THE MICROPROPAGATION OF COLEUS HYBRIDUS JUPITER VITROCULTURES UNDER DIFFERENT PARAFFIN OR SILICON OIL STRATUMS

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ABSTRACT. In the framework of this experiment was the study of the reaction of *Coleus hybridus Jupiter* vitrocultures in hypoxic conditions, using vitroplantlets having 2 weeks from initiation whereupon them was applied an oil stratum: paraffin or silicon oil. The oil stratum was of 1 cm^3, 2 cm^3 or 5 cm^3, the witness – lot vitroplantlets bare of oil. In the moment when the vitroplantlets surpassed the oil stratum was initiated a subculture bare of oil. After each 4 weeks the vitroplantlets was analyzed and was determined the assimilatory pigments in leafs. After the first 12 weeks witness lot grew up comparing to oil submersed vitrocultures, the witness lot over 48 experimental weeks were subcultivated 3 times. Using a stratum of 1 cm^3 of paraffin or silicon oil, after 24 of weeks in double stratum culture, the vitroplantlets surpassed the oil stratum, at this faze was no observed eminent inhibitory reaction. Covering the vitrocultures with a stratum of 2 cm^3 of paraffin or silicon oil, it was noted an inhibition of increase on a period of 32 of weeks, the content of assimilatory pigments being with 8,9 % lower than that earmarked to the witness lot to 4 weeks from inoculation (vitroplantule bare of oil). In this case – in subculture - was observed an inhibitory reaction for 9 weeks. Covering the vitroplantlets with 5 cm^3 of paraffin oil or silicon stratum was observed a prolongation of grow inhibition until 44 weeks (paraffin oil submersion) and 48 weeks (in silicon oil submention) – which determine a stronger inhibition in subcultures for 24 weeks. For inducing a long time grow inhibition - even in subculture - on *Coleus hybridus Jupiter* vitrocultures the best solution is using paraffin or silicon oil in a 5 cm^3 stratum of paraffin or silicon oil, the vitroplantlets reaction being similar.

Keywords: hypoxic conditions, oil stratum, paraffin oil, silicon oil, *Coleus hybridus Jupiter*

INTRODUCTION

The storage of vitroplantlets in vitro, like an alternative method for conservation of superior plants, generated a big interest over in vitro technologies using different method like: crioconservation, storage in oils submersion, using different alternative methods.

The in vitro storage purpose was and still is a challenge for many researches, using *Solanum tuberosum* vitroplantlets storage in mineral oil (Caplin, 1959), medium bare of sucrose (Jones, 1974) or abscisic acid (Henshaw et all., 1978), storage of *Lilium martagon* and *Lilium candidum* vitroplantlets in paraffin oil (Bolba, 2002), or low temperature 2-8°C on *Musa* vitroplantlets (Hassan, 2004), protoplst crioconservation in liquid azot (-196°C) (Walters et all., 2004).

Blidar and Cachiţă (2006) registered growth diminutions on *Cymbidium* protocorms cultivated on liquid medium using water with low deuterium level with only 37.5 ppm deuterium– in compare with 150 ppm in normal water. As result of this experiment was observed a good growth control, preserving the protocorms viability in subculture.

Radoveţ and Cachiţă (2007) study the reaction of *Coleus blumei Benth.* vitrocultures in hypoxic conditions, using vitroplantlets whereupon them was applied an oil stratum: paraffin or silicon oil. The oil stratum was of 1 cm^3, 2 cm^3 or 5 cm^3, the witness – lot vitroplantlets bare of oil. In the moment when the vitroplantlets surpassed the oil stratum was initiated a subculture bare of oil. After each 4 weeks the vitroplantlets was analyzed and was determined the assimilatory pigments in leafs. After the first 12 weeks witness lot grew up of the oil stratums, over 48 experimental weeks were subcultivated 3 times. Using a stratum of 1 cm^3 of paraffin or silicon oil, after 24 of weeks in double stratum culture, the vitroplantlets surpassed the oil stratum, at this faze was no observed any eminent inhibitory reaction. Covering the vitrocultures with a stratum of 2 cm^3 of paraffin oil, it was noted an inhibition of increase on a period of 32 of weeks, the content of assimilatory pigments being with 8,9 % lower than that earmarked to the witness lot to 4 weeks from inoculation (vitroplantule bare of oil). In this case – in subculture - was observed an inhibitory reaction for 9 weeks. Covering the vitroplantlets with 5

cm^3 of paraffin oil or silicon stratum was observed a prolongation of grow inhibition until 44 weeks – which determine a stronger inhibition in subcultures for 24 weeks. For inducing a long time grow inhibition on *Coleus blumei Benth.* vitrocultures the best solution is using paraffin or silicon oil in a 5 cm^3 stratum of paraffin or silicon oil, the vitroplantlets reaction being similar.

MATERIALS AND METHODS

In this experiment we used *Coleus hybridus Jupiter* vitrocultures having 2 weeks from vitroculture initiation on basic medium (BM) Murashige – Skoog (MS, 1962), modified by us, without fitohormons and glicine, with vitamins (thiamine HCl, pyridoxine HCl, nicotinic acid, each 1 mg/l), meso – inositol 100 mg/l, sucrose 20 mg/l (30 g/l in the original recipe) and agar – agar 7 g/l, without fitohormons. At this time whereupon vitroplantlets was applied an oil stratum: paraffin or silicon oil. The oil stratum was of 1 cm^3, 2 cm^3 or 5 cm^3, the witness lot – lot vitroplantlets bare of oil. After each 4 weeks the vitroplantlets was analyzed – measured the vitroplantule length and was determined total assimilatory pigments in leafs. In the moment when the vitroplantlets surpassed the oil stratum was initiated a subculture bare of oil: After 24, 32, 36, 44 and 48 weeks of submersion from the submerced under oil vitroplantlets ware taken minicuttings and subcultived on fresh culture medium bare from oil, this new resulting vitrocultures are: Subculture I: vitroplantlets resulting from minicutting taken after 24 weeks of submersion; Subculture II: vitroplantlets resulting from minicutting taken after 32 weeks of submersion; Subculture III: vitroplantlets resulting from minicutting taken after 36 weeks of submersion, Subculture IV: vitroplantlets resulting from minicuttings taken after 44 weeks of submersion; Subculture V: vitroplantlets resulting from minicuttings taken after 48 weeks of submersion.

The total assimilator pigments, were calculate by adding the values of a and b chlorophylls and, the yellow carotenoids pigments were evaluated by their elicitation in pure dimethylphormamyd (99,9%), (50 mg vegetal product filled in 5 ml solution, 72 hours maintained to 4ºC temperature) the total extract of assimilator pigments were resigned to spectrophotometric analysis, to a machine created by Carl Zeiss Jena, SPECOL 11 – tip, by the following wave lengthiness: 664 nm (for a chlorophyll), 647 nm (for b chlorophyll) and 480 nm (for carotenoids). The processing date was reformed after formulas:

a chlorophyll (mg/gsp) = 11,65 A_{644} – 2,69 A_{647}·v/sp

b chlorophyll (mg/gsp) = 20,8 A_{644} – 3,14 A_{664}·v/sp

carotinoids (mg/gsp) = (1000 A_{480} – 1,28 a chlorophyll – 56,7 b chlorophyll)/245 • v/sp

Where: A_{480} – value measured with a 480 nm filter; A_{647} – value measured with a 647 nm filter; A_{664} – value measured with a 664 nm filter; v – used solution (ml); sp – mg of vegetal material used for extraction/probe; a and b chlorophyll – amount in mg calculated in the first two formulas, graphically, the values were calculate in percent values, 100% were considered the registered values to the witness variant. All collected data ware analyzed in statistical program: STATISTICA 6.0 and represented on graphics and tables with the statistical significance.

RESULTS, DISCUSSION AND CONCLUSIONS

On a period of 12 weeks was observed that all submersed vitroplantlets had a similar reaction regardless oil type or applied stratum. So, at first 4 weeks of the double stratum oil submersion (using paraffin or silicon oil) already could be observed changes in the vitroplantlets behaviour (fig. 1 A and B, fig. 2). The growth suppress was observed regarding to vitroplantlets lengh, the submersed vitroplantlets maintained the same stem length of 1,8 cm, comparatively with the witness lot which attained a length of 5 cm, the difference between them being a significant increase with 177% (fig. 2-5, table 1). But the quantity of assimilatory pigments in oil submersed vitroplantlets was equal with witness lot: 3,8837 mg/g Sp regardless oil type or applied stratum (fig. 2-5). At assimilatory pigment measurements were established a non significant increment of 4.75 % comparing with initial moment – the oil submersion moment (fig. 2, table 1).

After 9 weeks of vitroculture in hypoxic conditions at all vitroplantlets level was observed a decrease of total assimilatory pigments by 6.56 %, a equal level at all variants of oil and oil stratum, this quantity being by 9.23% lower then at wetness lot – vitroplantlets bare of oil (fig. 2-5, table 1). The vitroplantlets length was match different at this moment, so the submersed vitrocultures registered a small increase by 33% from the submersion moment and a lower stem length by 70% in compare with witness lot, and (fig. 2-5, table 1).

A higher difference between oil submersed and bare of oil vitrocultures was observed after 12 weeks of culture, all submersed vitrocultures registered the same values: a difference of 9,2 cm (228 %) in stem length and a difference of 23.8% in quantity of assimilatory pigments. At this moment the witness lot had to be subcultured on new culture medium, over 48 experimental weeks this lot was subcultivated 3 times.

The behaviour of vitroplantlets in hypoxic conditions after first 12 weeks were different in depend of oil stratum and in depend of oil type.

So, using a 1 cm^3 oil stratum, the reaction of vitroplantlets were similar until 24 weeks of vitroculture. At this moment all vitroplantlets were 3,9 cm high with a positive difference by 116.67% on length level, but the submersed vitroplantlets in paraffin oil had a content of 3,4029 mg/gSp total assimilatory pigments with a negative difference by 8.9% in quantity of assimilatory pigments (the differences was reported to the measures realized before the oil submersion). The vitroplantlets submersed under silicon oil for 24 weeks had a better reaction, the stem length was 4,1 cm (with a positive difference of 2,3 cm) and a quantity of 3,4597 mg/gSp of assimilatory pigments (at this measure the difference was negative by 6.9%) – fig. 3. At this moment of experiment from vitroplantlets was taken minicuttings

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and subcultured on fresh culture medium bare of oil – resulting Subculture I. In subculture the resulted vitroplantlets had a normal growth, without persistence of growth inhibition, the vitroplantlets stem length were normal for 4 weeks of vitroculture (fig. 6 1-2).

Using 2 cm^3 oil stratums gave the possibility to extend conservation period to 32 or 36 weeks without operate new subcultures – only at this point the vitroplantlets overreached oil stratum. At these experimental variants also was observed a difference between vitroplantlets reaction on hypoxic conditions generated by submersion under paraffin or silicon oil. So the growth parameters at vitroplantlets covered by paraffin oil was 5.2 cm stem length (with a difference by 188% comparing with the moment before the oil add) and a quantity of 3.3902 mg/gSp assimilatory pigments (with a negative difference by 8.9 %) – fig. 4. From this variant was realized a new subculture: Subculture II. In subculture the resulted vitroplantlets had a growth inhibition until 12 weeks from the subculture (fig. 6: subculture II: vitroplantlets after 9 weeks of subculture resulting from minicutting taken after 32 weeks of submersion – 3 – paraffin oil and the same subculture after 12 weeks: 5. For reaction comparing between submertion in paraffin and silicon oil the subculture II was applied also at vitroplantlets covered with silicon oil fig. 6: subculture II: vitroplantlets after 9 weeks of subculture resulting from minicutting taken after 32 weeks of submersion – 4 – silicon oil – and the same subculture after 12 weeks: 6.

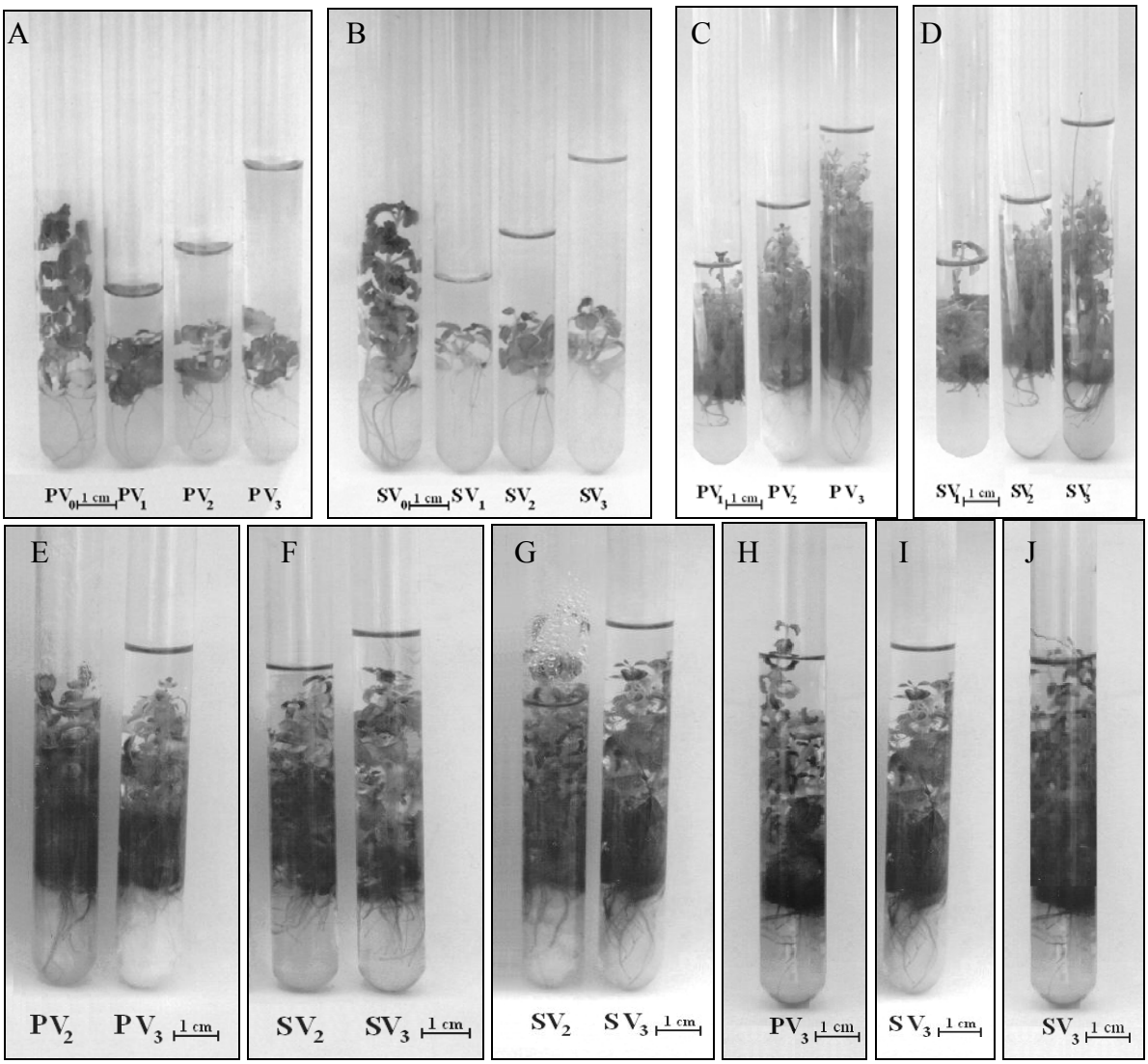

Fig. 1. Photographic details regarding the growth of ***Coleus hybidus Jupiter*** vitroplantlets submersed under paraffin (PV) or silicon (SV) oil, the stratum being of different height: V_0 – control lot, vitroplantlets bare of oil; V_1 – submersion oil stratum which cover the plantlets apex is 1 cm^3, V_2 –2 cm^3 and, V_3 –5 cm^3; where A and B – after 4 weeks of submersion; C and D – after 24 weeks of submersion; E and F – after 32 weeks of submersion; G – after 36 weeks of submersion, H and I – after 44 weeks of submersion, J – after 48 weeks of submersion

But using 2 cm^3 silicon oil submersion, the vitroplantlets overpass the oil stratum only after 36 weeks, witch had a larger stem: 5.3 cm (with a difference by 194%) and a content of 3,4009 mg/gSp assimilatory pigments (a negative difference of 8.9%) – fig. 4. At this moment of experiment from vitroplantlets was taken minicuttings and subcultured on fresh culture medium bare of oil – resulting Subculture III, the resulted vitroplantlets had a growth inhibition until 12 weeks from the subculture fig. 6 vitroplantlets after 24 weeks of subculture resulting

from minicutting taken after 36 weeks of submersion – 7 (paraffin oil) and 8 (silicon oil).

The longest period of oil preserving vitrocultures resulted in case of using a 5 cm^3 oil stratum – the preservation period being prolonged until 44 weeks using paraffin oil and 48 weeks using silicon oil. So, after 44 weeks under paraffin cover – the vitroplantlets have 5.8 cm and a 3.3599 mg/gSp (fig. 1 H and I, 5, table 1). From these vitrocultures was taken minicutings for new subculture nr. IV, the resulting vitroplantlets manifested a slow growth until the 24th week of subculture (fig. 6: vitroplantlets after 24 weeks of subculture resulting from minicuttings taken after 44 weeks of submersion 9 – covered with paraffin oil and 10 – covered with silicon oil – for reaction comparing).

The period of vitroplantlets slow growth using 5 cm^3 of silicon oil, at this moment these vitrocultures had 5.3 cm length and 3.4597 mg/gSp (fig. 1 I and J, 5, table 1) was 48 weeks. At this time was taken minicutings for new subculture nr. V, the resulting vitroplantlets manifested a slow growth until the 24th week of subculture (fig. 6: vitroplantlets after 24 weeks of subculture resulting from minicuttings taken after 48 weeks of submersion 11 – covered with paraffin oil – for reaction comparing and 12 – covered with silicon oil).

CONCLUSION

The prolongation of the storage period gave the possibility to avoid many subcultures especially using 5 cm^3 of paraffin or silicon oil, all new subculture involve costs, time and a lot of work, but it had also a negative influence over the subculture growth, the slow growth was manifested also in subculture.

So the increase of oil volume applied on top of vitrocultures, prolong the conservation under hypoxic condition - until 48 of weeks, but, with the increase of the storage period grow the inhibitory effects in storage conditions and in subculture for 24 weeks.

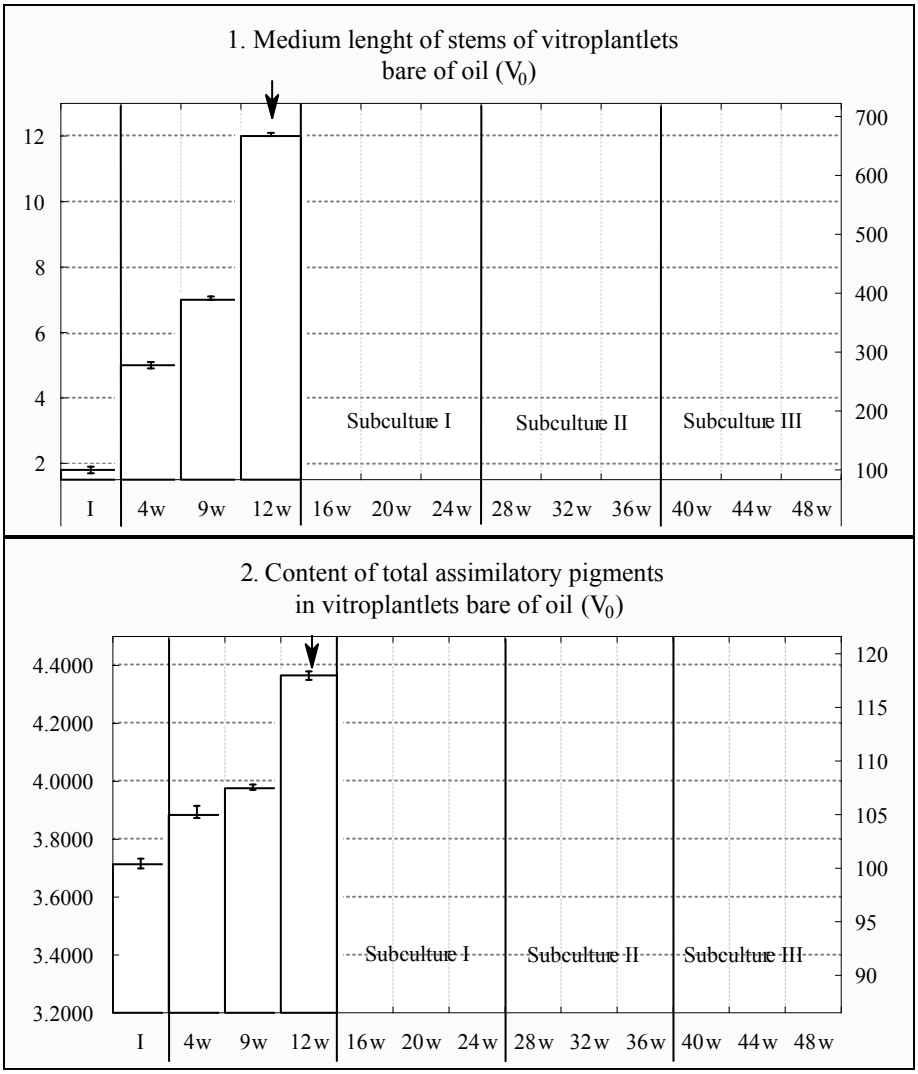

Fig. 2. The growth parameters of ***Coleus hybridus Jupiter*** vitroplantlets bare of oil: V_0 – control lot. After the first 12 weeks witness lot grew up comparing to oil submersed vitroplantlets, over 48 experimental weeks were subcultivated 3 times

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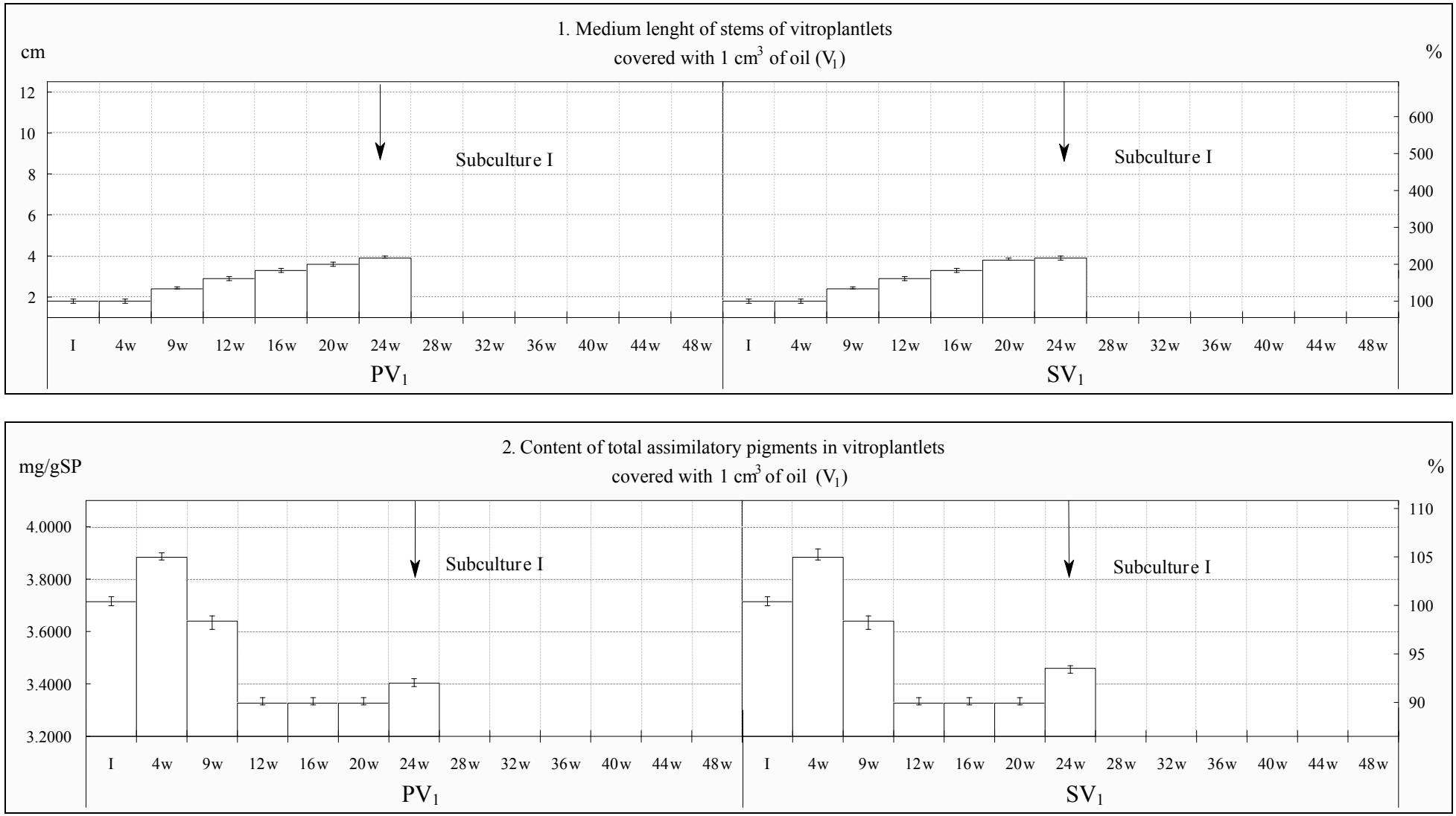

Fig. 3 The growth parameters of ***Coleus hybridus Jupiter*** vitroplantlets submersed under 1 cm^3 (V_1) of paraffin (P) or silicon (S) oil

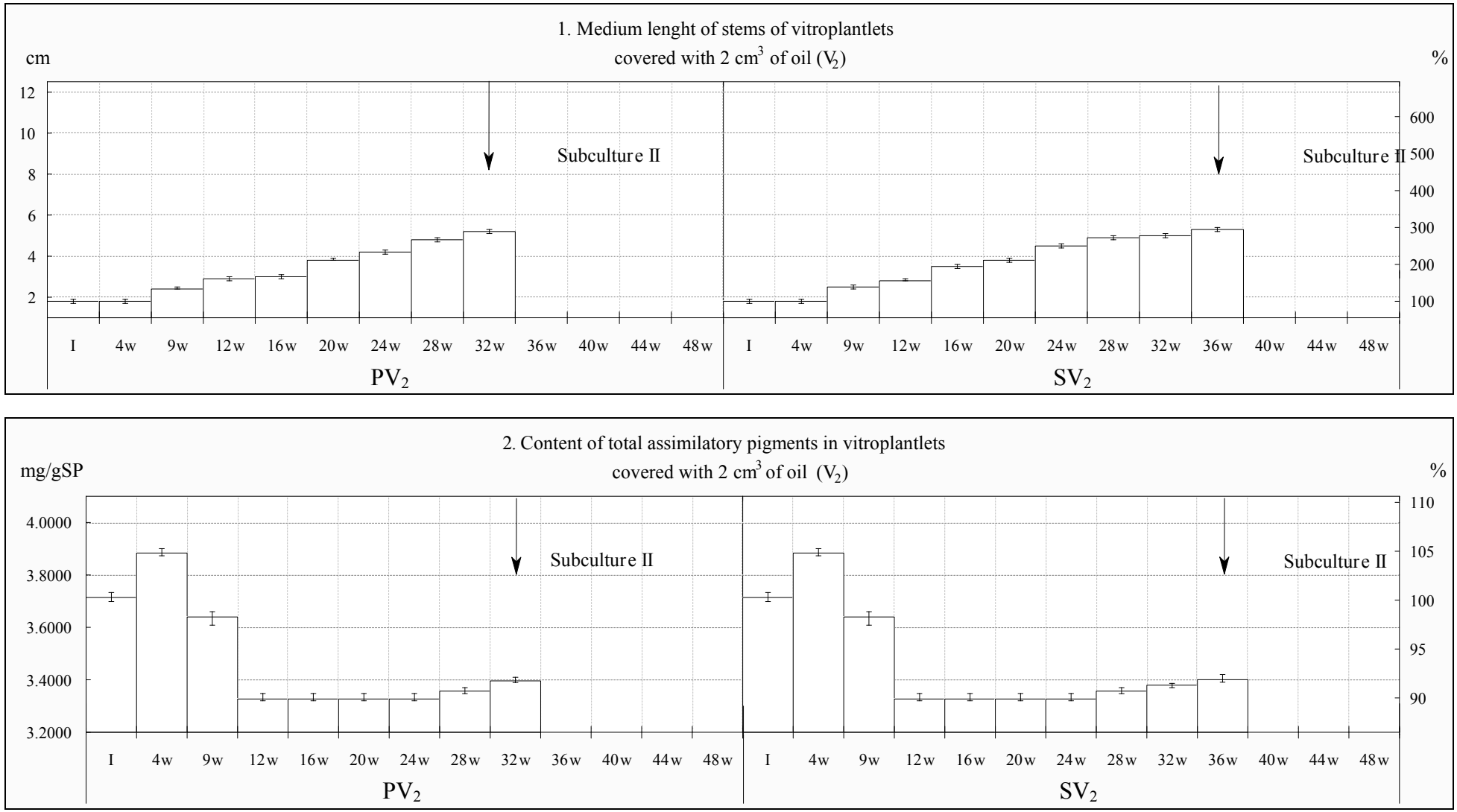

Fig. 4 The growth parameters of ***Coleus hybridus Jupiter*** vitroplantlets submersed under 2 cm^3 (V_2) of paraffin (P) or silicon (S) oil.

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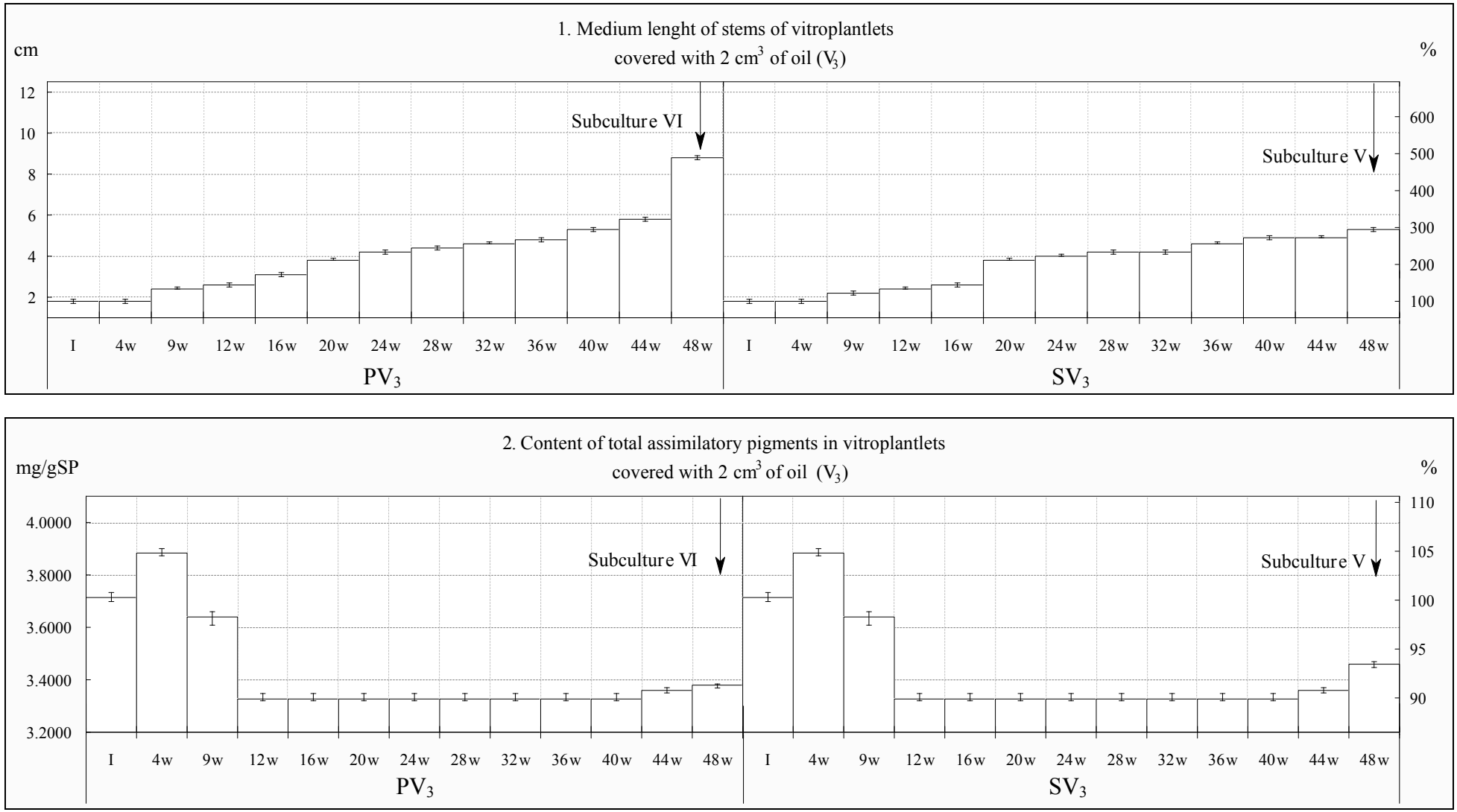

Fig. 5 The growth parameters of ***Coleus hybridus Jupiter*** vitroplantlets submersed under 5 cm^3 (V_3) of paraffin (P) or silicon (S) oil

Table 1

The absolute values and statistical significance regarding the growth of ***Coleus hybridus Jupiter*** vitroplantlets submersed under paraffin (PV) or silicon (SV) oil, the stratum being of different height: V_0 – control lot, vitroplantlets bare of oil; V_1 – submersion oil stratum which cover the plantlets apex is 1 cm^3, V_2 –2 cm^3 and, V_3 –5 cm^3, SP = g vegetal product, p = statistical significance: [**] - very significant ($p \leq 0{,}005$ and $p \leq 0{,}001$), [*] - significant ($p \leq 0{,}01$), [-] – non significant).

Growth parameter	Var. / weeks	Parameter value							Statistical significance (p)						
		V_0	PV_1	SV_1	PV_2	SV_2	PV_3	SV_3	V_0	PV_1	SV_1	PV_2	SV_2	PV_3	SV_3
Length of principal stem (cm)	I	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	*	*	*	*	*	*	*
	4w	5± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	1.8± 0.1	*	*	*	*	*	*	*
	9w	7± 0,1	2.4± 0.057	2.4± 0.1	2.4± 0.1	2.5± 0.057	2.4± 0.057	2.2± 0.057	*	**	*	*	**	**	**
	12w	12± 0.057	2.9± 0.1	2.9± 0.1	2.6± 0.1	2.8± 0.057	2.6± 0.057	2.4± 0.057	**	*	*	*	**	**	**
	16w	-	3.3± 0.1	3.3± 0.1	3± 0.057	3.5± 0.1	3.1± 0.057	2.6± 0.057	-	*	*	**	*	**	**
	20w	-	3.6± 0.1	3.8± 0.057	3.8± 0.057	3.8± 0.1	3.8± 0.057	3.8± 0.057	-	*	**	**	*	**	**
	24w	-	3.9± 0.057	3,9± 0.1	4.2± 0.057	4.5± 0.1	4.2± 0.057	4± 0.057	-	**	*	**	*	*	*
	28w	-	-	-	4.8± 0.1	4.9± 0.1	4.4± 0.057	4.2± 0.057	-	-	-	*	*	**	**
	32w	-	-	-	5.2± 0.057	5± 0.057	4.6± 0.057	4.2± 0.057	-	-	-	**	**	**	*
	36w	-	-	-	-	5.3± 0.1	4.8± 0.1	4.6± 0.057	-	-	-	-	*	*	*
	40w	-	-	-	-	-	5.3± 0.1	4.9± 0.1	-	-	-	-	-	*	*
	44w	-	-	-	-	-	5,8± 0.1	4.9± 0.057	-	-	-	-	-	*	*
	48w	-	-	-	-	-	8.8± 0.1	5.3± 0.1	-	-	-	-	-	*	*

Continuation of Table 1

Growth parameter	Var. / weeks	Parameter value							Statistical significance (p)						
		V_0	PV_1	SV_1	PV_2	SV_2	PV_3	SV_3	V_0	PV_1	SV_1	PV_2	SV_2	PV_3	SV_3
Content of total assimilatory pigments mg/g SP	I	3.7137± 0.0077	3.7137± 0.0077	3.7137± 0.0077	3.7137± 0.0077	3.7137± 0.0077	3.7137± 0.0077	3.7137± 0.0077	**	**	**	**	**	**	**
	4w	3.8837± 0.0101	3.8837± 0.0101	3.8837± 0.0101	3.8837± 0.0101	3.8837± 0.0101	3.8837± 0.0101	3.8837± 0.0101	**	**	**	**	**	**	**
	9w	3.9759± 0.0108	3.6399± 0.0092	3.6399± 0.0092	3.6399± 0.0092	3.6399± 0.0092	3.6399±0.0092	3.6399± 0.0092	**	**	**	**	**	**	**
	12w	4.3659± 0.0126	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	**	**	**	**	**	**	**
	16w	-	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267±0.0091	3.3267± 0.0091	-	**	**	**	**	**	**
	20w	-	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	-	**	**	**	**	**	**
	24w	-	3.4029± 0.0110	3.4597± 0.0143	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	3.3267± 0.0091	-	**	**	**	**	**	**
	28w	-	-	-	3.3567± 0.0091	3.3567± 0.0091	3.3267± 0.0091	3.3267± 0.0091	-	-	-	**	**	**	**
	32w	-	-	-	3.3902± 0.0142	3.3799± 0.0143	3.3267± 0.0091	3.3267± 0.0091	-	-	-	**	**	**	**
	36w	-	-	-	-	3.4009± 0.0101	3.3267± 0.0091	3.3267± 0.0091	-	-	-	-	**	**	**
	40w	-	-	-	-	-	3.3267± 0.0091	3.3267± 0.0091	-	-	-	-	-	**	**
	44w	-	-	-	-	-	3.3599±0.0085	3.3599± 0.0085	-	-	-	-	-	**	**
	48w	-	-	-	-	-	3.3799±0.0082	3.4597± 0.0111	-	-	-	-	-	**	**

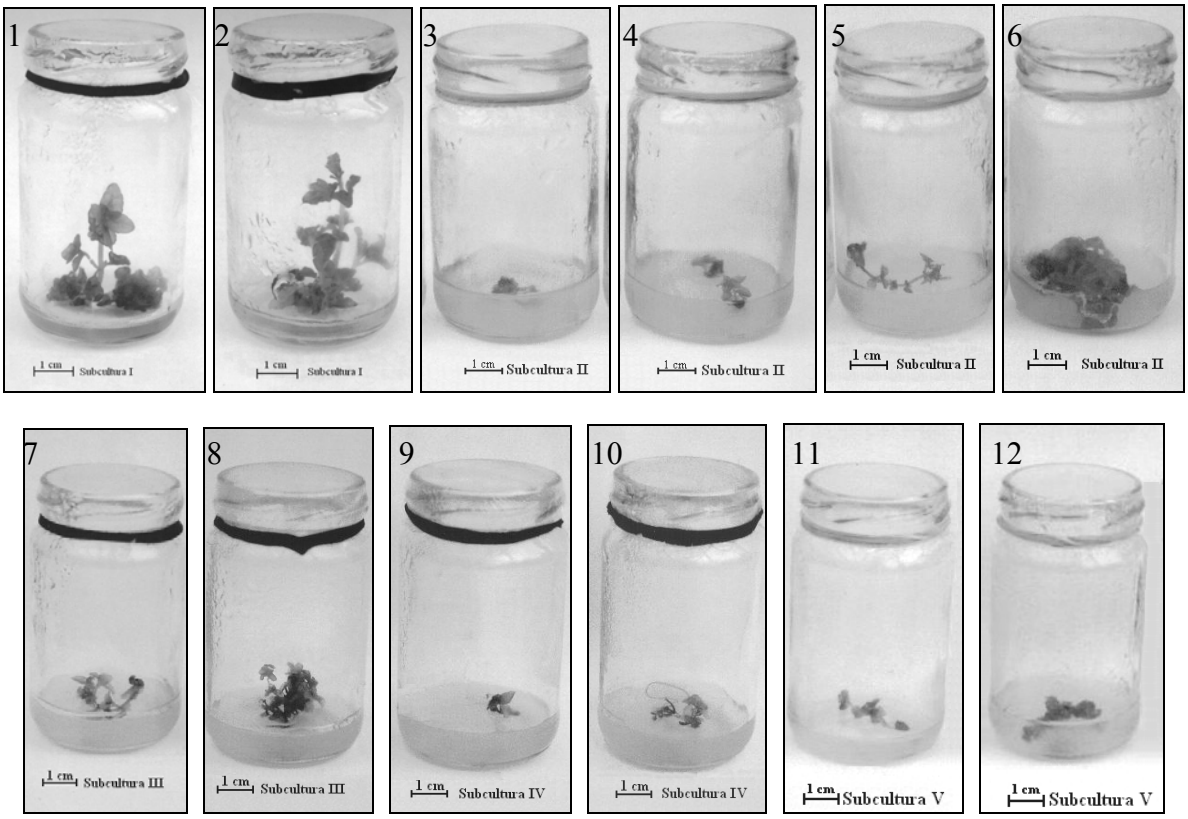

Fig. 6. After 24, 32, 36, 44 and 48 weeks of submersion from the submerced under oil vitroplantlets ware taken minicuttings and subcultived on fresh culture medium bare from oil, this new resulting vitrocultures are: Subculture I: vitroplantlets after 9 weeks of subculture resulting from minicutting taken after 24 weeks of submersion – 1 (paraffin oil) and 2 (silicon oil); Subculture II: vitroplantlets after 9 weeks of subculture resulting from minicutting taken after 32 weeks of submersion – 3 (paraffin oil) and 4 (silicon oil) and the same subculture after 12 weeks (5 and 6); Subculture III: vitroplantlets after 24 weeks of subculture resulting from minicutting taken after 36 weeks of submersion – 7 (paraffin oil) and 8 (silicon oil), Subculture IV: vitroplantlets after 24 weeks of subculture resulting from minicuttings taken after 44 weeks of submersion (9 and 10); Subculture V: vitroplantlets after 24 weeks of subculture resulting from minicuttings taken after 48 weeks of submersion (11 and 12).

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