RAPID INITIATION OF *IN VITRO* CULTURES AT *Viola Wittrockiana* Matrix F1 Red Blotch

Cristian Felix BLIDAR[*,**], Cristina ILEA[***]

[*] *University of Oradea, Faculty of Science, Biology Department, Oradea, Romania*
[**] *S.C. Sapient S.R.L., Research Department, Oradea, Romania*
[***] *Master's student, Faculty of Science, Biology Department, Oradea, Romania*
Corresponding author: Cristian Felix Blidar, University of Oradea, Faculty of Science, Department of Biology, 1 Universităţii Str., 410087Oradea, Romania, phone: +40722495933, fax: +40259408461, e-mail: cblidar@gmail.com

Abstract: *Viola wittrockiana* is one of the most popular ornamental plant, cultivated for its beauty, uniqueness and resistance. The global use of pansies in decorating outdoor spaces, such as gardens and parks, has determined the production and cultivation of an increasing number of varieties regarding flower's colour and recent researches are supporting achieving this feature. The main aim of this article is to investigate *in vitro* growth and development of pansies by using two different culture media: liquid culture medium provided with filter paper bridges (Blidar type), and culture medium supplemented with agar. Both cultured media are based on Murashige-Skoog formula of 1962. The control is represented by the classical solid culture medium, and the tested culture medium is represented by liquid culture medium (agar-agar free), provided with filter paper bridge. The role of this paper bridge is to maintain in close contact with constant nutrient concentration the seeds and then the vitro-plants of *Viola wittrockiana*. The analysis of the results of this experiment revealed the superior efficacy of using filter paper bridge *in vitro* pansies' growth compared with the solid culture medium. This result was achieved by monitoring 5 biometric and 2 gravimetric parameters for 60 days.

Keywords: *Viola wittrockiana*; filter paper bridge; *in vitro*; vegetable biotechnology; ornamental plants.

INTRODUCTION

Viola wittrokiana, common known as pansy, is an hybrid resulted from the breeding of three different species: *Viola ticolor, Viola lutea* and *Viola altaica.* This hybrid is cultivated as an ornamental plant especially in gardening. The genus is part of the Violaceae's family, and includes about 500 species, all known for their resistance to various environmental factors and also for their ornamental qualities given by special colours [25].

Pansies are perennial in cold climate, expressing two flowering periods: spring and autumn. The plants vary in heigh, between 10-25cm and may have either herbaceous or bushy aspect [1]. The flower of garden pansy has an unique geometry consisting of five petals: two superior, two lateral and one large inferior [23].

Plant breeding evolved from the traditional method to the use of biotechnology techniques. Thus, genetic engineering and plant biotechnology become important and inevitable in its future development, because using these new tools it can be achieved, in a short time, new hybrids or varieties in term of productivity resistance [17] or extraction of new compounds. In the last years more experiments were made on pansies using biotechnology. Thus, in 2007 it was developed a regeneration protocol starting from callus and using a traditional agar-agar culture media supplemented with 2,4-D and BA [22]. At pansies, it was also reported the influence of different sucrose concentrations for *in vitro* pollen's germination. When the sucrose concentration in agar medium reached 30%, the germination was at a maximum [13]. Other experiments were made on plant's anthers and the results showed that callus formation depends on the type and concentration of growth hormones that are used [14].

A very common method applied for *in vitro* technologies is using agar-agar solidified culture media. In the recent years new methods have been developed, such as using liquid media and different types of paper bridges.

Still, there are few references in scientific literature, regarding the use of filter paper bridges as an efficient method for *in vitro* cultivation of plant, and most of them are published in the last ten years. Two of the relevant aspects of using a liquid culture media provided with filter-paper bridge, in comparing to agar-agar culture media, are as following: reduced costs in experiments and decrease environmental pollution [4].

One of the first scientific articles that showed a comparison between using a liquid medium and an agar-agar culture media, was published in 1991, by Mohmand and Nabors. Wheat was used in that experiment and the results demonstrate, that total and embryogenesis callus was doubled and presented a significantly higher number of regenerants when wheat was cultivated on liquid culture medium provided with filter paper bridge, compared with agar-agar medium [16]. Zhongxu and al., in 2001, showed an improved *in vitro* rooting process in different fruits plants on filter-paper bridge compared with agar supplemented media [24]. One year later, an experiment using *Trapa japonica* revealed that the indirect regeneration process starting from callus was at the same intensity on both culture media: liquid and solidified [11]. Another study on *Litchi chinensis,* revealed that the germination was more efficient on paper bridge [12]. In 2004 Blidar promoted new type of filter paper bridges, and after a series of experiments on maize and wheat showed that plantlets grown on filter paper bridge had a significant development compared to the plantlets grown on agar medium [3, 7, 8]. Another experiment made in 2012 revealed that the highest bromelain enzyme from Pineapple was observed in leaf tissue after 4 months of *in vitro* culture in liquid medium with filter paper bridge, followed by agar supplemented medium [21].

Presently there are only two references regarding negative aspects of plantlets grown on liquid culture media with filter paper bridge compared to agar supplemented culture media. The first experiment was realized on *Ananas comosus* aiming caulogenesis on two substrates [2]. The second was about the effect of paper bridges, antioxidants and activated charcoal on development and growth of protocorm-like bodies of hybrid *Cymbidium*, and the results showed that the use of paper bridges effected the growth and development of protocorm-like bodies [20].

However this *in vitro* technique was used in many others experiments, and on different species. From these experiments it should be mentioned the conservation for three years of potato (*Solanum tuberosum*) micro-plants grown into *in vitro* at low light and temperature conditions [15], or regenerative capacity of callus of ornamental strawberry *Fragaria x Potentilla* [19]. Others experiments were realized on tabacco (*Nicotina tabacum*), alfalfa (*Medicago sativa*), poplar (*Populus euphratica*), and many others. A very interesting study, developed over several years, was realized on the orchids *Cymbidium hybridum.* The aim of this experiments was to observed some aspects such as: microelements deficiency effects on orchids grown into *in vitro* growth on filter paper bridges, the use of fructose in the nutrient medium and the caffeine effect on orchids grown [5, 6]. Another article published in 2011 showed the importance of filter paper bridge in secondary metabolism at micropropagated *Hypericum perforatum* grow. The results showed that the highest concentration of phenolic compounds and hypericine were observed in shoots grown on paper bridges and partial immersion [18].

The objective of this experiment was to continue previous experiments realized on filter paper bridges, using a liquid culture media, on another species namely *Viola wittrockiana*. This experiment also included the solidified culture medium supplemented with agar in order to obtain clear comparative data between the two techniques.

MATERIALS AND METHODS

Plant material. For this experiment were selected uniform and mature seeds of species *Viola wittrockiana* Matrix F1, red variety (*Viola wittrockiana* Matrix F1 Red Blotch), the seeds were purchased from commerce (producer: Agrosem Impex SRL Tg-Mureş, Romania).

Growth conditions and inoculations. The selected seeds were kept for 10 minutes under runnig tap water and disinfected using a sodium hypochlorite solution 5%, from a commercial bleach (ACE-automat). The disinfection was made for another 10 minutes, after that plant material was rinsed with sterile distilled water, for five times to remove disinfected solution. For inoculation were made two Murashige-Skoog (1962) modified mediua: agarized with 7 g/l agar-agar solution (**VR_A**) and liquid (agar-agar free) provided with filter paper bridge (**VR_{PB}**), Blidar type (BFPB) [3]. The agarized medium was considered as control experimental variant, and both types of media were free of hormons and aminoacids. The number of recipents and the number of inoculta were equal, 50 per both variant. After inoculation recipients were maintained at 23°C±1°C, in a growth chamber. The cultivated seeds were kept in the growth room for 60 days under 16-h photoperiod, light being produced by daylight white (6500K) flourescent tubes (20 μM $m^{-2}s^{-1}$ PAR).

Growth measurement. Plantlets grown, measurements and observations were made during 60 days, at an interval of 10 days each. Biometric parameters analyzed and compared to each plant are as following: length of roots, length of strain (hypocotyl and epicotyl), number of leafs, length of leafs, dry weight and fresh weight.

Statistical analysis. VR_A was considered the control group and the values registered were taken as references for experimental variant VR_{PB}. All statistical analyses were made using Microsoft Excel.

RESULTS

In terms of morphological, biometric and gravimetric parameters monitored in the experiment, it can be considered that overall, that using filter paper bridges and liquid medium (tested lot), compared to solid medium (control lot), improved plantlets grown as it can be seen in the following.

Measurements and observation at 10 days of experiments. Seed gemination rate, the first parameter analysed in the experiment, at 10 days after inoculation, presented a higher percentage for seeds disposed on filter paper bridge (VR_{PB}), compared with those on agarized medium (VR_A), and this trend is maintaining along the entire experimental period. In the observation made at 10 days it was observed the emergence of roots in both experimental lots. The highest value of *roots lengtht* were observed at the tested lot (VR_{PB}), compared with the control (VR_A), the percentage difference was 13.9% for liquid medium. Plantlets biomass expressed in grams had higher values on liquid medium compared to the classic solid medium for dry weight parameter (1.16 mg, with 5.45% superior than control).

Measurements and observation at 20 days of experiments. At 20 days the most significant change appeared in the *lengtht of roots*, the higher value being seen at plantlets on the solidified medium (22.31% compared with VR_{PB}). From morphological point of view it was observed the appearance of hypocotyl, epicotyl and leafs at the plantles cultivated on both types of culture media. For four biometric parameters: *root length*, *hypocotyl length*, *epicotyl length* and *leaf length* the percentage was equal on both media types (0.05/vitroplantlets).

Measurements and observation at 30 days of experiments. At 30 days it was observed differences between *epicotyl length* and the *number of leafs*: plantlets cultivated on agarized medium had a higher rate of growth than test lot VR_{PB}. Regarding *roots*

length, the values were superior for the vitroplantles cultivated on paper bridges compared to those cultivated on agarized medium, the percentage difference being over 6.86%. The same trend was observed for *hypocotyl length*, where the values were higher for the plantlets cultivated on liquid culture mediun, compared to the control (31.69% difference, 0.9 mm raported to the absolute values) (table 1).

Measurements and observation at 40 days of experiments. At 40 days, differences appeared at roots' and hypocotyls' length, the plantlets cultivated on filter paper bridge – VR_{PB} – had superior values compared to those cultivated on agarized substrate – VR_A – (at *root length* with 6.11mm or 58.9%, and *hypocotyl length* with 1.23 mm or 37.8% higher compared to the control lot), all these values were statistically significant (Table 1). Another difference was also observed when measuring *dry weight,* which was with 132.3% in favour of the vitroplantlets placed on paper bridge compared to agarized medium (Table 1). For all others parameters left the values were higher on control than the sample.

Measurements and observation at 50 and 60 days of experiments. In the observation made at 50 and 60 days was determined the same growth trend as above described. At 50 days the roots and hypocotyls length were higher at plantlets growth on liquid culture medium provided with filter paper bridge compared with the agarized medium. From statistic point of view the difference between this two lots was significant: *root length* with 6.59 mm (56.95%) higher for the sample compared to the control and *hypocotyl length* with 1.66 mm (48.53%) was superior for the plants placed on paper bridge compared to those cultivated on agar. For the following five parameters: *epicotyls length*, *leafs length*, *number of leafs per plantlet, fresh*

Table 1. Statistical processing of the data measured in the *in vitro* plants of pansys (*Viola Wittrockiana* Matrix F1 Red Blotch), cultivated on the following culture media: VR_A – agarized MS culture medium (control) and VR_{PB} - liquid MS culture medium with paper bridge

		VR_A (control) (agarized media)		**VR_{PB}** (liquid media with paper bridge)				
No. of days	**Statistical data**	**X ± Sx**	**s^2**	**X ± Sx**	**s^2**	**±d**	**%**	**Signifi-cance**
10	Germination faculty (%)	80 ± n/a	n/a	76 ± n/a	n/a	4	5	n/a
	Root length (mm)	1.23 ± 0.56	0.32	1.40 ± 0.93	0.86	0.17	13.83	ns
	Fresh weight (mg)	3.44 ± n/a	n/a	3.08 ± n/a	n/a	-0.36	-10.47	n/a
	Dry weight (mg)	1.1 ± n/a	n/a	1.16 ± n/a	n/a	0.06	5.45	n/a
20	Germination faculty (%)	81.8 ± n/a	n/a	82.6 ± n/a	n/a	0.8	0.9	n/a
	Root length (mm)	7.08 ± 0.49	0.22	5.5 ± 0.79	0.62	-1.58	-22.31	***
	Hypocotyl length (mm)	1.61 ± 1.24	1.53	1.5 ± 1.37	1.9	-0.11	-6.84	ns
	Epicotyl length (mm)	0.27 ± 0.44	0.20	0.25 ± 0.43	0.19	-0.02	-7.41	ns
	Leaf length (mm)	0.16 ± 0.37	0.13	0.12 ± 0.32	0.1	-0.04	-25	ns
	Leaf no.	0.05 ± 0.21	0.04	0.05 ± 0.22	0.05	0	0	ns
	Fresh weight (mg)	6.12 ± n/a	n/a	5 ± n/a	n/a	-1.12	-18.31	n/a
	Dry weight (mg)	1.12 ± n/a	n/a	1.18 ± n/a	n/a	0.06	5.35	n/a
30	Root length (mm)	9.62 ± 0.49	0.24	10.28 ±1.17	1.37	0.66	6.86	**
	Hypocotyl length (mm)	2.84 ± 0.37	0.14	3.74 ± 2.07	4.28	0.9	31.69	*
	Epicotyl length (mm)	4.81 ± 1.14	1.31	3.11 ± 0.8	0.98	-0.64	-35.35	***
	Leaf length (mm)	3.37 ± 0.71	0.51	2.17 ± 1.33	1.78	-1.2	-35.61	**
	Leaf no.	1.18 ± 0.39	0.15	0.74 ± 0.44	0.19	-0.44	-37.29	***
	Fresh weight (mg)	14.12 ± n/a	n/a	15.54 ± n/a	n/a	1.42	10.05	n/a
	Dry weight (mg)	1.72 ± n/a	n/a	2.42 ± n/a	n/a	0.7	40.69	n/a
40	Root length (mm)	10.37 ± 1.2	1.45	16.48 ±1.12	1.26	6.11	58.91	***
	Hypocotyl length (mm)	3.25 ± 0.51	0.26	4.48 ± 0.77	0.59	1.23	37.8	***
	Epicotyl length (mm)	5.59 ± 1.21	1.48	4.72 ± 1.36	1.87	-0.87	-15.57	**
	Leaf length (mm)	4.48 ± 1.12	1.26	3.93 ± 0.82	0.68	-0.55	-12.28	**
	Leaf no.	2.03 ± 0.88	0.77	1.44 ± 0.76	0.59	-0.59	-29.07	**
	Fresh weight (mg)	22.16 ± n/a	n/a	17.82 ± n/a	n/a	-4.34	-19.59	n/a
	Dry weight (mg)	1.98 ± n/a	n/a	4.6 ± n/a	n/a	2.62	132.32	n/a
50	Root length (mm)	11.57 ± 0.96	0.94	18.16 ± 1.02	1.04	6.59	56.95	***
	Hypocotyl length (mm)	3.42 ± 1.1	1.21	5.08 ± 1.28	1.66	1.66	48.53	***
	Epicotyl length (mm)	6.26 ± 0.93	0.87	4.92 ± 0.86	0.74	-1.34	-21.41	***
	Leaf length (mm)	6.31 ± 1.22	1.5	4.52 ± 1.35	1.84	-1.79	-28.37	***
	Leaf no.	3.1 ± 0.65	0.43	2.12 ± 1.45	2.12	-0.98	-31.62	**
	Fresh weight (mg)	67.66 ± n/a	n/a	24.06 ± n/a	n/a	-43.6	-64.44	n/a
	Dry weight (mg)	6.72 ± n/a	n/a	6.36 ± n/a	n/a	-0.36	-5.36	n/a
60	Root length (mm)	13.64 ± 0.7	0.49	23.15 ± 1.36	1.86	9.51	69.72	***
	Hypocotyl length (mm)	3.52 ± 1.12	1.26	6.2 ± 1.01	1.02	2.68	76.13	***
	Epicotyl length (mm)	8.52 ± 0.94	0.88	5.7 ± 0.86	0.74	-2.82	-33.1	***
	Leaf length (mm)	6.82 ± 1.12	1.26	5.6 ± 1.26	1.61	-1.22	-17.89	**
	Leaf no.	3.14 ± 0.53	0.28	2.85 ± 1.23	1.51	-0.29	-9.24	ns
	Fresh weight (mg)	68.7 ± n/a	n/a	25.98 ± n/a	n/a	-42.72	-62.19	n/a
	Dry weight (mg)	9.16 ± n/a	n/a	6.4 ± n/a	n/a	-2.76	-30.14	n/a

Note: X ± Sx [average (cm) ± standard deviation]; s^2 – variance; ±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 – not applicable.

weight, and *dry weight* the measured values were higher for plantlets cultivated on agarized medium compared to those cultivated on filter paper bridge (Table 1).

At the last observation made on this experiment – at 60 days – there were the same trends previously observed regarding the growth report of these two experimental groups. The roots and the hypocotyls length were higher at the plantlets placed on filter paper bridge compared to those cultivated on agarized medium. The absolute values for the *root length* parameter had 9.51 mm growth difference, with 69.7% more at the vitroplants cultivated on paper bridge compared to those cultivated on agarized substrate. The same characteristic aspect was observed for the *hypocotyl length* where the percentage was 76.1% higher for VR_{PB}, than VR_A, this values are statistical sustained (Table 1). All others biometric and gravimetric parameters had higher values for the control lot: one important percentage difference has been registered at *fresh weight* parameter, presenting a plus with 62.19% compared to those on liquid medium provided with filter paper bridge.

DISCUSSIONS

The results obtained in this experiment revealed the appearance of clear differences in the growth and development of *Viola wittrockiana* vitroplants starting from seed germination, all these depending on the used culture medium and the period in which the observations were made. Thus it can be considered that there are two important periods in growth and development of vitroplants namely one short term growth, under 30 days and a long term growth up to 60 days.

First 30 days. It was easily observed from the first days after the inoculation the presence of a higher germination rate for seeds placed on filter paper bridge compared to control medium, supplimented with agar-agar. This results can be explained considering the rapid access of vitroplants access to nutrient and minerals into the culture medium. Seed germination rate when seeds were inoculated on liquid medium provided with filter paper bridge had a higher value compared to the other experiments in which seeds germination was 90% [10].

Another important aspect observed in pansy plants development, had been the proliferation of the roots and strains much intense and faster at the tested vitroplants – VR_{PB} compared to the control lot – VR_A. In others experiment it was observed the same intense and fast growth of roots. In the scientific specialized literature similar data were registered, for example at wheat, where the cultivation on liquid medium provided with filter paper bridge has facilitated the growth of length of roots and leafs, leaf number, and high values of gravimetric parameters such as fresh and dry weight compared with the caryopsis cultivated in a solidified culture medium [7]. A superior increase of *in vitro* plants roots and stems initialized on filter paper bridge reported the agarized medium was observed in the experiment made on maize, where the difference reached after 21 days was 290.02% [8]. Also, at *Jasminum officinale* it was observed a more rapid growth of roots for vitroplants cultivated on liquid culture medium provided with filter paper bridge compared to agarized culture medium [9].

High values and beneficial aspects of plantlets growth and development on filter paper bridge were observed at the gravimetric parameters: fresh weight and dry weight. As expected, given the fact that through filter paper bridge the seeds hydration is facilitated the seeds germinated faster than on agarized support, this determining an accentuated and fast increasing of vegetative organs and in overall a growth in plant biomass. The same tendency was observed in the experiment conducted on *Helianthus annus* were the parameters such as: *fresh weight* and *dry weight* reported to vitroplantlets placed on agarized medium, had values of 25.5%, and 5.57% [4].

Growth in the medium term of *Viola wittrockiana* vitroplants, *after 30 days* from inoculation, presented some difference in development compared with to the fist 30 days period. The sample lot kept the same high growth at roots and hypocotyl, but were observed stagnation of other biometric and gravimetric parameters such as: length and number of leafs, fresh weight and dry weight. At plantlets grown on agarized medium the values of these parameters were higher compared with liquid medium.

An intense and accelerate growth of roots mainly favours the development of an intense vitroplants culture growth or can be used as a stage in some micropropagation experiments.

Faster *in vitro* seeds germination and vitroplants growth on Blidar type filter paper bridge (BFPB), leads directly to reducing the time until they reach the optimum dimension for subcultivation or for acclimatization, respectively is contributing to saving the electricity needed to ensure optimum conditions of temperature and lighting in vegetation rooms and through this decreasing participation at the environmental pollution.

REFERENCES

[1] Ajalin, I., Kobza, F., Dolezel, J., (2002): Ploidy identification of doubled chromosome number plants in *Viola* × *wittrockiana* Gams. M 1-generation. Horticultural Science, 29(1): 35-40.

[2] Barboza, S.B.S.C., Teixeira, J.B., Portes, T.A., Copati, L.A., Lédo, A.S., (2009): Cultivo inicial *in vitro* de gemas axilares de *Ananas comosus* (L.) Merr., em meio líquido/sólido, na presença/ausência de luz. Ciênc. agrotec., Lavras, Edição Especial, 33: 1832 -1836.

[3] Blidar, C.F., (2004): Evoluția protocormilor de *Cymbidium hybridum* cultivați „*in vitro*" pe medii lichide, pe punți din hârtie de filtru, în funcție de sezonul de inoculare. pp. 213-227. In: Cachiță, D.M., Ardelean A., Fati, V. (eds.): Lucrările celui de al XII-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale „Fitopatologia celulei vegetale în regim de vitrocultură", 5 iunie 2003 Jibou, Daya Press, Satu-Mare.

[4] Blidar, C.F., (2012): Eficientizarea biotehnologiilor *in vitro* la unele specii de plante de cultură utile în obţinerea de biocombustibili, factor de dezvoltare a bioeconomiei. Post-Doctoral Degree. POSDRU/89/1.5/S/63258: "Şcoala postdoctorală pentru biodiversitate zootehnică şi biotehnologii alimentare pe baza ecoeconomiei şi bioeconomiei necesare ecosanogenezei", 173 p.

[5] Blidar, C.F., Cachiţă, D.M., (2004): Reactivitatea protocormilor de *Cymbidium hybridum* în vitrocultură pe medii lichide carenţate în K şi I, susţinuţi pe suporturi din hârtie de filtru. Analele Universităţii din Oradea, Fascicula Biologie, 11: 157-162.

[6] Blidar, C.F., Cachiţă, D.M., Szabó, I., (2005): The testing of caffein „in vitro" reaction on *Cymbidium hybridum* protocorms subcultured on especially bridge of filter paper. Analele Ştiinţifice ale Universităţii „Alexandru Ioan Cuza" din Iaşi, Fasc. Genetică şi Biologie Moleculară, Tom. V, Ed. Univ. „Al. I. Cuza", ISSN: 1582-3571, Iaşi, pp. 145-150.

[7] Blidar, C.F., Ardelean, A., Turcuş, V., (2011). Efficient initiation of *in vitro* culture at wheat. Analele Universităţii din Oradea – Fascicula Biologie, 18(2): 176-181.

[8] Blidar, C.F., Ardelean, A., Turcuş, V., (2012). Using the filter paper bridge technique for the initiation of *in vitro* cultures of maize. Analele Universităţii din Oradea – Fascicula Biologie, 19(1): 17-22.

[9] Bhattacharya, S., Bhattacharyya, S., (2010): *In vitro* propagation of *Jasminum officinale* L.: A woody ornamental vine yielding aromatic oil from flowers. pp. 117-126. In Mohan Jain, S., Ochatt, S.J.: Protocols for *in vitro* propagation of ornamental plants. Humana Press, Springer New York Dordrecht Heidelberg London.

[10] Dhabhai, K., Sharma, M.M., Batra, A., (2010): *In vitro* clonal propagation of *Acacia nilotica* (L.) - a nitrogen fixing tree. Researcher, 2(3): 7-11.

[11] Hoque, A., Arima, S., (2002). Overcoming phenolic accumulation during callus induction an *in vitro* organogenesis in water chestnut (*Trapa japonica* Flerov). *In Vitro* Cellular & Developmental Biology, 38: 342-346.

[12] Khan, M.A., Ahmad, I., (2005). Multiple Shoot Induction and Plant Regeneration in Litchi (*Litchi chinensis* Sonn.). International Journal Of Agriculture & Biology, 7(3): 524-526.

[13] Li, C., Li, Q., Cheng, Z., Yu, A., Bao, M., Hu, H., (2011): Study on culture medium for *in vitro* pollen germination of *Viola wittrockiana* Gams. and *Viola tricolor* L. Northern Horticulture, Issue 3, 54-56

[14] Liu, H., Fu, Q., Chen, Y., (2011): Elementary Study on Anther Culture of Pansy (*Viola× wittrockiana*), Northern Horticulture, Issue 16, 161-163

[15] Mix-Wagner, G., (1999): The conservation of potato cultivars. Potato research, 42(3-4): 427-436.

[16] Mohmand, A.S., Nabors, M.W., (1991). Comparison of 2 methods for callus-culture and plant-regeneration in wheat (*Triticum aestivum*). Plant cell tissue and organ culture, 26(3): 185-187.

[17] Oksman-Caldentey, K.M., Barz, W., (2002): Plant biotechnology and transgenic plants. CRC Press, 720 p.

[18] Savio, L.E.B., Astarita, L.V., Santarèm, E.R., (2011): Secondary metabolism in micropropagated Hypericum perforatum L. grown in non-aerated liquid medium. Plant Cell, Tissue Organ Culture, 108: 465-472

[19] Şuţan, N.A., (2010a). Cercetări privind capacitatea de multiplicare *in vitro* şi stabilitatea genetică a unor hibrizi intergenerici *Fragaria* x *Potentilla*. U.B.B.Cluj, Fac. de Biologie şi Geologie, Conducător ştiinţific: prof. dr. Corneanu Gabriel, 260 p.

[20] Teixeira da Silva, J.A., (2013): Impact of paper bridges, activated charcoal, and antioxidants on growth and development of protocorm-like bodies of hybrid *Cymbidium*. *In Vitro* Cellular & Developmental Biology – Plant, 49: 414-420

[21] Vilanova Neta, J.L., da Silva Ledo, A., Lima, A.A.B., Santana, J.C.C, Leite, N.S., Ruzene, D.S., Silva, D.P., de Souza, R.R., (2012): Bromelain Enzyme from Pineapple: *In Vitro* Activity Study under Different Micropropagation Conditions. Applied Biochemistry and Biotechnology, 168: 234-246

[22] Wang, J., Bao, M.Z., (2007): Plant regeneration of pansy (*Viola wittrockiana*) 'Caidie' via petiole-derived callus. Scientia Horiculturae, 111: 266-270.

[23] Yoshioka, Y., Iwata, H., Hase, N., Matsuura, S., Ohsawa, R., Ninomiya, S., (2006): Genetic Combining Ability of Petal Shap in Garden Pansy (*Viola* × *wittrockiana* Gams) based on Image Analysis. Euphytica 151(3): 311-319

[24] Zhongxu, S., Jing, L., Yujun, W., Yanyi, Z., Zhilin, Q., (2001): Research on outer bottle rooting technique of fruit seedling *in vitro* with filterpaper bridge. Acta Horticulturae Sinica, 28(4): 345-347.

[25] Zhou, J.S., Gong, Q., Xing, F.W., (2008): *Viola nanlingensis* (Violaceae), a new species from Guangdong, southern China. Annales Botanici Fennici, 45: 233-236.

Received: 8 September 2014
Accepted: 14 November 2014
Published Online: 17 November 2014
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433