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In vitro root development under different IBA concentrations and culture media

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Abstract

In vitro root cultivation is a method that provides high production and volume of roots quickly due to controlled environmental conditions. However, few studies report the in vitro development of excised roots for the most diverse plant species. The objective was to evaluate the in vitro development of excised mint (Menta piperita L.) and tomato (Solanum lycopersicum L.) roots under different concentrations of IBA and verify tomato roots' growth in different cultivation media. Segments of 2 cm of roots were incubated in DSD1 culture medium with concentrations of 0, 0.25, 0.5, 1.0, and 2.0 mg. L^{-1} of AIB. Afterward, the 'M,' 'MSR', and DSD1 media were tested, supplemented with 1 mg L^{-1} of IBA. The cultivation of tomato roots shows greater in vitro development than mint, and 1 mg L^{-1} of IBA concentration was the most effective for tomatoes in DSD1 medium. The MSR medium resulted in a higher number and length of roots. Applying IBA increases the in vitro development of excised tomato roots compared to mint. For tomatoes, the concentration of 1 mg L^{-1} of IBA stimulates the most significant development of the roots. The MSR medium has the highest development of tomato roots at 60 days after in vitro introduction.

Keywords: *Menta piperita L.*, micropropagation, *Solanum lycopersicum L.*, tissue culture

Introduction

In *vitro* root cultivation is a method that, due to controlled environmental conditions and independence from seasonal variations, results in shorter organ development time, high yield, and optimization of physical space, providing high production and volume of roots in a short time (Cid & Teixeira, 2014).

Root cultivation can be employed to locate the biosynthesis site of compounds of the secondary metabolism of plants. Many medicinally important species synthesize pharmaceutical alkaloids as byproducts of normal metabolism in this organ. They efficiently accumulate large amounts of bioactive substances in the intercellular spaces, facilitating their extraction and isolation. (Baque et al., 2013).

In addition, in *vitro* root cultivation can be used to multiplicate Arbuscular Mycorrhizal Fungi (AMF). Currently, one of the obstacles to the production of AMF inoculants and their use by agricultural systems is the obligatory biotrophic nature of the fungus. An alternative to overcoming these obstacles is cultivating excised roots with subsequent inoculation of spores *in vitro*. This method can bring several advantages compared to the conventional method of growing in pots, such as producing pure inoculum with a high amount of contaminant-free propagules and in a small physical space (Ghorui et al., 2023).

An essential aspect of the success of *in vitro* cultivation is the composition of the culture medium and the supplementation with hormones that promote plant development. Auxins are a class of phytoregulators that are related to growth and development. Among the auxins, indolebutyric acid (IBA) acts in the formation of roots in several plant species, being widely used in plant propagation because it does not cause phytotoxicity in a wide range of concentrations and is efficient in a large

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number of plant species (Hartmann et al., 2017).

Likewise, the culture medium is responsible for providing the necessary conditions for explants to grow and develop *in vitro*, and there is a great diversity of media that can be employed, with different formulations and concentrations (Phillips & Garda, 2019). The roots are formed by cells specially adapted to absorb nutrients, developing better in culture media with low concentrations of ions. Thus, when multiplying micropropagated roots, the goal is root growth, the growing media should be more diluted (Danesh & Tufenkci, 2017).

Also, according to Pillai et al. (2015), few studies report the *in vitro* development of excised roots for the most diverse plant species.

Thus, the objective of the study was to evaluate the development of excised roots of mint (*Menta piperita*) and tomato (*Solanum lycopersicum*) under IBA concentrations and the growth of micropropagated tomato root segments in different culture media, plus the most efficient IBA concentration.

Material and methods

The study was conducted in two stages. The first experiment aimed to introduce and *in vitro* establishment of plant species in MS medium (Murashige & Skoog, 1962) subjected to different concentrations of IBA. In the second experiment, the aim was to promote the exclusive development of roots in DSD1 medium from the materials already established *in vitro* (Silva & Doazan, 1995), 'Minímo' (M) by Bècard & Fortin (1988), modified by Berbera & Fonseca (1996); 'MSR' medium, formulated by Declerck et al. (1998). The plant materials were mint (*Menta piperita* L.) and tomato (*Solanum lycopersicum* L.) seeds.

In vitro root production from mint and tomato root segments subjected to different concentrations of IBA

First, the germination of mint and tomato seeds was carried out. For this, the seeds were previously disinfested by immersion in 70% alcohol for 30 seconds and 2% sodium hypochlorite, plus a Tween-20 drop, for three minutes. Afterward, triple washing was performed in a sterile laminar flow hood with deionized and autoclaved water. Subsequently, they were seeded in 120 mL flasks containing MS medium with half its concentration, supplemented with 8 g L^{-1} of agar, 10 mg L^{-1} of sucrose, without phytoregulators, capped and stored in a growth chamber, at a temperature of 25 ±2ºC, 16 hours of photoperiod and 60-70% RH for 35 days.

After the emission of roots from the seeds, when they were more than 10 centimeters long, they were transplanted into Petri dishes. Thus, in a laminar flow hood, the roots were removed from the medium in which they were in the flasks and sectioned into segments of approximately 2 cm in length with a scalpel and tweezers. Subsequently, they were introduced into previously sterilized Petri dishes containing 25 mL of DSD1 culture medium, supplemented with 10 g L^{-1} sucrose, 8 g. L^{-1} of agar, different concentrations of IBA tested (0, 0.25, 0.5, 1.0 and 2.0 mg. L^{-1}) and the pH adjusted to 5.8 and, subsequently, autoclaved at a temperature of 121 °C and 1.2 atm, during 15 min, as recommended by Lopes (2003) for the root growth of the explants of these species.

The plates containing the root segments were sealed with PVC plastic film and packed in the dark in a growth room with the abovementioned conditions. After 20 days, the evaluation was performed by counting the number and length (cm) of the roots per explant with a magnifying glass. Lateral roots (branches) were not considered.

The experimental design was completely randomized, with two horticultural species, five concentrations of IBA, and four replications containing ten plates (with a root segment of 2 cm per plate). The results were submitted to analysis of variance, and the means were compared by Tukey's test ($p < 0.05$), in a factorial scheme species X concentration of IBA.

In vitro root production from tomato root segments under different growing media

In this step, three different formulations of culture media were tested: 'Minímo' (M), 'MSR' medium, and DSD1 medium, described above, plus 8.0 g L^{-1} of agar, 10 mg L^{-1} of sucrose and supplemented with 1 mg L^{-1} of IBA. Next, the tomato roots obtained in the previous experiment were transplanted. Thus, Petri dishes 10 cm in diameter, previously autoclaved and containing 25 mL of each of the culture media tested, receive a root segment of approximately 2 cm in a laminar flow hood. Afterwards, the plates were sealed with PVC plastic film and transferred to the growth room in the dark.

The number and average length of the three largest roots emitted per explant were measured using a magnifying glass. The first evaluation was 15 days after *in vitro* introduction, with an interval of assessments every 15 days, up to 60 days. The experimental design was completely randomized, with three culture media and four replications containing ten plates, containing one segment per plate. The results were submitted to analysis of variance, and the means were compared by Tukey's test ($p < 0.05$), in a two-factorial scheme (culture medium X days after *in vitro* introduction).

Results and discussion

In vitro root production from mint and tomato root segments subjected to different concentrations of IBA

The development of the root segments displayed significant differences between the two horticultural species evaluated, as well as for the doses of IBA, with no interaction between the factors. Mint obtained a lower number and average length of roots emitted per root segment than tomato. Regarding the concentrations of IBA in the culture medium, for mint, the addition of auxins did not influence the number of roots emitted per segment. Still, there was no emission of roots at the highest concentration of the phytoregulator. For the mean length, the concentration of 0.25 mg L^{-1} of IBA resulted in significantly larger radicals than the other treatments, although they were still relatively short. About the tomato, the number of roots emitted was higher in the culture medium supplemented with 1 mg L^{-1} of IBA, not differing statistically when 2 mg L-1 were added, while the other treatments were inferior. The longest roots were observed in the treatments containing 1 and 2 mg L^{-1} of IBA. In contrast, the concentration of 0.5 mg L-1 was lower than that of the other treatments since there was no root initiation process (**Table 1**).

The presence of IBA in the culture medium is reported to be very important for initiation and root development in plant tissue culture. Silva & Ferreira (2016) believe applying phytoregulators is crucial for the *in vitro multiplication* of numerous plant species. Its addition to nutrient media has as its primary objective to overcome possible deficiencies of endogenous levels of phytoregulators in the explants that are isolated from the producing regions in the mother plant; in addition to stimulating responses such as stretching or multiplication of the aerial part and roots.

Among the exogenous factors evaluated, auxin is a commonly used strategy to obtain adventitious rooting, as they act on the signaling of root-responsive cells. Among the auxins, IBA plays an important role in the initiation of root primordia, being one of the most used compounds also for root induction *in vitro*, as it does not cause phytotoxicity to explants in a wide concentration range (Hartmann et al., 2017). However, this inhibition caused by phytotoxicity is more often in the root elongation phase and results in the interruption of their growth, while in the initial phase, induction and initiation, sensitivity is lower (Cid & Teixeira, 2010). Thus, this may explain the shorter length of mint roots when exogenous auxins were used.

The best behavior of the tomato plant can be linked to several factors, one of them being genetic. The tomato plant belongs to the Solanaceae family, as well as the genera *Nicotiniana*, *Datura* and *Petunia*, which are recognized models for *in vitro* work, making this a suitable and very responsive material for tissue culture.

In this experiment, it was verified that most of the mint root segments (72%), after one week, showed signs of oxidation, observed by the characteristic appearance of dark color of the root segments. This problem may be related to the wound, which causes the release and oxidation of phenolic compounds, due to the action of oxidase enzymes, resulting in the inhibition of the growth of the explant, rapid darkening of the extremities, brown or black in color. Although the oxidation of phenolic compounds can harm the explant, these compounds are essential for the plant, controlling several metabolic pathways related to the plant's defense system (Taiz & Zieger, 2017). Cid & Teixeira (2010) recommend some techniques for oxidation control, such as washing the material before disinfestation, as it helps in the removal of phenolic compounds exudated on the surface. Likewise, activated carbon promotes the adsorption of the exudates released by the explants, in addition to reducing the light intensity incident in the culture medium, which can also result in oxidation. Using this substance is considered a standard procedure to avoid oxidation. Another possibility would be the addition of ascorbic acid or citric acid, which capture molecular oxygen and make the pH of the medium less suitable for the functioning of the oxidase enzyme.

Table 1. Number and length (cm) of mint and tomato roots subjected to different concentrations of IBA in DSD1 medium

IIBA concentration	Mint				Tomato			
(mg L^{-1})	Number of roots		Length of roots (cm)		Number of roots		Length of roots (cm)	
0.00	0.41	ns	0.28	b	0.39	b	0.55	ab
0.25	0.51		0.87	a	0.22	b	0.30	bc
0.50	0.66		0.28	b	0.00	b	0.00	c
1.00	0.50		0.07	b	2.89	a	1.25	a
2.00	0.00		0.00	b	1.81	ab	1.09	a
Average	0.41	b	0.30	b	0.87	a	0.64	a

Averages followed by the same lowercase letters in the rows and uppercase letters in the columns did not differ from each other according to Tukey's test ($p \leq 0.05$). ns- not significant

However, the addition of these compounds to the root segments of mint, in order to avoid oxidation, could influence the germination of AMF spores, also impair the symbiosis between *in vitro* roots and AMF, in a future use as a multiplier of exenic inoculums. Thus, as the root segments of tomato also showed more promise for root cultivation, it was decided to continue only with this species, using a concentration of 1 mg L^{-1} of IBA in the culture medium.

In vitro root production from tomato root segments under different growing media

All the culture media tested provided development of tomato roots. Regarding the number of roots emitted per root segment, in the first evaluation, at 15 days, the M medium presented significantly higher values, with an average of 8.26 roots emitted. On the other hand, the DSD1 medium had the lowest average, with 1.91 roots emitted per segment, and the MSR medium presented intermediate values (**Table 2**).

In the last evaluation, at 60 days, it was observed that there was a slight increase over time for the M medium, going to 9.32 roots emitted per segment. This increase over time was quite expressive for the MSR medium, going from 4.29 to 7.47 roots per segment between the first and last evaluation, not differing statistically from the M medium. In the formulation of the M culture medium, it is observed that it has only nitrate in its composition, while the MSR medium has nitrate and ammonium. Possibly, in medium M, the explant consumed all the nitrogen, with subsequent deficiency of this nutrient causing the emission of new roots to continue.

On the other hand, the DSD1 medium, even at 60 days, showed a small development in the number of roots over time, being lower than the others. There was no substantial increase for this variable after 45 days after *in vitro* introduction.

Cardoso (2005), working with excised tomato roots in growing media at 60 days, did not find significant differences in *in vitro root development* between DSD1, M, and MSR media. In addition, the author comments that although she has obtained good results with the DSD1 medium in her work, the MSR and M media are the most suitable for work with AMF, since they contain a low concentration of salts and phosphorus.

Regarding the average length of the three largest roots emitted per root segment, in the evaluation performed at 15 days, the length of the roots of the MSR medium was the one that presented the largest roots in relation to the others, with 6.91 cm, since the M and DSD1 media presented very short roots and did not differ statistically from each other, with 2.14 and 1.50 cm, respectively. At 60 days, the roots of the MSR medium continued with a high elongation rate, reaching 15.30 cm, which demonstrates a continuous growth in the roots introduced in this medium, being higher than the DSD1 medium with 10.21 cm and the M medium, which obtained the lowest values, with 6.56 cm. (**Table 3**). It should be noted that although the M medium developed a more significant number of roots, these were shorter.

In the MSR medium, the roots spread rapidly, generating many well-distributed branching roots throughout the plate. This length often made the evaluation difficult, since the plate had a radius of 5 cm, and the roots ended up tangling at the edges.

For the DSD1 medium, a balance between number and length of roots was observed. Thus, this medium is also interesting and conducive to the study of growth and multiplication of roots cultivated *in vitro*.

The culture media used *in vitro* are based on the requirements of the plants in relation to mineral nutrients and essential substances to meet the specific growth needs. The medium must provide not only micro and macronutrients, but also contain a source of carbohydrate to replace what the plant would fix from the atmosphere by the process of photosynthesis. Vitamins, amino acids, and growth regulators are examples of substances that can be added to the medium to provide greater growth of the plant organ, and changes in the cultivation medium must be specific to each work and the desired objective (Cid & Teixeira, 2010).

Table 2. Average number of roots at 15, 30, 45, and 60 days after in vitro introduction of tomato root segments in Petri dishes containing different culture media

Growing medium	Average number of roots			
	Days after *in vitro* introduction			
	15	30	45	60
"M"	8.26 a	9.17 a	9.29 a	9.32 a
"MSR"	4.29 b	5.41 b	7.13 ab	7.47 ab
"DSD1"	1.91 c	2.23 c	3.54 b	3.89 b

'Minímo' (M) from Bècard & Fortin (1988), modified by Berbera & Fonseca (1996); medium 'MSR', formulated by Declerck *et al.* (1998) and medium DSD1 (da Silva & Doazan, 1995). Medium followed by the same letters in the lines did not differ from each other by Tukey's test ($p \leq 0.05$). ns- not significant

Table 3. Average length of the three largest roots (cm) at 15, 30, 45, and 60 days after the introduction of tomato root segments in Petri dishes containing different culture media

Growing medium	Average length of the three largest roots (cm)			
	Days after *in vitro* introduction			
	15	30	45	60
"M"	2.14 b	4.62 b	5.99 b	6.56 c
"MSR"	6.91 a	13.65 a	14.90 a	15.30 a
"DSD1"	1.50 b	5.65 b	7.12 b	10.21 b

'Minímo' (M) from Bècard & Fortin (1988), modified by Berbera & Fonseca (1996); medium 'MSR', formulated by Declerck *et al.* (1998) and medium DSD1 (da Silva & Doazan, 1995). Medium followed by the same letters in the lines did not differ from each other by Tukey's test ($p \leq 0.05$). ns- not significant.

The DSD1 medium was successfully used in tomato and mint root multiplication works by Lopes (2003). Similarly, Rodrigues et al. (2007), studying the effects of silicon sources and different culture media on *the in vitro* propagation of orchids, found a greater number and length of roots, compared to MS medium. According to the authors, when there is an increase in the number of roots formed *in vitro*, the contact area between the root and the culture medium is also increased, resulting in greater absorption of macro and micronutrients. Additionally, in a substrate with less nutrients; as is the case with the DSD1 medium, increasing the length of the roots is a way to seek the nutrients necessary for their development, even if this must be done with the expenditure of energy.

The M medium, initially developed for tomato root cultivation, is a modification of the White medium. The macroelement composition of White medium is considerably lower than that of MS medium, commonly used for *in vitro* plant cultivation. However, this more dilute medium proved to be more suitable for root growth, following a study that compared the effects of different concentrations of elements on mycorrhizae formation (Ijdo et al., 2011).

MSR is a medium developed to optimize the growth of the intraradicular phase of AMF *in vitro*. The macroelement composition of MSR is similar to M medium. The differences between the two media occur in the concentrations of microelements and vitamins: MSR medium lacks iodide, myo-inositol, and glycine, and M medium lacks pantothenate, biotin, and cyanocobalamin. However, these components may not be essential, as their absence in both media has no apparent negative effect on symbiosis (Fortin et al., 2002).

One hypothesis for the longer length of the roots in the MSR medium is that because it is more dilute, the roots developed to increase the absorption area and, consequently, seek nutrients available in other places of the plate, resulting in a marked growth of the roots, which spread uniformly throughout the Petri dish, compared to the most concentrated media.

It is important that in studies with in *vitro* AMF, the culture medium has a minimum amount of phosphorus so that colonization is efficient, because when there are high concentrations the roots obtain nutrients more easily, and symbiosis does not occur. MSR and M media have lower amounts of nutrients than the other media usually used. Also, reduced carbon concentrations in the environment favor symbiosis (Danesh & Tufenkci, 2017).

Currently, some plant species that have been used as *in vitro hosts* of AMF, such as the roots of carrot (*Daucus carota*) that have been genetically transformed by the inclusion of the RiT-DNA plasmid with the aim of allowing a differentiated balance of phytohormones, which generates a greater volume of roots in culture media and consequently, allows the sporulation of AMF.

Thus, it is necessary to study the roots of other plant species that are not genetically transformed, since there is no need to work in an authorized laboratory, in addition to the bureaucracy that involves the authorization for the use of this type of organism. In addition, the use of AMF is an alternative where the rational use of fertilizers is sought, with techniques that optimize their absorption and use in agroecosystems, with the objective of providing sustainability through the benefits arising from the use of AMF. Using genetically transformed organisms would not be in line with what is proposed for the development of agriculture with lower environmental impacts. Thus, there is a need to evaluate other plant species that present continuous growth and rapid branching, and that are not genetically transformed.

Conclusions

The application of IBA increases the *in vitro* development of excised tomato roots, compared to mint. For tomato, the concentration of 1 mg L^{-1} of IBA is the one that results in the greatest development of the roots.

The MSR medium is the one with the highest development of excised tomato roots at 60 days after *in vitro* introduction.

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References

Baque, A., Murthy, H.N., Paek, K-Y. 2014. Adventitious root culture of *Morinda citrifolia* in bioreactors for production of bioactive compounds. In: Paek, K-Y., Murthy, H. N., Zhong, J-J. (Ed.) *Production of biomass and bioactive compounds using bioreactor technology*. Springer, New York, USA. p. 185-222.

Cardoso, J.T. 2005. *In vitro production of arbuscular mycorrhizal fungi in tomato roots*. 81f. (Master's Thesis) - Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Cid, L.P.B., Teixeira, J.B. 2010. *In vitro cultivation of plants*. Embrapa Technological Information, Brasília, BR. 49p.

Danesh, Y.R., Tufenkci, S. 2017. In Vitro Culturing of

Mycorrhiza and Mycorrhiza Like Fungi. *International Journal of Agricultural Technology* 13:1675-1689.

Declerck, S., Strullu, D.G., Fortin, J.A. (eds) 2005. *In vitro culture of Mycorrhizas*. Springer, Berlin, DE. 377p.

Declerck, S, Strullu, D.G., Plenchette, C.1998. Monoxenic culture of the intraradical forms of Glumus sp. Isolated from a tropical ecosystem: a proposed methodology for germoplasm collection. *Mycology* 90: 579-585.

Fortin, J.A., Bécard, G., Declerck, S., Dalpé, Y., St-Arnaud, M., Coughlan, A.P., Piché, Y. 2002. Arbuscular mycorrhiza on root-organ cultures. *Canadian Journal of Botany* 80: 1-20.

Ghorui, M., Chowdhury, S., Das, K., Sunar, K., Prakash, B. 2023. A review: in vitro cultivation of arbuscular mycorrhizal fungus for commercialisation. *Journal of Biological Methods* 46: e99010006.

Hartmann, H., Kester, D., Davies, F., Geneve, R., Wilson, S. 2017. *Hartmann and Kester's Plant Propagation Principles and Practices*, Pearson Education, New York, USA. 1004 p.

Ijdo, M., Cranenbrouck, S., Declerck, S. 2011. Methods for large-scale production of AM fungi: past, present, and future. *Mycorrhiza* 21:1-16.

Lopes, P. Z. 2003. In vitro cultivation of tomato, mint and grapevine roots for the production of inoculum of arbuscular mycorrhizal fungi. 68f. (Master's thesis) - Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tabacco tissue culture. *Physiologia Plantarum* 15: 473-497.

Phillips, G. C., Garda, M. 2019. Plant tissue culture media and practices: an overview. *In Vitro Cellular & Developmental Biology – Plant* 55: 242-257.

Pillai, D.B., Jose, B., Satheeshkumar, K., Krishnan, P.N. Optimization of inoculum density in hairy root culture of *Plumbago rosea* L. for enhanced growth and plumbagin production towards scaling-up in bioreactor. 2015. *Indian Journal of Biotechnology* 14: 264–269.

Rodrigues, J.D., Rodrigues, F.A., Villa, F., Pasqual, M., Santos, J.C.S., Silva, A.L.P. 2007. Effects of silicon sources and different culture media on in vitro propagation of orchids. *Ornamental Horticulture* 13: 810-813.

Silva, A.L.; Doazan, J.P. 1995. Une méthode d'irradiation aux rayons gamma appliqué a des porte-greffes de Vigne *in vitro*. *Journal International des Sciences de la Vigne et du Vin*. 29:1-9.

Silva, M. M. A.; Ferreira, L. T. 2016. In vitro cultivation of plants and their applications in cacti. *National Institute of the Semi-Arid Region*, INSA, Campina Grande, BR. 32 p.

Taiz, L., Zeiger, E. 2017. *Physiology and Plant Development*. Artmed, Porto Alegre.BR. 888 p.

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