THE MICROPROPAGATION OF *COLEUS BLUMEI BENTH.* VITROCULTURES UNDER DIFFERENT PARAFFIN OR SILICON OIL STRATUMS

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Summary: In the framework of this experiment was the study of the reaction of *Coleus blumei Benth.* 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 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.

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 or storage in oils submersion.

The vitrocultures, periodic had to be transferred on fresh culture medium, which involves, not only heaved costs for workers, chemical substances, culture vessels, but also a possible contamination or even loss of the existing vitrocultures. Moreover, this cultures type is inclined to adduce genetic changes, appearance of mutation. To avoid the big number of periodical subcultivation, was adopted different methods for grow rate reduction – subculture rate reduction (Bajaj, 1991).

One of such examples is the research of Halmagyi and Cachiţă (2002) witch studied the ultrastructural aspects of carnation callus (*Chrisanthemum leucantemun)* witch was storage about 19 months under 2 cm of paraffin oil. Only a six month period was benefit for the vitrocultures storage, without the decreasing of the regenerative capacity.

Bolba (2004) *studied the conservation of Lilium martagon* and *Lilium candidum* buds in hypoxic conditions – submersed under a paraffin oil stratum. The conservation period was 6 to 12 months, the storage in these conditions proved a negative effect through conservation method in *Lilium martagon* vitrocultures, but at *Lilium candidum* – after 6 storage months was observed a loss of the regenerative ability through the period of 4 weeks from subculture.

Petrus-Vancea and others (2005) experimented the *Dendrobium and Cattleya* protocorm conservation for 5 months, using distilled water, paraffin oil, castor-oil, flax oil and mixed in report of 1:1 of castor-oil with flax oil, the stratum height was between 0.5 cm and 2 cm. for both species the paraffin storage is the best storage method, other oils had a toxic effect on the vitrocultures.

MATERIAL AND METHODS

In this experiment we used *Coleus blumei Benth.* 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: Subculture I: minicutting taken after 24 weeks under oil submersion; Subculture II: minicutting taken after 32 weeks of submersion and Subculture III: minicutting taken after 48 weeks under oil 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) = $11.65\ A_{644} - 2.69\ A_{647} \cdot v/sp$

b chlorophyll (mg/gsp) = $20.8\ A_{644} - 3.14\ A_{664} \cdot v/sp$

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, DISCUSSIONS AND CONCLUSION

At first 4 weeks of the double stratum oil submersion (using paraffin or silicon oil) already could be observed modifications in the behaviour of the vitroplantlets (fig. 1 A and B, fig. 2-5). We observed that the of assimilatory pigments in submersed under oil vitroplantlets was equal with one from the moment before the hypoxic conditions: 3.6214 mg/g Sp regardless oil type or applied stratum, comparatively with the witness lot witch had a strong growth (fig. 2-5). At the witness lot the assimilatory pigments quantity was 3.6984 mg/g sp, with a non significant increment of 2% (fig. 2, table 1). The growth suppress was observed also 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 (fig. 2-5).

After 9 weeks of vitroculture in hypoxic conditions al the vitroplantlets level was observed a decrease of total assimilatory pigments by 5.8%, a equal level at all variants of oil and oil stratum, this quantity being by 13.8% lower then at wetness lot – vitroplantlets bare of oil (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 9.1 cm (505.55%) in stem length and a difference of 28.14% 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 16 weeks of vitroculture, the stem length became 3.3 cm and content a quantity of 3.3167 mg/gSp total assimilatory pigments (fig. 3). These vitroplantlets overreached oil stratum only after 24 of weeks of double stratum culture (fig. 1 C-D). At this moment the vitroplantlets submersed in paraffin oil were 3.9 cm high and content 3.3802 mg/gSp total assimilatory pigments – with a positive difference by 2.1 cm on length level, but a negative difference by 6.66% 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 3.4397 mg/gSp of assimilatory pigments (at this measure the difference was negative by 5.02%) – fig. 3. At this moment of experiment from vitroplantlets was taken minicuttings and subcultured on fresh culture medium bare of oil – resulting Subculture I. In subculture the resulted vitroplantlets had a normal growth, without persistence growth inhibition, the vitroplantlets stem length were normal for 4 weeks of vitroculture (fig. 1 I-J).

Using an oil stratum of 2 cm^3 gave the possibility to extend conservation period to 32 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 lower: they had 4.8 cm stem length (with a difference by 166% comparing with the moment before the oil add) and a quantity of 3.3702 mg/gSp assimilatory pigments (with a negative difference by 7%) – fig. 4. But the vitroplantlets high, witch was covered with silicon oil, had a larger stem: 5 cm (with a difference by 177%) and a content of 3.3962 mg/gSp assimilatory pigments (a negative difference of 6.3%) – fig. 4. At this moment of experiment from vitroplantlets was taken minicuttings and subcultured on fresh culture medium bare of oil – resulting Subculture II. In subculture the resulted vitroplantlets had a growth inhibition until 12 weeks from the subculture (fig. 1 K-L).

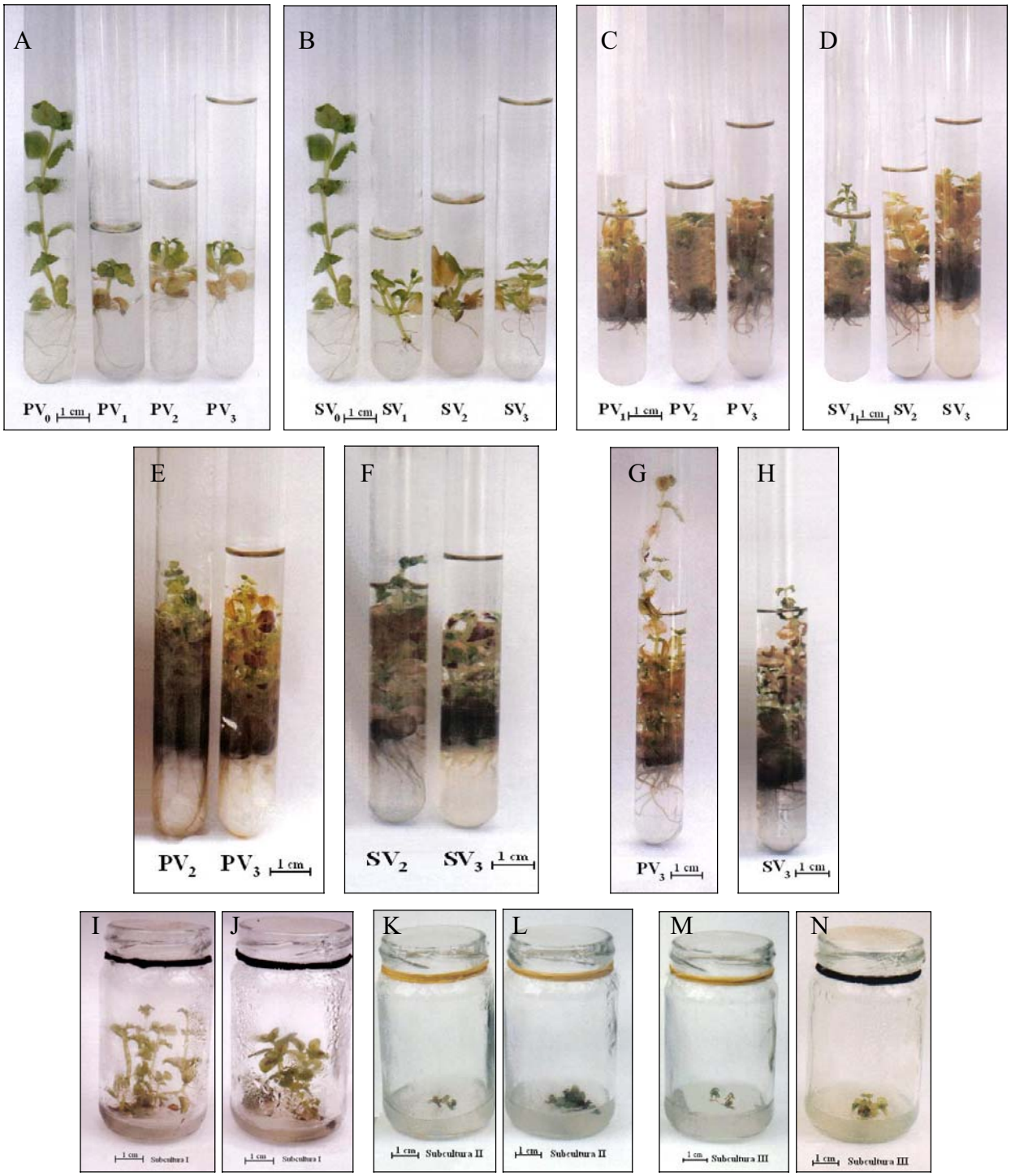

Figure 1. Photographic details regarding the growth of *Coleus blumei Benth.* vitroplantlets submersed under paraffin (PV) or silicon (SV) oil, the stratum being of different height: V_0 – control lot, vitroplantlets bare of oil; V_1 – submersion oil stratum which cover the plantlets apex is 1 cm^3, 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 and H – after 44 weeks of submersion. After 24, 32 and 48 weeks of submersion from these vitroplantlets ware taken minicuttings and subcultived on fresh culture medium bare from oil (I-N), this new resulting vitrocultures are: Subculture I: vitroplantlets after 9 weeks of subculture resulting from minicutting taken after 24 weeks of submersion – I (paraffin oil) and J (silicon oil); Subculture II: vitroplantlets after 12 weeks of subculture resulting from minicutting taken after 32 weeks of submersion – K (paraffin oil) and L (silicon oil); Subculture III: vitroplantlets after 24 weeks of subculture resulting from minicutting taken after 48 weeks of submersion – M (paraffin oil) and N (silicon oil).

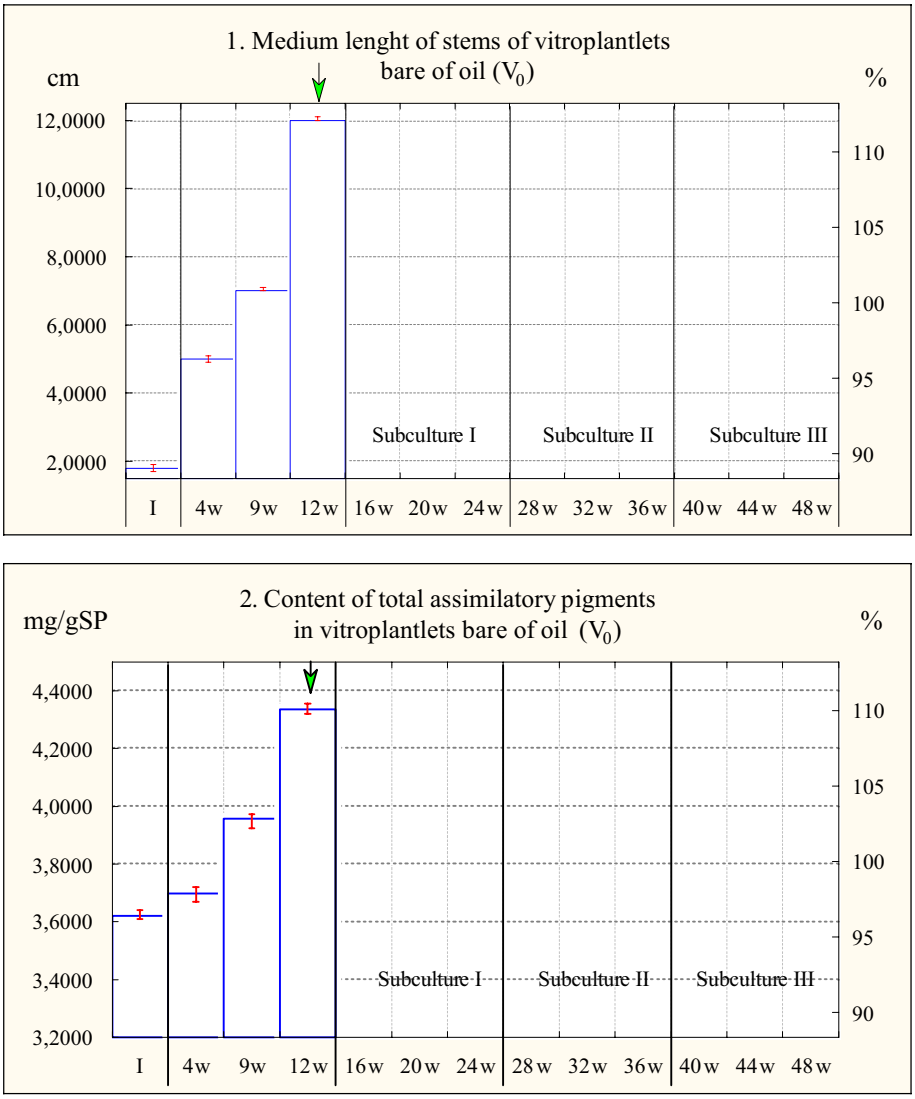

Figure 2. The growth parameters of *Coleus blumei Benth.* vitroplantlets bare of oil: V_0 – control lot. After the first 12 weeks witness lot grew up of the oil stratums, over 48 experimental weeks were subcultivated 3 times.

The longest period of oil preserving vitrocultures resulted in case of using a 5 cm^3 oil stratum – the preservation period being prolonged until 48 weeks, but at 44 weeks the vitroplantlets overreached the oil stratum (fig. 1 G-H), both variants registered the same quantity of assimilatory pigments, with a negative difference by 8.61% (fig. 5). The prolongation of the storage period had a negative influence over the subculture growth (Subculture III - at this moment of experiment from vitroplantlets was taken minicuttings and subcultured on fresh culture medium bare of oil). In subculture was observed a growth inhibition witch maintained until 24 weeks of subculture (fig. 1 M-N), with a low level of total assimilatory pigments.

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.

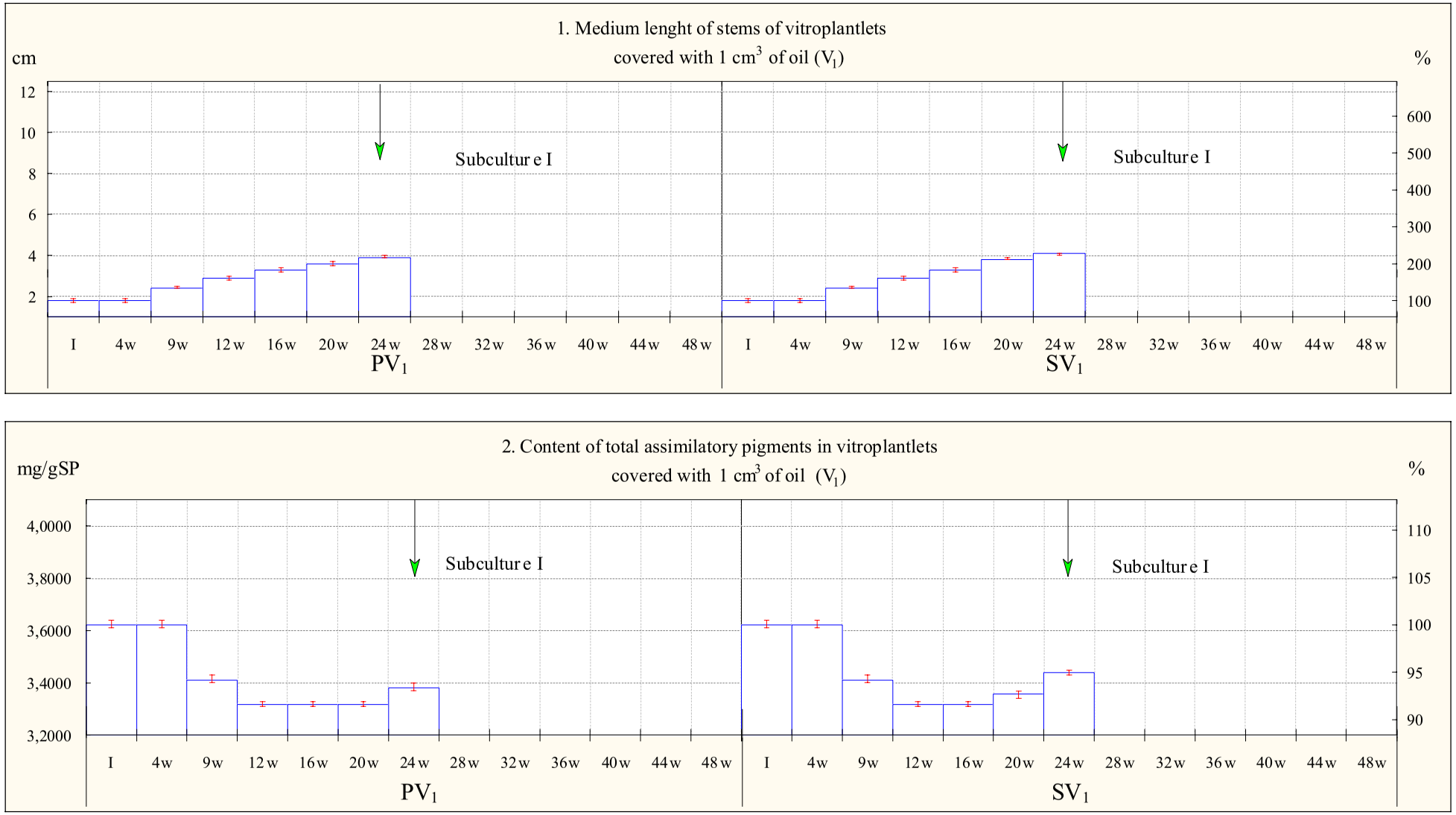

Figure 3. The growth parameters of *Coleus blumei Benth.* vitroplantlets submersed under 1 cm^3 (V_1) of paraffin (P) or silicon (S) oil.

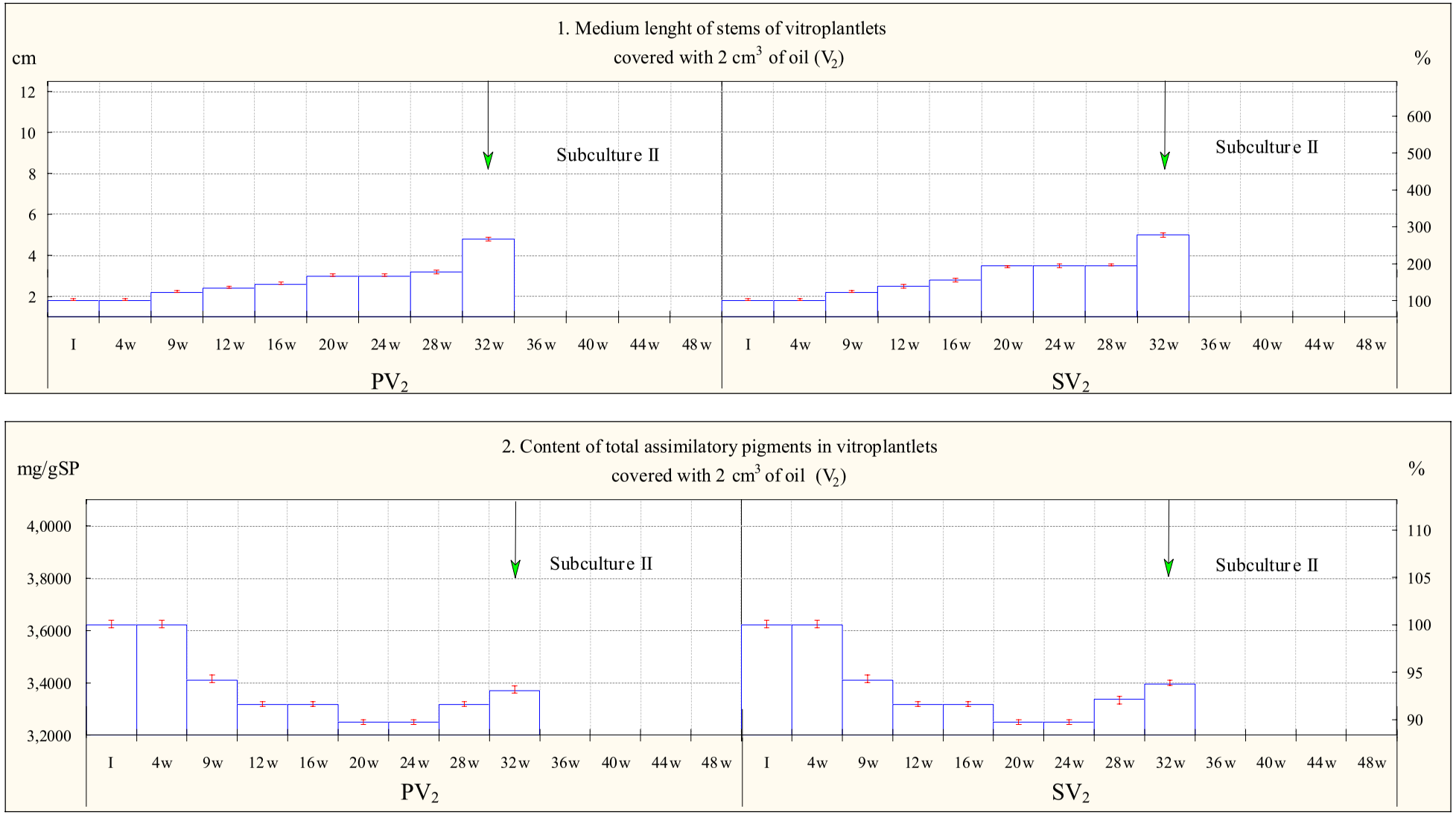

Figure 4. The growth parameters of *Coleus blumei Benth.* vitroplantlets submersed under 2 cm^3 (V_2) of paraffin (P) or silicon (S) oil.

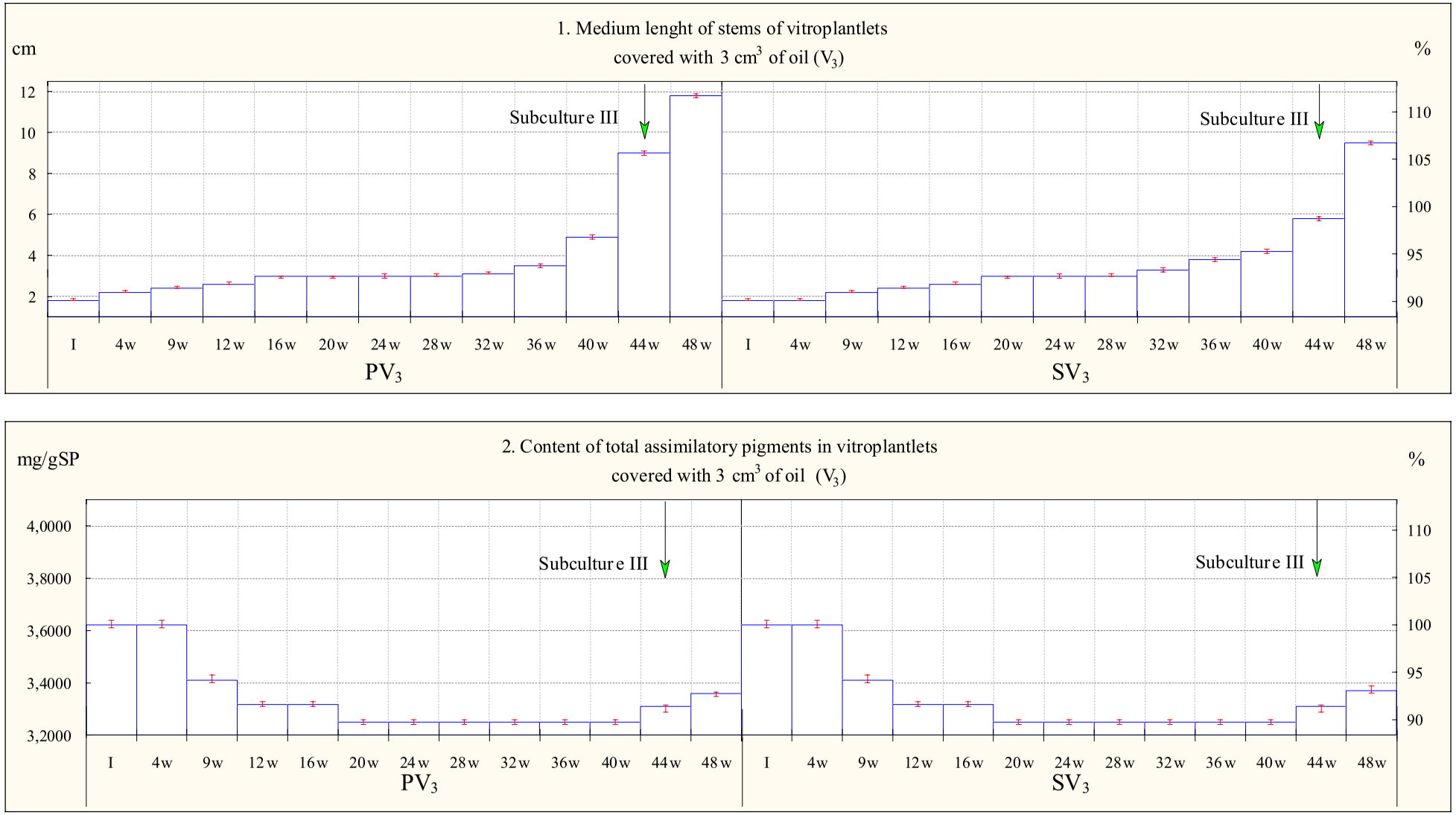

Figure 5. The growth parameters of *Coleus blumei Benth.* vitroplantlets submersed under 5 cm^3 (V_3) of paraffin (P) or silicon (S) oil.

Table 1. The absolute values and statistical significance regarding the growth of *Coleus blumei Benth.* 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	2.2± 0.057	2.2± 0.057	2.2± 0.057	1.8± 0.1	*	*	*	**	**	**	*
	9w	7± 0.057	2.4± 0.057	2.4± 0.057	2.4± 0.057	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± 0.057	2.8± 0.057	2.6± 0.057	24± 0.057	**	*	*	**	**	**	**
	16w	-	3.3± 0.1	3.3± 0.1	3± 0.057	3.5± 0.1	3± 0.057	2.6± 0.057	-	*	*	**	*	**	**
	20w	-	3.6± 0.1	3.8± 0.057	3± 0.057	3.5± 0.1	3± 0.057	3± 0.057	-	*	**	**	*	**	**
	24w	-	3.9± 0.057	4.1± 0.057	3.2± 0.057	3.5± 0.057	3± 0.1	3± 0.1	-	**	**	**	**	*	*
	28w	-	-	-	4.8± 0.057	5± 0.1	3± 0.057	3± 0.057	-	-	-	**	*	**	**
	32w	-	-	-	5.2± 0.057	5.2± 0.057	3.1± 0.057	3.3± 0.1	-	-	-	**	**	**	*
	36w	-	-	-	-	-	3.5± 0.1	3.8± 0.1	-	-	-	-	-	*	*
	40w	-	-	-	-	-	4.9± 0.1	4.2± 0.1	-	-	-	-	-	*	*
	44w	-	-	-	-	-	9± 0.1	5.8± 0.1	-	-	-	-	-	*	*
	48w	-	-	-	-	-	11.8± 0.1	9.5± 0.1	-	-	-	-	-	*	*

Continuation **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	3.6214± 0.0152	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	**	**	**	**	**	**	**
	4w	3.6984± 0.0258	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	3.6214±0 .0147	**	**	**	**	**	**	**
	9w	3.9564± 0.0242	3.4101±0 .0149	3.4101±0 .0149	3.4101±0 .0149	3.4101±0 .0149	3.4101±0 .0149	3.4101±0 .0149	**	**	**	**	**	**	**
	12w	4.3359± 0.0177	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	**	**	**	**	**	**	**
	16w	-	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 .0091	3.3167±0 0091	-	**	**	**	**	**	**
	20w	-	3.3167±0 .0091	3.3567±0 .0136	3.2502±0 .0090	3.2502±0 .0090	3.2502±0 .0090	3.2502±0 .0090	-	**	**	**	**	**	**
	24w	-	3.3802±0 .0142	3.4397±0 .0090	3.2502±0 .0090	3.2502±0 .0090	3.2502±0 .0090	3.2502±0 .0090	-	**	**	**	**	**	**
	28w	-	-	-	3.3167±0 .0091	3.3367±0 .0141	3.2502±0 .0090	3.2502±0 .0090	-	-	-	**	**	**	**
	32w	-	-	-	3.3702±0 .0142	3.3962±0 .0103	3.2502±0 .0090	3.2502±0 .0090	-	-	-	**	**	**	**
	36w	-	-	-	-	-	3.2502±0 .0090	3.2502±0 .0090	-	-	-	-	-	**	**
	40w	-	-	-	-	-	3.2502±0 .0090	3.2502±0 .0090	-	-	-	-	-	**	**
	44w	-	-	-	-	-	3.3099±0 .0140	3.3099±0 .0140	-	-	-	-	-	**	**
	48w	-	-	-	-	-	3.3599±0 .0082	3.3702±0 .0142	-	-	-	-	-	**	**

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