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In Vitro Propagation of Mature Carob Trees (*Ceratonia siliqua* L.) from the Axillary Buds

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Abstract

The influence of tree age and the effect of growth regulators on the micropropagation of the carob (*Ceratonia siliqua* L.) from the axillary buds of mature trees have been described. Significant differences ($P < 0.005$) in results are obtained in the stages of initiation, multiplication, and rooting according to their response to the various concentrations of different growth regulators examined, namely BA, IBA, AG_3. The use of 0.5 mg/l BA and 0.2 mg/l IBA was the most favorable for shoots neoformation. The leafy shoots are propagated in MS medium with BA at a concentration of 1.5 mg/l. The addition of gibberellic acid at 0.2 mg/l in the culture medium allows a good elongation and development of the shoots of the carob. The effect of the age of the plant material used has shown that explants taken from mature carob trees have a low capacity for bud sprouting and shoot proliferation compared to those taken from juvenile trees. Rooting has been successful when the plant material used is taken from young trees on an MS medium containing 2 mg/IBA, with an average number of 3 to 4, roots 1 to 2 cm long, then for the adult material, no rooting was observed. Based on these tests, it appears that micropropagation of the carob from the axillary buds is feasible, but additional work must be done to root this recalcitrant material.

Keywords

In Vitro, *Ceratonia siliqua*, Carob, Propagation, Axillary Bud, Rooting

1. Introduction

Morocco has an important source of wealth in plant genetic resources, thanks to its geographical location and its pedoclimatic diversity. Endemic to the Mediterranean region and enjoying a multitude of very specific characteristics, the carob tree (*Ceratonia siliqua* L.) is undoubtedly the most important species of arid and

semi-arid zones centered north of the Atlas chain, the Rif mountain and in some valleys in the southwest of the Anti-Atlas in humid and sub-humid bioclimates [1], this tree is remarkably resistant to climatic variability and well adapted to drought and salinity [2] and is frequently used for reforestation of Mediterranean regions [3] [4]. It is a very precious tree that plays an unequaled role in the cultural, socioeconomic, and ecological life of the local inhabitants, from this multi-use tree with pastoral, fodder, food, aromatic and medicinal values [4] [5].

The carob tree (*Ceratonia siliqua* L.) is widespread, in the form of spontaneous or planted stands. Carob trees may be male, female and hermaphrodite or polygamous inflorescences [6]. As the plant is dioecious, female feet bearing the seeds are the most sought after. Carob tree can be propagated by seed and vegetatively by stem cuttings, in populations resulting from seed propagation, the production is not uniform and the sex of the plants is not known until the appearance of the first flower buds [7] [8]. Whereas with vegetative propagation, it is possible to reproduce individuals of known sex and with desirable characteristics. Consequently, the necessary control of the sex ratio and the conservation of the characters of interest in a population, suggests the use of vegetative propagation techniques. Numerous studies and tests, using juvenile and adult explants, have been carried out to improve the micro-propagation of the carob tree [9]-[18].

The selection of explants, the composition of the culture medium [19] [20], the cultivar, and the application of exogenous phytohormones [21] are determining factors for the success of the *in vitro* culture of a plant species. Different types of growth regulators can specifically affect callus growth, stem elongation, and rooting. The effects of growth regulators vary with the stage of development, as low concentrations may favor a process, while higher concentrations have the opposite effect. However, little or no data are reported about *in vitro* propagation of carob mature tree in Morocco.

Furthermore, the adventitious root formation is essential to the success of vegetative propagation of woody plants. As in many tree species, rooting in the carob tree remains a major problem [22] [23]. The ability to root *in vitro* appears generally high on juvenile plant material, this decreases very rapidly with age [14] [24] [25].

Given the above difficulties, this study was carried out to investigate the influence of tree age, the effects of plant growth regulators on the *in vitro* propagation of mature C.siliqua trees. Experiments to evaluate the root formation of C. siliqua were tested.

2. Material and Methods

2.1. Preparation of Plant Material

Axillary buds were used as explants for the *in vitro* propagation of the carob (*C. siliqua*), axillary buds harvested from the spring shoots during (April, May) from mature trees in the Ouazzane region (AinBayda; Morrocco), selected and aged from

(12, 24 and 36 years). For comparison, 3-year-old grafting explants (branches of the mature tree were grafted onto seedlings used as rootstocks) were used. After cutting the leaves at the base of their petiole, the explants of the axillary buds whose size has been reduced to 2 cm in height and 0.5 cm in diameter, are first washed with running water with the addition of a few drops of Tween to remove as many microorganisms as possible, were sterilized in calcium hypochlorite solution (7.5% w/v) for 20 min and then in mercuric chloride solution (0.1% $HgCl_2$) for 6 min, followed by three rinses with sterile distilled water for 10 min each.

2.2. Condition and Cultivation

After disinfection, each explant, was placed in 100 ml flasks containing 50 ml of Murashinge and Skoog (MS) [26] base medium supplemented with 30 g/l of sucrose, 0.1 g/l myoinositol and solidified with agar (7 g/l). The pH of the medium was adjusted to 5.8 with a few drops of 0.1 N HCl or 0.1 N NaOH before sterilization by autoclave at 121˚C for 20 min. to reduce the browning of explants, compounds are added such as ascorbic acid and/or citric acid at a concentration of 100 mg/l.

The cultures were then placed in a culture chamber with lighting by fluorescent tubes 1500 Lux, a temperature of 25˚C ± 1˚C, and a photoperiodic regime with 16 hours of light for 8 hours of darkness. Results were evaluated after four weeks.

2.3. Shoot Culture

To test the effect of phytohormones on the shoot proliferation of axillary buds, the explants were cultured on solid MS [26] medium supplemented with BA at various concentrations (0.5; 1; 1.5 and 2 mg/l) alone or combined with IBA at a low concentration of 0.1 and 0.2 mg/l. Shoot proliferation was assessed by rate of bud sprouting, the mean of the number of shoots, and the mean length of the longest shoot produced per culture.

2.4. Multiplication Phase

After four weeks, the shoots obtained by axillary buds were separated from their basal callus and transferred into multiplication media, containing the basic medium (MS) with 30 g/l of sucrose, 0.1 g/l of Myo-inositol in the presence of BA at various concentrations (0.5; 1; 1.5 and 2 mg/l). The cultures were then placed for fours weeks in the culture chamber at 25˚C ± 1˚C and under a photoperiod of 16/8h. after fours weeks, the newly developed shoots were separated and subcultured into the same multiplication medium. Shoot multiplication was assessed by the multiplication rate, the mean of the number of shoots, and the mean length of the longest shoot produced per culture.

2.5. Elongation of Shoots

To improve the elongation of the new shoots developed, the shoots were maintained by subculturing for four weeks on a shoot multiplication medium con-

taining, MS salts supplemented with AG_3 at various concentrations (0.1; 0.2; 0.5 mg/l) in the presence of BA (1.5 mg/l). Shoot elongation was assessed by the mean of the number of shoots, and the mean length of the longest shoot produced per culture.

2.6. Rooting Phase

Young leafy shoots a few cms in length harvested at the end of the multiplication phase are cut about 2 mm at their base and transplanted into 100 ml flasks containing 50 ml of half-diluted MS culture medium. The rooting of shoots was approached in the presence of two IBA or NAAauxins at two concentrations (1 and 2 mg/l). Subsequently, two treatments were compared:

The first treatment (Ta) consists of growing the shoots on the medium MS diluted by half supplemented with IBA (2 mg/l) in the dark at two different temperatures (25˚C and 30˚C) for four days.

In the second treatment (Tb), the basal ends of the shoots were soaked in the IBAauxinic solution (2 mg/l) for five minutes and cultivated on the MS medium diluted by half and devoid of phytohormones in the dark at two different temperatures (25˚C and 30˚C) for four days.

For both methods, the shoots were transferred after four days in photoperiodic conditions from the culture chamber. The rooting induction was evaluated after one month and was expressed in terms of rooting percentage, root number, the longest root length per plantlet.

2.7. Statistical Analysis

A completely random experimental device was adopted and thirty explants were used per treatment, the results of different experiments were treated by analysis of variance (ANOVA) and the significantly different means were separated by Duncan's multiple-range test at $P < 0.05$. Results are represented by means ± standard deviations (SE).

3. Results

3.1. Effect of Plant Growth Regulators and Tree Age on Axillary Bud Sprouting of *Ceratonia siliqua*

The reaction of axillary buds after culture on modified MS medium supplemented with a various concentrations of cytokinin (BA) plus auxin (IBA) varies depending on the concentration of BA used and on the presence or absence of IBA. BA stimulated buds with a percentage between 21% and 34.66%, as regards IBA and whatever the combination used, the use of IBA is always better than the use of BA alone (Figure 1), the results obtained in Table 1 showed that the combination of BA and IBA, increased the percentage of new shoots of axillary buds between 23% and 46%, the best combination is 0.5 mg/l of BA and 0.2 mg/l of IBA, which increases the number of newly formed shoots 2.28 ± 0.17 and also allows a slight elongation of the stems 0.60 ± 0.06 cm (Table 1). BA and IBA re-

semble very effective for the stimulation and the proliferation of shoots from axillary buds from mature trees of *C. siliqua* (Figure 1).

The axillary buds collected from trees of different ages were grown on MS media supplemented with BA (0.5 mg/l) and IBA (0.2 mg/l) and GA_3 (0.2 mg/l), after four weeks, the results showed a significant difference in response between the different explants on the rate of bud sprouting, the number of shoots per bud and the size of the shoots. A very clear decrease in the explants response to the growth regulator used as stimulators for the growth of the shoots, depending on the age of the mother plant from which they originated, the explants dated 3 years old are classified in the first response lines which then gradually decreased with the age of the mother tree (Table 2).

Table 1. Effect of BA and IBA on axillary buds sprouting of mature trees of *C. siliqua* after four weeks of culture on modified MS medium.

BA (mg/l)	IBA (mg/l)	Number of shoots	Shoot lenght (cm)	% Budsprouting
0	**0**	**0.00 ± 0.00c**	**0.00 ± 0.00c**	**0.00 ± 0.00d**
	0	1.64 ± 0.17ab	0.48 ± 0.06ab	34.66 ± 3.44b
0.5	0.1	1.83 ± 0.17a	0.57 ± 0.06a	38.17 ± 3.44ab
	0.2	**2.28 ± 0.17a**	**0.60 ± 0.06a**	**46.00 ± 3.44a**
	0	1.44 ± 0.17ab	0.42 ± 0.06b	22.66 ± 3.44c
1.0	0.1	1.69 ± 0.17ab	0.40 ± 0.06b	25.48 ± 3.44c
	0.2	1.76 ± 0.17a	0.46 ± 0.06ab	30.66 ± 3.44bc
	0	0.92 ± 0.17b	0.32 ± 0.06bc	30.00 ± 3.44bc
1.5	0.1	1.35 ± 0.17ab	0.41 ± 0.06b	34.75 ± 3.44abc
	0.2	1.36 ± 0.17ab	0.35 ± 0.06abc	40.33 ± 3.44ab
	0	0.80 ± 0.17b	0.24 ± 0.06bc	21.00 ± 3.44c
2.0	0.1	1.16 ± 0.17ab	0.34 ± 0.06abc	21.75 ± 3.44c
	0.2	1.28 ± 0.17ab	0.36 ± 0.06abc	23.00 ± 3.44c

Values represent means ± SE of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$.

Table 2. Effect of the age of the mother plant on the buds sprouting of the carob tree explants cultivated on the MS medium added with 0.5 mg/l (BA), 0.2 mg/l (IBA), and 0.2 mg /l (GA_3).

Age (years)	Number of shoots	Shoot lenght (cm)	% Budsprouting
G1(3)	**7.32 ± 0.12a**	**2.30 ± 0.04a**	**78.00 ± 3.06a**
G2(12)	5.88 ± 0.12b	1.78 ± 0.04b	62.33 ± 3.06b
G3(24)	4.12 ± 0.12c	1.24 ± 0.04c	47.66 ± 3.06c
G4(36)	2.84 ± 0.12d	0.85 ± 0.04d	42.33 ± 3.06c

Values represent means ± of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$. (G: generation on years old).

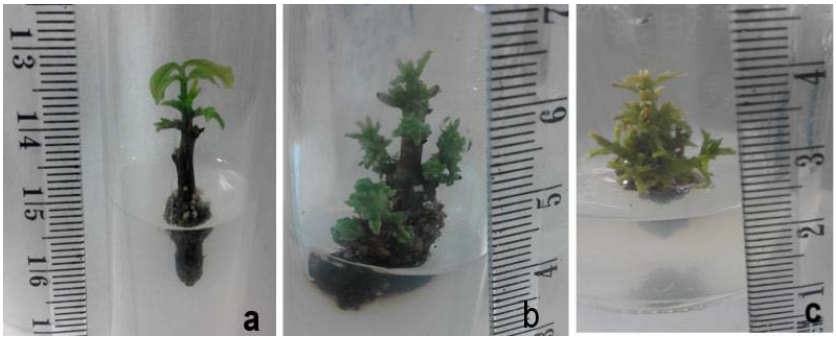

Figure 1. Sprouting from axillary carob buds maintained on MS media for four weeks, (a) (MS + 0.5 mg/l of BA), (b) (MS + 0.5 mg/l of BA + 0.2 mg/l of IBA), (c) elongation of shoots on GA_3 medium (MS + 0.5 mg/l of BA + 0.2 mg/l of GA_3).

3.2. Effect of Plant Growth Regulators and Tree Age on Shoots Multiplication of *Ceratonia siliqua*

After four weeks of culture, all the shoots produced in the first stimulation medium were transferred to the MS medium in the presence of different BA concentrations. During the multiplication phase, which takes place in three cultures, a difficulty of adaptation of the shoots was observed which translates in the formation of the cal, in some cases the whole implant transforms into cal which subsequently generates adventitious shoots (Figure 2). Besides, the number of glassy shoots is increasing and the miniature leaves have appeared in a characteristic form of *C. siliqua*. The shoots produced are all the better as the concentration of BA is 1.5 mg/l (Table 3), in this case, the number of shoots is equal to 5.24 ± 0.11, the size of the shoots is 1.54 ± 0.03 cm and the number of leaves is 3.28 ± 0.09, then the values are minimized at the lowest concentration (0.5 mg/l).

The use of GA_3 in the culture medium significantly affects the new formation of shoots and improves leaf development (Figure 2). The combination of GA_3 especially at 0.2 mg/l with 1.5 mg/l BA appears favorable for the development of shoots (Table 4), the best results in the averages on a number of shoots, shoot length, and a number of leaves are respectively; 6.28 ± 0.11, 2.92 ± 0.12 cm, and 5.28 ± 0.14. However, the use of a high concentration of 0.5 mg/l of GA_3 has caused a remarkable yellowing of the leaves which negatively affects the formation of the shoots.

3.3. Effect of Plant Growth Regulators and Tree Age on Rooting of *Ceratonia siliqua* Shoots

At the end of the multiplication phase, shoots of size 2.5 cm approximately (Figure 3) are individualized and transplanted onto the rooting medium containing 1/2 MS medium supplemented with auxin (IBA or NAA), after four weeks, all mediums used with both auxin give rise to a large callus at the base of the shoots. Regarding a Table 5, a significant influence of different auxins was observed, the presence of auxin in the medium increased the percentage of rooting induction about 10%, however, the IBA appeared more effective than NAA, 9.72% was the high percentage of rooting induction observed especially with IBA,

and NAA produced a low rooting percentage 3.48%. The best results obtained using IBA at a concentration of 2 mg/l in this case, the number of roots, and their length is, respectively, 3.48 ± 0, 15, and 1.51 ± 0.08 cm (Figure 3).

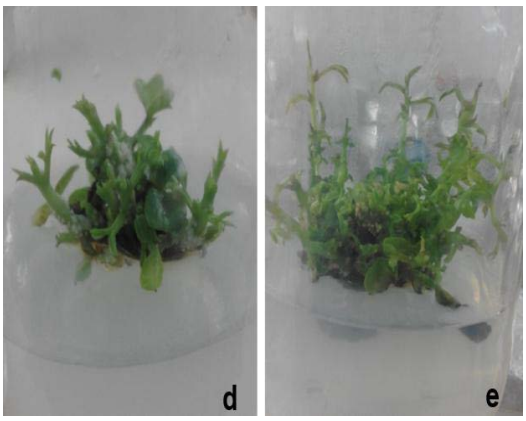

Figure 2. (d) Multiple shoot formation from shoot tip cultured on MS + 1.5 mg/l of BA; (e) shoots elongation after two weeks of culture on MS meduim + 1.5 mg/l of BA and 0.2 mg/l of GA_3.

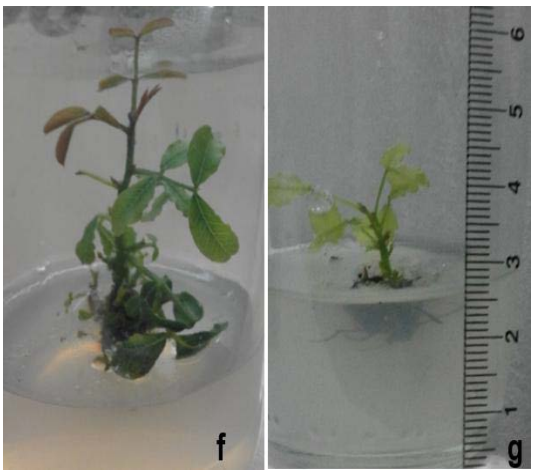

Figure 3. (f) Young carob seedlings aged 75 days from the *in vitro* culture of an axillary bud; (g): Root initiation was obtained after 45 days of culture on MS/2 + IBA 2 mg/l.

Table 3. Effect of different concentrations of BA on the multiplication of shoots derived from the axillary bud subculture on MS for four weeks.

BA (mg/l)	Number of shoots	Shoot lenght (cm)	Number of leaves
0.0	1.00 ± 0.11e	1.17 ± 0.03c	1.68 ± 0.09c
0.5	2.80 ± 0.11d	1.30 ± 0.03b	2.24 ± 0.09b
1.0	3.48 ± 0.11c	1.58 ± 0.03a	2.36 ± 0.09b
1.5	**5.24 ± 0.11a**	**1.54 ± 0.03a**	**3.28 ± 0.09a**
2.0	4.80 ± 0.11b	1.30 ± 0.03b	3.24 ± 0.09a

Values represent means ± of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$.

Table 4. Effect of BA associated with GA_3 on the number of shoots, elongation of the shoots and the number of leaves.

BA (mg/l)	GA_3 (mg/l)	Number of shoots	Shoot length (cm)	Number of leaves
1.5	0	5.36 ± 0.11c	1.80 ± 0.12c	3.84 ± 0.14b
	0.1	5.76 ± 0.11b	1.98 ± 0.12bc	3.92 ± 0.14b
	0.2	**6.28 ± 0.11a**	**2.92 ± 0.12a**	**5.28 ± 0.14a**
	0.5	6.12 ± 0.11a	2.28 ± 0.12b	5.16 ± 0.14a

Values represent means ± of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$.

Table 5. Effect of IBA and NAA at different concentrations on the rooting of *C.siliqua*.

Auxin	Concentration mg/l	% Rooting	Number of roots	Root lenght (cm)
IBA	1	5.26 ± 0.26 b	1.88 ± 0.14 b	1.41 ± 0.08 a
	2	**9.72 ± 0.27 a**	**3.48 ± 0.15 a**	**1.51 ± 0.08 a**
NAA	1	0.00 ± 0.27 d	0.00 ± 0.15 c	0.00 ± 0.08 c
	2	3.48 ± 0.27 c	1.76 ± 0.15 b	1.06 ± 0.08 b

Values represent means ± of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$.

To further promote maximum rooting, we tested the effect of the incubation temperature (25˚C, 30˚C) in the dark for four days with two ways of IBA treatment. The shoots are incubated at a temperature of 25˚C or 30˚C in the dark for four days in an MS medium containing 2 $mg{\cdot}l^{-1}$ of the IBA, before transferring them to light at a temperature of 25˚C. The results of Table 6 showed that the use of a temperature of 25˚C has produced the best results in a number of roots in 4.64 ± 0.15 with a maximum length of root in 1.74 ± 0.12 cm and a rooting percentage of 17.33%. compared to the use a temperature of 30˚C, have been decreased the percentage of rooted shoots and the number of roots and the quality of the shoots is slightly affected in the dark and at the temperature, then the leaves have lost their green color and have become yellow, these results showed that the temperature above 25˚C does not appear to be decisive for induction or initiation of roots for shoots from axillary buds.

Table 7 showed, the age of the mother's plant where it becomes explant has significantly influenced the rooting of the multiplicated shoots. The shoots from axillary buds taken from young trees (3 years old) had the highest percentage of rooting. Shoots derived from mature trees (12, 24, 36 years old) did not show a good response to rooting. It is clear that the rooting of cuttings decreases with the increase of the age of the mother's feet; the adventitious rooting is practically zero for mature trees with an age of 12 years, 24 years, and 36 years. But rooting is achievable for young plants 3 years old.

4. Discussion

The micropropagation of axillary buds derived from the adult tree of *C.siliqua*is

Table 6. Effect of incubation temperature in combination with two different treatments with IBA (2 mg/l).

Treatment	Temperature °C	% Rooting	Number of roots	Root lenght (cm)
Ta	25	**17.33 ± 0.98a**	**4.64 ± 0.15a**	**1.74 ± 0.12a**
	30	10.66 ± 0.98b	3.96 ± 0.15ab	1.78 ± 0.12a
Tb	25	8.66 ± 0.98b	2.52 ± 0.15b	1.75 ± 0.12a
	30	5.33 ± 0.98c	2.12 ± 0.15b	1.87 ± 0.12a
Tc	25	0.00 ± 0.98d	1.40 ± 0.15c	0.74 ± 0.12b
	30	0.00 ± 0.98d	1.12 ± 0.15c	0.60 ± 0.12b

Values represent means ± of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$. Ta: Culture on MS/2 medium supplemented by IBA; Tb: soaking 5 min in IBA before culture; Tc: control without treatment.

Table 7. Effect of the age of the explant on the rooting of the carob shoots on the MS/2 medium in the presence of 2 mg/l IBA.

Age	% Rooting
G1(3)	24.33 ± 0.33a
G2(12)	0.00 ± 0.33b
G3(24)	0.00 ± 0.33b
G4(36)	0.00 ± 0.33b

Values represent means ± of three replicates with 30 cultures. Values followed by the same letter are note significant at $p < 0.05$. (G: generation on years old).

not as easy as the use of young explants; several difficulties have been encountered in this work, such as the contamination of cultures, the browning of explants and the development of calluses. We chose the young shoots taken just after the buds of the tree burst in early spring (March, April), the stems of these shoots are still soft, charged with water and not lignified, they react better to grow in *in vitro* and the percentage of contamination has decreased by comparing it with the cultures of buds derived from lignified explants. Excessive contamination of crops has been reported by other authors [21] [27], working on tree-derived nodes have found that crop contamination was maximum during November and December, the explant browning is due to the oxidation of phenolic exudates, it is less significant in March and April, unlike other months of the year, this problem also observed in other woody plants can be overcome by transferring the cultures to a cool environment, while antioxidant treatments are generally ineffective [21] [28] [29] [30].

The development of shoots depends on the growth regulator used. The addition of BA, IBA, and GA_3 improve bud initiation and elongation of shoots. The use of BA alone has a positive effect on the initiation of budding of the axillary buds and the neoformation of shoots; likewise, its combination with low-concentration IBA (0.2 mg/l) improves the percentage of bud %, elongation of the shoots and the number of shoots per explant. The shoots obtained on me-

dium containing 0.5 mg·l^{-1} of BA alone or combined with 0.2 mg/l IBA had short internodes and quickly lost their elongation capacity, the leaves were curled and took on a characteristic shape. The addition of GA_3 in the culture medium improves the elongation of the shoots; the best elongation has been shown with 0.2 mg/l of GA_3. The results demonstrated that cytokinins induce the formation of axillary buds and therefore the proliferation of shoots. These results, which in favor with certain authors [31] obtained good results in MS medium supplemented with BA and IBA, however, [32] obtained the best results in MS medium supplemented with BA, IBA and GA_3.

The multiplication of micro-shoots produced of axillary buds on MS medium in the presence of different concentrations of BA had a tendency similar to that of shoots proliferation. In an MS medium without growth regulators, the axillary buds produced only one shoot. The best shoot multiplication rate was obtained on media supplemented with 1.5 mg·l^{-1} BA. the multiplication of shoots is hampered by the development of a basal callus which ends up invading the whole explant, however, adventitious buds reappear on this callus, the formation of a basal callus following the cultivation of carob shoots and its organogenic possibilities have been observed by other researchers [14] [21] [32].

The cutting ability of the shoots was also greatly affected by the age of the tree from which the explants were taken. The multiplication of shoots from axillary buds taken from young plants has shown a great capacity for shoot formation and rapid proliferation. [14] has shown that the explantation capacity decreases as the age of the tree from which the shoots were taken increases and that the best results have been obtained with explants from young plants. The herbaceous explants of *C. siliqua* are often found to be more optimal than the semi-woody explants for the regeneration of shoots; this can be explained by the differences in anatomical and physiological properties.

Rooting is one of the most critical key points in the production of rooted cuttings by vegetative propagation [33]. In *C. siliqua*, rooting corresponds to a difficult stage due to the low percentage of rooting of the shoots in response to the auxin tested. The application of IBA improved the percentage of rooting and the number of roots compared to the non-control treaty, all the rooting medium used, has led to the formation of the calluses, and in sometimes calluses have been formed with roots. Our results corroborate those obtained by [14] [21] who showed that IBA is among the auxins (IAA and NAA) the most favorable for rooting shoots. IBA has been reported to significantly increase adventitious root formation in many species [34] [35] [36]. Although, the best rooting percentage obtained using IBA in the culture medium does not exceed 15%, low rates of rhizogenesis have been found with other species including conifers of the genus Pinus [37], and eucalyptus [33]. According to the temperatures to which our cultures are subjected, the temperature of the culture chamber was maintained constant at 25˚C, according to [38] a high temperature (±30˚C) can favor the initiation of the primordial roots, while a lower temperature (±25) rather favors their development. In our case we tested the temperature effect during the incu-

bation of the seedlings four days in the dark, the results show that the temperature of 30˚C does not seem decisive in the induction or the initiation of the roots of the carob tree, the reason why it did not support significant root growth. With the carob tree, [10] demonstrates the importance of the multiplication medium on rhizogenesis and that the presence of GA_3 in the shoot multiplication medium inhibits rooting. According to our results, the potential of *C. siliqua* cuttings to form adventitious roots decreases with the increase in the age of the mother's feet on which the explants are taken. For many species, the rooting capacity of the shoots disappears beyond 10 or 20 years [39]. When the plants are young, adventitious rooting is possible but, it is practically zero for adult trees. This is in favor of the study conducted by [14]. Rooting experiments have demonstrated the good rooting capacity of micro-cuttings from the juvenile plant material of *C. siliqua* [16] [18]. The rooting of the carob is very difficult; it is limited by the age of the mother's foot which remains probably the most decisive parameter in the success of the technique of micropropagation. Rooting step was fundamental for the continuation of our work concerning the recovery of *in vitro* plants in the soil.

5. Conclusion

The control of *in vitro* production of carob requires the development of growing and rooting environments in order to have improved the quality of rooted shoots. Auxinic treatment, including IBA, appears necessary to improve root quantity and quality. The low rate of rooting remains the major problem in the micropropagation of the carob; obtaining a developed root system could remove any obscurity concerning the recovery of plants in the acclimatization greenhouse.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

BA: 6-Benzylaminopurine

GA_3: Gibberelicacid

$HgCl_2$: Bichlorure mercure

IBA: Indol-3-butyric acid

MS: Murashige and Skoog (1962)

NAA: 2-Naphthaleneacetic acid