PHYSICAL NATURE SUBSTRATE INFLUENCE ON *IN VITRO* GERMINATION OF *Ipomoea tricolor* Cav.

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Abstract. *Ipomoea tricolor* Cav. (morning glory) is one of the most popular outdoor ornamental plants, cultivated for its beauty, such as gardens and parks. Due to these qualities, it has determined the production and cultivation of an increasing number of varieties regarding the flower's colour and more recently it captured the attention for its potential pharmaceutical use. The main aim of this article is to investigate *in vitro* germination and the first stages regarding growth and development of morning glory by using four different culture media: solid (agarized) medium, liquid culture medium, double-layer culture medium with agarized basal layer and a liquid medium in the upper part and using Blidar filter paper bridges (FPB). All cultured media are based on Murashige-Skoog formula of 1962. The control was represented by the classical solid culture medium. The analysis of the results revealed that the best results were obtained on control culture medium and on FPB supporting the need for maximum possible aerated substrates for further growth and development of the morning glory.

Key words: *Ipomoea*; *in vitro*; seeds germination; physical nature of the substrate; filter paper bridge.

INTRODUCTION

For more than 30 years, plant species including ornamentals became important due to the presence of natural active principles acting for healing different diseases [55]. In this regard the chemical class of polyphenols became more studied for their high potential application for food preservation and therapeutic use as well as other chemicals [17].

Thus today, plant *in vitro* techniques became a core part of much more complex processes meant to improve products and services aiming the quality of life. We may add herewith new products with special properties, or next generation breeding programmes (i.e. new breeds to resists biotic and abiotic stress factors or produce novel substances with specific use) [59].

Plant biotechnology entered the era of bioeconomy after the Second World War, when have been developed micropropagation protocols at industrial level including for ornamentals. The main additional research studied subjects were mainly under the topics of plant physiology and development. During time more and more plant species became the object of research for *in vitro* micropropagation with the scope to include this technique as a core part into the breeding programs or under the broader technologies for producing products of specific use [56]. Additionally, *in vitro* plant technology, is also involved in obtaining pests-free varieties, trying to solve relevant phytosanitary problems [42]. The nowadays challenges of these *in vitro* techniques of plant micropropagation are represented by cost efficiency and energy reducing costs [11, 36].

Among the ornamental plant species *Ipomoea tricolor* Cav. (family Convolvulaceae) known as morning glory became of high interest due to the beauty of blooming flowers. Generally, species belonging to the genus *Ipomoea* are often called „Morning Glory" because most of them bloom in the morning. Nowadays, along with other ornamental plants, *I. tricolor* is one of the most promising species in terms of color and study cancer diseases [30, 57]. However, the species of the genus *Ipomoea* are long time known for their use in the traditional medicine for the treatment of various diseases such as: diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephalus, meningitis, kidney diseases, digestive disorders and inflammations [34]. Due to the interaction with some specific fungi species such as *I. tricolor* can develop hallucinogenic properties due to the synthesis of LSD' similar compounds [53, 57]. Due to specific acyl sugar content in Mexico, *Ipomoea* species are used as green manure and weed control, in sugarcane fields farmers promote the cultivation of *I. tricolor* before sugarcane cultivation. The allelopathic potential of the *Ipomoea* genus was described by Pereda-Miranda and Mata (1993) [45], Anaya *et al.* (1995) [3] and Bah and Pereda-Miranda (1997) [4] and they identified the *tricolorin A* substance from the active fractions of glycosidic resins of plants as the main plant growth inhibitory agent in monocotyledons, having an effect similar to herbicide. The effect of tricolorin A obtained from *I. tricolor* seeds on breast carcinoma cells was studied by a series of researchers who described the high cytotoxicity of the compounds in its structure on human breast cancer cell cultures [12, 45], and in 2017, Lawson *et al.* [31] conducted studies on the chloroform extracts obtained from *I. alba* and *I. tricolor* seeds, noting a strong antibacterial activity on *Enterococcus faecalis* and *Bacillus cereus*, antifungal on *Cryptococcus neoformans*, also highlighting the cytotoxic action on cancerous breast cells. El Hawary *et al.* (2021) [18] performed comparative phytochemical and biological studies of the lipoidal

profile of the *I. tricolor* and *I. fistulosa* leaves, species that grow in Egypt, identifying high percentages of unsaponifiable fraction with a high content of fatty acids (linoleic and palmitic), and the extracts in petroleum ether at different concentrations showed an important increase in cholinesterase inhibition activity, as well as significant anti-inflammatory activity.

Therefore, a relatively high range of studies is noted, which have been done over the years on the species of the *Ipomoea* genus, but the research based on the *in vitro* culture techniques applied to the *I. tricolor* species is relatively limited. One of them describes a protocol for the regeneration of the embryogenic callus in the *I. purpurea* and *I. tricolor* species using culture medium supplemented with 1 mg/L 4-fluoro-phenoxy acetic acid and 6% sucrose, obtaining numerous somatic embryos when the callus is transferred to lacking media of growth regulators [22].

The particular ornamental importance of these plants, as well as their potential for pharmaceutical use, was the main reason for starting the present study. The scope of this research is to develop optimal conditions regarding *in vitro* germination of morning glory seeds. In this regard a secondary focus was the study of the very first stages of growth and development related to *in vitro* multiplication of the species, for the fast, efficient and highly productive creation of a germplasm useful in studies of improvement or effective multiplication in species of the *Ipomoea* genus. In this regard using of liquid and solid culture media were studied to analyze to morphogenetic changes and to different intensities of organogenesis.

MATERIALS AND METHODS

Plant material. The plant material consisted of commercial seeds of *Ipomoea tricolor* Cav. 'Heavenly Blue' provided from the market.

Preliminary seeds germination test. In Petri Dishes of 10 cm were prepared as humid chambers in dark conditions at 20°C using sterile tap water and filter paper to evaluate the germination rate of the commercial seeds of *I. tricolor*, this having the value of 94% reached on the 9th day from the germination start.

Culture media and *in vitro* germination test. All tested culture media were based on the original formula of Murashige-Skoog (1962) (MS62) [39] and modified by removing, B6, B1 and PP vitamins. The cultivation media were solid and liquid, depending on the experimental variants tested; with a pH of 5.7 (with a WTW – Multi 3630 IDS pH meter equipped with the SenTix® 950 sensor) before agar was added to the variants that required this component.

The experiment for *in vitro* germination was organized by using four culture media as following:

- **V_0** – solid culture medium MS62 - as control;
- **V_L** – liquid culture medium MS62 (without agar);
- **V_{DL}** – double-layer culture medium, where the basal layer was represented by solidified MS62 medium (V_0), and the upper layer by the liquid culture medium (V_L);
- **V_{PB}** – liquid culture medium and using „Blidar" type filter paper bridges [9] in contact with 5 mL of liquid medium as a support for germinating seeds.

A volume of 5 mL of culture medium was used per each test tube, ensuring a medium column with a height of 10 mm in the case of the V_0 and V_L variants. In case of V_{PB} variant, a culture medium column of 15 mm was applied due to the introduced paper bridges and in case of V_{DL} variant, a culture medium column of 20 mm was applied, due to the double-layer culture medium (i.e. 5 mL of solid medium and 5 mL of liquid medium on the top of the solid).

Culture media sterilization. The sterilization of the culture media was realized by autoclaving for 20 minutes at 121 ºC and 1 atmosphere [13]. The jars containing sterile culture medium were passed into the sterile chamber of laboratory to reach room temperature. In the case of the double-layer variant, before placing the seed on the solid substrate, the liquid nutrient layer was introduced.

Seeds sterilization and inoculation for *in vitro* germination. The entire sterilization process too place in the sterile chamber under aseptic conditions in the hood with laminar flow of sterile air (BL 1200), and following the laboratory protocol [10]. Four gauze bags were made containing 130 seeds each of *I. tricolor*, were kept in a container, under running tap water for 30 minutes. Seeds sterilization was carried out in the hood with laminar flow, by placing the gauze bags in a 5% sodium hypochlorite solution as a chemical agent supplemented with Tween 80, and under continuous hand shake for 6 min. [8], after which they were rinsed 6 times with sterile bidistilled water, at the room temperature, for 1 minute each under continuous hand shake.

The inoculation consisted on placing a single seed per jar (35 mL). In case of solid culture media, each seed was slightly pushed into the medium, avoiding its submergence; in case of liquid culture medium, each seed was placed at the surface of the culture medium followed by sinking to the bottom of test tubes. In the case of the paper bridge variant, each seed was placed on the middle of the bridge, avoiding its sinking. For the double-layer culture medium variants, the liquid layer was poured into the test tube after placing each seed on the surface of solidified culture medium.

After inoculation, the containers were closed with transparent and colorless ethylene film and passed in the growth room, at 22-24 ºC; under fluorescent white light, with a color temperature of 9000 K, light intensity of 27.6 $\mu M \cdot m^{-2} \cdot s^{-1}$ (measured with a LI-COR LI-250A photoradiometer equipped with a LI-COR QUANTUM photosensitive cell Q 108519) and the photoperiod was 16 hours light / 24 hours [40].

Observations and morphometric measurements. The observations of *in vitro* cultures were made weekly, until the third week). Observations consisted

in their general appearance; morphometric measurements consisted in evaluating several main parameters such as: root growing in length, number of secondary roots, hypocotyl length development, number of leaves, leaf length and epicotyl length. The morphometric parameters were measured using a caliper (mm), while the numerical values (for secondary roots and leaves) were recorded as pieces/plantlet.

Statistical analysis. All statistical analyses were made using Microsoft Excel; values are significantly different at $P<0.05$ according to the Student's *t*-test. The experiment was repeated three times.

Accessed data bases. For establishing the correct names of plant species, we accessed the International Plant Names Index [61].

RESULTS

At 7 days of *in vitro* culture, the best results were recorded in the control variant (V_0) and the one with filter paper bridge (V_{PB}), where the seedlings had a rapid growth, in some cases exceeding the height of the tube test; the plantlets had light green or more intense green leaves and a well-represented root system, both by ramifications and in some cases by absorbent root hairs (i.e. V_{PB} variant). The roots were colorless in all variants similar with other results [35]. In case of control, the roots were not covered by root hairs as the mineral nutrition and water intake were sufficient [27]. Plantlets grown on the double-layer system (V_{DL}) had a height lower compared to control (V_0) and filter paper bridge variant (V_{PB}), but higher compared to the liquid variant (V_L) (table 1).

At 14 days, the best results were also recorded in the control variants (V_0) and on the filter paper bridge (variant V_{PB}). In case of V_L and V_{DL} variants, a delayed growth compared to control group was noted. However, a general height difference was observed between the two variants, the plantlets grown on V_{DL} variant being larger as a result of earlier access to aeration, thus avoiding the installation of vitrification [48], conferred by the hydrogel physical support of the basal layer in which they were trapped, thus being able to overcome the liquid layer more easily (table 1).

Similar to the previous date of the experimental observations, **at 21 days**, the best results were recorded on the control plantlets (V_0) and those germinated on filter paper bridges (V_{PB}) variants (fig. 1, table 1). Even if the seedlings on the V_{PB} variant had a lower height than the control, they showed a slightly higher development, a fact noted especially in the case of roots and leaves. The explanation consist in the selective access to the nutrients of the culture medium as well as in ensuring the aeration of the roots provided by the filter paper bridges [7, 8]. The liquid medium variants that led to hypoxia at inoculum level (V_L and V_{DL}) delayed the growth and development of the morning glory plantlets. This technique can be used in the case of crop conservation [16], as vitroplants harmoniously developed a root and caulinar system, but their growth speed was at most one third of that provided by the environment agarose (V_0).

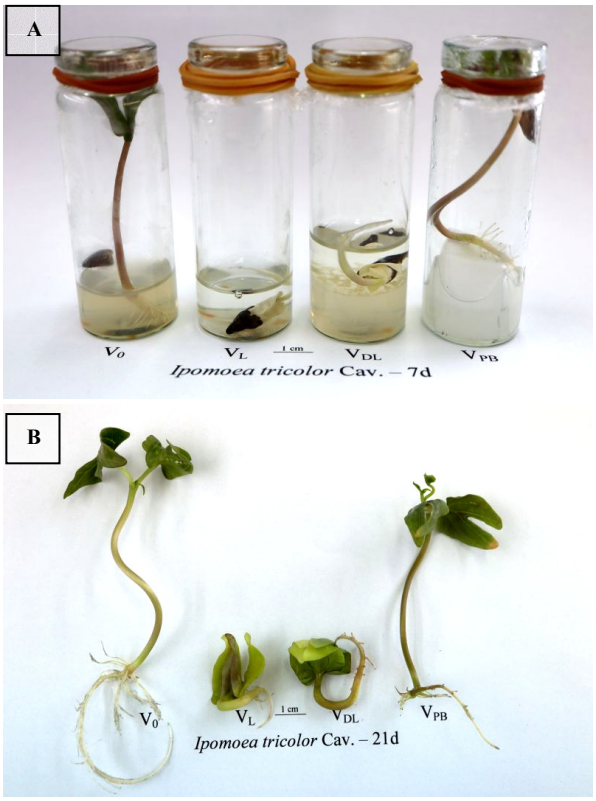

Figure 1. General comparative aspects of *in vitro* plants of the *Ipomoea tricolor* grown in different MS62 culture medium conditions: $\mathbf{V_0}$ – solid culture medium (control lot), $\mathbf{V_L}$ – liquid culture medium, $\mathbf{V_{DL}}$ – dual layer culture medium (solid at the bottom and liquid in the upper part), and $\mathbf{V_{PB}}$ - liquid culture medium provided with filter paper bridges, at 7 days (**A**), and 21 days (**B**) after the implementation of the experiments.

DISCUSSION

The very first stage for plant *in vitro* micropropagation is the introduction of healthy plant inoculum into *in vitro* conditions and it is one of the outmost importance for the further developments during micropropagation, especially at the industrial level [6]. The start of the micropropagation protocols for inoculation, micropropagation acclimatation and further elite stages highly depends on the initiation of *in vitro* culture [19].

By accessing the Claviarte database over 75,000 articles have been published including the subject of seeds germination. Among these, 25 review articles related to germination were published. However, only 12 article specifically includes studies related to germination of *I. tricolor* [61]. This was the main reason to start a germination study to this species as it was not possible to have study results for *in vitro* germination yet.

It is well established generally that the physical nature of the germination substrate for *in vitro* further cultivation is particularly important [33]. Also it was

proved that under bad circumstances related to electrolyte disturbances seeds germination can lead to the expression of morphological anomalies, and sometimes to revealing some genetic disorders [21], given also the specific microclimatic condition (i.e. high humidity, low light levels and hetero- or mixotrophic conditions).

Nevertheless, in case of *in vitro* culture initiations such situations cannot be brought into discussion, the difference between the physical types of substrate is leading to different plantlets growths [7, 8], or however, in certain situations, with similar results [19]. On the other hand the electrolyte equilibrium of model culture media such as the MS62, even under the *in*

Table 1. Statistical processing of the *primary root length* (**A**), *secondary root number* (**B**), *hypocotyl length* (**C**) *epicotyl length* (**D**), *leaves number* (**E**) and *leaf blade length* (**F**) measured in the *in vitro* seedlings of *Ipomoea tricolor* on different MS62 culture medium conditions: $\mathbf{V_0}$ – solid culture medium MS62 culture medium (control lot), $\mathbf{V_L}$ – liquid culture medium, $\mathbf{V_{DL}}$ – dual layer culture medium (solid at the bottom and liquid in the upper part), and $\mathbf{V_{PB}}$ - liquid culture medium with filter paper bridges.

	No. of days	Control lot	Statistical data X ± Sx	Variants	Statistical data X ± Sx	±d	±%	Significance
A. Primary root length (mm)	**7**	$\mathbf{V_0}$	22.83 ± 8.02	$\mathbf{V_L}$	6.80 ± 3.15	-16.03	-70.21	***
				$\mathbf{V_{DL}}$	5.90 ± 3.56	-16.93	-74.16	***
				$\mathbf{V_{PB}}$	17.40 ± 6.42	-5.43	-23.78	**
	14		40.05 ± 11.27	$\mathbf{V_L}$	11.30 ± 6.72	-28.75	-71.79	***
				$\mathbf{V_{DL}}$	18.00 ±11.23	-22.05	-55.06	***
				$\mathbf{V_{PB}}$	28.65 ± 8.44	-11.4	-28.46	***
	21		53.68 ± 16.90	$\mathbf{V_L}$	14.76 ± 5.93	-38.92	-72,5	***
				$\mathbf{V_{DL}}$	23.57 ± 7.81	-30.11	-56,09	***
				$\mathbf{V_{PB}}$	25.32 ± 9.76	-28.36	-52,83	***
B. Secondary root number (no.)	**7**	$\mathbf{V_0}$	14.88 ± 8.90	$\mathbf{V_L}$	0.00 ± 0.00	-14.88	-100	***
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-14.88	-100	***
				$\mathbf{V_{PB}}$	8.10 ± 5.22	-6.78	-45,56	***
	14		20.72 ± 6.22	$\mathbf{V_L}$	0.85 ± 2.08	-19.87	-95,9	***
				$\mathbf{V_{DL}}$	2.05 ± 2.74	-18.67	-90,11	***
				$\mathbf{V_{PB}}$	15.35 ± 6.33	-5.37	-25,92	**
	21		28.00 ± 13.20	$\mathbf{V_L}$	2.82 ± 2.53	-25.18	-89,93	***
				$\mathbf{V_{DL}}$	6.78 ± 5.56	-21.22	-75,79	***
				$\mathbf{V_{PB}}$	14.36 ± 9.97	-13.64	-48,71	***
C. Hypocotyl length (mm)	**7**	$\mathbf{V_0}$	43.50 ± 16.86	$\mathbf{V_L}$	15.00 ± 22.79	-28.5	-5.52	***
				$\mathbf{V_{DL}}$	12.50 ± 2.11	-31	-71.26	***
				$\mathbf{V_{PB}}$	40.70 ± 10.28	-2.8	-6.44	ns
	14		65.88 ± 16.36	$\mathbf{V_L}$	13.85 ± 3.31	-52.3	-78.98	***
				$\mathbf{V_{DL}}$	14.55 ± 2.60	-51.3	-77.91	***
				$\mathbf{V_{PB}}$	53.95 ± 12.49	-11.3	-18.11	**
	21		67.00 ± 15.97	$\mathbf{V_L}$	16.52 ± 5.90	-50.8	-75.34	***
				$\mathbf{V_{DL}}$	14.21 ± 3.72	-52.9	-78.79	***
				$\mathbf{V_{PB}}$	50.00 ± 13.05	-17	-25.37	***
D. Epicotyl length (mm)	**7**	$\mathbf{V_0}$	0.00 ± 0.00	$\mathbf{V_L}$	0.00 ± 0.00	0	n/a	ns
				$\mathbf{V_{DL}}$	0.00 ± 0.00	0	n/a	ns
				$\mathbf{V_{PB}}$	0.00 ± 0.00	0	n/a	ns
	14		0.44 ± 0.51	$\mathbf{V_L}$	0.00 ± 0.00	-0.4	-100	***
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-0.4	-100	***
				$\mathbf{V_{PB}}$	0.10 ± 0.30	-0.4	-77.27	**
	21		3.00 ± 5.03	$\mathbf{V_L}$	0.00 ± 0.00	-3	-3	**
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-3	-3	**
				$\mathbf{V_{PB}}$	2.16 ± 3.50	-0.4	-0.84	ns
E. Leaves number (no.)	**7**	$\mathbf{V_0}$	0.00 ± 0.00	$\mathbf{V_L}$	0.00 ± 0.00	0	n/a	ns
				$\mathbf{V_{DL}}$	0.00 ± 0.00	0	n/a	ns
				$\mathbf{V_{PB}}$	0.00 ± 0.00	0	n/a	ns
	14		0.44 ± 0.51	$\mathbf{V_L}$	0.00 ± 0.00	-0.44	-100	***
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-0.44	-100	***
				$\mathbf{V_{PB}}$	0.15 ± 0.30	-0.29	-65.91	**
	21		1.68 ± 1.17	$\mathbf{V_L}$	0.00 ± 0.00	-1.68	-100	***
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-1.68	-100	***
				$\mathbf{V_{PB}}$	1.12 ± 1.16	-0.56	-33.33	ns
F. Leaf blade length (mm.)	**7**	$\mathbf{V_0}$	0.00 ± 0.00	$\mathbf{V_L}$	0.00 ± 0.00	0	n/a	ns
				$\mathbf{V_{DL}}$	0.00 ± 0.00	0	n/a	ns
				$\mathbf{V_{PB}}$	0.00 ± 0.00	1.12	n/a	ns
	14		0.88 ± 1.13	$\mathbf{V_L}$	0.00 ± 0.00	-0.88	-100	***
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-0.88	-100	***
				$\mathbf{V_{PB}}$	0.15 ± 0.48	-0.73	-82.95	**
	21		1.68 ± 1.17	$\mathbf{V_L}$	0.00 ± 0.00	-1.68	-100	***
				$\mathbf{V_{DL}}$	0.00 ± 0.00	-1.68	-100	***
				$\mathbf{V_{PB}}$	1.64 ± 2.23	-0.04	-2.38	ns

Note: X ± Sx [average (cm) ± standard deviation]; ±d – difference to the control lot in absolute values; ±% – difference to the control lot in percentage values; based on *p* values (significance of difference to control lot): ns – no significant difference ($p>0.1$), * - low significant difference ($0.05<p\leq0.1$), ** - significant difference ($0.01<p\leq0.05$), *** - very significant difference ($p\leq0.01$); n/a – non applicable.

vitro stress conditions, support germination and further development for the very first stages of *in vitro* culture as well as afterwords at a pH under 5.6 [58]. All our experiments have been conducted at a pH of 5.6 after autoclavation and based on MS62 culture medium well established for its electrolytes composition. By including sterile seeds into *in vitro* culture it is also possible to avoid virus contamination [20] and the success of *in vitro* culture at the industrial level is also highly depending on the absence of viruses also [1].

The scope of this study was to establish the effectiveness of the physical nature of the growing substrate during *in vitro* seed initiation of *Ipomoea tricolor*, as well as its influence on vegetative organ growth during the first 3 weeks of culture. The four types of culture media had a visible impact on seed germination and further on the very first growth and development stages that is in line with other studies on different species [25, 41].

At a practical level, the development of a cost-efficient experimental protocol that allows the increase with *in vitro* improved efficiency of plant species, means a gain of time necessary for reaching the desired stage, optimal for the next stages, along with a related economic gain, respectively of the production yield [32, 51].

The most effective experimental variant regarding the size, but also the number of secondary roots and leaves, was represented by the control (V_0). Similar results were obtained by Lozzi *et al.* (2019) [32] for the *Ceratonia siliqua* L. species, regardless of the amount of minerals in the culture media. There are also reports that indicate in certain species an *in vitro* reactivity that was higher compared to the solidified medium with agar, in case of using other solid substrates, such as AMPTMA-based PE-PH substrate. In this case there where was identified better reaction in all studied morphometric indicators when comparing the number of shoots or roots developed, the length of shoots and roots, or the number of leaves developed in chickpea [26]. In case of morning glory, the seedlings developed upon the germination on solidified culture medium, showed the highest rate of growth and development, is supporting the use of this type of physical substrate. It was well established that the MS62 culture media may further influence roots development based on the natural requirements of the species [51]. Thus, for certain tropical plants species it is possible to use liquid culture medium with better results regarding rooting and shooting and for other origin plant species we need to use mainly solidified culture medium [37].

Based on these results, FPB induced a better development of aerial part of the seedling compared to control, even the seedlings lengths where higher for control compared to those obtained on FPB. In this case it appears that the new seedlings are more adapted for further *in vitro* cultivation.

In case of using the FPB, a strong root branching and formation of root hairs was noted, similar to study's results of Perveen *et al.* (2015) [46] for *Murraya koenigii* and Blidar *et al.* (2016) [7] for *Viola witrokiana*. It was concluded that due to FPB porosity it is facilitated through capillarity the increased absorption of nutrients through its entire surface area [54]. This should be a major advantage to be also used for acclimation [9]. Better results in using FPB to other types of substrates were also obtained for sub-cultivation and shoot elongation of *Schleichera oleosa* [49], *Stevia rebaudiana* [5], or even in successful micrografting of roses [14]. Moreover, filter paper bridge technique is also effective in frequently subcultivating nodal cuttings for bud induction without any damage in *Litchi chinensis* [29], or even in the induction and subcultivation of callus tissue in *Trifolium pratense* [28].

Using the liquid culture medium, or the medium in a double-layer (solid medium covered by liquid medium), seedlings with the lowest degree of development were obtained, most probably due to hypoxia conditions in line with other authors results [50]. It appears that the contact of the new formed seedling with a solid substrate for seed germination process under further developed mechanical stress is essential for roots growth and further development [2, 23].

Thus, a frequent problem encountered in liquid culture media is the induction of hyperhydration [32, 47]. This physiological negative reaction of plantlet formation might have been due to different *in vitro* factors. Thus, in certain cases it can be prevented by using cotton plugs to seal test tubes and flasks because it is mainly associated with a lack of gas exchange [6, 48]. However, in some studies it was demonstrated the high efficiency use of the liquid culture medium, compared to the solid one, i.e. for root initiation in the case of *Alocasia amazonica* [24] and *Catharanthus roseus* [43], or of double-layer media, such as in embryogenesis and plant regeneration from maize zygotes [38]. In our case, it was demonstrated that the liquid and double-layer medium variants are an advantage for the conservation of the seed-initiated morning glory seedlings, as they harmoniously developed an efficient root and caulinar system, even their growth process was lower at most one third of that observed for seedlengs obtained on the control as classic solid MS62 medium. Preservation in aqueous liquid media or represented by mineral oils have also been obtained with good results in other plant species, such as: *Bacopa monnieri* [52], *Lippia alba* [44], *Pfaffia glomerata* and *Lippia filifolia* [16].

Finally it can be considered that depending on the purpose pursued, different types of physical supports of the culture media can be used for *in vitro* plantlets growth of *Ipomoea tricolor*. These results also may substantiate further research that aims increasing the efficiency of plant micropropagation conditions in this species.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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