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Full Length Article

Improvement of Germination, Rooting and Elongation Capacities *In Vitro* of Four Argan (*Argania spinosa*) Genotypes under the Effects of pH and Activated Carbon

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Abstract

Argan tree (*Argania spinosa* (L.) Skeels), an important species in southwestern Morocco, is the mainstay of socio-economic development in Morocco. However, difficult climatic conditions, overgrazing, excessive harvesting of seeds for oil extraction and above all, embryonic and integumentary dormancy of the seeds have led to the absence of natural regeneration of this species. In this work, the application of recent biotechnological tools for the conservation of this critically endangered species is outlined. The aim of our work was to evaluate and improve germination, rooting and growth capacities of four argan genotypes, namely Bouizakarne, Agadir, Admine and Ighrem, under the control of activated carbon and pH via *in vitro* culture. The results showed that the germination in the presence of 5 $g.L^{-1}$ of activated carbon was more important and faster in ½ MS medium (92.99%). In addition, the pH between 5 and 7 was more favorable to the germination which varied between 71.87 and 81%. For *in vitro* plant development, the highest levels were obtained at ½ MS in the presence of activated carbon (58.73% of rooted seedlings with main roots of 16.29 cm, dense lateral roots, stems of 5.95 cm and leaf number of 6.68) and at pH 4, 5, 5.8 and 10, which showed a rooting rate ranging from 43.18 to 60.98%, a main roots length, with secondary roots, between 14.35 and 15.99 cm, a stems length estimated between 4.68 and 5.67 cm and leaf number ranging from 5.59 to 8.12. However, pH 7 inhibited rooting and aerial growth of Ighrem and Bouizakarne genotypes. Similarly, the Admine genotype development reacted negatively with activated carbon. A high percentage of acclimatization is of great importance for a possible propagation of this endangered species. As our protocols have solved the major problem of the argan tree by optimizing its germination and rooting, they could be recommended for the regeneration of this plant.

Keywords: Argan tree; Germination; Development; *In vitro* culture; Activated carbon; pH

Introduction

The argan tree (*Argania spinosa* (L.) Skeels), from the Sapotaceae family endemic to the arid and semi-arid regions of southwest Morocco, plays an essential role whether on an economic, medicinal or environmental level. This species is a unique example of a multi-purpose fruit-bearing woody species in Morocco *i.e.*, production of quality oil, fodder, fuel (M'hirit *et al*. 1998). Indeed, due to the oil extracted from its kernels and its foliage, which feed the herds of goats and sheep, 90% of the local population's income depends on an agroforestry system based on this tree (Majourhat *et al.* 2007). Its main interest lies in the medicinal and nutritional value of its kernels, which give a precious oil that is the best-known production of the argan tree. Due to its richness in nutritional compounds with antioxidant activities, argan oil could have an antiproliferative action against some cancers. It is also receiving a lot of attention as a preventive nutritional approach to prevent cardiovascular risk and to fight against rheumatic and articular pain (Chenuet 2007). In addition, this Moroccan species ensures a more profitable agriculture by fighting against wind and water erosion while maintaining soil moisture and fertility which are effective against desertification (Mezghenni *et al*. 2014; Badaouini 2015). Its biological and ecological interest therefore lies in its remarkable adaptation to very constraining climatic

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conditions characterized by drought and soil poverty due to its great genetic variability (M'hirit *et al*. 1998; El Mandouri *et al*. 2020). With all its interests, UNESCO classified the Moroccan argan tree as a Biosphere Reserve in 1999. In addition, the production of the argan tree, next to the Arab country has attracted the curiosity of several countries Mexico, China, France. Similarly, several studies have investigated the possibility of domesticating *Argania spinosa* in the desert in Israel in order to take advantage of this species (Nerd *et al*. 1994).

The ecological and medicinal properties of this tree therefore contribute to an economic development in Morocco and worldwide. However, the populations of argan trees 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). As most woody plants, the argan tree can be reproduced by sowing seeds. However, due to the loss of their germination power, notably linked to the problems of drought, grazing by livestock, excessive harvesting of seeds for oil extraction, and above all the embryonic and integumentary dormancy of argan seeds, several studies, unfortunately, have shown that its seeds are difficult to germinate in their natural state (Kéchaïri and Abdoun 2013; Mezghenni *et al*. 2014). This state highlights an urgent intervention to preserve the argan tree plantation by launching massive reforestation programs based on conventional techniques by sexual and asexual propagation by cuttings and grafting (Nouaim *et al*. 2002). However, due to the poor root quality of the seedlings produced, these techniques are unable to meet the high demand and overcome the difficulties of establishment in the field. In this sense, *in vitro* cultivation could be a very interesting alternative to the traditional propagation of the argan tree in order to maintain its biological, medicinal, ecological and economic values.

Argan tree seeds are characterized by embryonic and tegmental dormancy, which causes a significant loss of seed viability. Successful germination and development of the argan tree via *in vitro* culture is influenced by several factors, namely the explant (Mezghenni *et al*. 2014) and (El Mandouri *et al*. 2020), the genotype (Mezghenni *et al*. 2014), the composition of the culture medium (Mezghenni *et al*. 2014) and the effect of activated carbon (Khelifi *et al.* 1996; Bousselmame *et al*. 2001). However, the influence of water potential on the germination and growth of argan trees has not received any attention.

Although argan tree is known for its positive effect in the adsorption of polyphenols and products of their synthesis inhibiting root growth, activated carbon is also little used to overcome dormancy and rooting of argan trees. The objective of this work is to study for the first time the capacity of germination and development of four argan genotypes of Moroccan origin, namely Bouizakarne, Agadir, Admine and Ighrem under the control of activated carbon and pH. In this case, different culture media namely MS and ½ MS containing activated carbon and different pH values are needed to optimize the germination, rooting and elongation of the seedlings of our argan genotypes.

Materials and Methods

Plant material

During their period of optimal morphological and physiological maturity (from mid-June to July), the fallen argan fruits are collected from under the trees, then air-dried and preserved until the time of sowing. Four argan genotypes of Moroccan origin were tested *in vitro* in this work, namely Bouisakarne, Agadir and two genotypes from the province of Taroudant, specifically Admine, lowland area and Ighrem, mountain area. Since argan seeds never germinate *in vitro* (Khelifi *et al*. 1996), it was essential to crush the seeds in order to bring out the kernels to develop efficient *in vitro* germination protocols (Fig. 1).

Disinfection of the kernels

The kernels were disinfected, under a laminar flow hood, by soaking for 1 min in ethanol at 70°C followed by 15 min in sodium hypochlorite at 12°C titrant. Then, the kernels were rinsed 3 to 4 times with sterile distilled water and dried on sterile filter paper before sowing. The seed count was carried out daily while observing the emergence of the radicle, considered as an indicator of germination.

Effect of activated carbon

Very well known for its positive effect in the adsorption of polyphenols and products of their synthesis inhibiting root growth, activated carbon at a concentration of 5 $g.L^{-1}$ was tested in order to verify its effect on the optimization of germination, rooting and elongation of argan trees. Activated carbon was added to two culture media: MS medium and ½ MS medium (Murashige and Skoog 1962) where the macronutrients of the MS medium were diluted by half. Both media are supplemented with 30 $g.L^{-1}$ sucrose, 1$g.mL^{-1}$ thiamine and solidified with 10 $g.L^{-1}$ agar. Before adding 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 characterized by a temperature of 25 °C +/-1°C and a photoperiod of 16 h light/8 h darkness.

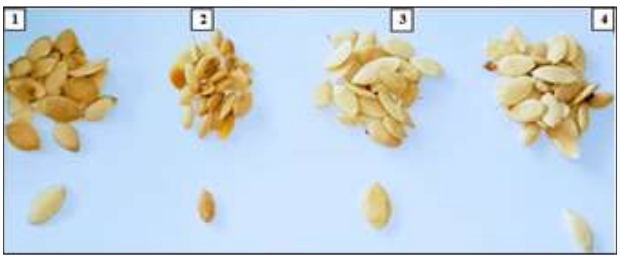

Fig. 1: Kernels of the four argan genotypes: 1, Bouizakarne; 2, Agadir; 3, Admine; 4 Ighrem

Effect of pH

Many factors influence the germination and seedling establishment processes. The potential hydrogen (pH) is one of these factors. However, this influence of pH on germination has received little attention in horticulture. In this sense, we tested the *in vitro* germination and development of argan kernels on ½ MS culture medium adjusted to five pH values (4, 5, 5.8, 7 and 10), with the aim of identifying the effect of pH on the germination capacity of argan kernels, the rooting and the elongation of *in vitro* plants.

After 8 weeks of cultivation, the rooted in vitro plants were transferred to pots filled with growing medium (½ sand and ½ peat) for acclimatization in a greenhouse (temperature: 25°C and natural light) and watered with Hoagland's solution (Hoagland and Snyder 1933).

Statistical data analysis

Design of the experiments was completely randomized with three replications. Data were analyzed using SPSS software 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

Effect of activated carbon on argan germination

Effect of the addition of activated carbon: After adding activated carbon at 5 $g.L^{-1}$ in both MS and ½ MS media, the analysis of variance showed significantly that the addition of activated carbon did improve the germination of argan kernels. Indeed, the activated carbon allowed to obtain a better germination rate of up to 87.6% in comparison with the medium without activated carbon (70.64%) (Fig. 2a). Similarly, the presence of activated carbon accelerated the germination of argan kernels. It is only on the 3rd day that the kernels started to germinate and after 12 days, the maximum germination rate stabilized compared to the medium without activated carbon (Fig. 2b).

Effect of genotype in the presence of activated carbon: All genotypes reacted positively to activated carbon. However, the germination percentage varied significantly among genotypes; being the highest in Agadir (98.99%) followed by Ighrem and Admine (89.6 and 86.99%, respectively). The Bouizakarne genotype showed the lowest level of germination (Fig. 3a). The addition of activated carbon accelerated germination for Admine and Ighrem while it showed a long lag phase of about 6 and 8 days for Agadir and Bouizakarne, respectively (Fig. 3b).

Effect of medium in the presence of activated carbon: The results showed very different reactions of germinating kernels in the presence of activated carbon depending on the basic medium used. Germination was more progressive and faster in the presence of activated carbon on the ½ MS medium compared to the MS medium to reach the optimal germination rate (92.99%) on day 16 (Fig. 4a–b).

Interaction of pH × genotype × activated carbon: The germination percentage varied differently according to the genotype and the medium used in the presence of activated carbon. Maximum germination was observed on ½ MS medium in all genotypes. For Agadir, difference was shown between the MS and ½ MS media wherein germination rate was always 98.99% (Fig. 5a). Similarly, germination curve throughout 36 days varied according to the genotype and the medium used. In the presence of activated carbon, the ½ MS medium showed a short latent phase especially for the Ighrem and Agadir genotypes (2 days). However, the argan kernels germinated less quickly in the presence of activated carbon on MS medium with a long lag phase of up to 8 and 12 days in all genotypes except the Ighrem genotype (4 days). The lag phase, whether short or long, the presence of activated carbon produced always a short exponential phase (10 days maximum) equilibrating at very satisfactory rates in all genotypes (Fig. 5b–c).

Effect of pH on argan germination

Effect of pH: The number of germinated kernels was significantly affected by the pH of the medium. Indeed, pH 5.8 recorded the highest germination (80.99%) followed by pH 5 (77.53%) and pH 7 (71.87%). However, the lowest germination was noted at pH 4 and 10 with 67.8 and 67.48%, respectively (Fig. 6a). In general, the lowest and highest pH levels were less favorable to argan germination. Moreover, all pH values showed a short lag phase of 3 days except pH 10 which allowed the germination of the kernels on the 5th day. In addition, at pH 7 the kernels germinated later in the first days than at other pH values, especially at pH 5 which was the fastest pH value (Fig. 6b).

Genotype effect: Maximum germination was observed in the Ighrem genotype. The other genotypes Agadir, Admine and Bouizakarne recorded the lowest germination levels (Fig. 7a). In addition, all genotypes responded positively and rapidly with all pH values studied. Indeed, the kernels germinated on day 5 for the Bouizakarne and Admine genotypes. The other two genotypes, Agadir and Ighrem, were quicker (4th day) with a more remarkable reactivity in Ighrem (Fig. 7b).

Genotype × pH interaction: Results showed different reactions in the behavior of the kernels depending on the pH of the medium and the genotype used. The latter affected the germination rate. Indeed, at any pH value, the highest level was observed in Ighrem with a germination percentage between 90 and 98.99%. However, the lowest germination in Admine were found at pH 7 and 10 with 62.5 and 50%, respectively. For the genotypes Bouizakarne and Agadir, the lowest germination rates were recorded at pH 4 with 50 and 55.55%, respectively (Fig. 8a). Data recorded over time

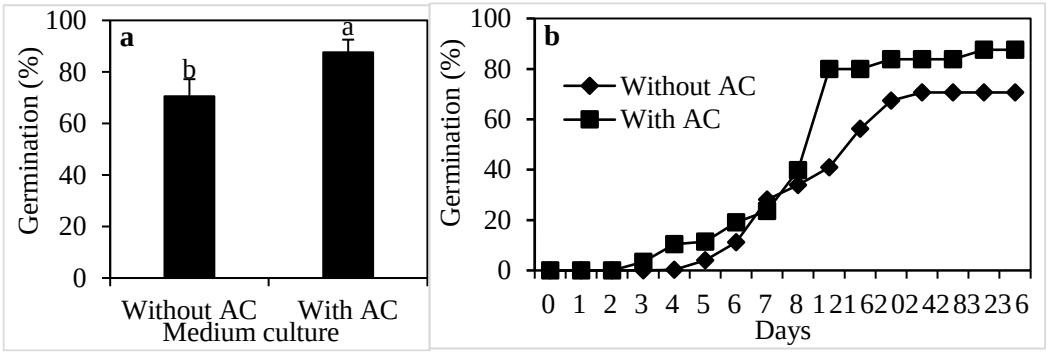

Fig. 2: Effect of activated carbon (AC) on the *in vitro* germination of argan trees (a) and evolution of *in vitro* germination of argan trees in the presence of activated carbon (b)

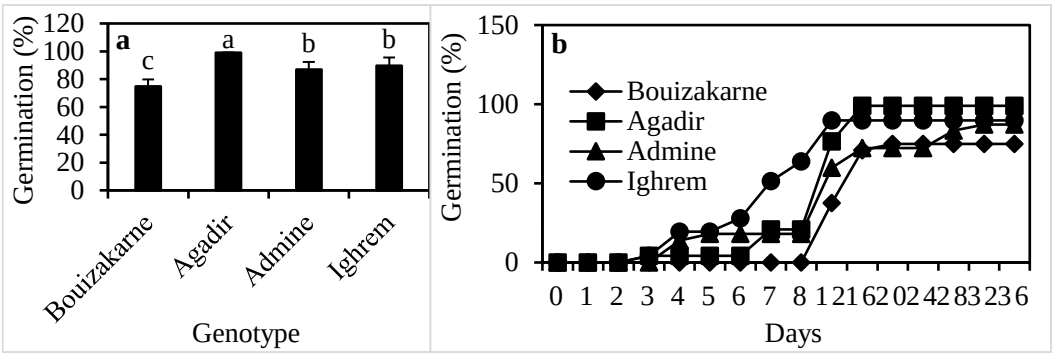

Fig. 3: Effect of genotype on *in vitro* germination in the presence of activated carbon (a) and evolution of *in vitro* germination of four argan genotypes in the presence of activated carbon (b)

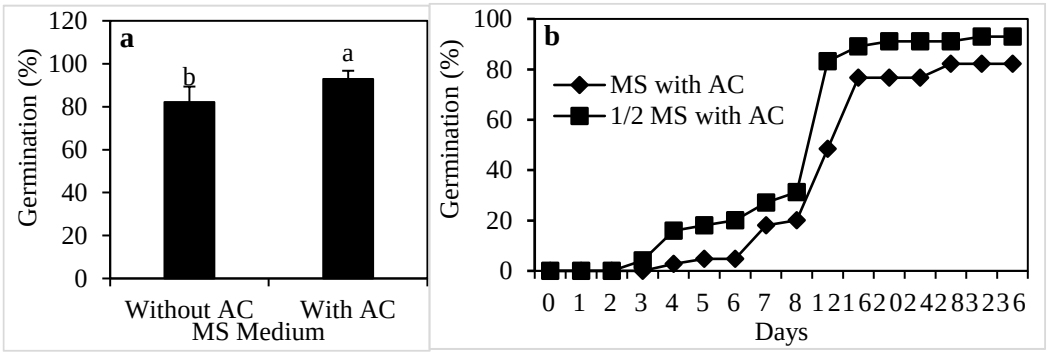

Fig. 4: Effect of MS and ½ MS medium with activated carbon on argan germination (a) and evaluation of *in vitro* germination of argan tree on MS and ½ MS media with activated carbon (b)

showed that Ighrem was the fastest genotype to germinate at all five pH values studied. Indeed, it was only on day 4 at pH (5, 5.8 and 7) and on day 5 at pH (4 and 10) that the kernels germinated and it was only after 10 days that they reached the maximum germination rate. On the other hand, despite showing a low germination rate, pH 4 and 10 recorded a short lag time of about 4 days in the Admine and Ighrem genotypes. However, pH 7 showed in all genotypes a lag phase of up to 7 days with the exception of the Ighrem genotype. Similarly, the germination time of the kernels of the Agadir and Admine genotypes was spread out until the 8th day at pH 5.8. However, pH 5 showed a long latency phase of about 7 days in the Bouizakarne genotype (Fig. 8b1–b4).

Effect of activated carbon on rooting and quality of vitroplants

Effect of the medium with activated carbon: Activated carbon added to the culture medium stimulated the rooting and development of the vitroplants. However, this effect varied according to the culture medium studied. Indeed, the

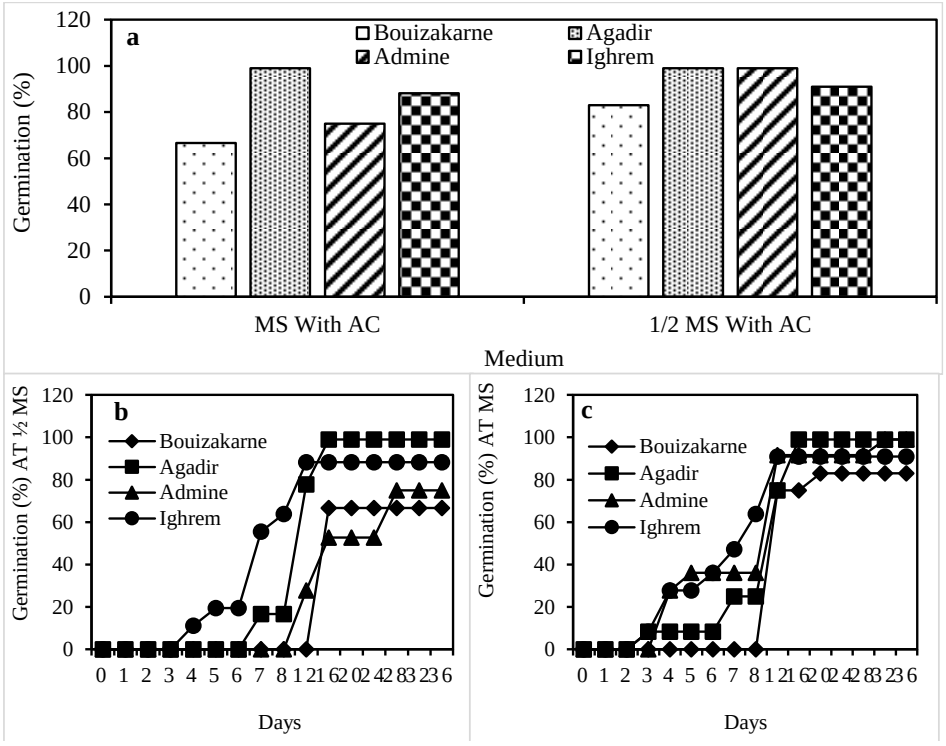

Fig. 5: Effect of MS and ½ MS medium with activated carbon on germination of four argan genotypes as a function of time on MS with activated carbon (a) and ½ MS with activated carbon (b)

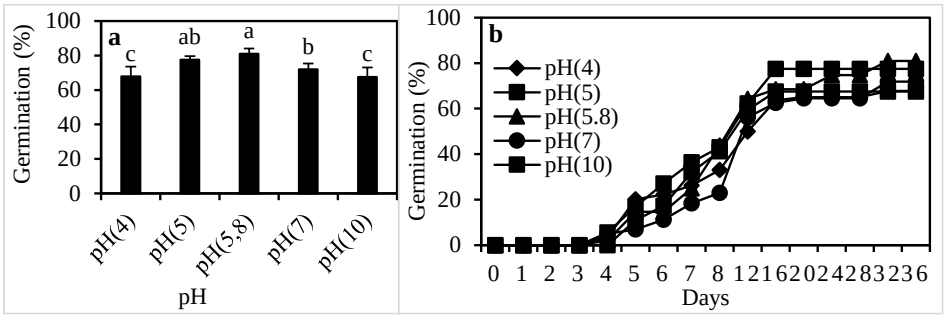

Fig. 6: Effect of pH on argan germination *in vitro* germination of argan tree on ½ MS medium adjusted to five pH values (a) and as a function of time (b)

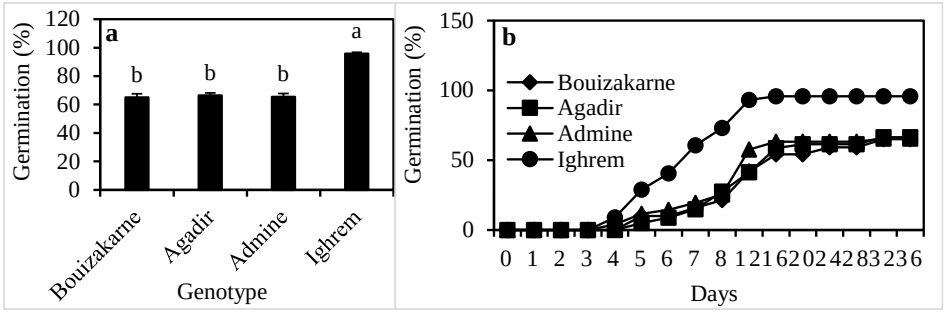

Fig. 7: Effect of argan genotype under pH control (a) and evaluation of *in vitro* germination under pH control as a function of time (days) in four argan genotypes (b)

½ MS medium was considered the most notable medium compared to the MS medium in the presence of activated carbon. It showed the highest rooting with 58.73% of the *in vitro* plants rooted. In addition, ½ MS medium resulted in the longest main root (16.29 cm), the densest secondary root, the longest stem (5.95 cm) and the highest number of leaves

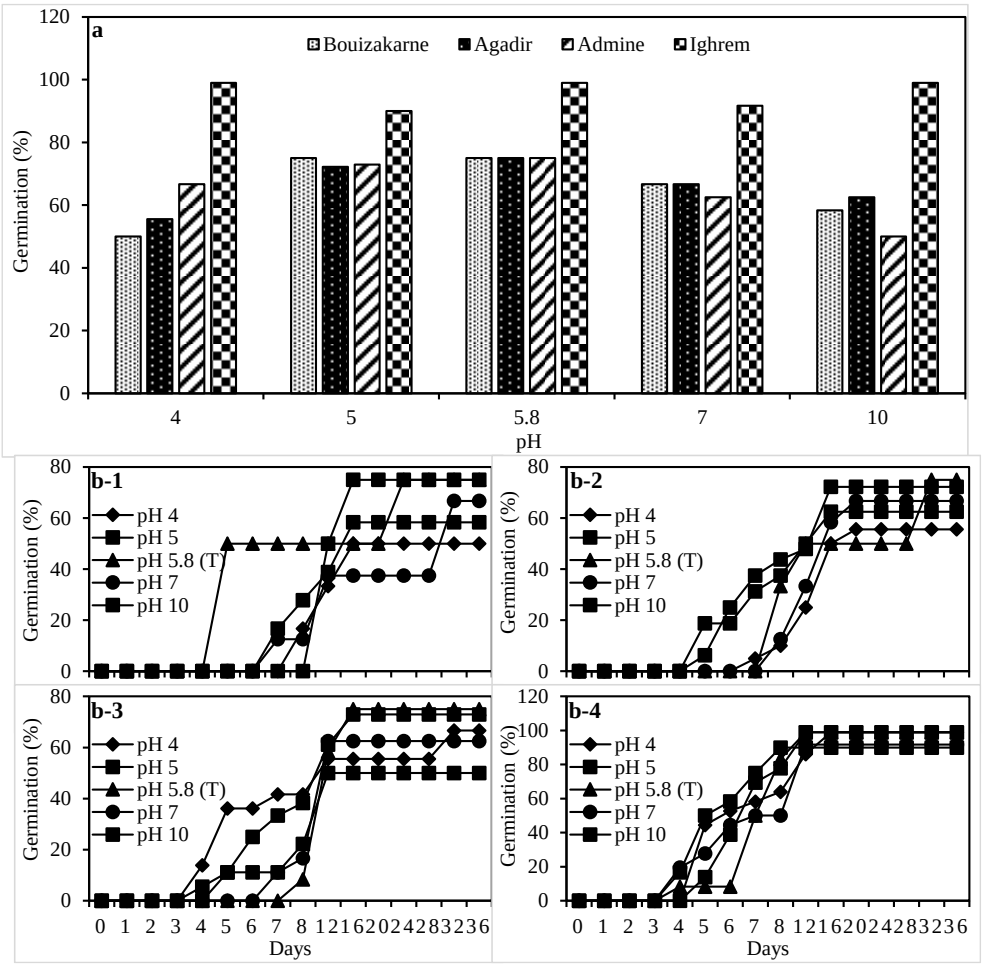

Fig. 8: Germination capacity of the four argan genotypes Bouizakarne, Agadir, Admine and Ighrem on ½ MS medium added to the five values of pH (a); evaluation of *in vitro* germination of the four argan genotypes as a function of time on ½ MS medium adjusted to five pH values: (4, 5, 5.8, 7 and 10): b-1, Bouizakarne; b-2, Agadir; b-3, Admine and b-4, Ighrem (b)

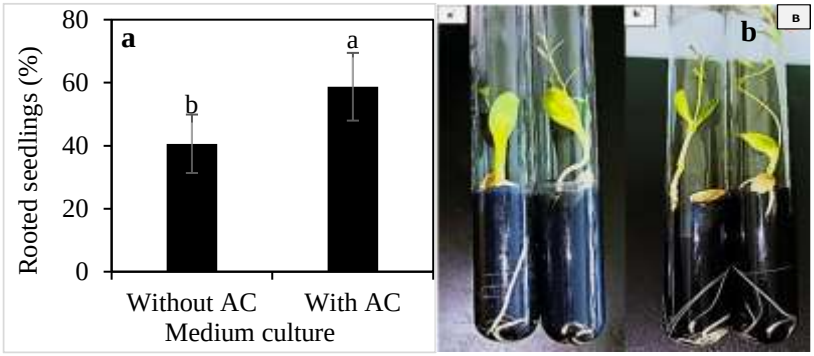

Fig. 9: a: Effect of medium with activated carbon on the rooting rate in the argan tree; b: Picture showing the effect of medium with activated carbon on rooting in argan tree: a: MS with carbon; b :1/2 MS with carbon

(6.68) compared to MS medium (Fig. 9a–b; Table 1).

Effect of genotype: The analysis of the results showed that the rooting and development of *in vitro* plants in the presence of activated carbon were significantly different among the genotype. In fact, the longest roots were recorded in the Ighrem genotype with a 68.88% of the *in vitro* rooted plants, with main root length of 17.75 cm and prolific secondary roots. In contrast, the Admine genotype showed the least rooting (21.6% of *in vitro* plants rooted and main roots of 12.58 cm). The other two genotypes Bouizakarne and Agadir

Table 1: Effect of activated carbon on rooting and growth of *in vitro* plants of the four argan genotypes

pH	Average length of main roots (cm)					Average appearance of secondary root (cm)				
	4	5	5.8	7	10	4	5	5,8	7	10
Bouizakarne	14.83a	15.16b	14.66b	0b	13b	+(denses)	+	+	-	-
Agadir	14.5a	15b	15.5b	14a	14.57ab	+	+	+(denses)	+(denses)	-
Admine	14.25a	16.5ab	14.83b	15.25a	14.33ab	+	+(denses)	+	+(denses)	-
Ighrem	16.2a	17.25a	18a	0b	15.5a	+	+(denses)	+(denses)	-	-
Mean	14.94±1.15a	15.99±1.3a	15.74±1.63a	7.31±7.67b	14.35±1.26a	+	+	+	+	-

showed an average rooting level with 52.67 and 55.55% of *in vitro* plants rooted and with main roots of 14.86 and 15.47 cm, respectively (Fig. 10a–b). On the other hand, the elongation of the *in vitro* plants and the number of leaves were higher in the Ighrem and Agadir genotypes with a stem length of 6.33 and 5.35 cm and a number of leaves of 6.74 and 7.04, respectively. Admine genotype also showed the lowest aerial growth *i.e*., 2.83 cm stem length and three leaves (Fig. 10c).

Genotype × activated carbon interaction: The results showed that the rooting and subsequent development of the *in vitro* plants were affected by the activated carbon added to the culture medium. The activated carbon added to ½ MS medium improved rooting and development of *in vitro* plants in all genotypes compared to MS medium. For the two genotypes Bouizakarne and Agadir, the addition of activated carbon to ½ MS medium increased the percentage of rooted *in vitro* plants by 62.5 and 66.66%, respectively. Furthermore, it stimulated the length of the main roots, the presence of dense adventitious roots, the length of the stems and the number of leaves in these two genotypes. However, activated carbon strongly stimulated the percentage of rooted *in vitro* plants of the Ighrem genotype on ½ MS medium with 75%, the length of roots, which amounted to 18.85 cm, the presence of lateral roots, the length of the stem which amounted to 6.83 cm and the number of leaves which was 7.33. Ighrem was the most active genotype regardless of the medium used. In contrast, activated carbon had a different effect on the rate of rooted *in vitro* plants and their subsequent development in the Admine genotype depending on the medium used. Activated carbon added to MS and ½ MS media recorded the lowest rate of rooted seedlings (15.2 and 28%, respectively), due to a blockage of kernels at rooting. Adding activated carbon to MS medium inhibited the root length (10.5 cm) as well as the growth of the aerial part (no stems or leaves appeared); while on ½ MS medium, activated carbon was notable for aerial growth (with an average stem of 5.66 cm and number of leaves of 6), for root length (14.66 cm) and especially for the presence of dense secondary roots (Fig. 11; Table 1).

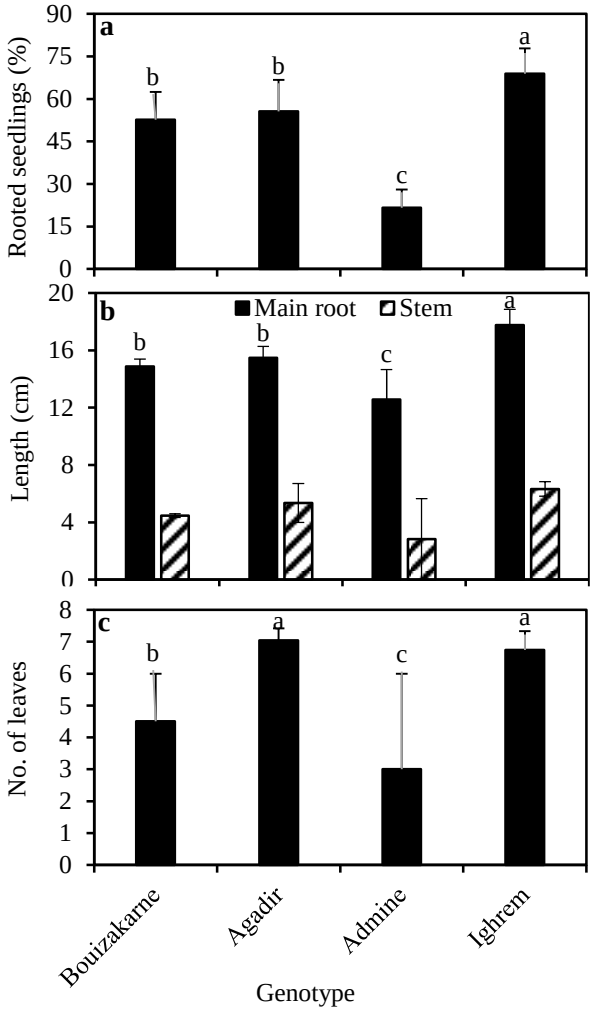

Fig. 10: a: Effect of argan genotype on rooting under the control of activated carbon; b: Effect of argan genotype on main root length and stem length under activated carbon control; c: Effect of argan genotype on leaf number under activated carbon control

Effect of pH on rooting and *in vitro* plant quality

Effect of pH: The effect of pH was significant on the rooting and development of *in vitro* plants. Indeed, as it recorded the highest level of germination, pH 5.8 also showed the highest rooting rate (60.98%) followed by pH 5 and 10 with 54.18 and 47.09%, respectively. Conversely, the lowest rooting percentage was revealed by pH 7 with 24.99%, while pH 4 showed an average rooting i.e., 43.18%. (Fig. 12). Furthermore, pH 7 negatively affected the further development of the *in vitro* plants. Indeed, it showed the shortest main root of 7.31 cm, the shortest stem of 2.18 cm and the lowest average number of leaves (2.87). The pH 4, 5, 5.8 and 10 showed no significant difference on root length and stem elongation. However, pH 4 followed by pH 5 showed the

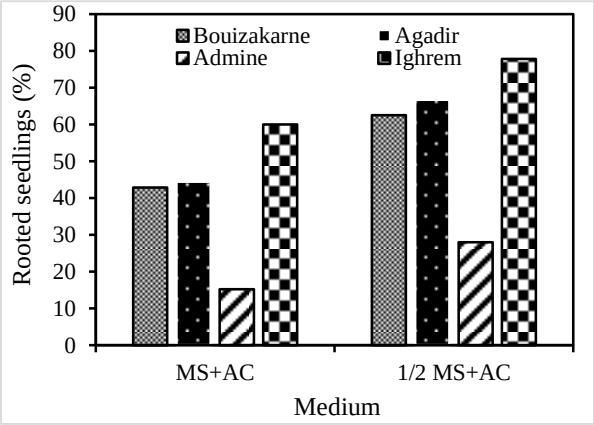

Fig. 11: Medium × argan genotype interaction on the rooting rate under the control of activated carbon

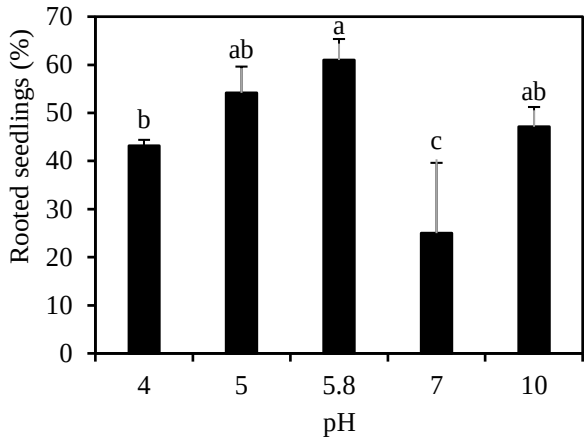

Fig. 12: Effect of pH on the rooting rate in the argan tree

showed no lateral roots (Table 2 and 3).

Effect of genotype: Data showed that rooting and development of *in vitro* plants were significant in the genotype studied. Indeed, the lowest level of rooting was found in the Bouizakarne genotype with a low percentage of rooted *in vitro* plants (37.47%) and a low length of main roots (11.53 cm). On the other hand, the Agadir genotype recorded the highest rooting rate with 53.62% and the highest length of main roots with 14.71 cm. However, this genotype also showed the highest level of aerial growth (6 cm stem length and 7.17 leaf number) compared to the other genotypes (Fig 13a–c).

pH × genotype interaction: Effect of pH on rooting percentage, root length, lateral root emergence, stem length and number of leaves was significant depending on the argan genotype studied. Firstly, pH 5.8 improved the rate of rooted *in vitro* plants in all genotypes. Indeed, the highest level of rooting was recorded in the Ighrem genotype with 71.42% of rooted *in vitro* plants, dense lateral roots and long main roots of 18 cm. In addition, stem elongation and leaf number were not different among genotypes. The pH 5 positively affected the length of main roots in all genotypes as well as the presence of dense lateral roots in Ighrem and Admine genotypes. However, it showed a low rate of rooted seedlings in the Admine genotype (40%) with short stems (4 cm) and low number of leaves (4.25). While the Agadir genotype showed the highest level of aerial growth. On the other hand, the pH 7 was inadequate for rooting and development of the *in vitro* plants in both Bouizakarne and Ighrem genotypes. Indeed, no rooting (neither true root nor secondary root) and no aerial growth (neither stem nor leaves) were observed. However, this pH stimulated rooting, elongation and leaf number of the *in vitro* plants of the other genotypes Agadir and Admine. Additionally, it appeared that negative effect of

Table 2: Effect of pH of the medium ½ MS on the rooting of *in vitro* plants of the four argan genotypes

pH	Average stem length (cm)					Average number of leaves				
	4	5	5.8	7	10	4	5	5.8	7	10
Bouizakarne	6a	4.5bc	4.66a	0b	4b	7.16bc	6.83b	6.33a	0b	5.5a
Agadir	7a	8a	4.5a	4.5a	6a	8.75ab	9.6a	5.5a	6a	6a
Admine	3.5b	4c	5a	4.25a	4b	5.5c	4.25c	5.83a	5a	5a
Ighrem	6.2a	6b	6a	0b	4.75ab	9.2a	7.5b	7.42a	0b	6.5a
Mean	5.67±1.61a	5.62±1.83a	5.04±1.04a	2.18±2.36b	4.68±1.2a	8.12±1.74a	7.04±216ab	6.27±1.14b	2.75±2.95c	5.59±1.03b

Table 3: Effect of pH on aerial growth of *in vitro* plants of the four argan genotypes

Genotypes	Average length of main roots (cm)		Appearance of secondary root (cm)		Average stem length (cm)		Average number of leaves	
	MS+AC	½ MS+AC	MS+AC	½ MS+AC	MS+AC	½ MS+AC	MS+AC	½ MS+AC
Bouizakarne	14.33b	15.4b	+	+(dense)	4.33ab	4.6b	3b	6a
Agadir	14.66b	16.28b	+	+(dense)	4b	6.71a	6.66a	7.42a
Admine	10.5c	14.66b	+	+	0c	5.66ab	0c	6a
Ighrem	16.66a	18.85a	+	+(dense)	5.83a	6.83a	6.16a	7.33a
Mean	14.03±2.57a	16.29±1.82b	-	-	3.54±2.49b	5.95±1.04a	3.95±3.09b	6.68±0.79a

highest average leaf number (8.12 and 7.04, respectively). In addition, all pH values allowed the emergence of secondary roots with a high density for the exception of pH 10, which low and high pH values was more remarkable on germination than on rooting and subsequent growth of *in vitro* plants. Although pH 4 and 10 showed the lowest germination, they

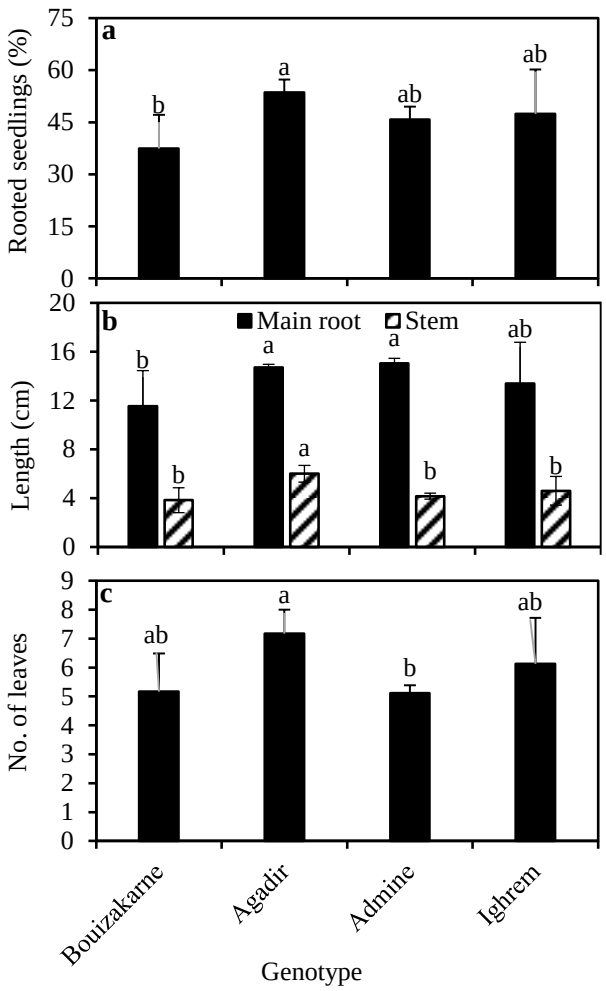

Fig. 13: Effect of pH on argan genotype rooting. Control (a) and effect of argan genotype on root and stem length under pH control; c: Effect of argan genotype on leaf number under pH control (b)

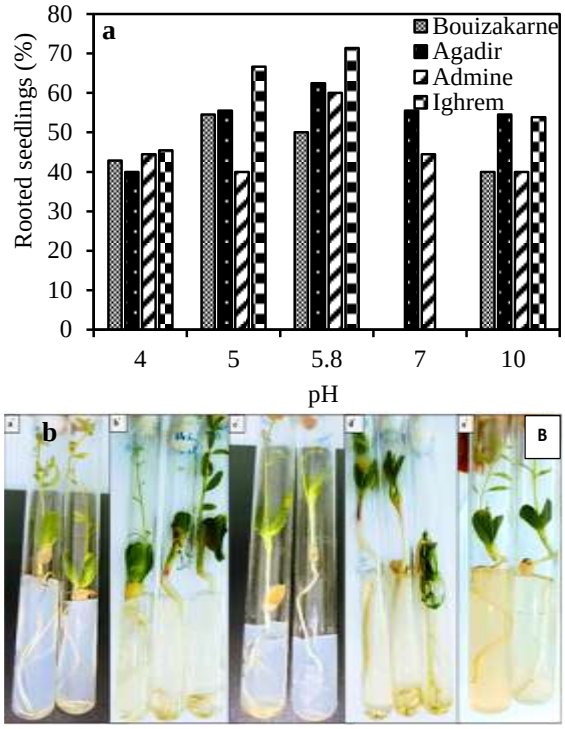

Fig. 14: a: pH × genotype interaction on rooting rate under pH control; b: Effect of pH values on the rooting and quality of argan seedlings after 8 weeks of culture: a': pH 4; b': pH 5; c': pH 5,8; d': pH 7; e': pH 10

Fig. 15: Acclimatization of rooted argan in vitro plants after 8 weeks

positively affected rooting (ranging from 40 to 54.54% of the rooted *in vitro* plants with roots of 13 to 16.2 cm in length) and the growth of the above-ground part (stems of 3.5 to 7 cm and number of leaves of 5 to 9.2) of the *in vitro* plants in all the genotypes (Fig. 14a–b; Table 2–3).

Acclimatization of *in vitro* rooted plants was done immediately in the greenhouse, after carefully removing the very fragile seedlings from the culture tubes and removing the agar adhering to the roots under a very weak water current, at the rate of one plantlet per pot. Under the optimal conditions of germination and development of the argan tree that were determined, we obtained a recovery rate of 90.3% of the acclimatized seedlings (Fig. 15).

Discussion

The germination and development of the argan tree *in vitro* was significantly different under the control of activated carbon and pH. Data showed that in vitro culture of the argan tree in the presence of activated carbon was possible and effective. Indeed, the presence of activated carbon positively affected the germination rate and time of our four argan genotypes (Fig. 2). This could be explained by its capacity to reduce the chemical substances inhibiting morphogenesis due to its effect exerted by the adsorption of the toxic substances produced and released by the explant in the culture medium (Benderradji *et al*. 2007). Furthermore, the

addition of activated carbon stimulated the rooting and growth of *in vitro* plants in Ighrem, Agadir and Bouizakarne genotypes, especially on ½ MS medium. This favorable effect of activated carbon on root growth by eliminating the inhibitory effect of high concentrations of auxins was also explained by the work of Bu and Chen (1988) who approved that 1 mg of activated carbon can absorb 100 μg of NAA. However, Damiano (1980) and Bettaieb *et al*. (2007) found that in the presence of 1–2 $g.L^{-1}$ of activated carbon, general rhizogenesis of shoots grown *in vitro* was observed, reducing the time required for root emission and accelerating their growth. Bousselmame *et al*. (2001) found that 12% of the cuttings cultured with secondary roots of an average length of 10 cm in the presence of 5 $g.L^{-1}$ of activated carbon. Its adsorbing effect of growth regulators inhibiting root growth is also affirmed with increasing rooting rate and duration of the rooting phase (Maene and Debergh 1985; Desjardins *et al*. 1987; Gübbük and Pekmezci 2006). Furthermore, the stimulating effect of activated carbon on rooting and development of *in vitro* plants was greater with ½ MS medium than with MS medium (Fig. 4–9). This could be explained by the low ionic concentration of the medium which allows the stimulation of buds as well as root formation, and these findings agree with previous reports (Kulchetschi *et al*. 1995; Brhadda *et al*. 2003). Similarly, Benderradji *et al*. (2007) reported that ½ MS medium is often advantageous to induce rhizogenesis. However, Khelifi *et al*. (1996) unfortunately could not explain the negative effect of activated carbon. Indeed, they found that the addition of activated carbon to the culture medium proved unfavorable for the germination of argan embryos as well as causing very marked cotyledonary deformations and vitrification of the leaflets leading to the death of the seedling in most cases. We also obtained this inhibitory effect of activated carbon in the Admine genotype, which had poor rooting on both MS and ½ MS media and no aerial growth on MS media (Fig. 10; Table 1). In general, it seems that there is a relation between the activated carbon, the genotype studied, and the culture medium used. Indeed, the interaction of these three factors is probably explained by the reaction of the activated carbon to the genetic factor and the nutritional requirements of the genotype studied or by the ionic concentration of the medium used or even by the concentration of activated carbon adopted.

Concerning the effect of pH, a highest germination percentage in argan genotypes was recorded at pH between 5 and 7. However, the lowest and highest pH values showed the lowest germination levels in argan (Fig. 6). This pH range (pH 5 and 7) was also recommended by Carlson and Rowley (1980) for bedding plant production. Bailey and Hammer (1986) showed that germination of tomato and petunia (*Petunia* × hybrids) was not affected when their seeds were irrigated with water at various acidities. On the other hand, the rooting and development of the *in vitro* plants of our four argan genotypes were not prevented by the acidity or even the basicity of the culture medium (Fig. 13; Table 2–3). However, Comtois *et al*. (2004) and Lamhamedi *et al*. (2006) reported that alkaline pH can adversely affect the availability and uptake of mineral elements in the rhizosphere, especially microelements. According to Deccetti *et al*. (2005), the reduction of pH from 7.0 to 5.0 allowed the best response to root formation of *A. glabra* L. by increasing the rooting percentage and the average number of roots produced per tigelle. Harbage *et al*. (1998) found that root formation in apple cuttings was stimulated only when the pH of the rooting medium was reduced from 7.0 to 4.0. Indeed, the latter work may reinforce the inhibitory effect of pH 7 on germination and root formation in our certain argan genotypes. Youmbi *et al*. (1998) found that *in vitro* germination of *Dacryodes edulis (Burseraceae)* pollen decreased considerably and became minimal (15%) at pH 7. This could be due to the inhibition of the synthesis or action of a certain enzyme necessary for germination resulting from the neutrality of the medium. On the other hand, the stimulating effect of pH is also observed on the appearance of dense secondary roots in all four genotypes in majority of the cases. Similarly, Harbage *et al*. (1993) also showed the effect of pH on the induction phase of lateral root formation. However, this effect is not limited to the formation of lateral roots but also influences their growth.

Conclusion

The addition of activated carbon to the ½ MS medium significantly improved the germination and development of *in vitro* grown plants of the argan genotypes except Admine genotype. The effect of pH on germination and development of *in vitro* plants was significantly different among the genotype, while pH 5 and 5.8 showed the highest germination and development of all genotypes. Rooting and aerial growth of the seedlings in the Ighrem and Bouizakarne genotypes were inhibited at pH 7. However, the lowest and highest pH values retarded the germination and development of some genotypes. Concerning the acclimatization of the rooted *in vitro* plants, a normal growth was observed with a high survival rate (~100%). The protocols adopted for *in vitro* culture were effective in the genetic improvement, possible introduction and implantation program of the argan tree.

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Author Contributions

All authors have contributed equally to this work and give consent to publish the work.

Conflict of Interest

The Authors declare that there is no conflict of interests that could possibly arise.

Data Availability

The data presented in this study will be available on request from the corresponding author.

Ethics Approval

Not applicable for this study.

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