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ORIGINAL ARTICLE

A novel regeneration system through micrografting for *Argania spinosa* (L.) Skeels, and confirmation of successful rootstock-scion union by histological analysis

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Abstract

A novel regeneration system through micrografting is reported for the endangered *Argania spinosa* (L.) Skeels. Rootstocks were obtained from in vitro germinated seeds of the argan genotype G27. It was found that the storage time significantly affects the seed germination capacity, and that the seeds cultured immediately after harvest exhibit the highest germination percentage (91.6%). Besides, transferring seedlings to half-strength Murashige and Skoog (½MS) medium supplemented with 1 mg l^{-1} gibberellic acid (GA_3) and 1 mg l^{-1} 6-benzylaminopurine (BAP) resulted in the highest shoot and root lengths (2.05 and 5.73 cm, respectively). Scions were taken from axillary shoots developed in vitro from microcuttings of genotype G41. Micrografting was performed by using the wedge technique. Afterwards, the micrografted plants were transferred to media supplemented with different plant growth regulators (PGRs). After 2 months of culture, 65–100% of the micrografted plants survived, and no difficulties were observed during the formation of the rootstock-scion union. Interestingly, the use of GA_3 at concentrations ranging from 0.1 to 1 mg l^{-1} was essential for successful micrografting (85–100%) and subsequent growth and development of shoots (2.40–2.72 cm length). After micrografting, scions produced one, two or multiple shoots, depending on PGRs. Histological analysis clearly demonstrated the successful union between rootstocks and scions, with active cell division and vascular tissue formation in the grafting region. After transferring the micrografted plants to the glasshouse, a survival rate of 80% was observed, and the plants showed normal growth and development.

Key message

An efficient micropropagation system through in vitro grafting is reported for the first time ever for *Argania spinosa* (L.) Skeels, and successful micrografting is confirmed by histological analyses.

Keywords Acclimatization · *Argania spinosa* (L.) skeels · Histology · In vitro · Micrografting

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Introduction

Argan (*Argania spinosa* (L.) Skeels) is a multipurpose and endangered agroforestry tree species, producing one of the most expensive edible oils in the world (El Kharrassi et al. 2018; Koufan et al. 2018; Lybbert et al. 2011). Argan belongs to the family Sapotaceae and the genus *Argania* (El Babili et al. 2010), and is endemic to Morocco (Moukrim et al. 2019). It is naturally found in a restricted geographical area of the Atlantic coast of Morocco, between Safi and Saharan coast (Zrira 2017). Due to the nutritional and health-promoting properties of argan oil, this species was introduced to other countries such as Kuwait (Al-Menaie et al. 2007), Argentina (Falasca et al. 2018) and Spain (Martínez-Gómez et al. 2018).

In Morocco, argan is cultivated in an area of around 800,000 ha (Zrira 2017). It plays an important socioeconomic role since it creates employment opportunities for the local population, particularly in the sectors of edible oil production, and nutraceutical and cosmetic product manufacturing, thus contributing to poverty alleviation (Lybbert et al. 2011). Argan also has a great ecological importance as it guarantees soil protection, and erosion and desertification control. Furthermore, argan contributes to biodiversity conservation and water quality improvement (Moukrim et al. 2019; Zrira 2017).

Despite the high agro-ecological and socio-economic importance of argan, this tree is threatened by many factors, including successive years of drought, human activities, goat overgrazing, climate change and habitat destruction, among others. As a way to preserve this plant species, a collection orchard was established by the National Institute of Agronomic Research of Morocco (INRA). This collection is located in the experimental station called Melk Zhar (Agadir, Morocco) and contains 150 argan genotypes. Now, it is necessary to develop efficient regeneration systems for mass-propagation of the best trees of this population.

Argan can be propagated in vivo either by seeds, by stem cuttings or by grafting. Generally, propagation by seeds is least desired because of the long juvenile period and as it hampers the production of true-to-type plants due to the heterozygous nature of argan. Besides, successful germination of argan seeds depends on many factors such as seed size and weight, storage period and pretreatments (Al-Menaie et al. 2007; Nouaim et al. 2002). Thus, this technique is inappropriate for large-scale propagation or the establishment of argan orchards (Metougui et al. 2017). Vegetative propagation of argan through either stem cuttings or grafting could be used for the propagation of selected genotypes with desirable traits. Accordingly, some investigations were carried out in order to develop efficient propagation systems through these two techniques. The use of stem cuttings for argan propagation may be hampered by rooting difficulties. In fact, the rooting ability depends on many factors such as the genotype, the cutting type (semi-hardwood, lignified or softwood), the substrate used and pretreatments. Moreover, fungal contamination, irregular shape of plants and the formation of a fascicle root system in young plants were also reported (Metougui et al. 2017; Nouaim et al. 2002; Taoufiq et al. 2011). On the other hand, the success of in vivo grafting in argan depends strongly on the genotype, the grafting technique and the degree of compatibility between rootstocks and scions (Metougui et al. 2017; Taoufiq et al. 2011).

In the recent years, in vitro propagation of argan through seed germination and microcuttings was attempted. In vitro seed germination resulted in high germination percentages as well as successful shoot development and acclimatization (Justamante et al. 2017). Nevertheless, the use of in vitro germination as a means of propagation will probably lead to the same constraints as in vivo germination, mainly the loss of genetic stability of regenerants. Propagation through microcuttings from adult tree-derived shoots could be employed to produce true-to-type plants of argan. However, the main drawback of this technique is the difficulty to induce adventitious roots (Koufan et al. 2018; Lamaoui et al. 2019). Micrografting (i.e. in vitro grafting) of argan can be envisaged as an alternative method for vegetative propagation of this species. In fact, micrografting allows to benefit from the advantages of both the above-mentioned techniques and thus overcoming the rooting difficulties associated with argan propagation through microcuttings. To the best of our knowledge, propagation through micrografting has never been reported in argan.

Micrografting refers to the technique in which a shoot tip taken from a selected plant, is inserted under aseptic conditions into a rootstock obtained from in vitro seed germination (Jonard 1986). Successful micrografting depends strongly on the formation of vascular connection between the scion and the rootstock (Ribeiro et al. 2015). Micrografting was first described by Murashige et al. (1972) as a tool to recover virus-free citrus plants. Since then, this technique was used for many purposes. For example, to elucidate plant systemic signaling (Tsutsui and Notaguchi 2017), to study interactions between rootstocks and scions of different species (Estrada-Luna et al. 2002), to describe the mechanism of graft compatibility and development (Pina and Errea 2005), to rejuvenate adult tissues (Perrin et al. 1994), and to eliminate pathogens from plants (Sharma et al. 2008). Besides, micrografting was used for virus diagnosis and indexing (Singh et al. 2019), for somatic embryo rescue (Raharjo and Litz 2005) and for cryopreservation and genetic transformation purposes (Almeida et al. 2003; Volk et al. 2012). Micrografting was also suggested to overcome the rooting recalcitrance of some plant species, for large-scale production

of commercial cultivars and to renew the old orchards (Yıldırım et al. 2010).

The purpose of this work was to develop an efficient regeneration system through micrografting for the endangered *Argania spinosa* (L.) Skeels. Accordingly, the effects of various culture conditions on in vitro seed germination, shoot elongation and in vitro grafting were evaluated. In addition, histological analyses were performed to confirm the success of in vitro grafting.

Materials and methods

Rootstock preparation

Mature fruits of *Argania spinosa* (L.) Skeels were collected from an argan tree (genotype G27: G-A2L7) located in the argan orchard of Melk Zhar of INRA (Agadir, Morocco; 30° 02′ 33.0″ N 9° 33′ 04.0″ W). This tree has been previously used by our group as a source of rootstocks for in vivo grafting and showed high germination capacity and the development of a good root system (more than 85% germination, data not shown). The fruits were thoroughly washed with tap water then the fruit pulp was manually removed. The kernels were either used immediately or stored for several months (6, 12 or 24 months) under dark conditions at 24 °C.

For disinfection, the seeds were extracted mechanically then washed thoroughly with sterile distilled water. They were surface-sterilized for 10 min in a solution of 50% commercial bleach (containing 5% sodium hypochlorite), followed by three rinses (10 min each) in sterile distilled water. Afterwards, the seeds were cultured for one month on germination medium, consisting of 6 g l^{-1} agar (Sigma, St. Louis, MO, USA) dissolved in distilled water (Koufan et al. 2020). Seeds were considered germinated once the radicle had emerged. After germination, the seedlings were cultured for 2 months on four different culture media to evaluate their effects on shoot and root elongation: plant growth regulator (PGR)-free half-strength Murashige and Skoog medium (½MS; Murashige and Skoog 1962); ½MS medium supplemented with 1 mg l^{-1} 1-naphthaleneacetic acid (NAA) and 1 mg l^{-1} 6-benzylaminopurine (BAP), ½MS medium supplemented with 1 mg l^{-1} indole-3-butyric acid (IBA) and 1 mg l^{-1} BAP and ½MS medium supplemented with 1 mg l^{-1} gibberellic acid (GA_3) and 1 mg l^{-1} BAP (All PGRs were purchased from Sigma).

Scion preparation

Semi-hardwood stem cuttings (one-year-old, 10–12 cm length) were taken from the argan genotype G41 (G-A4L1) located in the argan orchard of Melk Zhar and characterized by fast growth and short juvenile period. After removing leaves, the cuttings were thoroughly washed with tap water and then surface-sterilized by immersion in a solution of 50% commercial bleach for 10 min, followed by three rinses in sterile distilled water. The cuttings were cut into small segments (microcuttings) of 1.5–2 cm length, each containing two axillary buds. The microcuttings were cultured on PGR-free ½MS medium for one month. After this initiation period, they were transferred to ½MS medium supplemented with 1 mg l^{-1} GA_3 as suggested by Koufan et al. (2018) to promote axillary bud development.

Micrografting

Before in vitro grafting, the leaves and cotyledons of seedlings were removed. The seedlings were decapitated then a vertical cut of 0.5 cm was made from the apical end.

Axillary shoots arising from microcuttings were used as a source of scions. The shoots were cut into small sections of 1–1.5 cm length (scions) and then two wedge-shaped cuts were made in the basal portion of scions.

Grafting was performed by inserting the wedge-shaped end of scions into the vertical cut of rootstocks. After in vitro grafting, the plants were cultured for 2 months on ½MS medium supplemented with either 0.1 mg l^{-1} GA_3, 0.5 mg l^{-1} GA_3, 1 mg l^{-1} GA_3, or 0.1 mg l^{-1} GA_3 combined with 0.4 mg l^{-1} IBA.

Plantlet acclimatization

Two months after in vitro grafting, the micrografted plants were taken from culture vessels and their roots were gently washed with tap water to remove residual agar. The plants were then potted in a mixture of peat and sand (1:1, w/w), covered with a transparent plastic cover and left in the culture room for 15–30 days (i.e. pre-acclimatization, 16 h photoperiod, 40 µmol m^{-2} s^{-1} light intensity, 24 °C). Afterwards, the plants were transferred to the glasshouse and covered with a polyethylene bag for 15 days to maintain high humidity. The bag was gradually opened to allow acclimatization to glasshouse conditions (27 °C, 70% relative humidity).

Culture conditions

The basal medium ½MS consisted of ½MS macro-elements, MS microelements, MS vitamins and was supplemented with 3% sucrose (Sigma, Steinheim, Germany). All culture media were solidified with 6 g l^{-1} agar. The pH was adjusted to 5.7 before autoclaving at 121 °C for 20 min. All the cultures were maintained under 16 h photoperiod

(40 µmol m^{-2} s^{-1} light intensity) at 24 °C and were transferred to fresh medium at 4-week intervals.

Histological analysis

Histological analysis were performed as follows: samples of the grafting region (1 cm above and 1 cm below the graft union) were collected 2 months after micrografting, fixed in FAA (formalin, acetic acid, ethyl alcohol) for 48 h, dehydrated in a graded ethyl alcohol series for 60 min each (10, 30, 50, 70, 90 and 95%) and then left in 100% ethyl alcohol overnight (Mazri et al. 2013). The samples were embedded in paraffin then cut into thin sections of 7 µm by rotary microtome (Leica RM2245, Germany). The thin sections were double-stained with safranin and fast green. Observations were made with an optical microscope (Leica DMLS, Germany).

Data recording and statistical analysis

For rootstock preparation, each argan seed was cultured in a test tube (2.5 cm in diameter, 15 cm in height) containing 13 ml of culture medium, which was considered as one replicate, and each treatment was replicated 120 times. During this phase, the effect of seed storage on germination was evaluated after one month of culture on germination medium. Afterwards, the effect of different PGRs on shoot and root elongation was evaluated after 2 months of culture. In this case, each seedling was cultured in a test tube, and each treatment was repeated 24 times. For scion preparation, two microcuttings were cultured per jar (6.5 cm in diameter, 12 cm in height), each jar contained 40 ml culture medium and a total of 96 jars were used. After in vitro grafting, the micrografted plants were cultured in test tubes. Each tube was considered as an experimental unit, and each treatment was replicated 20 times. After 2 months of culture, the micrografting success rate, the necrosis rate, the percentage of scions producing one, two or more than two shoots, and the average length of shoots were evaluated. During acclimatization, the survival rate was calculated after one month in the glasshouse.

A completely randomized design was used in this study. Data were analyzed by ANOVA followed by Student–Newman–Keuls (SNK) comparison test at the 5% level of significance. All percentage data were arcsine transformed prior to analysis. All analyses were performed using SPSS (v. 26, IBM, Chicago, IL, USA).

Results

Rootstock preparation

After one month of culture on germination medium (Fig. 1a), no bacterial contamination was observed. The germination rate varied significantly depending on the storage period. The highest germination rate (91.6%) was observed when seeds were used immediately after harvest.

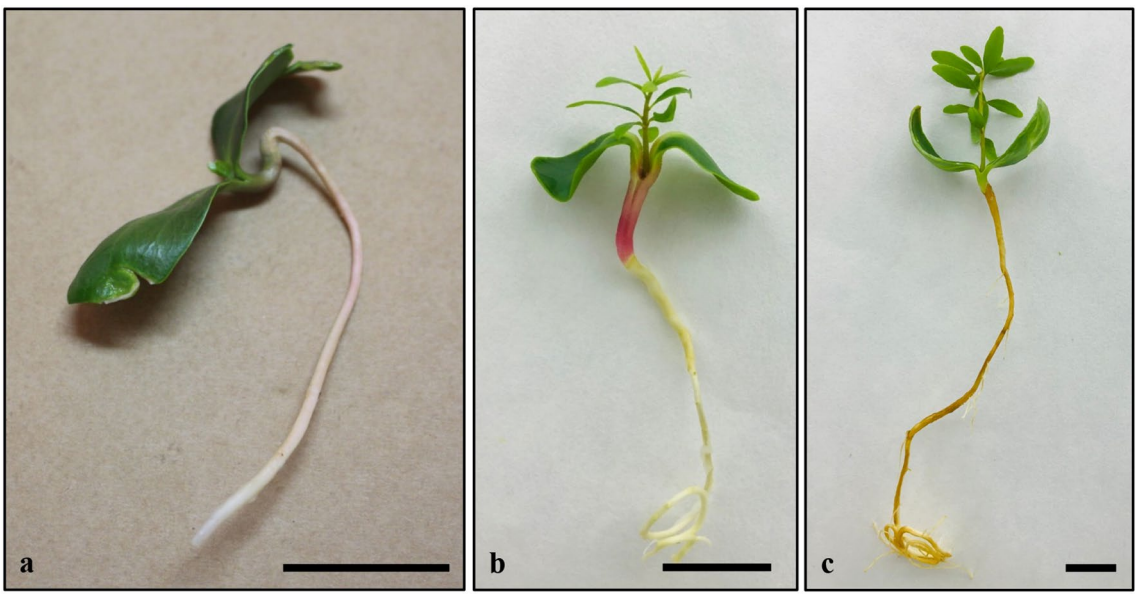

Fig. 1 Seed germination of *Argania spinosa* (L.) Skeels. **a** Seedling after one month of culture on germination medium (6 g l^{-1} agar dissolved in distilled water). **b** Seedling after one month of culture on germination medium and one month of culture on ½MS medium supplemented with 1 mg l^{-1} GA_3 and 1 mg l^{-1} BAP. **c** Seedling after one month of culture on germination medium and 2 months of culture on ½MS medium supplemented with 1 mg l^{-1} GA_3 and 1 mg l^{-1} BAP. Bars correspond to 1 cm

The seeds stored for 6–24 months showed germination rates ranging from 65 to 73.3%, with no significant differences among them (Table 1). Our results suggest to use argan seeds immediately after harvest for efficient germination.

After germination, the seedlings were transferred to four different culture media to promote shoot and root elongation. After 2 months of culture, the highest shoot length (2.05 cm) was observed on ½MS medium supplemented with 1 mg l^{-1} GA_3 and 1 mg l^{-1} BAP (Table 2). The seedlings cultured on PGR-free ½MS medium showed a shoot length of 1.37 cm. Surprisingly, when the culture medium was supplemented with NAA, the average shoot length was 0.21 cm. Regarding root elongation, the seedlings cultured on media supplemented with PGRs showed significantly longer roots (4.88–5.73 cm) than those cultured on PGR-free ½MS medium (2.25 cm). These findings highlight the promotive effect of PGRs on root growth of argan seedlings.

Our results showed no significant differences in shoot and root lengths when the seedlings were cultured on media containing either IBA or GA_3 (Table 2). However, the use of GA_3 resulted in slightly longer shoots and roots (Fig. 1b, c). Thus, ½MS medium supplemented with 1 mg l^{-1} GA_3 and 1 mg l^{-1} BAP is recommended since longer shoots will make micrografting easier.

Table 1 Effect of storage time on seed germination after one month of culture on germination medium

Storage time	Germination rate
No storage	91.6 ± 27.7 a
6 months	65.0 ± 47.8 b
12 months	71.6 ± 45.2 b
24 months	73.3 ± 44.4 b

Data are means ± standard deviation (n = 120). Data followed by the same letter are not significantly different at the 5% level (Student–Newman–Keuls)

Scion preparation

After one month of culture on initiation medium, an acceptable disinfection rate of 58.33% was observed. Besides, microcuttings showed a beginning of axillary bud development (Fig. 2a). After 3 months of culture on ½MS medium supplemented with 1 mg l^{-1} GA_3, the shoot length varied from 2.2 to 5 cm. Unexpectedly, it was found that the majority of microcuttings (71.42%) showed the development of only one axillary shoot (Fig. 2b).

Micrografting and plantlet acclimatization

After 2 months of culture, 65–100% of the micrografted plants survived, and no difficulties were observed during the formation of the rootstock-scion union (Fig. 3a). In fact, callus formation was observed in the grafting region, which was a sign of a good scion-rootstock junction. The highest survival rate (100%) was observed in the plants grown on ½MS medium supplemented with 0.1 or 1 mg l^{-1} GA_3 (Table 3). However, statistical analysis showed no significant difference among the culture media containing PGRs.

Our results showed that micrografting failure was mainly due to necrosis. In fact, up to 35% of the grafted plants showed scion necrosis or necrosis of the rootstock-scion junction (Fig. 3b; Table 3). In very limited cases, rootstocks turned brown and died (Fig. 3c). The highest necrosis rate was observed in the plants cultured on PGR-free ½MS medium, whereas those cultured on ½MS containing 0.1 mg l^{-1} GA_3, 1 mg l^{-1} GA_3 and the combination of 0.1 mg l^{-1} GA_3 and 0.4 mg l^{-1} IBA did not show necrosis.

All the plants that survived to micrografting successfully produced new shoots. These plants showed normal growth and development. In fact, shoot elongation was observed and new leaves were formed. Shoot elongation was influenced by PGRs. After 2 months of culture, the highest shoot length (2.72 cm) was observed in the medium containing 0.1 mg l^{-1} GA_3, with no significant difference with the other PGR-containing media (2.40–2.68 cm; Table 3). The shoots produced by the micrografted plants cultured on PGR-free ½MS medium showed an average length of 0.56 cm. Besides, it is

Table 2 Effect of plant growth regulators on seedling's shoot and root elongation after 2 months of culture

Culture medium	Average root length (cm)	Average shoot length (cm)
½MS	2.25 ± 2.02 a	1.37 ± 1.29 b
½MS + 1 mg l^{-1} NAA + 1 mg l^{-1} BAP	5.30 ± 2.68 b	0.21 ± 0.38 a
½MS + 1 mg l^{-1} IBA + 1 mg l^{-1} BAP	4.88 ± 1.79 b	1.91 ± 1.23 b
½MS + 1 mg l^{-1} GA_3 + 1 mg l^{-1} BAP	5.73 ± 2.56 b	2.05 ± 1.37 b

Data are means ± standard deviation (n = 24). Data in the same column followed by the same letter are not significantly different at the 5% level (Student–Newman–Keuls). *BAP* 6-benzylaminopurine; *GA_3* gibberellic acid; *IBA* indole-3-butyric acid; *½MS* half-strength Murashige and Skoog medium; *NAA* 1-naphthaleneacetic acid

Fig. 2 Axillary shoot development from microcuttings. **a** Microcuttings after one month of culture on PGR-free ½MS medium. **b** Axillary shoot development after 3 months of culture on ½MS medium supplemented with 1 mg l^{-1} GA_3. Bars correspond to 1 cm

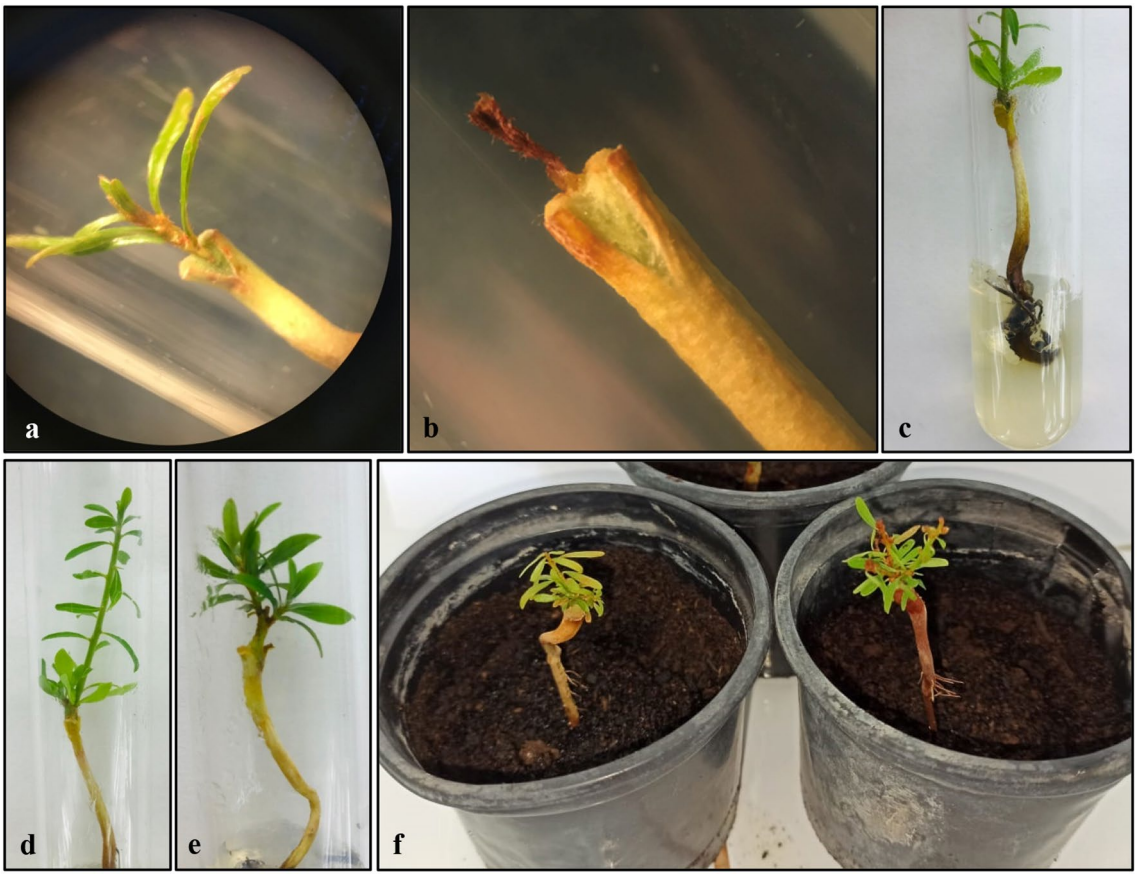

Fig. 3 Argan micrografting. **a** Rootstock-scion union after micrografting. **b** Scion necrosis after micrografting. **c** Rootstock necrosis after micrografting. **d** Micrografted plant producing one shoot after 2 months of culture on ½MS medium supplemented with 1 mg l^{-1} GA_3. **e** Micrografted plant producing 2 shoots after 2 months of culture on ½MS medium supplemented with 1 mg l^{-1} GA_3. **f** Plantlet acclimatization

Table 3 Effect of plant growth regulators on argan micrografting after 2 months of culture

Culture medium	Successful micrografting (%)	Percentage of necrosis (%)	Percentage of plants producing new shoots after micrografting (%)	Percentage of plants producing one shoot (%)	Percentage of plants producing two shoots (%)	Percentage of plants producing more than two shoots (%)	Average length of shoots (cm)
½MS	65 ± 48.9 a	35 ± 48.9 a	65 ± 48.9 a	65 ± 48.9 a	0 ± 0 a	0 ± 0 a	0.56 ± 0.10 a
½MS + 0.1 mg l^{-1} GA_3	100 ± 0 b	0 ± 0 b	100 ± 0 b	65 ± 48.9 a	35 ± 48.9 a	0 ± 0 a	2.72 ± 0.65 b
½MS + 0.5 mg l^{-1} GA_3	90 ± 36.7 b	10 ± 30.7 b	90 ± 30.7 b	60 ± 50.2 a	30 ± 47.0 a	0 ± 0 a	2.68 ± 0.53 b
½MS + 1 mg l^{-1} GA_3	100 ± 0 b	0 ± 0 b	100 ± 0 b	45 ± 51.0 a	30 ± 47.0 a	25 ± 44.4 b	2.40 ± 0.37 b
½MS + 0.1 mg l^{-1} GA_3 + 0.4 mg l^{-1} IBA	85 ± 36.6 b	0 ± 0 b	85 ± 36.6 b	15 ± 36.6 b	70 ± 47.0 b	0 ± 0 a	2.64 ± 0.22 b

Data are means ± standard deviation (n = 20). Data in the same column followed by the same letter are not significantly different at the 5% level (Student–Newman–Keuls). *GA_3* gibberellic acid; *IBA* indole-3-butyric acid; *½MS* half-strength Murashige and Skoog medium

worth noting that after micrografting, scions may produce one, two or multiple shoots, depending on PGRs (Fig. 3d, e). The production of multiple shoots from a single scion was observed in the culture medium containing 1 mg l^{-1} GA_3 (25%; Table 3). Based on our results, GA_3 is recommended for argan micrografting.

After one month in the glasshouse the survival rate ranged from 50 to 80%, depending on the pre-acclimatization period. The highest survival rate was observed after 30-day pre-acclimatization. The micrografted plants showed normal growth and development under the glasshouse conditions (Fig. 3f).

Histological analysis

Histological observations clearly demonstrated the successful union between scion and rootstock (Fig. 4). Indeed, vascular tissue formation was observed in the grafting region. Thus, micrografting can be considered as a novel and efficient system for argan propagation.

In addition to vascular connection, the histological observations showed active cell division in the grafting region, which may reflect callus formation. Besides, xylem, which is a water and nutrient conductive tissue, was also observed.

Discussion

In the present work, a novel regeneration system through micrografting is reported for argan, an endangered agroforestry species highly valued for its nutritional and medicinal properties, and for its ecological benefits. Despite the many difficulties that hamper the propagation of argan through the conventional techniques such as in vivo grafting and

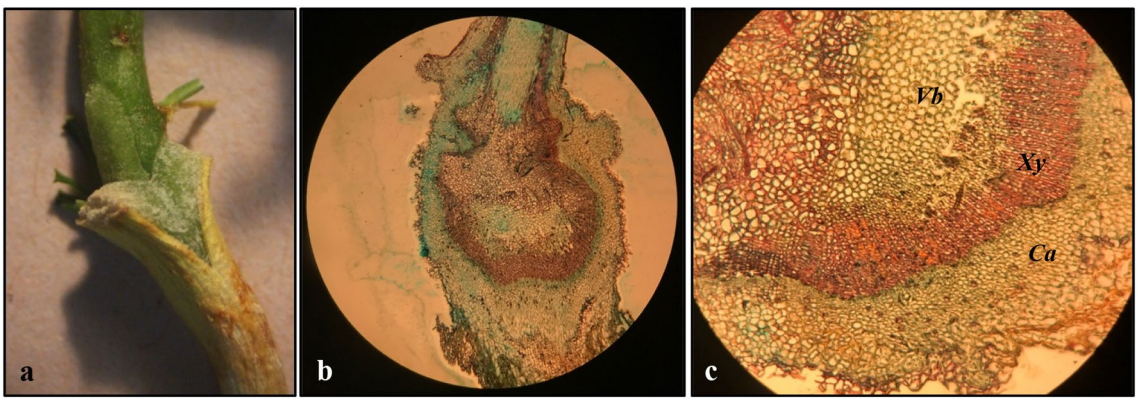

Fig. 4 Histological observations of the grafting region after 2 months of culture. **a** Grafting region before histological analysis. **b** Vertical section (×4) of the grafting region demonstrating complete junction between rootstock and scion. **c** A close-up view (×10) from the grafting region. *Ca* cell division indicating callus formation in the junction region, *Vb* vascular bundles, *Xy* xylem

stem cuttings, very few studies were reported regarding its micropropagation (Justamante et al. 2017; Koufan et al. 2018; Lamaoui et al. 2019). To the best of our knowledge, this is the first report describing successful propagation of argan through micrografting.

In this study, rootstocks were obtained from seeds germinated in vitro. It was found that seed germination depends on the storage period, and that the freshly harvested seeds exhibit the highest germination percentage. Thus, our results recommend the use of fresh seeds. Our findings are consistent with those of Berka et al. (2018), indicating that argan seeds lose their germination capacity due to prolonged storage. Similar results were observed in other plant species such as *Pterocarpus santalinus* L. and *Calanthe tricarinata* Lindl. (Chaturani et al. 2006; Godo 2010). This may be due to several factors including RNA degradation, which was reported to correlate with storage time, and the increase of moisture content of seeds during storage (Fleming et al. 2017; Shelar et al. 2008). These factors lead to a progressive loss of the germination capacity of seeds and to seed deterioration. Moreover, Berka et al. (2018) reported that the oxidation products of seed oil may be toxic to argan embryos and thus affect their germination capacity.

Our findings showed that the elongation of seedling-derived shoots varies depending on PGRs, and that 1 mg l^{-1} GA_3 resulted in the highest shoot length after 2 months of culture. Regarding the scions used in the present study, they were excised from axillary shoots derived from micro-cuttings cultured on a medium also supplemented with 1 mg l^{-1} GA_3 as suggested by Koufan et al. (2018). This GA_3 concentration resulted in a rapid growth of axillary shoots with up to 5 cm length after 3 months of culture. All these results highlight the beneficial effect of GA_3 on the elongation of argan shoots. Such effect was reported in many other plant species (Moshkov et al. 2008). GA_3 belongs to the gibberellin family of growth regulators. It is the most frequently used gibberellin in plant micropropagation and is involved in numerous developmental processes in vitro, including shoot growth and elongation (Moshkov et al. 2008).

The genotype G41 was chosen as a source of scions because of its agronomic and phytochemical characteristics. A previous study by our group showed that the leaves of this genotype gave an essential oil yield of 45 mg/1.5 g dry matter while the seeds gave a yield of 770 mg/1.5 g dry matter. The total phenolic content of essential oils ranged from 91.73 to 123.70 mg/g dry weight gallic acid equivalent. The main fatty acids found in the essential oils extracted from leaves were eicosenoic and behenic acids while the essential oils extracted from seeds are rich in oleic and linoleic acids. Besides, the essential oils of G41 exhibited a high antioxidant activity (> 88% radical scavenging activity) (Koufan et al. 2020).

In vitro propagation through micrografting was reported in many plant species, including *Pyrus elaeagrifolia* (Dumanoğlu et al. 2014), *Olea europaea* L. (Farahani et al. 2011) and *Ceratonia siliqua* L. (Hsina and El Mtili 2009). In all these cases, the wedge technique was used and resulted in satisfactory results. However, Estrada-Luna et al. (2002) reported that this technique was not suitable for prickly pear (30% viability), and suggested the horizontal graft technique instead (90% viability). This indicates that the suitability of the micrografting technique depends on plant species. In the present work, the wedge grafting technique seems highly appropriate since it gave interesting results, with a survival rate of up to 100%. On the other hand, it was found that the percentage of plants producing new shoots and the number of shoots produced by scions after micrografting depend on the presence/absence of GA_3 in the culture medium. Besides, the combination of 0.1 mg l^{-1} GA_3 and 0.4 mg l^{-1} IBA also showed interesting results. This PGR combination was suggested by Córdova-Risco et al. (2017) for *Pouteria lucuma* micrografting, a plant species belonging to the same family as argan (Sapotaceae). The effect of PGRs on micrografting was reported by other researchers (e.g. Yıldırım et al. 2010; Pahnekolayi et al. 2019). Furthermore, it is well known that PGRs play a major role in plant propagation in vitro. In fact, the exogenous plant hormones interact with the endogenous ones. This results in cell division, proliferation, differentiation and morphogenesis (Gaj 2004; Jiménez 2001). Similarly to in vitro seedling and axillary shoot development of argan, the use of GA_3 showed very good results after micrografting, with more than 85% of the micrografted plants producing new shoots, as well as normal growth and development of these plants.

The high survival rate observed after in vitro grafting may reflect a high compatibility between the two genotypes used, which was confirmed by histological observations. In fact, histological analysis showed vascular tissue formation in the grafting region, which is a criterion of successful micrografting (Ribeiro et al. 2015). On the other hand, the experiments carried out in this investigation showed that tissue necrosis was the leading cause of plant mortality after micrografting. Necrosis was mainly observed in scions, which may reflect a bad insertion of the scion into the rootstock. Generally, tissue necrosis is a common problem encountered in many plant species when cultured in vitro, regardless of the technique used (Bairu et al. 2009; Gow et al. 2009; Meziani et al. 2016).

In the present study, the acclimatization rate of the micrografted plants ranged from 50 to 80%, depending on the pre-acclimatization period. The results reported in the literature show that the survival rate of micrografted plants during acclimatization varies among species. For example, in almond, the survival rate reached 100% (Yıldırım et al. 2010), while it was 58% in cherry (Bourrain and

Charlot 2014). In Khasi mandarin, a survival rate of 72% was reported (Singh et al. 2019), whereas in Kinnow mandarin, the survival rate ranged from 45.83 to 70.83%, depending on the rootstock used (Chand et al. 2016). Our findings showed that a 30-day pre-acclimatization increased the survival rate during acclimatization. Justamante et al. (2017) maintained rooted microcuttings of argan for almost the same period of time (4 weeks) under the controlled conditions of the growth chamber before transferring them to the greenhouse. The results of the present study indicate that in vitro grafting can be considered as an efficient method for argan propagation.

Conclusions

An efficient regeneration system through micrografting was established for the endangered *Argania spinosa* (L.) Skeels. Rootstocks were obtained from in vitro seed germination while scions were derived from microcuttings. Successful micrografting was observed when the plants were cultured on media supplemented with GA_3, and histological observations confirmed the successful union between rootstocks and scions. Our results will be useful for the multiplication of argan, a highly valuable plant species recalcitrant to vegetative propagation. We are currently evaluating the growth and development patterns of the micrografted plants. Further studies will be carried out to assess the genetic conformity of the plants by using morphological traits and molecular markers.

Author contributions MK and IB conceived the idea. IB and LER supervised the work. MK performed seed germination experiments. MAM performed microcuttings experiments and carried out statistical analysis. MK and MAM wrote the manuscript. MK, AE and SM performed micrografting experiments. SM, AE and IT performed histological analysis. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

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