

High-throughput in vitro rooting in caper (*Capparis spinosa* L.) via a paper-based system

Margarita Pérez-Jiménez

margarita.perez3@carm.es

Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA), La Alberca (Murcia)

José Manuel Gambín

Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA), La Alberca (Murcia)

Miriam Romero-Muñoz

Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA), La Alberca (Murcia)

Carmen López-Sierra

Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA), La Alberca (Murcia)

Research Article

Keywords: Adventitious rooting, mineral nutrition, root morphology, filter paper, micropropagation, acclimatization

Posted Date: August 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10662982/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Caper (*Capparis spinosa* L.) is a promising Mediterranean crop, but the lack of reliable clonal propagation protocols limits the large-scale multiplication of selected genotypes. This study aimed to develop a practical rooting procedure for micropropagated shoots of a genotype selected for commercial shoot production, with particular emphasis on rooting speed and root quality. The effects of auxin treatment, Murashige and Skoog (MS) mineral salt concentration, physical support and ultrasound were evaluated, together with a paper-based rooting system. On agar-solidified media, reducing MS salts accelerated root induction independently of the auxin treatment. Root emergence occurred earliest on one-third-strength MS, followed by half-strength MS, whereas full-strength MS delayed rooting. After 90 days, more than 90% of shoots rooted on one-third- and half-strength MS, compared with approximately 70% on full-strength MS. Replacing agar with vermiculite or applying ultrasound did not significantly improve rooting percentage or root morphology. In contrast, the paper-based system under the lowest nutrient availability promoted earlier rooting, the highest final rooting response, and the formation of more abundant, predominantly basal and downward-growing roots. These results show that rooting efficiency in caper depends not only on root induction but also on the nutritional and physical environment surrounding developing roots. Combining reduced mineral availability with a non-gelled paper support provides a simple approach for improving rooting performance and may facilitate the scaling-up of caper micropropagation.

Key message

Reducing mineral salt concentration accelerates caper rooting, while a paper-based non-gelled system improves root morphology, providing a simple approach for scalable clonal propagation.

Introduction

Caper (*Capparis spinosa* L.) is a perennial xerophytic shrub native to the Mediterranean basin and valued for its edible flower buds, immature fruits, and young shoots. Its ability to grow under high temperatures, limited water availability, and poor soils has renewed interest in the species as a crop for the diversification of Mediterranean agriculture under increasingly arid conditions (Chedraoui et al. 2017; Kereša et al. 2019; Koufan et al. 2022). In south-eastern Spain, tender caper shoots are traditionally pickled and consumed as a regional product. This traditional use is part of a broader and increasingly attractive international market for caper products; however, further commercial development remains constrained by the still limited domestication and professionalization of the crop, including the scarcity of selected cultivars and reliable propagation systems (Chedraoui et al. 2017; Sottile et al. 2021). Production for this market requires plants with vigorous resprouting, thick and tender shoots, small and sparse leaves, and few or no thorns. These traits define a selection objective that differs from the more widely studied production of flower buds and fruits and makes the identification and preservation of suitable local genotypes especially important.

The domestication of caper has nevertheless been constrained by its propagation. Plants are commonly raised from seed, but germination is often irregular and the resulting progenies are genetically heterogeneous. Seed propagation is therefore unsuitable when the objective is to reproduce selected plants with a particular combination of commercial traits (Foschi et al. 2022; Koufan et al. 2022). This limitation is particularly relevant for shoot production, because the suitability of a seed-derived plant cannot be fully assessed until it has completed its juvenile development and expressed its vegetative characteristics. Propagation by stem cuttings would retain the selected genotype, but rooting is generally erratic and is strongly affected by the physiological condition of the donor plant, the type of cutting, and the genotype (Gianguzzi et al. 2019; Koufan et al. 2022). Consequently, the lack of a dependable clonal propagation method remains an important obstacle to the establishment of uniform caper plantations.

Micropropagation provides a means of overcoming this limitation by enabling the rapid multiplication of selected plants under controlled conditions. Previous studies have demonstrated that caper can be propagated from nodal segments, seed-derived shoots, and floral explants, and that multiplication can also be intensified through liquid-culture and temporary-immersion systems. However, the conversion of multiplied shoots into rooted plants remains one of the least consistent stages of the process. Root induction has been achieved using different auxins, reduced-strength basal media, short auxin pulses, and, in some genotypes, media without exogenous auxins (Chalak et al. 2003; Musallam et al. 2011; Carra et al. 2012a; Kereša et al. 2019). The diversity of these responses reflects the strong influence of genotype and culture history and explains why published procedures are not always transferable to newly selected material (Koufan et al. 2022). Moreover, treatments that stimulate root initiation may also induce basal callus, which can delay root formation and compromise the suitability of the resulting root system for subsequent ex vitro transfer (Carra et al. 2012b; Gianguzzi et al. 2020). Prolonged rooting periods may additionally extend the production cycle and delay the onset of acclimatization. An effective rooting protocol must therefore combine reliable root formation with rapid induction, appropriate root morphology, and straightforward plant handling.

Most caper rooting procedures have been developed on agar-solidified media, however, other techniques have arisen. Liquid culture and temporary-immersion systems have already shown that the multiplication stage of caper can be simplified while reducing the use of gelling agents and manual operations (Gianguzzi et al. 2019; Contreras-Loera et al. 2021).

Accordingly, the aim of this study was to develop a practical and scalable rooting procedure for micropropagated shoots of a caper genotype selected in the field for commercial shoot production. The effects of plant growth regulators, mineral salt concentration, physical support, and ultrasound treatment on root induction were evaluated. In addition, a paper-based system supplied with liquid rooting solutions was assessed as an alternative to conventional agar-based culture. Rooting was monitored over time to compare both the capacity for root formation and the speed of the response.

Material and methods

Plant material and experimental conditions

Plant material was collected from wild caper plants (*Capparis spinosa* L.) growing in south-eastern Spain (Murcia, Spain). A single genotype was selected for superior shoot traits (shoot thickness, absence of thorns, small and sparse leaves, and high sprouting capacity). Selected mother plants were georeferenced (GPS), and shoots were transferred to the tissue culture laboratory. This genotype had been previously evaluated under laboratory and commercial conditions to confirm its suitability for clonal propagation.

Shoots were excised into nodal segments and surface-sterilized following a protocol previously developed in our laboratory for wild and creeping species. Briefly, nodal segments were washed in a commercial soap solution, immersed in 70% (v/v) ethanol for 5 min, and then treated with 2% (v/v) sodium hypochlorite supplemented with 0.1% (v/v) Tween 20 for 2 h on an orbital shaker under a laminar flow hood.

In vitro cultures were established and subcultured monthly on multiplication medium for 3 months. The multiplication medium consisted of Driver and Kuniyuki Walnut (DKW) basal salts (Driver and Kuniyuki 1984) supplemented with 0.5 mg L⁻¹ 6-benzylaminopurine (BA), 3% (w/v) sucrose and 0.6% (w/v) Plant Propagation Agar (Condalab, Madrid, Spain). The pH was adjusted to 5.7 with 0.1 N KOH before autoclaving at 122°C for 16 min. Cultures were maintained in growth chambers at 25 ± 1°C under a 16 h photoperiod (white LED light, 380–780 nm; 45 µmol m⁻² s⁻¹).

Root induction with different PGRs

Rooting was induced using 9 media formulations in micropropagated caper shoots. The basal medium consisted of Murashige and Skoog (MS) salts (Murashige and Skoog 1962), 3% (w/v) sucrose, and 0.6% (w/v) Plant Propagation Agar (Condalab, Madrid, Spain), supplemented with plant growth regulators (PGRs) as indicated in Table 1. A volume of 120 mL was dispensed per glass vessel. The pH was adjusted to 5.7 with 0.1 N KOH prior to autoclaving at 122°C for 16 min. Cultures were incubated at 25 ± 1°C under a 16 h photoperiod (white LED light, 380–780 nm; 45 µmol m⁻² s⁻¹) (Fig. 1A). Rooting response was recorded at 30, 45, 60, 75 and 90 days after culture initiation.

Table 1
Composition
of the
culture
media used
during the
induction of
roots in
*Capparis
spinosa* L.
shoots.

Root induction with vermiculite and ultrasounds

Rooting was also tested using a modified TN medium (Table 1) combined with vermiculite (TNV; Table 2) (Fig. 1C). Vermiculite was added to the vessels at a ratio of 40 g vermiculite per 80 mL culture medium, and vessels were autoclaved at 122°C for 16 min. In a separate treatment, caper shoots cultured on TN medium were exposed to ultrasound (Branson M 2800, Emerson, Saint Louis, MO, USA) for 15 min to assess its effect on rooting (Fig. 1B). Shoots cultured on TN medium without ultrasound served as the control. All cultures were maintained at 25 ± 1°C under a 16 h photoperiod (white LED light, 380–780 nm; 45 µmol m⁻² s⁻¹). Rooting response was recorded at 30, 45, 60, 75 and 90 days after culture initiation.

Table 2
Composition of the culture media used during the induction of roots in *Capparis spinosa* L. shoots.

Media	Salts	NAA	IBA	Sucrose	Agar	Vermiculite
		mg l ⁻¹		g l ⁻¹		
TNV	1/3 MS	1.50	0.00	10.00	3.00	500.00
TNP	1/3 MS	1.50	0.00	10.00	0.00	0.00
QNP	1/5 MS	1.50	0.00	10.00	0.00	0.00
WNP	-	1.50	0.00	10.00	0.00	0.00
MS Murashige and Skoog salts, NAA a-naphthalene acetic acid; IBA indolebutyric acid.						

Root induction via a paper-based system

For the paper-based rooting system, shoots were placed on sterile filter paper inside Magenta boxes (Duchefa Biochemie, Haarlem, The Netherlands) (Fig. 1D). Filter paper squares were autoclaved at 122°C for 16 min. Under a laminar flow hood, three liquid treatments were applied to the filter paper: TNP, QNP and WNP (Table 2). Rooting response was recorded at 30, 45, 60, 75 and 90 days after culture initiation.

Statistical analysis

Each treatment consisted of 20 vessels (glass vessels or Magenta™ boxes), with 10 shoots per vessel. Data were tested for normality and homogeneity of variance prior to analysis. Differences among treatments were assessed by analysis of variance (ANOVA). Mean separation was performed using Duncan’s new multiple range test at P < 0.05. Analyses were conducted using Statgraphics Centurion® XVI (StatPoint Technologies Inc., The Plains, VA, USA).

Results

All agar-solidified media induced adventitious rooting in micropropagated caper shoots. However, the resulting root systems generally showed poor morphological quality. Roots were thin and frequently emerged not only from the basal cut surface but also from aerial portions of the shoots. In many cases, they grew horizontally along the surface of the medium or upwards into the vessel headspace rather than penetrating the agar, resulting in weak and poorly organized root systems (Fig. 1E).

Figure 2 shows the changes over time in the percentage of rooted shoots cultured on agar-solidified media supplemented with IBA, NAA, or a combination of both auxins. In all three auxin treatments, the onset of rooting was progressively delayed as the concentration of MS salts increased. Root emergence was first observed in one-third-strength MS medium, followed by half-strength MS medium, whereas shoots cultured on full-strength MS medium showed the latest rooting response. Although one-third-strength MS consistently produced the earliest numerical response, no significant differences were detected between one-third- and half-strength MS media at the end of the experiment (90 days). This pattern was consistent irrespective of whether IBA, NAA, or a combination of both auxins was used. After 90 days, more than 90% of the shoots had rooted in both one-third- and half-strength MS media, whereas approximately 70% of those cultured on full-strength MS medium had rooted in each auxin treatment.

The physical support and ultrasound treatment did not significantly affect the rooting percentage after 90 days of culture (Fig. 3). Similar responses were obtained when shoots were cultured on agar-solidified medium or vermiculite, or when they were subjected to ultrasound treatment before culture, with approximately 90% of the shoots developing roots in all three treatments. Nevertheless, neither the replacement of agar with vermiculite nor the application of ultrasound improved root morphology, as the roots remained predominantly thin and irregularly oriented (Fig. 1F).

In the paper-based system, shoots cultured on paper without culture medium showed the highest rooting response (Fig. 4). This treatment promoted both an earlier onset of rooting and the highest percentage of rooted shoots at the end of the experiment. By contrast, the inclusion of MS salts delayed root emergence and reduced the final rooting percentage. Roots formed on paper without culture medium also showed a visibly improved morphology compared with those produced on agar or vermiculite. They were visually more abundant, emerged predominantly from the basal region of the shoots, and grew downwards, forming a denser and better-oriented root system (Fig. 1F, G and H).

Discussion

Adventitious rooting is considered one of the most variable and critical stages of caper micropropagation, and markedly different responses have been reported depending on genotype, culture medium, plant growth regulators and culture conditions (Koufan et al. 2022). In the present study, rooting percentages above 90% were obtained after 90 days when shoots were cultured on one-third- or half-strength MS medium, irrespective of whether IBA, NAA or their combination was used. These values are within the upper range previously reported for *Capparis spinosa*. Chalak et al. (2003) obtained 87%

rooting after a 4-h pulse treatment with 100 mg L⁻¹ IAA followed by culture on half-strength MS medium, whereas Musallam et al. (2011) reported 80% rooting on half-strength MS supplemented with 5 mg L⁻¹ IAA. Carra et al. (2012a) obtained up to 93.7% rooted shoots with IBA or the synthetic phenylurea 2,3-MDPU, while Contreras-Loera et al. (2021) reported 80% rooting on basal MS medium without auxins.

Although rooting percentages close to or even above 90% have been reported in caper, these high values contrast with the persistent reputation of the species as difficult to propagate. This apparent contradiction probably reflects not an intrinsic inability of caper shoots to form roots, but rather the poor reproducibility of rooting responses among different plant materials and culture conditions. Indeed, strong genotype-dependent responses have been reported even among accessions from the same geographical origin, and a rooting protocol that performs well with a particular accession may not be transferable to others (Gianguzzi et al. 2020; Sottile et al. 2021). Sottile et al. (2021) therefore argued that caper should not necessarily be considered a difficult-to-propagate crop *sensu stricto*, since satisfactory propagation can be achieved when appropriate techniques are applied to responsive and well-selected genotypes. Furthermore, successful root induction does not necessarily imply the formation of a root system capable of supporting subsequent plant establishment. In caper, roots induced under some in vitro conditions have been described as weak and unsuitable for successful ex vitro transfer (Carra et al. 2012b). Thus, the efficiency of a rooting protocol should be considered not only in terms of the percentage of shoots forming roots, but also in terms of root quality and their subsequent performance during acclimatization.

Within this context, identifying the specific culture conditions that allow caper shoots not only to root efficiently but also to do so rapidly becomes particularly important. In the genotype evaluated here, mineral salt concentration emerged as one of the most consistent factors affecting the rooting response. In all three auxin treatments, root emergence occurred first on one-third-strength MS, followed by half-strength MS, whereas full-strength MS delayed root induction. Notably, the time required for root emergence increased progressively with the initial mineral salt concentration. This temporal pattern is consistent with the possibility that root induction was favored as nutrient availability declined during culture, with media containing lower initial salt concentrations reaching a permissive nutritional threshold for rhizogenesis earlier than full-strength MS. Although nutrient depletion was not directly measured, this interpretation would explain the progressive delay in rooting observed as the initial salt concentration increased. Moreover, at the end of the experiment, one-third- and half-strength MS resulted in more than 90% rooted shoots, compared with approximately 70% on full-strength MS. The fact that this response was repeated with IBA, NAA and IBA + NAA suggests that, under our experimental conditions, reducing the mineral salt concentration was more decisive for accelerating root induction than the auxin source itself. Thus, adjustment of mineral nutrition appears to be a key component of a genotype-specific rooting protocol aimed at shortening the rooting phase in caper.

The favorable effect of reducing mineral salt concentration during rhizogenesis is not restricted to caper and has been reported in several woody species. In *Ceratonia siliqua*, Gonçalves et al. (2005) increased rooting to 50% by reducing MS salts to half strength, while further adjustment of the macronutrient

composition increased rooting to 80%. Similarly, in the drought-tolerant wild pear *Pyrus boissieriana*, the best rooting response was obtained on one-sixth-strength MS supplemented with a low concentration of IBA, resulting in 100% rooting without intermediate callus formation (Zakavi et al. 2016). An even stronger dependence on mineral concentration was reported in American chestnut (*Castanea dentata*), where shoots failed to root on full-strength DKW medium despite the presence of auxins, whereas reducing the basal salts to half strength markedly improved root induction, reaching 83% with 10 μ M IBA and 100% after further optimization of the auxin treatment (Liu et al. 2022). These examples suggest that the mineral requirements of the rooting phase may be considerably lower than those required to sustain active shoot proliferation in some woody species.

A possible physiological basis for this response lies in the ability of plants to adjust resource allocation and root development according to mineral availability. Mineral nutrients, particularly nitrate, act not only as metabolic resources but also as signals regulating root system development (Lay-Pruitt and Takahashi 2020). Under reduced nitrate availability, plants may increase their relative investment in below-ground structures as part of a nutrient-foraging response. In *Arabidopsis thaliana*, for example, low nitrate supply increased the root-to-shoot biomass ratio and promoted lateral root and root-hair development, thereby increasing the exploratory capacity of the root system (De Pessemier et al. 2022). Nitrate signalling is also closely interconnected with auxin metabolism and transport; the nitrate transceptor NRT1.1/NPF6.3 participates in the regulation of auxin biosynthesis and distribution during root development (Maghiaoui et al. 2020). Although these mechanisms have mainly been characterized for the development and architecture of an already established root system rather than for adventitious root initiation, they provide a plausible physiological framework for the faster rhizogenesis observed here under reduced mineral supply.

Importantly, the percentage of rooted shoots alone did not adequately describe rooting performance in the present study. Although approximately 90% of shoots produced roots when cultured on agar or vermiculite or after ultrasound treatment, the resulting roots were predominantly thin and irregularly oriented. A similar distinction between root induction and root quality has previously been recognized in caper. Carra et al. (2012b) reported that roots induced under some auxin treatments were weak and unsuitable for subsequent ex vitro transfer. Thus, the efficiency of a rooting protocol should be evaluated not only by the percentage of shoots forming roots, but also by the morphological quality of the root system and its potential to support subsequent plant establishment.

The unusual orientation of roots observed on agar-solidified medium, where roots frequently grew along the medium surface or towards the vessel headspace rather than penetrating the gel, suggests that the physical properties of the support may also influence root development. Gelling agents are not biologically neutral; Scholten and Pierik (1998) showed that adventitious rooting was among the developmental processes particularly sensitive to agar characteristics. More recently, increasing agar concentration and the associated mechanical impedance were shown to reduce root penetration and rooting depth (Bello et al. 2022). Although mechanical resistance was not experimentally measured in

our study, these observations are consistent with the possibility that the agar matrix imposed a physical constraint on root growth and orientation.

In contrast, the paper-based system under the lowest nutrient availability provided a non-gelled environment in which developing roots could grow without being embedded in a solid matrix. Shoots rooted earlier, reached the highest final rooting percentage and developed a more abundant and better-oriented root system, with roots emerging predominantly from the basal region and growing downwards. This indicates that the response cannot be attributed solely to the elimination of agar, but rather to the combination of a favorable nutritional environment and a physical support that does not constrain root growth. Besides its biological advantages, the absence of a gelling agent may also simplify subsequent handling by avoiding the need to remove agar from the roots before transfer.

Conclusions

The present results indicate that efficient *in vitro* rooting of caper depends not only on inducing a high percentage of rooted shoots, but also on providing nutritional and physical conditions that favor rapid formation of a well-organized root system. In the genotype studied here, reducing mineral salt concentration accelerated root induction, while the paper-based system promoted the development of more abundant and better-oriented roots without the need for a gelled medium. The combination of these two factors therefore provides a simple, gelling-agent-free approach that could facilitate the handling and scaling-up of caper rooting. More broadly, these findings support the idea that the apparent recalcitrance of caper can be alleviated through genotype-specific optimization of both mineral nutrition and the physical environment surrounding the developing roots. Further acclimatization studies will be required to determine whether the improved root morphology observed *in vitro* translates into higher survival and faster establishment *ex vitro*, as well as to assess the transferability of the system to other caper genotypes.

Declarations

Funding

This work was supported by the Region of Murcia.

Author information

Authors and Affiliations

Departament of Biotechnology, Genomics and Plant Breeding, Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA), C/Mayor s/n, 30150, La Alberca, Murcia, Spain

Margarita Pérez-Jiménez, José Manuel Gambín, Miriam Romero-Muñoz, Carmen López-Sierra.

Contributions

M.P.-J., supervised the research, analyze the data and wrote, reviewed and edited the manuscript. J. M.G. carried out the majority of the experiments, M.R.-M. participated in the statistical analysis and paper editing. C.L.-S. participated in plant material evaluation and lab work. All authors read and approved the final manuscript.

Corresponding author

Correspondence to Marga Pérez-Jiménez

Ethics declarations

Ethical approval

Not applicable

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Bello Bello E, Rico Cambron TY, Ortiz Ramírez LA, Rellán Álvarez R, Herrera-Estrella L (2022) ROOT PENETRATION INDEX 3, a major quantitative trait locus associated with root system penetrability in *Arabidopsis*. *J Exp Bot* 73:4716–4732. <https://doi.org/10.1093/jxb/erac188>
2. Carra A, Del Signore MB, Sottile F, Ricci A, Carimi F (2012a) Potential use of new diphenylurea derivatives in micropropagation of *Capparis spinosa* L. *Plant Growth Regul* 66:229–237. <https://doi.org/10.1007/s10725-011-9645-3>
3. Carra A, Sajeva M, Abbate L, Siragusa M, Sottile F, Carimi F (2012b) In vitro plant regeneration of caper (*Capparis spinosa* L.) from floral explants and genetic stability of regenerants. *Planr Cell Tissue Organ Cult* 109:373–381. <https://doi.org/10.1007/s11240-011-0102-9>
4. Chalak L, Elbitar A, Cordahi N, Hage C, Chehade A (2003) In vitro propagation of *Capparis spinosa* L. *Acta Hort* 616:335–338. <https://doi.org/10.17660/ActaHortic.2003.616.50>
5. Chedraoui S, Abi-Rizk A, El-Beyrouthy M, Chalak L, Ouaini N, Rajjou L (2017) *Capparis spinosa* L. in a systematic review: a xerophilous species of multi values and promising potentialities for agrosystems under the threat of global warming. *Front Plant Sci* 8:1845. <https://doi.org/10.3389/fpls.2017.01845>
6. Contreras-Loera F, Chávez-Ortiz LI, Morales-Domínguez JF, Pérez-Molphe-Balch E (2021) Micropropagation of the caper in semi-solid medium and in temporary immersion bioreactors. *Revista Mexicana de Ciencias Agrícolas* 12:37–50
7. De Pessemier J, Moturu TR, Nacry P, Ebert R, De Gernier H, Tillard P, Swarup K, Wells DM, Haseloff J, Murray SC, Bennett MJ, Inzé D, Vincent CI, Hermans C (2022) Root system size and root hair length

- are key phenes for nitrate acquisition and biomass production across natural variation in *Arabidopsis*. *J Exp Bot* 73:3569–3583. <https://doi.org/10.1093/jxb/erac118>
8. Driver JA, Kuniyuki AH (1984) In vitro propagation of Paradox walnut rootstock. *HortScience* 19:507–509
 9. Foschi ML, Juan M, Pascual B, Pascual-Seva N (2022) Collection guidelines to achieve a viable caper commercial propagation. *Agronomy* 12:74. <https://doi.org/10.3390/agronomy12010074>
 10. Gianguzzi V, Inglese P, Barone E, Sottile F (2019) In vitro regeneration of *Capparis spinosa* L. by using a temporary immersion system. *Plants* 8:177. <https://doi.org/10.3390/plants8060177>
 11. Gonçalves S, Correia PJ, Martins-Loução MA, Romano A (2005) A new medium formulation for in vitro rooting of carob tree based on leaf macronutrients concentrations. *Biol Plant* 49:277–280. <https://doi.org/10.1007/s10535-005-7280-4>
 12. Kereša S, Stanković D, Batelja Lodeta K, Habuš Jerčić I, Bolarić S, Barić M, Bošnjak Mihovilović A (2019) Efficient protocol for the in vitro plantlet production of caper (*Capparis orientalis* Veill.) from the East Adriatic coast. *Agronomy* 9:303. <https://doi.org/10.3390/agronomy9060303>
 13. Koufan M, Belkoura I, Mazri MA (2022) In vitro propagation of caper (*Capparis spinosa* L.): a review. *Horticulturae* 8:737. <https://doi.org/10.3390/horticulturae8080737>
 14. Lay-Pruitt KS, Takahashi H (2020) Integrating N signals and root growth: the role of nitrate transceptor NRT1.1 in auxin-mediated lateral root development. *J Exp Bot* 71:4365–4368. <https://doi.org/10.1093/jxb/eraa243>
 15. Liu Z, Bi W-L, Shukla MR, Saxena PK (2022) In vitro technologies for American chestnut (*Castanea dentata* (Marshall) Borkh) conservation. *Plants* 11:464. <https://doi.org/10.3390/plants11030464>
 16. Maghiaoui A, Bouguyon E, Cuesta C, Perrine-Walker F, Alcon C, Krouk G, Benková E, Nacry P, Gojon A, Bach L (2020) The *Arabidopsis* NRT1.1 transceptor coordinately controls auxin biosynthesis and transport to regulate root branching in response to nitrate. *J Exp Bot* 71:4480–4494. <https://doi.org/10.1093/jxb/eraa242>
 17. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
 18. Musallam I, Duwayri M, Shibli RA (2011) Micropropagation of caper (*Capparis spinosa* L.) from wild plants. *Funct Plant Sci Biotechnol* 5:17–21
 19. Scholten HJ, Pierik RLM (1998) Agar as a gelling agent: differential biological effects in vitro. *Sci Hort* 77:109–116. [https://doi.org/10.1016/S0304-4238\(98\)00165-4](https://doi.org/10.1016/S0304-4238(98)00165-4)
 20. Sottile F, Caltagirone C, Peano C, Del Signore MB, Barone E (2021) Can the caper (*Capparis spinosa* L.) still be considered a difficult-to-propagate crop? *Horticulturae* 7:316. <https://doi.org/10.3390/horticulturae7090316>
 21. Zakavi M, Askari H, Irvani N (2016) Optimizing micropropagation of drought resistant *Pyrus boissieriana* Buhse. *Physiol Mol Biology Plants* 22:583–593. <https://doi.org/10.1007/s12298-016-0387-6>

Table 1

Table 1 is available in the Supplementary Files section.

Figures

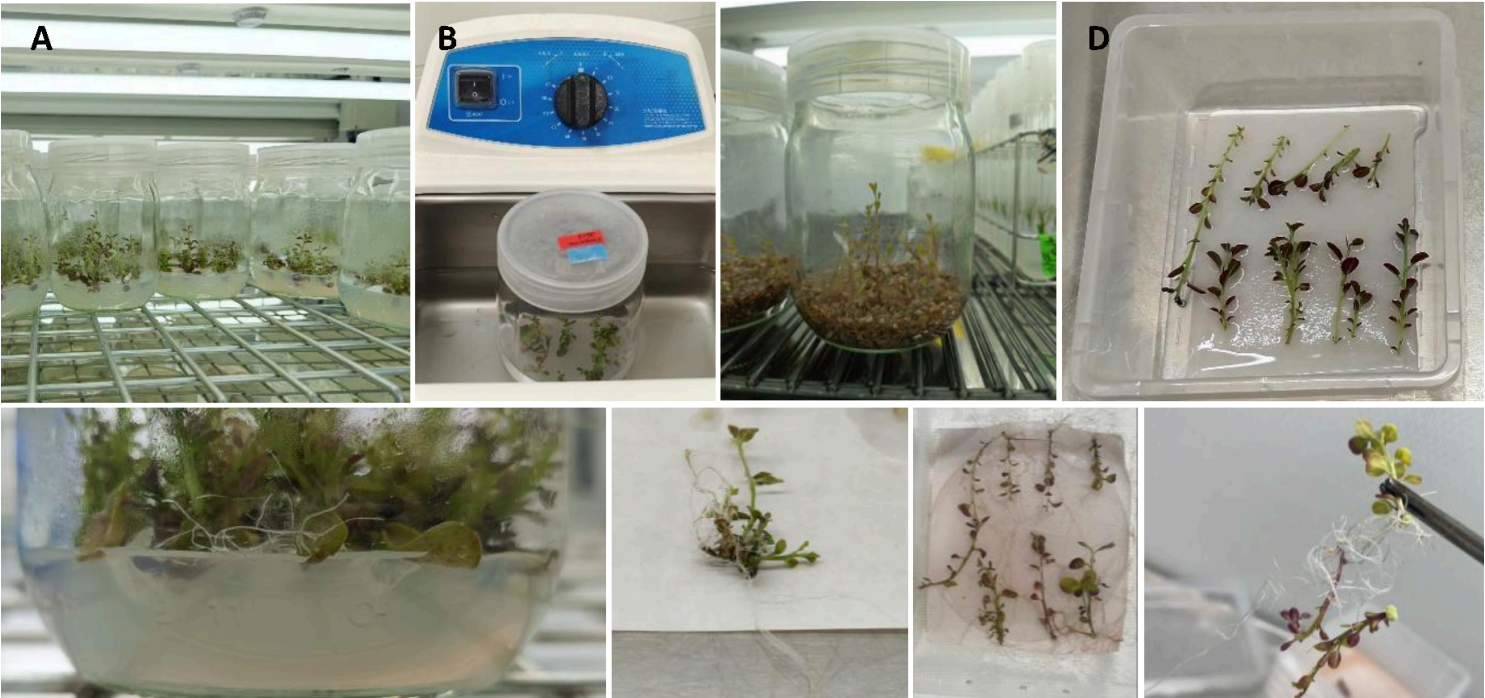


Figure 1. Caper (*Capparis spinosa* L.) shoots in the process of rooting. Caper shoots in the different culture media with agar to induce rooting (A), in the ultrasound equipment (B), in culture media with vermiculite (C) and in paper with water inside of a magenta box (D). Roots arising from the bottom and aerial parts of the caper shoots when cultured in media with agar (E-F), basal roots in shoots over paper with culture media (G-H).

Figure 1

See image above for figure legend

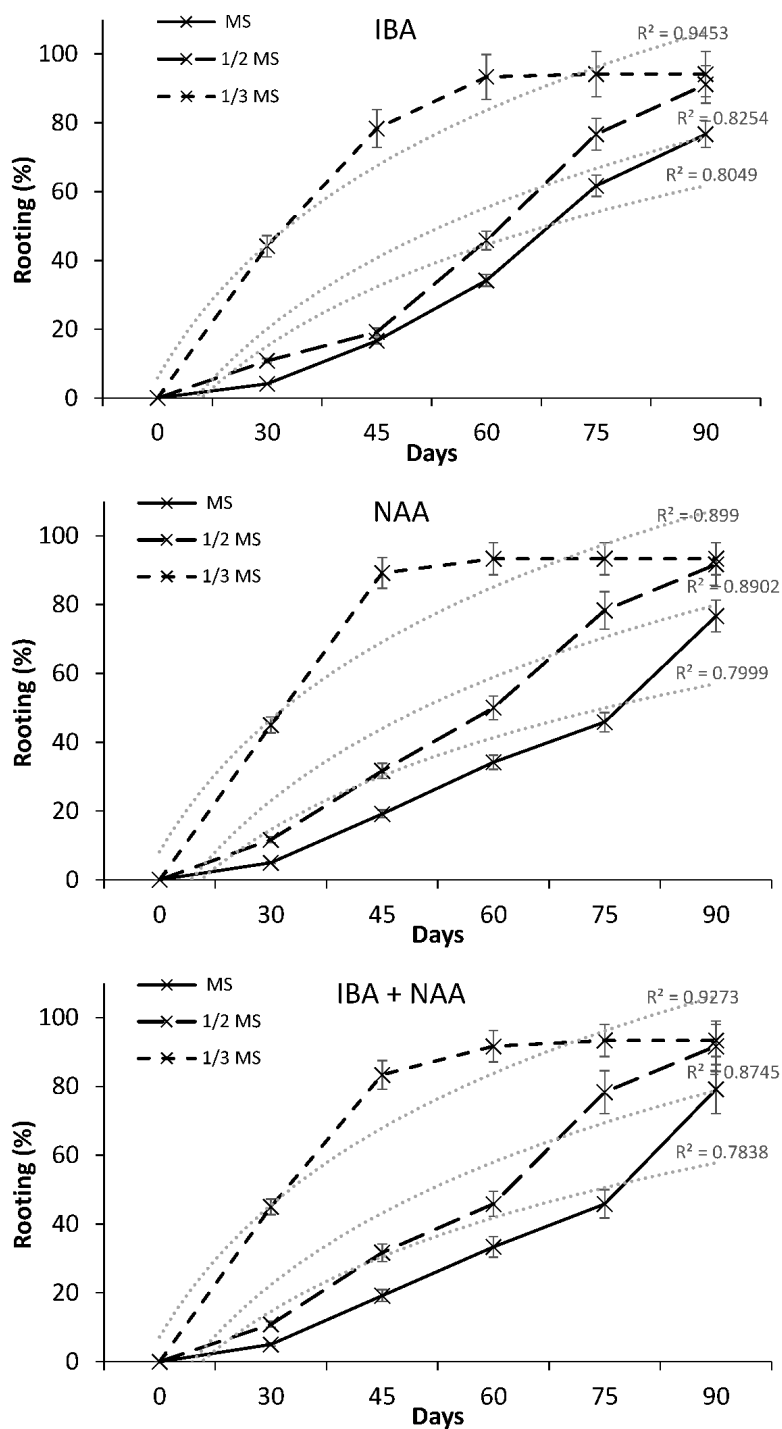


Figure 2. Percentage of rooted shoots of caper (*Capparis spinosa* L.) in culture media containing IBA, NAA and IBA+NAA at 30, 45, 60, 75 and 90 days after culture. Data represent average $\pm$ SD values.

Figure 2

See image above for figure legend

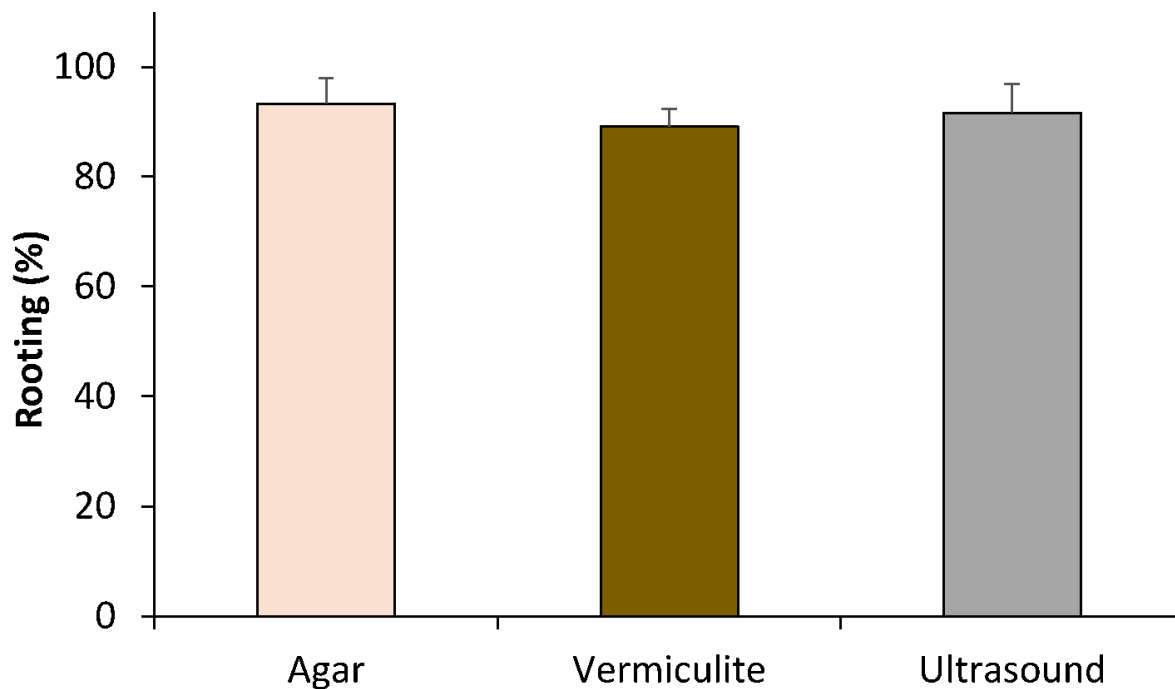


Figure 3. Percentage of rooted shoots of caper (*Capparis spinosa* L.) in different treatments after 90 days of culture. Data represent average $\pm$ SD values. Bars with different lower case letters indicate a significant difference by Duncan's new multiple range test ($P \leq 0.05$).

Figure 3

See image above for figure legend

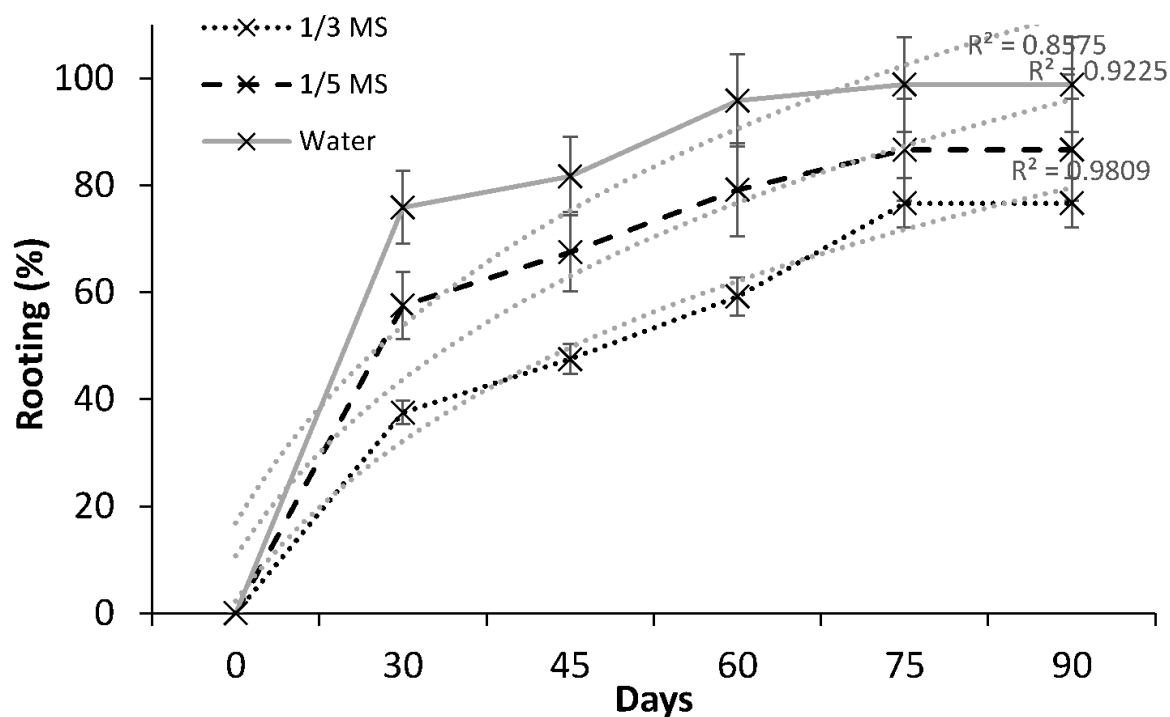


Figure 4. Percentage of rooted caper (*Capparis spinosa* L.) shoots cultured in a paper-based system supplied with solutions containing different MS salt concentrations, measured at 30, 45, 60, 75 and 90 days. Data represent average values.

Figure 4

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)