts between 1.5 and 3.0 g. Such a distribution was found for all studied trees in this forest. We also found similar distribution in different regions (unpublished). This distribution has been reported between populations, between plants within population and within individual plants in many other species. Sources of variations that may contribute to within-plant variation of individual nut mass include competition between developing ovules within fruits, time of season at which an ovule is fertilised, position on the shoot, water regime during fruit growth, the origin of fertilizing pollen, etc. The weight of the nuts (average or median) was not related to the 'f' or 'd' criterion.

Nuts sown a few weeks after harvest germinated quickly. Large nuts exhibited the greater germination percentage (> 80%). Seeds with weight greater than 3.5 g germinated one week after sowing and each seed gave two to three plantlets. For seeds harvested from the same trees and stored two years before sowing, the germination percentage was only 2%.

A comparison of 'f' and 'd' types of argan tree from Ademine forest showed that this criterion can affect germination. The germination rates of nuts varied from 33 to 70% for 'f' trees and from 0 to 13% for 'd' trees (Figure 1). Statistic tests showed that the germination percentage of nuts from 'f'

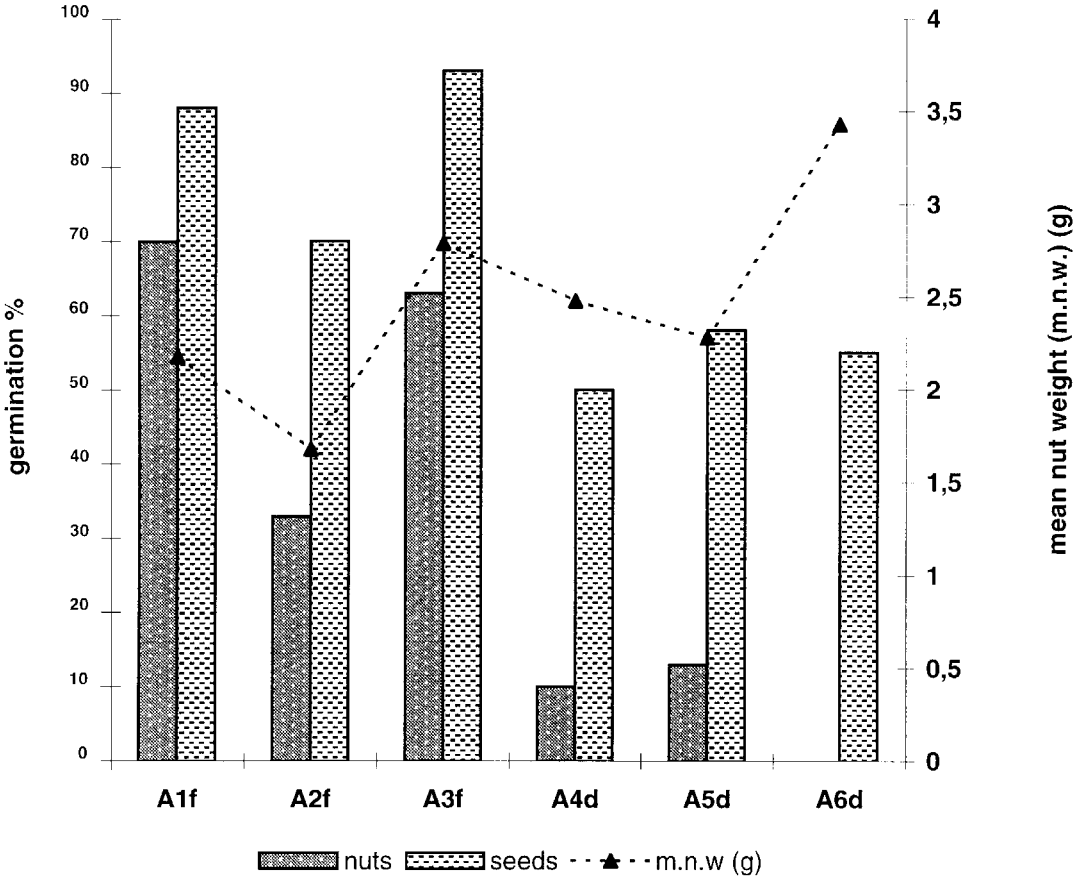

Figure 1. Nuts and seeds germination rate of six *A. spinosa* trees from Ademine forest in southwestern Morocco. 'f' trees have a 'facile' seed wall to break, 'd' trees have a 'difficile' seed wall to break.

tree type is significantly higher than for nuts from 'd' tree type. These results could be related to wall thickness. When using the seeds obtained after breaking the nuts of the same trees, the results showed a much higher germination percentage: 70 to 93% for 'f' trees and 50 to 58% for 'd' trees (Figure 1). Seeds of tree 6d showed a 55% germination percentage whereas the corresponding rate for nuts was 0%. Its seed/nut ratio (0.06) was the lowest observed among studied trees. The high weight of nuts for this tree is due to their thick wall.

Germination rate of seeds was more rapid for 'f' type than for 'd' type. The lag period was the greatest for tree 6d: 10 days after sowing, germination percentage was only 15%. For tree 1f, 92% of seeds had germinated on the same period. The difference between 'f' and 'd' types was significant at 1% level one week after sowing and at the 5% level at the end of the experiment.

2. *Propagation by cuttings*

The experiment with peat/terragreen mixture showed that vegetative propagation of argan tree by lignified cuttings collected from adult trees is possible, although they have a rather low rooting capacity. The highest value, 80%, was for tree 10 of population I (Table 2).

The experiment carried out with softwood cuttings showed that among the tested substrates, Terragreen gave the highest level of rooting (33%), whereas it was low on vermiculite (17%) and zero on 'Oil dri' siliceous substrate. For a tree with high rooting capacity, 75% of the cuttings

Table 2. Number of rooted cuttings from 10 *A. spinosa* trees belonging to two different populations from southwestern Morocco (20 cuttings per tree).

Tree	1	2	3	4	5	6	7	8	9	10
Population I (Aït Baha)	4	1	1	2	0	0	2	3	3	16
Population II (Kerdous)	6	2	6	6	2	4	0	0	6	6

Table 3. Number of shoots formed on Murashige and Skoog medium by four *A. spinosa* different clones.

Clone	Initial number	First transfer	Second transfer
17	307	340	460
1	319	579	790
21	192	208	214
28	80	110	210

(transfers were made after 3 weeks of growth).

were rooted on Terragreen. This substrate exhibits both good water retention capacity and good aeration.

Our results show that vegetative multiplication of argan tree by cutting is possible, but confirm the difficulty of rooting old lignified cuttings. Our results also show that this technique can be optimised by using softwood cuttings and axenic conditions to avoid contamination by fungi. However, it must be pointed out that this propagation technique may lead to architectural problems. When shoots grow from auxillary buds of cuttings, the growth remains often plagiotropic for several months. It is presumably due to the fact that buds growing from cuttings which were originally branches do not always have the entire architectural differentiation sequence of the species. A fascicle root system at an early stage may also represent a handicap for young transplanted plantlets. For successful transplantation in arid areas, it is desirable to have tap roots able to reach quickly the deep layers of soil.

3. In-vitro *micropropagation*

Proliferation. The choice of MS propagation medium was done after several preliminary trials in which we compared propagation media already used for commercial propagation of trees (chestnut, pear tree, oak, raspberry bush . . .) by Agri-Obtention laboratories, INRA Dijon. On most of these media, plantlets lost their leaves and showed necrotic apex. The MS medium induced a good shoot development and a large number of shoots longer than 5 cm (Table 3). Vitamin E addition improved plantlets aspect and multiplication level reached 2–2.5 after three weeks. Prolongation of the photoperiod to 16 h had no effect on the proliferation level. The most important effect was obviously the origin (mother tree) of the plantlets, i.e., the clone effect. Some clones showed low multiplication rates, forming necrotic apex on all media.

Only 5 *A. spinosa* clones of 28 tested had sufficiently high proliferation rates on MS medium to allow the study of rooting.

Rooting. In the first experiment, rooting failed on MS medium whatever the hormone used. On LP medium, rooting percentage of plants grown without a dark period was 17% when IBA was added, 14.8% in the presence of IBA and BAP, and 6.9% with IBA and Phloroglucinol. When plants were stored for one week in the dark, rooting percentage was 16.7% on IBA medium, 6.7% on IBA+BAP medium and 0% on IBA+Phloroglucinol medium. Phloroglucinol always induced lower rooting level and was dropped for the second experiment.

Comparison of hormonal and vitamin E effects in 5 clones of *A. spinosa* showed that the clonal effect was again important and that this varied depending on the clone/hormone interaction. In clones 3 and 28, for example, rooting was absent with IBA but occurred with IBA+BAP. However, in clones 0 and 17, the addition of BAP reduced the rooting percentage, when compared to the IBA medium.

Vitamin E improved rooting on IBA medium but had a complex effect on IBA+BAP medium. For clones 3 and 28, in which BAP had a positive effect, the addition of vitamin E eliminated this benefit, while in clones 0 and 17, the negative effect of BAP was reduced by vitamin E. No rooting was observed whatever the medium for clone 1. In general, rooting levels were low and interactions were common between the supplements used (vitamin E and hormones). Other factors not controlled in this experiment may have

contributed to these results, such as plant physiological status or environmental conditions.

In the second experiment, microcuttings of clone 17 exhibited a high rooting level in media I, II and V, whereas those of clone 1 (as in experiment 1) did not root on any of tested agar media (Table 4). Clone 17 did not root on medium III (where the pH was the lowest) or medium VI, which contained naphtalene acetic acid. When micro-cuttings from the two clones were rooted in tubes containing Terragreen instead of agar, the rooting level was higher (clone 17: 82%; clone 1: 74%). This indicates that the argan tree is more sensitive to substrate properties (and especially aeration) than it is to chemical composition.

4. *Root growth*

Seedlings. Root growth of seedlings grown for two month in minirhizotrons was much greater than shoot growth (Table 5), but subject to great variability between seedlings. Except for seedling six, for which shoot growth stopped, the taproot/shoot ratio varied from 2 to 13. Total root length was 15–60 fold that of the shoot length. In two seedlings, taproot lengths were 8 and 12 cm when the cotyledons opened. After 38 days, shoot lengths were 12.5 and 8 cm when their taproots reached 48 and 53 cm respectively. At the same time, total root length measured 157 and 229 cm, 20 times more than the shoots (Figure 2). However, the roots contained much more water than the shoots and the shoot dry weight was greater than the root dry weight. Roots represented 30–45% (average: 38%) of the dry matter for two month old plantlets. Conversely, the root system is an important reservoir of water, which could be used during short dry periods.

In-vitro *produced plantlets.* The growth pattern of these plantlets was similar to that for seedling plantlets. However, since they have no photosynthetic cotyledons, initial growth of roots was much lower. Usually, a two week lag phase was observed, then a growth phase started and one or two taproots rapidly developed, with an average growth rate of 1 cm/day.

Table 4. Rooting percentage of two micropropagated *A. spinosa* clones (1 and 17) on different media.

Medium	I	II	III	IV	V	VI	Terragreen
Clone 17	10	15	0	5	16	0	82
Clone 1	0	0	0	0	0	0	74

Composition of media are given in Table 1.
80–120 microshoots were used for each media.

Discussion

Germination. Many authors have reported the difficulty of seed germination for the argan tree and they recommended either scarification or acid treatment. Our experiment showed that these treatments are not necessary. Soaking nuts in water is sufficient, as commonly done in forest nurseries. Disinfection of nuts is useful because it avoids microbial contamination. It is very important to use large, fresh nuts with thin shells. For a given tree, our results showed that germination percentage is related to nut weight. However, between

Table 5. Length and dry weight of shoots and roots of eight *A. spinosa* seedlings.

Plant	Length (cm)				Dry weight (g)	
	Shoots	Taproot	Secondary roots	Total	Shoots	Roots
1	19.8	141	562	703	1.03	0.76
2	16.0	95	831	926	0.85	0.57
3	12.0	57	340	397	0.55	0.32
4	6.0	13	81	94	0.14	0.06
5	20.0	264	560	824	1.07	0.87
6	1.0	79	365	444	0.64	0.35
7	26.0	47	730	777	0.92	0.51
8	11.5	124	175	299	0.51	0.28

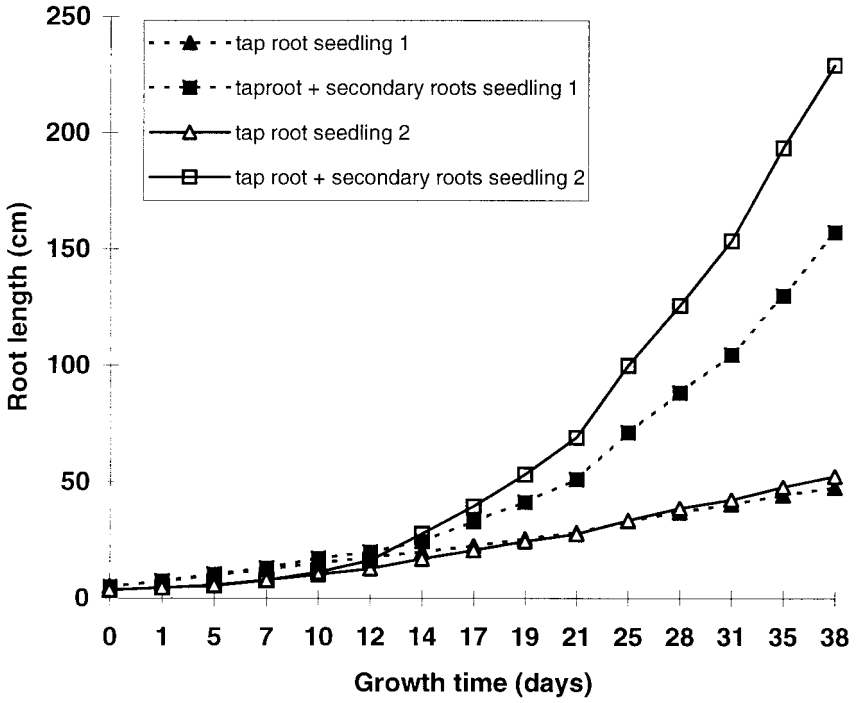

Figure 2. Root growth of two *A. spinosa* seedlings (plants 1 and 2) in a minirhizotron.

trees, the 'f' or 'd' criterion is of major importance, the germination rate being mainly related to wall thickness. Our results also agree with the suggestion that oil seeds lose viability during storage.

These results could be important in reducing the costs of seedling production in nurseries, for reforestation programs. However, for domestication purposes, the selection of economically interesting genotypes must be associated with a propagation method able to capture the selected characteristics of the mother tree, such as vegetative propagation.

Cuttings. Cutting propagation of lignifed shoots of argan tree was first conducted by Franclet (personnal communication), who concluded that it was difficult. Our results showed that the rate and level of rooting varied between clones, as was observed with *Quercus* (Moon and Yi, 1993), hybrid larch (Pâques and Cornu, 1991) and *Leucaena leucocephala* (Dick et al., 1999). In *L. leucocephala*, this was attributed to differences in the morphology of the cuttings. We observed that the argan tree is sensitive to root aeration. Such observation was made for other dry land species: Badji et al. (1991) found that among four tested substrates, well drained crushed basalt powder placed on a bed of gravel was the best rooting medium for stem cuttings of *Acacia senegal*. Shiembo et al. (1997) compared the effects of different solid media (saw dust, sand, gravel) on the vegetative propagation of the african fruit tree *Ricinodendron heudelotii*; the good results on saw dust were attributed to its relatively high air/water ratio and water content. Similar results were obtained by Ofori et al. (1996) with an other moist forest african tree, *Milicia excelsa*. However, other species such as *Cordia alliodora* (Mesen et al., 1997) require a better aeration of the medium for cutting survival.

Vegetative propagation through cuttings is usually restricted to juvenile material because ageing reduces the ability of cuttings to root. Cutting back and hedging mature oak trees before cutting was necessary to have a good rooting level (Chalupa, 1993). Rejuvenation of semi-hard shoots obtained from clones of *Quercus acutissima* has been acheived through grafting twice onto two-year-old rootstocks (giving 60% rooting), whereas after grafting only once the average rooting frequency of the cuttings was 11% (Moon

and Yi, 1993). Pâques and Cornu (1991) also showed that cuttings from two-year old hybrid larch, had a greater growth rate and rooting capacity than cuttings from 16-year old trees. The use of *in vivo* rejuvenation treatments to mature chestnut trees allowed Sanchez et al. (1997) to obtain shoots with juvenile characteristics exhibiting maximum rooting rates. The period of harvest can also affect rooting capacity. Lignified cuttings from *Acacia senegal* harvested during a drought season did not root, while young cuttings harvested during the wet season all rooted (Badji et al., 1991).

Micropropagation. *A. spinosa* micropropagation has been tested without success by several authors. Aaouine and Bazagra (1990) worked on callous regeneration because they have found that microcutting was very difficult or impossible, but no success was reported for callous regeneration until now. We report here the first positive results for microcutting propagation of argan tree. However, multiplication rates of *A. spinosa* showed large variation between clones. Obviously, the high genetic variability of this tree is an obstacle to the use of a same medium for several clones. This phenomenon is frequent with trees and medium have to be adapted to meet the requirements of different clones or varieties. Meier-Dinkel et al. (1993) observed large variations between 52 clones of *Quercus robur* regarding shoot productivity under the same multiplication conditions. Juncker and Favre (1989) also found important differences between 16 oak clones derived from juvenile seedlings.

MS medium is convenient for the proliferation of *A. spinosa*. This medium was also the most effective for bud induction and normal growth of the microshoots of *Dittrichia viscosa* (Boonne et al., 1992). Vitamin E stimulation, as observed here for *A. spinosa*, was already reported for other trees such as eucalyptus, larch and oak (Nkanka, 1982).

The rooting step still need to be optimized for *A. spinosa*. As for cuttings, microcuttings of *A. spinosa* exhibited the best rooting level on Terragreen, whereas rooting was absent for several clones in agar media. The necessity of well drained rooting medium was also reported for micropropagation of *Acacia senegal* (Badji et al., 1991). It may be an important criterion for trees from arid areas.

For acclimatization of *A. spinosa*, moisture must be progressively reduced from saturation to 80% during a three to six weeks period in order to avoid plant mortality. It is important that relative moisture is always maintained above 80%.

Root growth. The growth of argan plantlets in minirhizotrons showed that the taproot from seeds can reach 27 cm when the cotyledons have just opened. The taproot of a 15 cm plantlet can reach more than 100 cm. These observations could explain a large part of the failing of plantlet transplantations. We have observed that in forest nurseries, no care was taken about the root system. Plantlets, several months old, were transplanted with their total shoots, but only with a small part of their root system, representing < 10% of total root length. Large root growth variation has also been found for many tree species (Ahée and Duhoux, 1994; Scaltsoyiannes et al., 1994). In arid areas, soil ecological conditions are often adverse and the transplanted plantlets have more chance to survive if their root system can rapidly reach deep moist layers in order to meet the water demand of the shoots.

Practical implications

Failing of argan tree regeneration is often attributed to multiplication difficulties. However, our experimental results have shown that this tree can be propagated by various techniques. Due to its mainly allogamous reproduction (Msanda, 1993), different multiplication methods could be used, according to the objective. Reforestation by seedlings is the best method because it maintains the great genetic variability of the species and confers ecological resistance. We showed that mother-tree selection (use of large fresh, thin shelled nuts) could improve germination rate and reduce production costs in forest nurseries. *In situ* experiments are still required to compare advantages and disadvantages of direct seedling and transplantation of plantlets from nursery. Our results suggest that early transplantation of plantlets is preferable to avoid transplantation difficulties. Seeds could be transplanted just after

germination and a good soil preparation could favor root growth and plantlet installation.

We have also shown that vegetative multiplication of the argan tree is possible. The aim of our work was not to define ideal methods or media for micropropagation, but was to check the feasibility of this technique. Our results represent the first report of *in-vitro* culture and regeneration for argan tree. This technique allows mass production of selected trees. Once clones have been tested in field trials, it would be possible to predict the future production of argan trees in agroforestry systems. The drought resistance could be another important criterion. Our experiments showed that *in-vitro* propagation could allow early selection of clones on their root/shoot ratio. This was also reported for *in-vitro* cuttings of *Acacia albida* (Ahée and Duhoux, 1994). Other tests, such as nutritional characteristics (Boone et al., 1992), as well as water stress or salinity tolerance, could also be performed early during *in-vitro* experiments on genotypes first selected on their potential oil production ('f' type trees). Vegetative propagation by cuttings or micro-cuttings already allows homogeneous plant material for research as, for example experiments dealing with mycorrhizal dependency (Nouaïm and Chaussod, 1994; Nouaïm et al., 1994).

Whatever the multiplication method used, disinfection of the parent material (seeds or shoots) is important. We observed that it is an important criterion to have a good multiplication rate. Also, great care should be taken about the root system. It is vital for the argan tree whose drought resistance is due to its exceptional root development (El Aboudi et al., 1991). Root efficiency highly depends on its architectural and mycorrhizal status. Drained substrate improves root ramification and root symbiosis (Nouaïm and Chaussod, 1994).

For agricultural improvement of argan tree-based agroforestry systems, a three-step program can be suggested. First, trees should be selected on different criterion (oil yield or drought resistance). Then they could be rejuvenated by cuttings to form a collection of mother trees. Finally, microcuttings could be used for propagation and mass production.

In the argan forest, rural populations often need additional income from immigration to survive. This points out the imbalance of traditional agroforestry system, which are now clearly threatened. The only way to avoid irreversible damage to the environment and to promote sustainable management of the argan forest is to improve the agricultural income. It seems now possible to optimize argan tree based agroforestry systems, through the production of a high quality exotic oil, for which there is already a large demand, exceeding present supplying capacities. 'Planting trees that yield a good profit' is the best way to fight against desertification. Leakey and Simons (1998) showed that increasing the quality, number and diversity of domesticated trees could provide a wide array of non-timber forest products (NTFPs). NTFPs could enhance the capacity of agroforestry to fulfill its ultimate potential as a way to alleviate poverty and to mitigate deforestation and land depletion. Domestication of trees for agroforestry to produce NTFPs can therefore benefit both the farmer and the environment. Vegetative propagation, enabling argan tree domestication, is a real chance for development (Sasson, 1993). We showed that argan tree domestication is technically feasible. However, dissemination of this knowledge is necessary for a true sustainable development.

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