Article

Micropropagation of the caper in semi-solid medium and in temporary immersion bioreactors

Fabián Contreras-Loera
Lucía Isabel Chávez-Ortiz
José Francisco Morales-Domínguez
Eugenio Pérez-Molphe-Balch[§]

Center of Basic Sciences-Autonomous University of Aguascalientes. Av. University 940, ZC. 20131 Aguascalientes, Mexico. (ibq.fabcl@gmail.com: luciachavez75@gmail.com; jfmoral@correo.uaa.mx).

[§]Corresponding author: eperezmb@correo.uaa.mx.

Abstract

The objective was the development of an efficient system for the micropropagation of the caper (*Capparis spinosa* L.), a woody shrub of great interest due to its products, its remarkable resistance to drought and its tolerance to high temperatures. *In vitro* cultures were first established through disinfection and seed germination. A scarification of these with concentrated H_2SO_4 (98% v/v) was necessary to break dormancy. Only 15% of the seeds germinated. Nodal segments were obtained from the germinated seedlings, which were cultivated in basal medium of Murashige and Skoog (MS) semisolid added with benzyladenine (BA), 2-isopentenyladenine (2iP) and metatopoline (MT), this in order to induce multiple sprouting. The best response was obtained with 2 mg L^{-1} of these cytokinins, with an average number of well-differentiated sprouts per nodal segment of 3.6 with MT, 2.3 with BA and 1.5 with 2iP, this after 54 d of incubation. The combination of cytokinins with an auxin, naphthaleneacetic acid, was also tested. This combination improved the BA response, reaching an average of 3.2 sprouts per nodal segment. With the other cytokinins, it did not show a positive effect, maintaining very similar values with and without auxin. In addition to well-differentiated sprouts, masses or clusters of numerous small sprouts were generated, unsuitable for transfer to the rooting medium. These masses were transferred to RITA type temporary immersion bioreactors, where an average of 89 well-differentiated sprouts were generated per original explant, this in a medium with 1 mg L^{-1} of BA with 2 min immersions every 6 h. The sprouts took root with an efficiency of 80% in the basal environment, generating well-differentiated plants suitable for transfer to the ground. The survival already in the external environment of the plants generated *in vitro* was 85%, showing an apparently normal development already in *ex vitro* conditions.

Keywords: *Capparis spinosa*, cytokinins, RITA®.

Reception date: January 2021
Acceptance date: February 2021

Introduction

The development of *in vitro* propagation methods for plant species of agricultural, pharmaceutical or forestry interest, has taken a considerable boom in recent years. This is even more noticeable in perennial species where conventional multiplication methods are not very efficient. An example of this is the caper (*Capparis spinosa* L.), a species of the Capparaceae family cultivated in the Mediterranean and adjacent regions. It is a species with several traditional and potential uses in the food, medicinal, cosmetic, pharmacological and ornamental industries (Khalil *et al*., 2012; Carra *et al*., 2012a; Farhoudi and Makezadeh, 2013).

The caper also stands out for its resistance to drought derived from various anatomical adaptations (Gan *et al*., 2013) and its ability to maintain a high photosynthetic rate under extreme arid conditions and high temperatures (Levizou *et al*., 2004), reason for which it is a species of interest to be introduced in hot regions with low water availability. However, one of the greatest difficulties in achieving this is the availability of plants. The propagation of this species by conventional methods is inefficient due to the low seed germination rate (Hessam *et al*., 2012; Carra *et al*., 2012a) and low rooting efficiency of its vegetative cuttings (Chalak and Elbitar, 2006; Khalil *et al*., 2012).

Given the above, there are already reports of *in vitro* propagation of this species; for example, Rodríguez *et al*. (1990) propagated the caper from nodal segments grown in Murashige and Skoog (MS) medium added with combinations of benzyladenine (BA), indoleacetic acid (AIA) and gibberellic acid (GA_3). Musallam *et al*. (2011) mention that the best means for the establishment of plants was Lloyd and McCown (WPM) supplemented with 0.8 mg L^{-1} of kinetin (Cin), 0.05 mg L^{-1} of AIA and 0.1 mg L^{-1} of GA_3, obtaining an average sprout elongation of 1.89 cm, a number of nodes of 4.07 and a number of leaves of 8.45.

In addition, they obtained 80% rooting with 50% MS medium supplemented with 5 mg L^{-1} of AIA and 0.1 mg L^{-1} of GA_3. The *in vitro* generated seedlings were acclimatized with 63% efficiency. For their part, Al-Safadi and Elias (2011) report that the average number of sprouts generated per explant was 5.5 when the MS growth medium contained 2 mg L^{-1} of zeatin riboside, 0.1 mg L^{-1} of GA_3 and 1 mg L^{-1} of naphthaleneacetic acid (ANA).

They also report indirect organogenesis from callus, this in a MS medium with 1 mg L^{-1} of Cin and 0.1 mg L^{-1} of AIA, producing two plants for each piece of callus. About 86% of the plants survived when transferred to pots placed in a greenhouse. Finally, Carra *et al*. (2012a) introduced the use of synthetic cytokinins of the diphenyl group in the *in vitro* multiplication of the species, finding that N-phenyl-N'-benzothiazol-6-yl-urea (FBU) and N-phenyl-N´-(1,2,3-thidiazol-5-yl-urea, also called thidiazuron (TDZ) were able to generate multiple sprouting in combinations with indole butyric acid (AIB), although with lower efficiencies than BA.

On the other hand, the use of Temporary Immersion Bioreactors (BITs) has recently gained momentum as part of the protocols for the massive *in vitro* propagation of plants of interest. These devices have shown their ability to notably increase efficiency and shorten the times required in other systems (Etienne and Berthouly, 2002).

Temporary immersion systems are designed so that the plant tissue remains immersed in liquid culture medium for certain periods alternated by dry periods in which the tissues are in contact with air. The frequency and duration of these periods must be established for each species in particular. There are several designs of BITs, one of the most efficient being the Récipient à Immersion Temporaire Automatique (RITA®), Vitropic, SA, France.

This consists of a container divided into two sections, the upper part contains the seedlings and the lower part the growing medium. The pressure applied to the lower compartment by the injection of sterile compressed air pushes the medium to the upper part, where it comes into contact with the plant tissue, submerging it as long as pressure is applied. During the immersion period, the air generates bubbles that gently agitate the tissues and renew the atmosphere of the upper space within the culture chamber, with the overpressure escaping through outlet tubes protected by sterile filters in the upper part of the apparatus. Once the air pressure ceases, the culture medium falls by gravity to the lower compartment drying the plant tissue (Teisson *et al*., 1996).

In this work, the development of complete *in vitro* propagation schemes for capers is reported, both in conventional semisolid media and in RITA® type temporary immersion bioreactors. This technology can be applied to generate enough plants for the introduction of this species as an alternative crop in regions of Mexico where high temperatures are combined with low water availability.

Materials and methods

Disinfection and rupture of seed dormancy: caper (*Capparis spinosa* L.) seeds were obtained from a commercial company. The seeds were subjected to three different treatments in order to disinfect them and break dormancy. In treatment 1, the seeds were soaked in concentrated H_2SO_4 for 20 min and rinsed under running water.

Subsequently, they were treated with 70% ethanol for 45 s, rinsed again and disinfected with commercial bleach based on sodium hypochlorite (Cloralex®) 15% for 15 min. In the laminar flow hood, they were rinsed three times with sterile distilled water. Finally, they were inoculated in culture containers with 30 ml of basal medium from Murashige and Skoog (1962) or MS, at pH 5.7, with 30 g L^{-1} of sucrose and 8 g L^{-1} of agar as gelling agent.

Four seeds were placed per culture container and incubated at 25 ±2 °C, under white LED light (LED) with a photoperiod of 16 h light, 8 dark. In treatment 2, the seeds were washed three five minutes each, with running water and Dermoclean® liquid soap (10 ml L^{-1}), rinsed with running water, placed in 70% ethanol for 45 s and rinsed again. They were then disinfected with commercial bleach based on sodium hypochlorite (Cloralex®) at 15% for 15 min. In the laminar flow hood, they were rinsed three times with sterile distilled water and placed in a solution of 500 mg L^{-1} of GA_3 for 24 h.

After this time, they were placed in flasks with 30 mL of basal MS medium and incubated under the same conditions as in treatment 1. Treatment 3 was similar to treatment 2, only instead of leaving the seeds for 24 h in GA_3, these were left for 120 h, after which they were again disinfected with 10% Cloralex® and finally placed in flasks with 30 mL of basal MS medium and incubated under the mentioned conditions.

The seedlings that germinated 67-100 days after inoculation, were transferred to basal MS medium with 0.5 mg L^{-1} of benzyladenine (BA) in order to accelerate their development and stimulate the sprouting of the lateral buds to obtain the necessary material for subsequent experiments.

The sprouts that were generated in this medium were transferred to MS medium added with 1.5 g L^{-1} of activated carbon in order to grow and differentiate. The nodal segments of these seedlings were used as explants for the multiplication experiments described below.

Effect of plant growth regulators (RCV) on the *in vitro* multiplication of capers: 0.5 cm long nodal segments were used as explants, taken from plants kept in the medium with activated carbon and obtained from *in vitro* germinated seeds. In later experiments the nodal segments were taken from the sprouts generated in previous experiments. In a first experiment, the effect of five different cytokinins on the generation of sprouts from the nodal segments was analyzed.

The cytokinins tested were benzyladenine (BA), 2-isopentenyladenine (2iP), kinetin (Cin), metatopoline (MT), and thidiazuron (TDZ), all from Phytotechnology Labs. The concentrations tested for each cytokinin were 0.5, 1, 1.5 and 2 mg L^{-1}, generating 20 different treatments. Basal MS medium without cytokinins was used as a control. The explants were inoculated vertically, taking care that the yolk remained at the level of the nutrient medium. Five culture vessels with three nodal segments in each one of them were used for each treatment (n= 15).

The entire experiment was performed twice. In a second experiment the combination of a cytokinin with an auxin was analyzed. Only the cytokinins BA, 2iP, Cin and MT were tested, as TDZ showed toxic effects for this species in the first experiment. 1, 2 and 3 mg L^{-1} of each cytokinin were tested in combination with 0.5 mg L^{-1} of the auxin naphthaleneacetic acid (ANA). The explants were inoculated in the same way as in the first experiment.

Six culture vessels with three nodal segments each were used for each treatment (n= 18) and the entire experiment was performed twice. In both experiments, the incubation conditions were the same as those used for seed germination. The number of sprouts per explant was recorded at 54 days of incubation.

Statistical analysis of the results: given that the data generated did not comply with the assumption of normality, a non-parametric analysis of longitudinal data was used in factorial experiments to determine the relative effect of each of the treatments tested in the generation of sprouts. The statistical analysis was performed with the R program version 3.2.3, using the nparLD version 2.1 library (Noguchi *et al*., 2012).

Multiplication in bioreactors: RITA® temporary immersion bioreactors were used. The explants that were inoculated were poorly differentiated sprout masses, generated in semisolid media added with BA, 2iP and MT. Three explants were inoculated per bioreactor. The conditions that were tested were the cytokinin added to the culture medium and the number of dives scheduled per day. The media were basal MS with 1 mg L^{-1} of BA or 2iP. The immersion cycles were every 6 or every 4 h, each lasting two minutes. The bioreactors were placed in the incubation room for 60 or 90 days under the same conditions as the cultures in semisolid medium.

Rooting: rooting tests were carried out with sprouts generated in the multiplication stage that had a length of at least 5 mm, an axillary bud and that were entirely green in color. The media tested were the basal DM and DM added with 1.5 g L^{-1} of activated carbon. Two types of lighting were also tested, white light LEDs and photosynthetically active radiation (RFA) LEDs, which are the combination of red and blue LEDs in a 7 to 1 ratio. Incubation was done at 25 ±2 °C. 80 sprouts were placed in each of the treatments and the entire experiment was performed twice. At 50 days of incubation, the presence or absence of roots in the inoculated sprouts was determined.

Acclimatization and transfer to the soil of the generated plants: in the acclimatization stage, four protocols were used: 1) culture vessels containing rooted sprouts were selected, from which the seal was removed and the lid was loosened. They were left for 22 d with the lid loose under the same incubation conditions. After this time the plants were removed from the culture flask and the roots were washed with running water to remove remains of the culture medium, they were placed in nursery bags with commercial ProMix substrate and placed in a bioclimatic chamber at a temperature of 22- 24 °C, 50-60% relative humidity and 16/8 photoperiod (light/dark) for 15 days, then they were taken to the greenhouse; 2) the same steps indicated in protocol 1 were followed until they were placed in bags with substrate, but they were taken directly to the greenhouse and remained in it, covered by the transparent bag for 17 d. On day 24 they were taken out of the greenhouse and placed outdoors; 3) similar to the previous one, but they were removed from the greenhouse on day 77, when they had already developed several new leaves inside it; and 4) the plants remained only three days covered by the plastic bag and at 63 d they were removed from the greenhouse. In all the protocols, the final survival results were taken 90 days after they started.

Results and discussion

Regarding the *in vitro* germination of the seeds, only treatment 1, with H_2SO_4, was able to break the dormancy of the seeds, obtaining 15% germination (Figure 1a). In the GA_3 treatments that were tested (treatments 2 and 3) there was no germination. Furthermore, germination was very asynchronous, since it occurred between 67 and 234 d of incubation. The germination percentage obtained with the use of H_2SO_4 is lower than that obtained by Al-Safadi and Elias (2011) with 32% and Bhoyar *et al*. (2010) with 33% and it is higher than that mentioned by Hessam *et al*. (2012) who mention that this treatment is ineffective. Regarding the use of GA_3 to break dormancy, Hessam *et al*. (2012) obtained a germination percentage of 42%, but used a four times higher concentration of gibberellin (2000 mg L^{-1} of GA_3 for 24 h).

Regarding the multiplication in semisolid media, when only cytokinins were used, the best results were observed with MT, BA and 2iP at a concentration of 2 mg L^{-1}, with an average number of sprouts per explant of 3.6, 2.3 and 1.5, respectively (Figures 1c, 1d and 1e). Cin generated values less than 1 sprout per explant, similar to the control without cytokinins, while TDZ generated very few sprouts (0.13 per explant), this in addition to generating necrosis in 30% of the treated explants. Therefore, these cytokinins were not used in the following experiment.

It is worth highlighting the good response generated by metatopoline (MT), a cytokinin that, to our knowledge, had not been tested before with this species. This is an aromatic cytokinin with high activity in plant tissues (Strnad, 1997). It has been proposed as an alternative to the use of

BA in those processes that require this activity, such as multiplication from buds or nodal segments. This is due to its ability to stimulate the generation of sprouts with an efficiency comparable to BA, but without showing the inhibitory effect on their rooting that is typical of BA (Werbrouck *et al*., 1996).

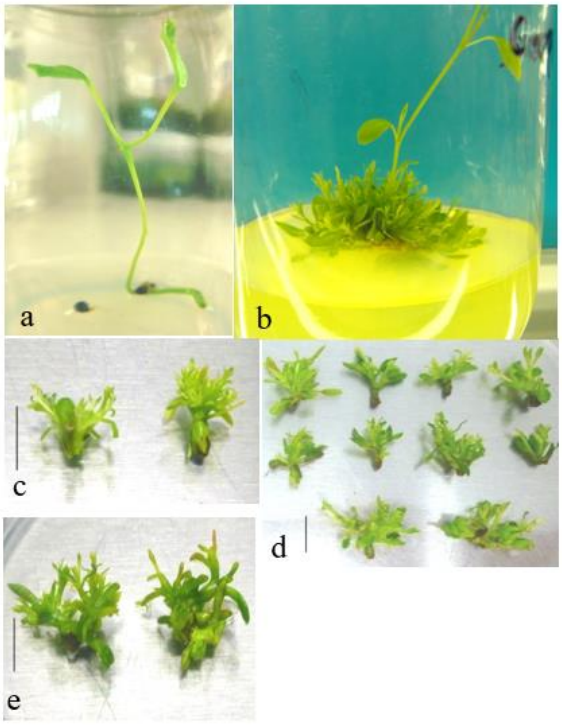

Figure 1. ***In vitro*** **propagation of capers in a semi-solid medium. a) seedling obtained from seed disinfected and germinated** ***in vitro*****; b) response observed at 90 d in a nodal segment cultured in medium with 2 mg L^{-1} of MT. Note the presence of a well differentiated sprout and a mass of poorly differentiated sprouts at the base; c) response to the 54 d of incubation of nodal segments exposed to BA; d) at 2iP; and e) to MT.** Bar= 1 cm.

In this work, only well-differentiated sprouts were quantified, of at least 8 mm in length and with at least one axillary bud, which were collected for rooting. However, in some cases at the base of the explants, masses of extraordinarily little differentiated sprouts were obtained and of very small size to be transferred to the rooting medium (Figure 1b). This type of response was also reported by Rodríguez *et al*. (1990). These sprouts, as they were not suitable to be transferred to the rooting medium, were not quantified for the results of this stage.

This response was observed in the BA, 2iP and MT treatments. In similar experiments, Musallam *et al*. (2011) obtained the best results using Lloyd and McCown culture medium (WPM) added with BA at 1.2 mg L^{-1} with an average number of sprouts per explant of 4.6 to 4.8. For the Capparis decidua species, Deora and Shekhawat (1995) produced the highest number of sprouts per explant (7.2) in MS supplemented with 5 mg L^{-1} of BA and Tyagi and Kothari (1997) obtained the best multiplication in MS medium supplemented with 5 mg L^{-1} of BA with an average per explant of six sprouts.

In the tests where cytokinins combined with auxin (ANA) were used, the values for the number of sprouts per explant were maintained, except in the case of BA, where the combination with ANA increased the efficiency to 3.2. Carra *et al*. (2012a) used MS medium with 1.4 mg L^{-1} of BA and 0.1 mg L^{-1} of IBA for multiplication, obtaining 8.9 sprouts per explant. In another report, (Carra *et al*., 2012b) placed explants in MS supplemented with 1.4 mg L^{-1} of BA and 0.05 mg L^{-1} of AIB generating 8 to 10 sprouts per explant. In the *Capparis decidua* species, Deora and Shekhawat (1995) achieved the maximum number of sprouts using MS supplemented with 5 mg L^{-1} of BA, 0.1 mg L^{-1} of ANA, 50 mg L^{-1} of ascorbic acid, 25 mg L^{-1} of L-arginine and citric acid, producing 4 to 7 sprouts per explant. Tyagi *et al*. (2010) achieved maximum sprout formation in MS medium supplemented with 2 mg L^{-1} of BA and 0.5 mg L^{-1} of ANA, with an average of 20 sprouts per explant. The best treatment of Vijay *et al*. (2014) for the induction of sprouts was DM with 4 mg L^{-1} of BA, 0.1 mg L^{-1} of ANA, 50 mg L^{-1} of ascorbic acid and 30 mg L^{-1} of citric acid and adenine sulfate, obtaining 3 to 4 sprouts per explant.

As can be seen, the results regarding the number of sprouts per explant, which were obtained in this work, were lower than some of those reported. However, in this case only the well-differentiated sprouts generated in each cycle were counted: that is, those directly capable of rooting and becoming plants. Poorly differentiated sprout groups, or sprout masses, were not included in these results. In most of the cited studies, all the sprouts generated were counted, regardless of their degree of differentiation, which makes the values not comparable.

As mentioned above, the germination rate in this species is low, making it difficult to have seedlings that serve as a source of nodal explants. However, with the propagation system proposed here, the sprouts that are generated in a multiplication cycle can be used as a source of the nodal explants necessary for subsequent cycles, thus maintaining plant production indefinitely. In this way, it is only necessary to carry out the establishment of *in vitro* cultures starting from seeds once.

Regarding the numerical analysis of the results, Figure 2 shows the graphs obtained with the R software (ver. 3.2.3), which handles a relative scale (without units) from 0 to 1 and shows the effect of each of the growth regulators tested with their respective concentrations. The statistical analysis shows that there are differences between the different treatments based on an ANOVA-TypeStatisc (ATS) with a value $\alpha = 0.05$. For the experiment only with cytokinins (Figure 2a) the p values of 3.48 e-14, 8.69 e-03 and 1.08 e-09 are obtained, for the effect of cytokinin, concentration and cytokinin: concentration interaction, respectively. In the experiment where cytokinins are combined with ANA (Figure 2b), the p-value for cytokinin is 1.71 e-10, for the concentration it is 5.64 e-09 and for the interaction cytokinin: concentration is 1.59 e-05.

The above shows that in both experiments there is at least one treatment that is statistically different. In the first case it is TDZ, which has a null effect on the production of sprouts, while in the second case it is the combination of CIN with ANA, which is the one that least affects the production of sprouts. This analysis showed that statistically there is an influence on the cytokinin used, its concentration and the interaction of the hormone with the concentration, in the number of sprouts produced.

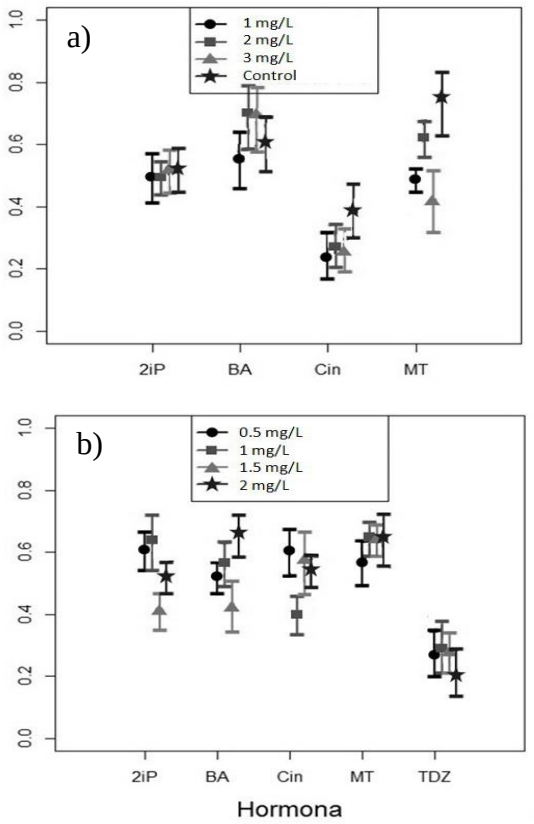

Figure 2. Relative effects of the different treatments according to a non-parametric analysis of longitudinal data performed with the R program version 3.2.3, using the nparLD library version 2.1 ($p \leq 0.05$): a) Cytokinins at different concentrations; and b) Cytokinins combined with ANA.

The sprout masses generated in this work are another aspect to highlight. First, when these are transferred to a semi-solid medium without plant growth regulators, some of the sprouts begin to differentiate and grow, being able to be collected and transferred to the rooting medium, leaving the mass of sprouts in the same medium and container so that they are starting from it, more sprouts will be differentiated.

This occurs when removing the first sprouts that differentiated, since apparently these exert an apical dominance that stops the differentiation of other sprouts. In this way, in a culture container, continuous production can be maintained for up to 200 d. On the other hand, these sprout masses can be inoculated in a temporary immersion bioreactor, as described below, where the differentiation and growth of the sprouts will occur simultaneously, without the delay and asynchrony due to apical dominance observed in the semi-solid medium.

In the RITA systems, the best treatment was MS with BA (1 mg L^{-1}) with immersions every 6 h, producing an average of 38.5 and 89 well-differentiated sprouts per explant, this at 60 and 90 d of incubation, respectively (Figures 3a and 3b). Under the same conditions, treatment with 2iP (1 mg L^{-1}) generated an average of 52 and 67 sprouts per explant. The higher frequency of immersion, every 4 h, generated averages of slightly lower sprouts, but with symptoms of hyperhydration, for which an adequate treatment was not considered.

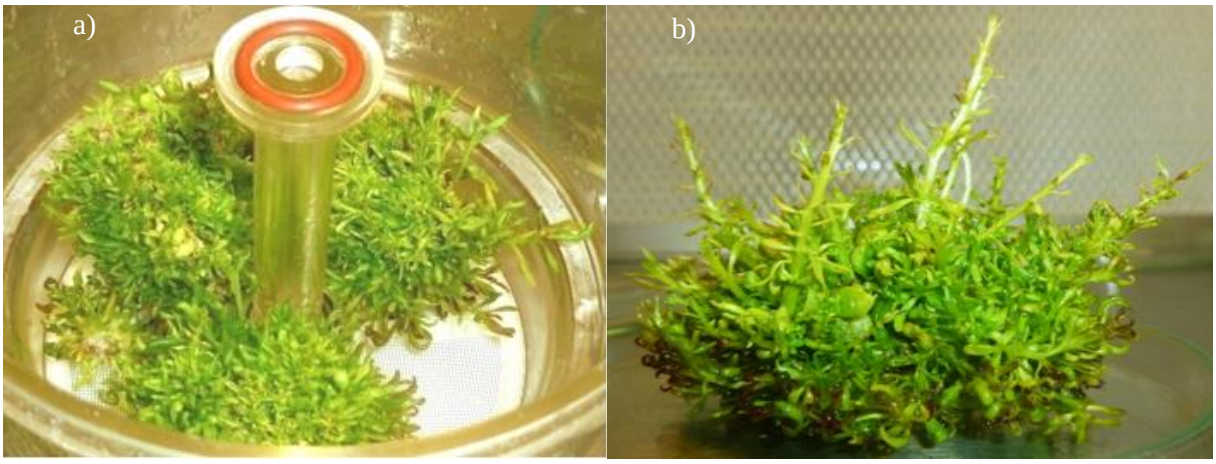

Figure 3. Differentiation of caper sprouts in temporary immersion bioreactors (RITAs). a) response of the explants at 60 days; and b) at 90 days of these, subjected to 2 min immersions every 6 h of medium enriched with 1 mg L^{-1} of BA.

This good response in RITA systems was obtained with cytokinin levels lower than those used in semi-solid media. In preliminary experiments, cytokinins were tested at concentrations equal to those used in semisolid media. In these cases, little differentiation of the sprouts and high hyperhydration were observed, so the concentration of cytokinins was reduced by half, giving the good results shown. No antecedents were found on propagation in bioreactors in this species, but it was observed that the production of well-differentiated sprouts in these systems was considerably higher than that observed in semi-solid media, this coinciding with reports made in other species in which the semi-solid medium is compared with these systems (Etienne and Berthouly, 2002).

In this work a hybrid system is proposed, where the nodal explant is grown in a semi-solid medium added with MT, BA and 2iP, where masses of exceedingly small and poorly differentiated sprouts are generated, which are then developed in a RITA, eventually producing, after these two stages, an average of 89 sprouts suitable to be rooted for each original nodal explant.

Rooting of the sprouts generated occurred with a frequency of 80% in basal MS medium (Figures 4a, 4b and 4c). The response was similar regardless of the treatment and culture system in which the multiplication stage was carried out (semi-solid medium or RITA). In the medium with activated carbon, the rooting efficiency was lower, ranging between 51 and 61%. Rodríguez *et al*. (1990); Chalak and Elbitar (2006); Musallam *et al*. (2011) used indoleacetic acid to induce rooting in this species, and obtained 80%, 87% and 70% efficiency, respectively.

For the *Capparis decidua* species Tyagi and Kothari (1997) used indolebutyric acid with a rooting percentage of 65%. In this work, a similar efficiency was obtained without the need to incorporate auxins into the culture medium. This is important, since in other woody species it has been reported that auxins can generate, in addition to roots, callus tissue that can affect the *ex vitro* survival of plants, perhaps because it causes an interruption in the connection of the vascular systems between the sprout and the root (Delgadillo-Díaz de León *et al*., 2013).

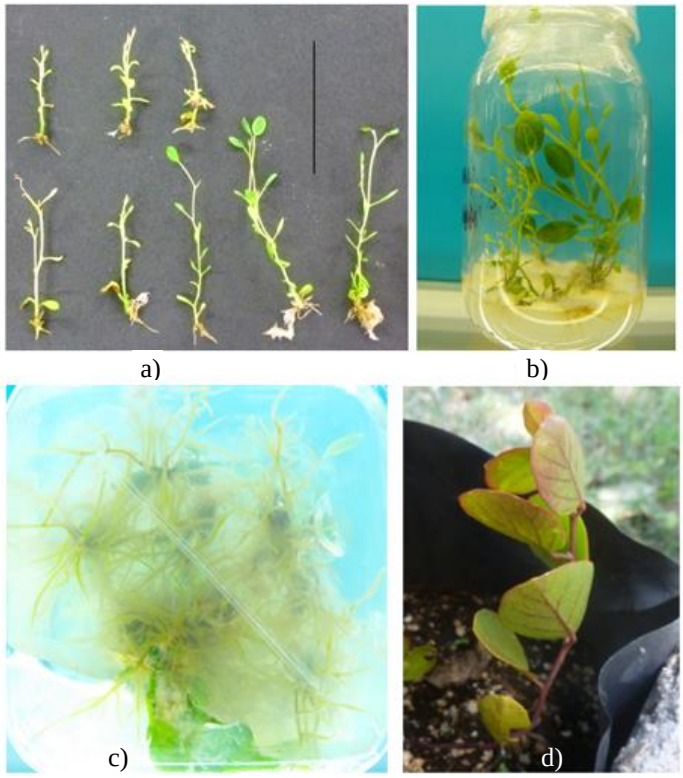

Figure 4. Rooting and growth in soil of caper plants generated *in vitro*. a) beginning of rooting and growth of caper sprouts 25 d after being separated and placed in a basal medium (bar= 5 cm); b) plants generated *in vitro* after 50 d of incubation in basal medium, ready for adaptation and transfer to soil; c) appearance of the radical systems generated at 50 d; and d) plant generated *in vitro,* already growing in the soil and outdoors 50 d after leaving the *in vitro* system.

The quality of the light, white or RFA, did not affect the rooting rate, nor the development of the aerial part of the seedlings at this stage. However, since the energy consumption of RFA lamps is lower than that of white light, their use could be an advantage from the point of view of production cost. This type of lighting has shown beneficial effects on the photomorphogenesis and productivity of various plant species, both under greenhouse conditions and *in vitro* (Goins *et al*., 1997; Dutta Gupta and Jatothu, 2013).

Finally, with respect to the process of adaptation and transfer to soil of the plants generated *in vitro*, the best results were obtained with the second of the tested protocols, with 85% survival (Figure 4d). In protocols 3 and 4, survival was lower (65 and 47%). Regarding protocol 1, in which a bioclimatic chamber was used to regulate environmental conditions, it was inefficient, since all the plants died when they were removed from the chamber and taken to the greenhouse. The literature reports survival rates similar to those obtained in this research, Chalak and Elbitar (2006); Al-Safadi and Elias (2011); Carra *et al*. (2012a); Vijay *et al*. (2014), the latter for the species *C. decidua*, report survivals of 86%, 82%, 92% and 80%, respectively.

Conclusions

The low efficiency of caper multiplication through seeds was confirmed, due to its low germination rate. This justifies the development of *in vitro* multiplication systems that allow the mass production of plants. The cytokinins MT, BA and 2iP were found to be capable of generating multiple sprouts and sprout masses in semi-solid medium. The well-differentiated sprouts were rooted and turned into seedlings, while the sprout masses were transferred to a RITA system, where they generated 89 well-differentiated sprouts per original explant. The rooting and adaptation and transfer to the soil of the plants obtained *in vitro* occurred with efficiencies of 80 and 85%, respectively. Although there were already reports on the *in vitro* propagation of this species, this work contributes to the use of TM and RITA systems, which notably increases the efficiency of the system.

Cited literature

Al-Safadi, B. and Elias, R. 2011. Improvement of caper (*Capparis spinosa* L.) propagation using *in vitro* culture and gamma irradiation. Scientia Horticulturae. 127:290-297. Doi: 10.1016/j.scienta.2010.10.014.

Bhoyar, M. S.; Mishra, G. P.; Singh, R. and Singh, S. B. 2010. Effects of various dormancy breaking treatments on the germination of wild caper (*Capparis spinosa*) seeds from the cold arid desert of trans-Himalayas. Indian J. Agric. Sci. 80:621-625.

Carra, A.; Del Signore, M. B.; Sottile, F.; Ricci, Ada. and Carimi, F. 2012a. Potential use of new diphenylurea derivatives in micropropagation of *Capparis spinosa* L. Plant Growth Regulators. 66:229-237. Doi: 10.1007/s10725-011-9645-3.

Carra, A.; Sajeva, M.; Abbate, L.; Siragusa, M.; Sottile, F. and Carimi, F. 2012b. *In vitro* plant regeneration of caper (*Capparis spinosa* L.) from floral explants and genetic stability of regenerants. Plant Cell, Tissue and Organ Culture. 109:373-381. Doi: 10.1007/s11240-011-0102-9.

Chalak, L. and Elbitar, A. 2006. Micropropagation of *Capparis spinosa* L. subsp. *rupestris* Sibth. & Sm. by nodal cuttings. Indian J. Biotechnol. 5:555-558.

Delgadillo-Diaz, L. J. S.; Morales-Dominguez, J. F.; Santos-Diaz, M. S. and Pérez-Molphe, B. E. 2013. *In vitro* propagation of Mexican oaks (*Quercus* spp.). Polibotánica. 35:85-97.

Deora, N. S. and Shekhawat, N. S. 1995. Micropropagation of *Capparis decidua* (Forsk.) Edgew. A tree of arid horticulture. Plant Cell Reports. 15:278-281. Doi:10.1007/BF00193736.

Dutta-Gupta, S. and Jatothu, B. 2013. Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. Plant Biotechnol. Reports. 7:211-220. https://doi.org/10.1007/s11816-013-0277-0.

Etienne, H. and Berthouly, M. 2002. Temporary immersion system in plant Micropropagation. Plant Cell, Tissue and Organ Culture. 69:215-231. Doi:10.1023/A:1015668610465.

Farhoudi, R. and Makezadeh, T. M. 2013. The effect of seed dormancy breaking methods on caper (*Capparis spinosa* L.) germination and growth. Sci. J. Agron. Plant Breed. 1:20-25.

Gan, L.; Zhang, C.; Yin, Y.; Lin, Z.; Huang, Y.; Xiang, J.; Fu, C. and Li, M. 2013. Anatomical adaptations of the Xerophilous medicinal plant, *Capparis spinosa*, to drought conditions. Hortic. Environ. Biotechnol. 54:156-161. Doi:10.1007/s13580-013-0162-3.

Goins, G. D.; Yorio, N. C.; Sanwo, M. M. and Brown, C. S. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. Exp. Bot. 48:1407-1413.

Hessam, A. I.; Khani-Nejad, S. and Kafi, M. 2012. Roles of duration and concentration of priming agents on dormancy breaking and germination of caper (*Capparis spinosa* L.) for the protection of arid degraded areas. Pak. J. Bot. 44:225-230.

Khalil, S. M.; Ramachandra, B. N.; Jacob, S. and Rachel, T. R. 2012. Effect of rooting hormones (IBA and NAA) on rooting of semi hardwood cuttings of *Capparis spinosa*. J. Agric. Biod. Res. 1:135-139.

Levizou, E.; Drilias, P. and Kyparissis, A. 2004. Exceptional photosynthetic performance of *Capparis spinosa* L. under adverse conditions of Mediterranean summer. Photosynthetica. 42:229-235. https://doi.org/10.1023/B:PHOT.0000040594.85407.f4.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15:473-497. Doi:10.1111/j.1399-3054.1962.tb08052.x.

Musallam, I.; Duwayri, M. and Shibli, R. A. 2011. Micropropagation of caper (*Capparis spinosa* L.) from wild plants. Functional Plant Sci. Biotechnol. 5:17-21.

Noguchi, K.; Gel, Y. R.; Brunner, E. and Konietschke, F. 2012. nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments. J. Statistical Software. 50:1-23. Doi: 10.18637/jss.v050.i12.

Rodríguez, R.; Cuozzo L. M. R. and Ancora, G. 1990. *In vitro* propagation of caper (*Capparis spinosa* L.). *In vitro* Cellular Development Biology-Plant. 26:531-536. Doi: https://doi.org/10.1007/BF02624097.

Strnad, M. 1997. The aromatic cytokinins. Physiologia Plantarum. 101:674-688. Doi: 10.1111/j.1399-3054.1997.tb01052.x.

Teisson, C.; Alvard, D.; Berthouly, B.; Cote, F.; Escalant, V.; Etienne, H. and Lartaud, M. 1996. Simple apparatus to perform plant tissue culture by temporary immersion. Acta Hortic. 440:521-526. https://doi.org/10.17660/ActaHortic.1996.440.91.

Tyagi, P. and Kothari, S. L. 1997. Micropropagation of *Capparis decidua* through *in vitro* shoot proliferation on nodal explants of mature tree and seedling explants. J. Plant Biochem. Biotechnol. 6:19-23. Doi: 10.1007/BF03263003.

Tyagi, P.; Khanduja, S. and Kothari, S. 2010. *In vitro* culture of *Capparis decidua* and assessment of clonal fidelity of the regenerated plants. Biologia Plantarum. 54:126-130. Doi: 10.1007/s10535-010-0019-x.

Vijay, N.; Arya, S. and Arya, L. D. 2014. Rapid and mass propagation of the economically important desert plant *Capparis decidua* for its afforestation program. J. Arid Land Studies. 24:33-36.

Werbrouck, S. P. O.; Strnad, M.; Van-Onckelen, H. A. and Debergh, P. C. 1996. Meta-topolin, an alternative to benzyladenine in tissue culture? Physiologia Plantarum. 98:291-297. https://doi.org/10.1034/j.1399-3054.1996.980210.x.