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Full Length Article

Establishment of Optimal Protocols for Germination, Rooting and Elongation of four Moroccan Argan (*Argania spinosa*) Genotypes

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Abstract

The establishment of several protocols to lift integumentary and embryonic dormancy is necessary to achieve and hasten the germination of argan tree [*Argania spinosa* L. (Skeels)], which faces several obstacles, including difficult climatic conditions, overgrazing and excessive harvesting of seeds for oil extraction threatening its natural regeneration. The present work aims to improve the germination and root power under greenhouse, *In vitro* and on petri dishes in four genotypes of argan tree namely Bouizakarne, Agadir, Admine and Ighrem. The germination under greenhouse is optimized after five pre-treatments of seeds: including the pulping of seeds, the mechanical scarification, the cold storage (4°C) for 1 month, the chemical scarification with sulfuric acid (95%) for 20 min and finally the chemical scarification with hydrogen peroxide (33%) for 24 h. The germination and the *in vitro* development of the argan tree are evaluated under the control of the medium effect (full and ½ strength MS) and the light effect. The results obtained showed that seed pulping was the most effective treatment under greenhouse with 66.66%. For *in vitro* culture, ½ strength MS medium recorded the highest germination rate (77.19%). Moreover, the argan tree was indifferent to light in triggering germination. However, light incubation improved germination on full strength MS medium more than on ½ strength MS medium. Yet, ½ strength MS medium incubated in the darkness revealed the highest percentage of germination *in vitro* with 81%. Thus, *in vitro* culture was shown to be the most favorable condition compared to other culture conditions. Moreover, it was not only attributed to the improvement of the germination rate but also to the reduction of the latency phase and to a good development of the *in vitro* plants. Indeed, it was not until day 4 that the kernels started to germinate with 18 days under glass. It was not until two months later that it developed a stem of 5.04 cm and a main root of 15.74 cm in length with possible adventitious roots. This was approved for all genotypes but was very remarkable in Ighrem the most recent genotype.

Key words: Argan tree; Pretreatment; Germination; *In vitro* culture; Light; Rooting; Elongation

Introduction

Argan [*Argania spinosa* (L.) Skeels] tree is the endemic Sapotaceae plant in southwest of Morocco. It occupies the upper valley of Oued Grou, southeast of Rabat and the Mediterranean side of the Beni Snassen Mountain range, north of Oujda (Metougui *et al.* 2017). With about 20 million trees on an area of about 800,000 ha and by a longevity of over 48,300 years, the argan is considered an important forest tree in Morocco (Elmandouri *et al.* 2020). It is distinguished by a resilience to difficult environments characterized by drought, risk of erosion and soil poverty due to its high genetic variability (Elmandouri *et al.* 2020). Similarly, the argan tree plays an essential role for Morocco whether on an economic, social or environmental level. Due to their biological and ecological properties, this Moroccan species generates 800 thousand workdays, guarantees the daily needs of three million people and ensures a more profitable agriculture by fighting against wind and water erosion while maintaining the moisture and fertility of the soil, which are effective against desertification (Mezghenni *et al.* 2014; Badaouini 2015). In addition, the populations of argan tree have begun to regress intensively resulting in a decrease of about 600 ha per year and an average density of 30 trees per hectare (Mdarhri *et al.* 2011). Due to harsh climatic conditions, overgrazing and excessive harvesting of seeds for oil extraction, the natural regeneration capacity of the argan tree is very low or even difficult (Mezghenni *et al.* 2014).

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Like most woody plants, the argan tree can be reproduced by seed sowing. Due to the loss of their germinative power, especially related mainly to the problems of drought or grazing by livestock, several studies have shown that the seeds of argan tree are difficult to germinate in their natural state (Bani-Aameur and Alouani 1999). In order to maintain the economic value of the argan tree and its strategic position in the agrarian systems of southwestern Morocco, it is essential to improve its production and regeneration potential (Mezghenni *et al*. 2014). Germination is a critical stage in plant development. Although they are put under optimal growth conditions, many species exhibit varying degrees of dormancy and generating problems with germination inhibition (Mezghenni *et al*. 2014; Kermiche and Merabti 2018).

Given that the Agran seeds are characterized by embryonic and integumentary dormancy, which causes a significant loss of seed viability. The success of argan germination *via in vitro* culture is influenced by several factors, notably the explant, the genotype and the composition of the culture medium. Our work was aimed to study, for the first time, the germination capacity of four argan genotypes of Moroccan origin, namely Bouizakarne, Agadir, Admine and Ighrem. Thus, different germination conditions under greenhouse, *in vitro* and in Petri dishes, were used to optimize the germination rates of our argan genotypes. We worked on the development of an empirical model to improve the germination capacity of argan seeds according to their competitiveness as well as the rooting and elongation of the seedlings under the control of different factors namely appropriate method of disinfection, light, cultivation method, as well as the effect of genotype and seed type of four Moroccan genotypes.

Materials and Methods

Plant materials

During their period of optimal morphological and physiological maturity (from mid-June to July in 2021 and 2022), the fruits of fallen argan trees were collected under the trees and then dried in the open air and kept until the time of sowing. Four genotypes of Moroccan origin were tested in this work namely Bouisakarne, Agadir and two genotypes from the province of Taroudant specifically Admine, plain area, and Ighrem, mountain area. Whole seeds (with endocarp) were obtained after pulping of the fruit for germination under greenhouse, and kernels (without endocarp) resulting from the crushing of seeds for *in vitro* culture and for petri dishes, in order to develop the effective germination protocols (Fig. 1).

Disinfection of the kernels

The kernels were disinfected, under a laminar flow hood, by soaking for one min in ethanol at 70°C followed by 15 min in sodium hypochlorite per 12° titrant. The kernels were then rinsed 3 to 4 times with sterile distilled water and dried on sterile filter paper before sowing.

Germination *in vitro*

Effect of medium: The culture media used were full-strength and ½ strength MS media where the macro-elements in full strength MS medium were diluted by half (Murashige and Skoog 1962). Both media were added with 30 g/L sucrose and 1 g/mL thiamine and solidified with 10 g/L agar. Before the addition of the latter, the pH of the medium was adjusted to 5.7 ± 0.1. Sterilization was done by autoclaving at 120°C for 20 min. The disinfected kernels were placed in tubes or flasks filled with culture medium (25 mL) and finally placed in a controlled culture chamber.

Effect of light: On both full- and ½ strength MS media, the kernels were incubated in the darkness and in the light until they were germinated, in order to examine the effect of light in promoting germination and the development of seedling. Then the crops were finally arranged in a controlled culture chamber characterized by a temperature of 25 ± 1°C and a photoperiod of 16/8 h light/dark. The count of germinated kernels was carried out daily while observing the emergence of radicle was considered as indicator of the germination.

Germination on petri dishes: The petri dishes lined with filter paper and distilled water were autoclaved for 20 min at 120°C. Then, the kernels were put to germinate in sterile petri dishes lined with two layers of filter paper soaked with sterile distilled water.

Germination under greenhouse: In view of the seed dormancy in argan seed, we applied various seed pre-treatment before sowing. In this sense, five pre-treatments preceded the germination of seeds: pulping of seeds (T1), mechanical scarification (T2), cold storage (4°C) for 1 month (T3), chemical scarification with 95% sulfuric acid (H_2SO_4) for 20 min (T4) and chemical scarification of seed with 33% hydrogen peroxide (H_2O_2) for 24 h. Seeds were then germinated in trays 12 cm in diameter and 15 cm deep filled with soil and peat (50/50) at a rate of one seed per tray and placed under controlled conditions of 30 ± 2°C with natural light of 16/8 h in a greenhouse. As needed, watering was done once or twice a week with Hoagland's solution.

Statistical analysis

Design of the experiments was completely randomized with three replications. Data were analyzed using SAS software (Statistical Analysis System version 9.1 and version 5.5), and subjected to analysis of variance (ANOVA). Means were compared using Duncan's Multiple Range (DMR) test at the 5% significance level of probability.

Results

Germination *in vitro*

Effect of medium: The number of germinated kernels was significantly affected by the full and ½ strength MS media (Fig. 2). Indeed, the analysis of variance showed that the highest germination was obtained on ½ strength MS medium. The evaluation of the germination curve showed the existence of the same latency time of 3 days for both media (Fig. 3). Nevertheless, on day 12 the kernels grown on ½ strength MS medium continued to germinate actively and reached 62.49%, while the kernels germinated on full strength MS medium reached 49.9% only. Both media increased germination progressively to reach their maximum (77.19 and 64.09% for ½ strength MS and full-strength MS media, respectively) after 20 days.

Effect of light: The results showed non-significant difference in the rate and kinetics of germination between light and darkness (Fig. 4–5). Indeed, the argan kernels also showed a short lag phase of three days in both darkness and light. Thus, although light accelerated germination in the first few days, the kernels showed a non-significant germination rate between darkness (70.06%) and light (71.22%). The argan tree was indifferent to the presence or absence of light to trigger germination. However, the kernels germinated in the darkness were transferred to a chamber with a 16/8 h light/darkness for a good rooting and a subsequent growth.

Effect of genotype: The germination rate of genotypes varied significantly. A highest germination percentage was observed in Ighrem genotype (89.85%) followed by Admine and Bouizakarne (67.17 and 65.83%, respectively). Agadir genotype recorded the lowest (59.72%) germination (Fig. 6). Moreover, the germination rate differed considerably from one genotype to another with time (Fig. 7). From the germination kinetics curve, it can be deduced that germination of Admine and Agadir genotypes after 36 days was delayed during the first days of germination than the other genotypes. Ighrem and Admine genotypes reached maximum germination in only 16 days.

Medium-light interaction: *In vitro* germination was highly affected by the medium used and the light (Fig. 8). Indeed, light negatively affected the germination percentage on ½ strength MS medium (73.39%). However, it was positive on full strength MS medium (increased from 59.13% in darkness to 69.05% in light). On the other hand, the highest percentage of germination was recorded on ½ strength MS medium in the darkness (81%).

Medium-genotype interaction: Depending on the medium used, germinating kernels of different genotypes reacted differently (Fig. 9). On ½ and full-strength MS media the maximum germination rate was observed in the Ighrem genotype with 98.99 and 80.71% respectively, while it was the lowest in Agadir genotype irrespective of culture medium (66.94% on ½ strength MS and 52.5% on full-strength MS). Generally, ½ strength MS medium was highly improved compared to full strength MS medium for all genotypes.

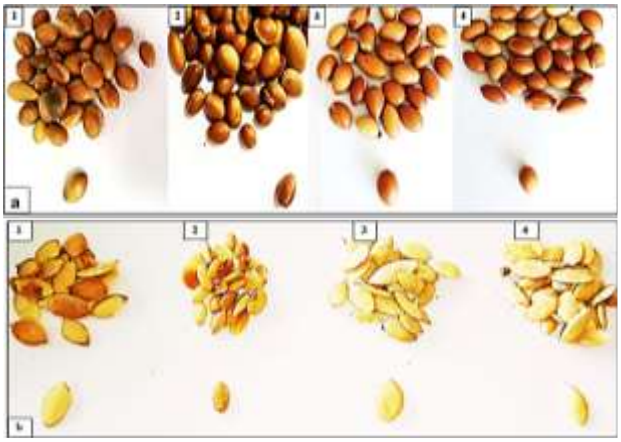

Fig. 1: Seeds of the four genotypes of argan tree (**a**); Kernels of the four genotypes of argan tree (**b**) 1, Bouizakarne; 2, Agadir; 3, Admine and 4, Ighrem

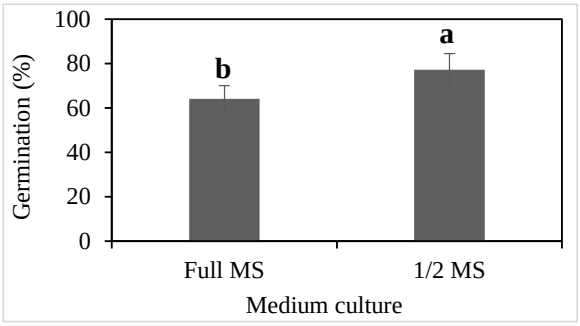

Fig. 2: Effect of full strength and ½ strength MS media on *in vitro* germination of argan tree

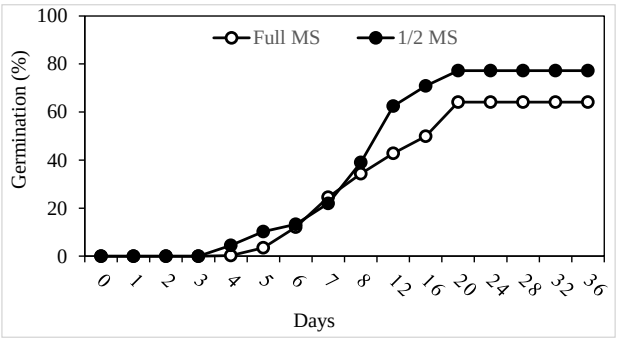

Fig. 3: Evolution of *in vitro* argan germination on MS and ½ MS media as a function of time (days)

Genotype-light interaction: The kernels of the different genotypes reacted differently to light (Fig. 10). In this case, the Ighrem genotype showed the highest germination rate in both light and darkness but with a remarkable improvement in light (93.6%). However, darkness was more favorable (62.5%) for the Agadir genotype than

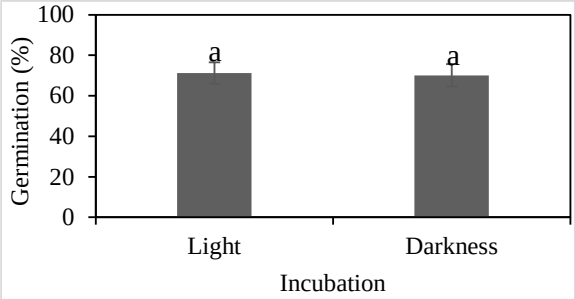

Fig. 4: Effect of light on *in vitro* germination of argan tree

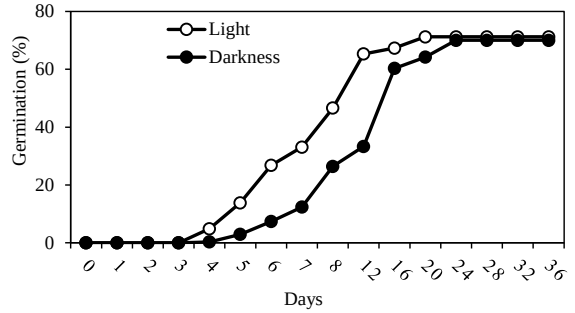

Fig. 5: Evolution of *in vitro* germination of argan tree in light and darkness as a function of time (days)

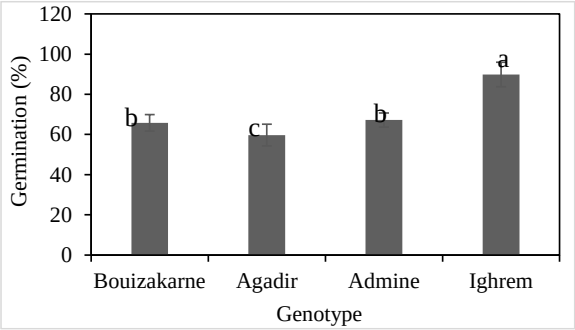

Fig. 6: Effect of genotype on *in vitro* germination of argan tree

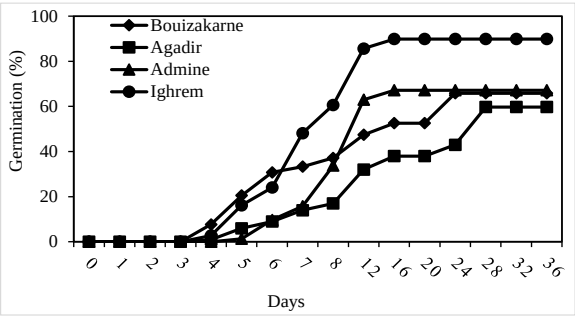

Fig. 7: Evolution of *in vitro* germination of four argan genotypes as a function of time (days): Bouizakarne; Agadir; Admine; Ighrem

light (56.94%). For Bouizakarne and Admine genotypes, the germination rate recorded no significant

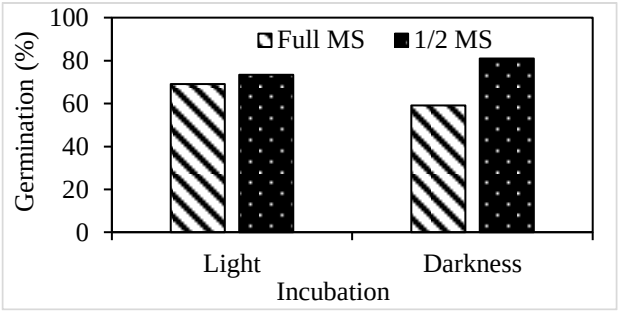

Fig. 8: Light-medium interaction effect on *in vitro* germination of argan tree

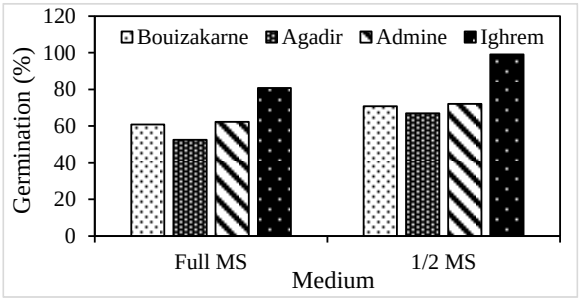

Fig. 9: Medium-genotype interaction effect on *in vitro* germination in the four argan genotypes: Bouizakarne; Agadir; Admine; Ighrem

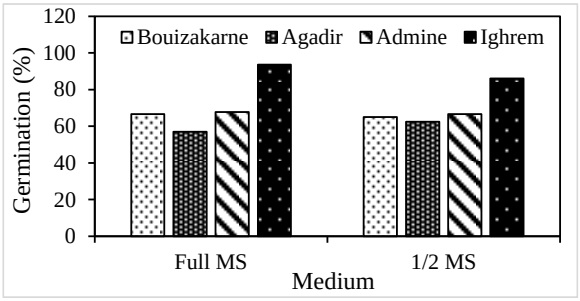

Fig. 10: Light-genotype interaction effect on *in vitro* germination in the four genotypes: Bouizakarne; Agadir; Admine; Ighrem

difference between light and darkness.

Medium - genotype - light interaction: The medium ½ strength MS improved the germination rate in all genotypes in the darkness as well as in the light, but with a good improvement of the germination rate in the darkness (between 75 and 98.99%) than in the light (between 58.88 and 98.99%). On the other hand, on the full-strength MS medium all the genotypes recorded a higher rate in the light which amounted to 88.21% in the Ighrem genotype. Contrarily, Agadir recorded the lowest percentage regardless of the medium and the incubation condition used (Fig. 11).

Germination under greenhouse

All chemical treatment seemed not to release the seeds

dormancy although they made the seeds burst. The H_2SO_4 (T5) completely prevented germination. H_2O_2 (T4) showed a low germination rate (16.66%). Similarly, mechanical scarification (T2) presented a low germination (22.91%). This negative effect of these treatments was in the majority of cases due to the penetration of the watering solution by the cracks seed coat causing the damage of the embryo. Contrarily, cold storage (T3) recorded an average germination rate (37.5%). However, direct sowing of pulped seeds (T1) revealed the highest germination (66.66%). Pulping of the seeds gave the best result, so this treatment can be used to optimize germination in the greenhouse of all genotypes (Fig. 12).

Germination in petri dishes

In petri dishes study, the kernels germinated quickly and reached 60.37% of germination. The only problem was the volume of soaking water. The kernels did not germinate anymore and became infected when they were highly imbibed.

Incubation conditions and argan germination

Since the best germination *in vitro* was observed on the ½ strength MS medium in the darkness and the best pre-treatment was the pulping of seeds in the greenhouse. These germination conditions were used to compare with the germination in petri dishes. The analysis of variance showed that the percentage of germination was significantly different among the genotypes according to culture conditions (Fig. 13). Indeed, the best germination percentage was revealed by the *in vitro* culture with 81%. When the seeds were germinated in Petri dishes, the rate was 60.37% followed by the greenhouse conditions, which recorded the lowest germination (57.46%). The evaluation of germination over time showed the sigmoidal curve after 52 days of culture for each culture condition (Fig. 14). For petri dishes and *in vitro* culture, the kinetic curves showed a very short lag phase. Indeed, it was only on the 3rd day in the petri-dishes and the 4th day *in vitro* culture that the first kernels germinated, and it was only after 18 days that the first seeds started to emerge radicles in the greenhouse with a long exponential phase of germination of one month against only 10 days *in vitro* and on the dishes.

Effect of genotype: Data showed that the highest germination percentage was noted in the Ighrem genotype (91.03%), while Bouizakarne genotype recorded the lowest rate (43.7%). The other two genotypes, Agadir and Admine showed similar germination i.e., 65.07 and 65.28%, respectively (Fig. 15).

Condition-genotype interaction: The results showed that the germination rate was dependent upon the genotype and conditions used (Fig. 16). For all genotypes, the highest germination was recorded from *in vitro* culture (75 to 98.99%). For petri-dishes and greenhouse culture, the

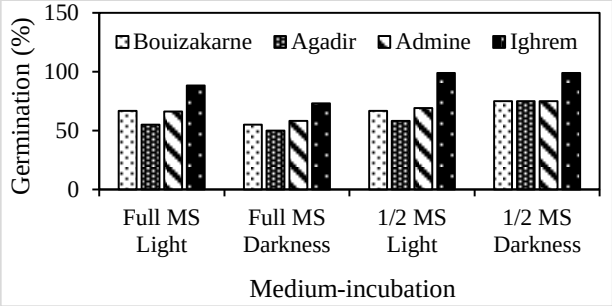

Fig. 11: Medium-light interaction effect on *in vitro* germination in the four genotypes: Bouizakarne, Agadir, Admine and Ighrem

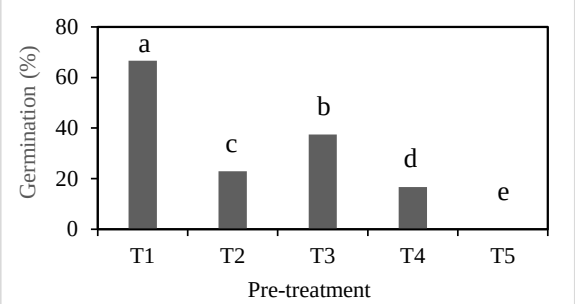

Fig. 12: Effect of treatments on the germination of argan seeds under greenhouse: T1: Depulping of seeds; T2: Mechanical scarification; T3: Cold storage (4°C) for 1 min; T4: Chemical scarification with sulfuric acid (H_2SO_4) for 20 min; T5: Chemical scarification with hydrogen peroxide (H_2O_2) for 24 h

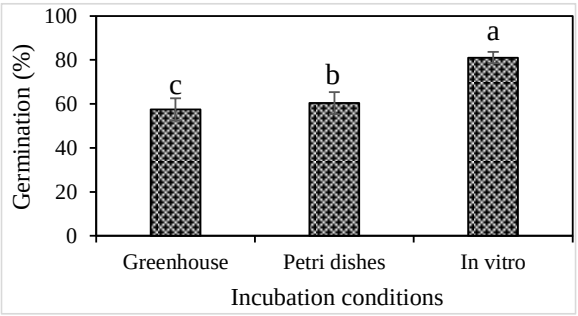

Fig. 13: Germination rate in greenhouse, petri dishes and *in vitro* condition of argan tree

Bouizakarne genotype recorded the lowest germination (31.11 and 25%, respectively). Similarly, Agadir genotype recorded a lower germination on petri dishes (54.75%) while for Admine genotype a lowest percentage was found under greenhouse with 54.16% (Fig. 17). In all conditions, the highest germination was displayed by Ighrem genotype ranging from 84.12 to 98.99%.

Effect of *in vitro* culture medium on root and shoot growth of argan *in vitro* plants

Effect of the medium: Data revealed that rooting was significantly affected by the culture medium. Indeed, the

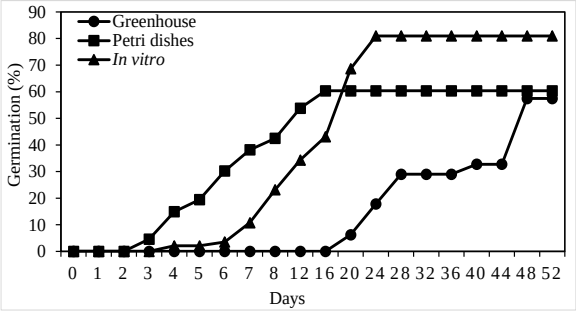

Fig. 14: Evolution of germination under greenhouse, on petri dishes and *in vitro* of argan tree according to time (days)

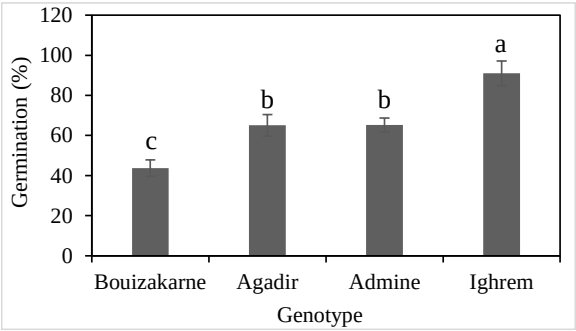

Fig. 15: Effect of the four argan genotypes: Bouizakarne, Agadir, Admine and Ighrem on germination on the three conditions

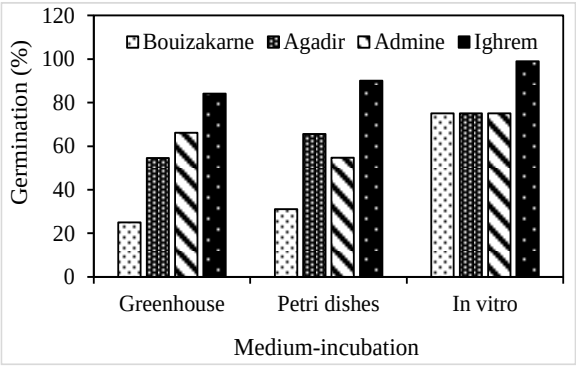

Fig. 16: Germination capacity of the four argan genotypes: Bouizakarne, Agadir, Admine and Ighrem under three growing conditions: greenhouse, petri dishes and *In vitro*

dilution of macro-elements by half in the MS medium also induced a good rooting of argan *in vitro*. After 2 months of culture, ½ strength MS medium showed the highest rooting percentage (60.98%) and quality of *in vitro* plants as compared to that noted in full-strength MS medium (42.7%) (Fig. 18–19). In addition, it produced 15.74 cm long main roots and it showed prolific adventitious roots in some genotypes. However, both ½ and full-strength MS media showed no significant difference on the above-ground growth. They generated *in vitro* plants with stems of 4.38 and 5.04 cm, and average leaf numbers by 5.6 and 6.27, respectively (Table 1).

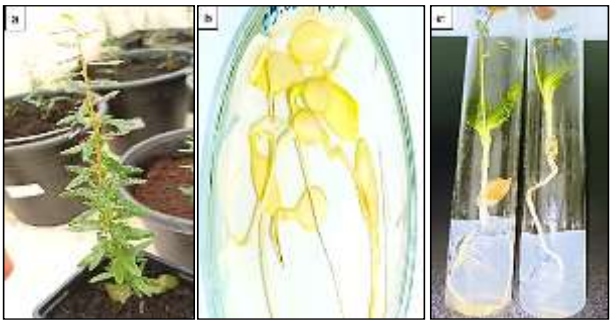

Fig. 17: Effect of culture condition on the germination of argan tree
a: Greenhouse culture; **b:** Culture on petri dishes; c: *In vitro* culture

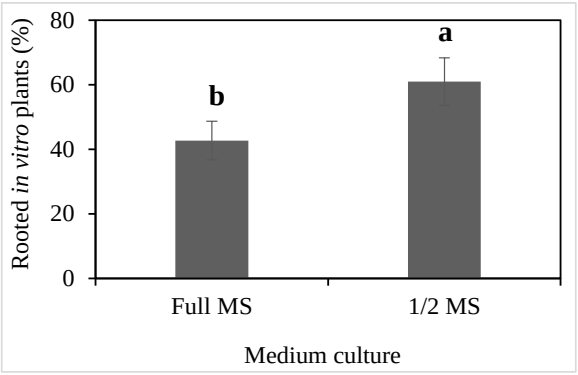

Fig. 18: Effect of full strength and ½ strength MS media on the percentage of rooted *in vitro* plants of argan genotypes

Fig. 19: Effect of MS and ½ MS media on the percentage of rooted *in vitro* plants of argan genotypes: **a**: full strength MS medium; **b**: ½ strength MS medium

Effect of genotype: The rooting rate was significantly different in the genotypes. Ighrem genotype showed the highest rooting (65.71%) of *in vitro* grown plants and also showing the longest root (17.08 cm). Bouizakarne recorded the lowest rooting of *in vitro* grown plants (41.66%) with

Table 1: Effect of MS medium and ½ MS medium on rooting and growth of *in vitro* plants of four argan genotypes after 2 months: Bouizakarne; Agadir; Admine; Ighrem

Genotypes	Average length of main roots (cm)		Appearance of secondary root (cm)		Average stem length (cm)		Average number of leaves	
	MS	½ MS	MS	½ MS	MS	½ MS	MS	½ MS
Bouizakarne	11.50c	14.66b	+	+	4.0a	4.66a	5.50ab	6.33a
Agadir	13.00bc	15.50b	+	+	4.5a	4.50a	6.00ab	5.50a
Admine	13.50b	14.83b	+	+	4.25a	5.00a	4.33b	5.83a
Ighrem	16.16a	18.00a	+	+(dense)	4.78a	6.00a	6.57a	7.42a
Mean	13.54 ± 1.31b	15.74 ± 1.12a			4.38 ± 0.25a	5.04 ± 0.48a	5.60 ± 0.68a	6.27 ± 0.6a

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

13.08 cm root length. The genotypes Admine and Agadir showed average root length i.e., 14.16 and 14.25 cm, respectively (Fig. 20–21). All genotypes showed non-significant difference for shoot length (Table 1).

Medium-genotype interaction: Data revealed that ½ strength MS medium was more effective for rooting and *in vitro* plant quality in all genotypes compared to full-strength MS medium. Indeed, ½ strength MS medium recorded the highest rooting rate (50 to 71.42%) in all genotypes (Fig. 22). Similarly, it also stimulated the main root length (14.66 to 18 cm) of *in vitro* grown plants and the presence of secondary roots in all genotypes. On the other hand, the genotype Ighrem recorded the highest rooting percentage of *in vitro* grown plants (71.42%), with the longest root (18 cm), the densest adventitious root, the longest stem (6 cm) and the largest number of leaves (7.42) on ½ strength MS medium (Table 1).

Discussion

In this study, the germination and development of the argan tree were significantly affected by the growing conditions and the factors studied. Indeed, the germination percentage under greenhouse was improved by pulping the seeds (Fig. 6–7). This positive effect of pulping was also proven by Mezghenni *et al*. (2014) on the *ex vitro* multiplication of some other argan genotypes. Similarly, Nouaim (1994) found that acid treatments are useless. Other authors have revealed that mechanical scarification as a pre-treatment countered the problem of argan seed dormancy by making seed coat permeable to water and gases, and initiation of process of germination (Nouaim 1991; Chaussod and Nouaim 1994; Nouaim *et al*. 1995). Contrarily, Elmandouri *et al*. (2020) reported that treatment with 1 mg/L gibberellic acid at 4°C for 48 h increased the germination of argan seeds. Kechairi and Lakhdari (2002) and Miloudi (2006) found that the germination rate was satisfactory after a simple soaking of the seeds in water at room temperature. For *in vitro* culture, our results showed that the germination of argan tree was important on the ½ strength MS medium compared to the full-strength MS medium (Fig. 14). In this context, Mezghenni *et al*. (2014) reported that the use of ½ strength MS medium was largely sufficient for *in vitro* argan germination, especially for Smimou and Bouisakarme genotypes. In addition, the dilution of macro elements by half of the MS medium also induced a good rooting and

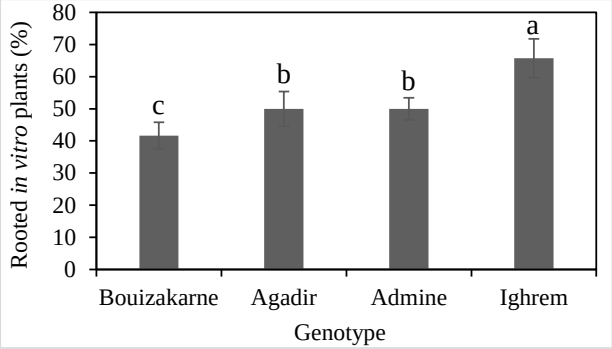

Fig. 20: Effect of genotype on the percentage of rooted seedlings of four argan genotypes: Bouizakarne; Agadir; Admine; Ighrem

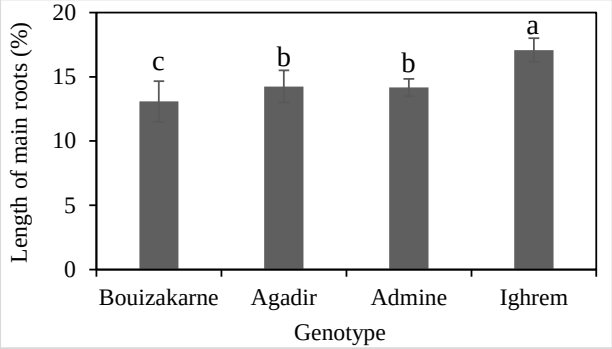

Fig. 21: Effect of genotype on the length of the main roots (cm) of four argan genotypes: Bouizakarne; Agadir; Admine; Ighrem

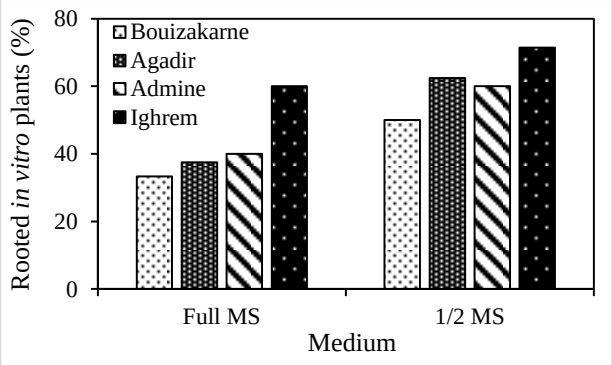

Fig. 22: Effect of MS and ½ MS media on the percentage of rooted seedlings in the four argan genotypes: Bouizakarne; Agadir; Admine; Ighrem

vigor of the argan vitroplants. This rooting stimulating effect could be explained by the low ionic concentration of the culture medium which allows to stimulate buds as well as root formation in stems (Kulchetschi *et al.* 1995). Similarly, Benderradji *et al*. (2007) reported that ½ strength MS medium is often advantageous for inducing root formation. Murashige and Skoog medium diluted 952 by half has also been used as a basal medium for several species such as *Moringa oleifera* (Quashie *et al*. 2012), *Argania spinosa* (Bousselmame *et al.* 2001), and *Olea europeae* cv. Picholine Marocaine (Brhadda *et al.* 2003).

Concerning the effect of light, this factor did not record any significant difference on the germination and development of argan trees. However, it was strongly dependent upon the culture medium used. Indeed, on ½ strength MS medium, darkness was highly reactive compared to light (Fig. 13). Malik and Born (1987) showed that light inhibited germination for *Galium spurium* seed due to the intensity and duration of light exposure. Thus, light inhibited germination on Petri dishes in *Cynara syriaca* and 'Camus' in the work of Basnizki and Mayer (1985), whereas germination was optimal in the darkness in *C. syriaca*. On the other hand, on the full-strength MS medium, light allowed us to record the highest germination percentage in the four argan genotypes (Fig. 13). This is also revealed by Bonnewell and Pratt Koukkari (1983) for *in vitro* germination of *Typha latifolia* and by Mairone and Geslot (1987) for the germination of *Jasminum fruticans*, which required, along with high temperature and low O_2, a long exposure to light to achieve high germination percentages.

Generally, there is a relation between light and the culture medium used. Indeed, the interaction of these two factors is probably explained by the reaction of the ionic concentration of the medium used to the light or by the genetic factor and the nutritional requirements of the species studied. On the other hand, Ighrem was the most active and fastest genotype regardless of the factor studied (Fig. 13). This could be due to the recent harvesting of its seeds, their maturity, their viability, their genetic resources or even their provenance (characterized by an average altitude of 1700 m). In this sense, it has been shown that higher the germination rate, higher is the viability of the recently harvested seeds (Zahidi and Fouzia 1997; Alouani and Bani-Aameur 2004). On the other hand, when comparing the growing conditions, *In vitro* culture was the most improving condition for all genotypes compared to culture on petri-dishes or in the greenhouse (Fig. 6–7). In this context, Mezghenni *et al*. (2014) using four argan genotypes concluded that the germination rate *in vitro* is higher than that obtained in soil. In our case, the latency phase of the germinated seeds *ex vitro* is long, compared to that of *in vitro* which was characterized by a shorter latency time (Fig. 3). These finding corroborate with the findings of Brhadda *et al*. (2020) in two genotypes of *Lycopersicum esculentum* namely Campell 33 and Rio Grande, who showed that *in vitro* and petri dishes culture also showed better germination compared to greenhouse culture.

Conclusion

Germination and rooting of four Moroccan argan genotypes were evaluated and improved in the order: Bouizakarne, Agadir, Admine and Ighrem under different factors. The pulping of seeds improved the germination of argan seeds under greenhouse conditions compared to other treatments. *In vitro* culture was the most notable condition compared to the petri dishes and greenhouse, in which ½ strength MS medium showed the best germination and rooting in all genotypes. Light had no great influence on the germination and growth of argan genotypes, while ½ strength MS medium under darkness had positive influence on argan seed germination. Among the genotypes, Ighrem recorded the highest percentage of germination and rooting. The establishment of *in vitro* protocols is a pragmatic strategy for achieving satisfactory and rapid germination.

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Author Contributions

All authors have read and agreed to the published version of this manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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