# THE INFLUENCE OF THE SEASON AND CULTURE MEDIUM ON MICROPROPAGATION OF TWO INTERGENERIC *Fragaria X Potentilla* VARIETIES

Anca Nicoleta ŞUŢAN[*], Aurel POPESCU[*],Valentina ISAC[**]

[*] *University of Piteşti, Faculty of Sciences, Department of Biology and Horticulture, Piteşti, Romania*
[**] *Research Institute for Fruit Growing, Piteşti, Romania*
*Corresponding author: Anca Nicoleta Sutan, University of Piteşti, Faculty of Sciences, Department of Biology and Horticulture, 1 Târgu din Vale, 110040 Piteşti, Romania, tel.: 0040348453100, fax: 0040348453123, e-mail: ancasutan@yahoo.com*

**Abstract.** In order to develop a protocol for high efficiency *in vitro* propagation of two intergeneric *Fragaria x Potentilla* varieties, 'Serenata' and 'Pink Panda' respectively, the influence of season on the rate of multiplication was investigated in shoot cultures on Murashige and Skoog (MS) and Lee and Fossard (LF) media, supplemented with different combinations of growth regulators. *In vitro* performance of explants indicated a positive correlation between shoot proliferation and season in both genotypes of ornamental strawberry. The mean number of shoots formed per explant was higher when 'Serenata' and 'Pink Panda' varieties were subcultured on MS or LF media, in the active growing season, irrespective of the culture medium composition. In both ornamental strawberry varieties, the mean number of shoots formed per explant was slightly higher when subcultured on MS medium, in the spring and summer season, as compared to LF medium, which was proven to be the most effective in the cold season.

**Keywords:** *in vitro* culture, season, culture media, intergeneric hybrids, *Fragaria x Potentilla*.

## INTRODUCTION

Sexual compatibility of *Potentilla palustris* with some *Fragaria* species [15, 19], allowed the occurrence of a large range of *Fragaria x Potentilla* intergeneric hybrids, combining the ornamental value given by the beauty of their flowers and prolonged blossoming season (May - October) with production of edible fruits. 'Pink Panda', 'Lipstick', 'Red Ruby' and 'Serenata', are the most common ornamental strawberry varieties, which together the new released varieties such as 'Rosalyne', 'Rosseberry' [8], 'Tarpan', 'Tristan', 'Roman', 'Pikan' and 'Merlan' [21], 'Rosana', 'Pink Panther', 'Viva Rosa', 'Wild Fire', 'Pretty in Pink', are gaining the interest of the breeders for either improvement of fruit qualities in ornamental strawberry varieties [10] or improvement of the main ornamental traits, such as early blossoming, compact habit, atractive flowers, and capacity of propagating by seeds [2].

As the *Fragaria* x *Potentilla* hybrids, such as 'Serenata' and 'Pink Panda' meet the trend in ornamental horticulture, large quantities of planting material are needed to be available at any time of the year. The conventional propagation of these varieties does not allow the obtention of high number of stolons of guaranteed authenticity and biological value in a very short time. Since ornamental strawberries are highly heterozigous and segregation in their progenies is unforeseeable, the *in vitro* micropropagation, of these elite plants, provides an advantage for their multiplication, without sexual recombination and in a very short time.

The comparison of responses on various culture media and choosing the most appropriate for obtention of a high efficiency of shoot multiplication in *Fragaria x Potentilla* hybrids, irrespectively of the season, is not a simple task primarily due to their different genetic origin.

In this work, the efficiency of micropropagation in different seasons and using different culture media for the initiation of shoot cultures and maintenance of subcultures have been studied, in order to elaborate an reliable protocol for the high rate *in vitro* propagation of the ornamental strawberry.

## MATERIALS AND METHODS

The research work was carried on within Biotechnology Laboratory, at the Research Institute for Fruit Growing, Piteşti – Maracineni (Romania). Two varieties of ornamental strawberry (*Fragaria x Potentilla*), named 'Pink Panda'and 'Serenata', respectively, were established in the *in vitro* culture starting from meristems with 2-3 leaf primordia, of 0.1-0.3 mm in size, excised from runners formed by field plants and then cultured on Lee and Fossard [11] medium supplemented with Murashige and Skoog [14] vitamins. Regenerated shoots were subcultured successively on Murashige - Skoog [11] or Lee - Fossard [8] solidified with agar at 7.0 g $l^{-1}$ concentration. As carbon source in all culture media was used dextrose, at 40 g/l concentration. The pH was adjusted to 5.8.

Three treatments with different combinations and concentration of 6-benzylaminopurine (BAP), indolylacetic acid (IAA), 3-indolylbutiric acid (IBA) and giberellic acid ($GA_3$), added to both MS and LF basic culture media, were used in order to find an adequate medium for obtaining a high rate of micropropagation while maintaining a good vigor of micropropagated shoots, as following: 1.0 mg/l BAP + 0.2 mg/l IBA + 0.1 mg/l $GA_3$ as M1/L1 variant or 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l $GA_3$ as M2/L2 variant or 2.0 mg/l BAP + 1.0 mg/l IAA as M3/L3 variant. The concentration of cytokinins in the experimental treatments covered the range currently used with commercial strawberry, thus allowing the establishment of that inducing the best morphogenetic response.

The cultures have been incubated in a growth chamber at the temperature of 22-24°C, with a

photoperiod of 16 hours light/8 hours darkness, and a light intensity of about 40 μmol $m^{-2}$ $s^{-1}$.

The observations were carried out at every 28 days, respectively at the moment of subculturing the micropropagated shoots. The micropropagation rate was calculated as the average number of shoots regenerated on each primary explant cultured *in vitro*, on each of the media tested.

To avoid statistical errors, at least 5 culture flasks with 5 shoots per flasks were used as repetitions in each of the experimental treatment investigated. Statistical analysis of the data obtained with 'Pink Panda' and 'Serenata' varieties on the MS [14] and LF [11] media supplemented with various combinations of growth regulators were performed using Statistical Package for the Social Science (SPSS) statistical software (ver. 16.0), using Duncan's Multiple Range Test, at $p < 0.05$.

## RESULTS

A shoot regeneration frequency of 100% was found for the apical meristems inoculated on the *in vitro* culture initiation medium in both May and October. However, as shown in Figures 1 – 4, the statistical analysis of the data obtained after the transfer of shoots on the multiplication media revealed significant and very significant differences between the shoot's proliferation capacity during May, June, July and August, as compared to October, November, December, and January, respectively, these being closely dependent on the used basal medium, and number of subcultures.

In 'Pink Panda' variety, the inoculation of shoots on MS [14] medium led to higher and significantly higher rates of multiplication after the first subculture, when carried out in May, compared to those observed after first subculture, when carried out in October. Thus, the highest rate of multiplication (10.9 shoots formed per initial explant), recorded in May, in the case of shoots whose multiplication was stimulated on the MS [14] basal medium supplemented with 1.0 mg/l BAP + 0.2 mg/l AIB + 0.1 mg/l $GA_3$ (M1 variant), was significantly higher than that recorded in October (6.05 shoots per initial explant).

If in 'Pink Panda' variety the statistical analysis of the obtained results did not revealed significant differences between the values for multiplication recorded in the two seasons, for the shoots inoculated on the LF [11] basal medium, the interaction season × culture medium was observed in the case of 'Serenata' variety, the process of shoot formation on LF [11] basal medium being more active in October, as compared to May, irrespective of the combination and concentration of growth regulators. Similar to the results obtained with 'Pink Panda' variety, the highest multiplication rate of shoots inoculated on the MS [14] basal medium (19.3 shoots formed per initial explant) was recorded in May with the M1 experimental variant. About the same values were calculated for the M2 and M3 variants in May, respectively 18.45 and 19.2 shoots formed per initial explant, significantly higher to those recorded for October.

Nevertheless, we found a favourable influence of the increased concentration of BAP in the MS [14] basal medium, in October, indicated by the higher values of multiplication rate in the M3 experimental variant, for the both genotypes investigated. On the other hand, during the period of active growing, it was revealed the favourable effect of supplementing the MS [14] basal medium with cytokinins and auxins in a relatively balanced ratio, as shown in Figure 1 for the M2 and M3 experimental variants.

Separation of shoots regenerated after the first subculture and their transfer on the corresponding variants of medium, in June, and respectively November, revealed a better multiplication ability of the explants during the months of active growing, especially for 'Serenata' variety. Thus, only in the case of 'Pink Panda' variety, the values of multiplication rate recorded in L2 and L3 medium variants were higher in November, as compared to June. Similarly to the results presented previously, LF [11] basal medium was proven to have a mineral composition adequate for stimulation of axilary shoot formation, out of the season of active growing. Moreover, as for the case of first subculture, calculation of the average number of shoots formed per initial explant showed higher values for the L3 (2.0 mg/l BAP + 1.0 mg/l IAA) variant in November, as compared to all the other experimental variants. If the favourable influence of the increased concentrations of BAP on the *in vitro* proliferation capacity in ornamental strawberry genotype during the months of autumn is not significant (as shown by the Duncan test), the favourable effect of a lower cytokinin/auxin ratio during the period of active growing, characteristic for strawberry genotypes, was found to be statistically proved (Fig. 2).

Comparative analysis of data obtained after the third subculture, on one hand for July, and on the other hand for December, revealed the lower and, respectively, significantly lower potential of proliferation (depending on the culture medium composition) of the explants from 'Pink Panda' and 'Serenata' varieties, during the cold season. As in the case of previous subcultures, significant differences between December and July were calculated for 'Serenata' variety, irrespective of the culture medium composition. The interaction between season × culture medium induced the same distribution of the average number of shoots formed per initial explant, for December the highest rates significantly higher being induced onto LF [11] basal medium, in comparison with MS [14] basal medium (Fig. 3).

Also, the season's influence on the *in vitro* multiplication capacity of *Fragaria x Potentilla* intergeneric hybrids, was observed even during the period of active growing in strawberry, the decrease of the number of shoots formed per initial explant in June, and then in July, being significant in this context.

Similarly, in our experiments, as shown by the Duncan test, the average number of shoots formed per initial explant was higher and significantly higher in August, as compared to January, dependent on the culture medium composition (Fig. 4).

The measurements of the length of shoots regenerated after the forth subculture have shown also a significant influence of the season, in close correlation with the genotype and culture medium. Thus, if during the period of active growing the rate of *in vitro* multiplication of the investigated genotypes was significantly higher in comparison with the corresponding values for the cold season, the length of shoots have had lower values in August, compared to January. This finding was confirmed by the overall results presented in Table 1, which shows that the interaction between the investigated factors led to an average length of the newly formed plantlets ranging between 0.7 and 2 cm in August, while the values for this characteristic were ranging between 1.6 and 3 cm in January, with the highest values recorded in 'Pink Panda' variety.

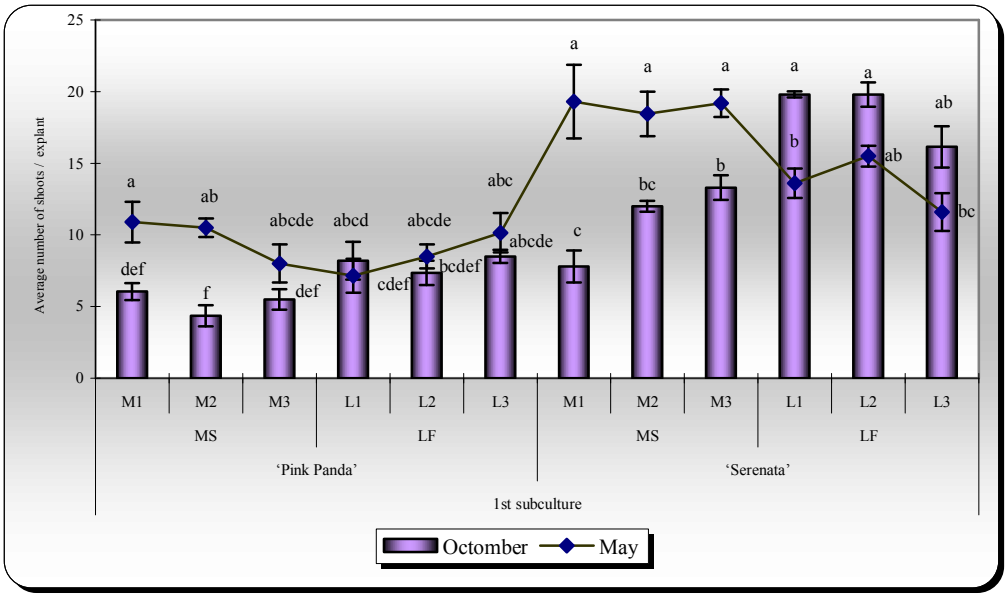


**Figure 1.** on the capacity of *in vitro* multiplication of shoots regenerated from meristems in the *Fragaria x Potentilla* intergeneric hybrids; comparison between May and October; M1/L1 - 1.0 mg/l BAP + 0.2 mg/l IBA + 0.1 mg/l $GA_3$; M2/L2 - 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l $GA_3$; M3/L3 - 2.0 mg/l BAP + 1.0 mg/l IAA (bars represents the standard deviation; a, b, c, d, e, f: assessment of the significance of differences, by Duncan's Multiple Range Test, $p<0.05$).

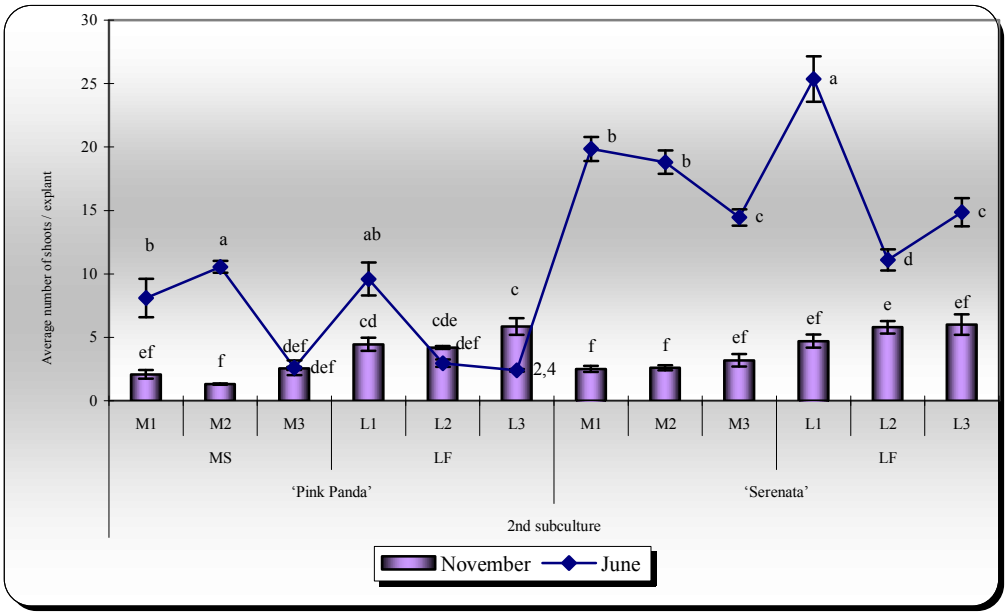


**Figure 2.** The influence of interaction between season × culture medium on the *in vitro* multiplication capacity through axilary shoot formation in the *Fragaria x Potentilla* intergeneric hybrids; comparison between June and November; M1/L1 - 1.0 mg/l BAP + 0.2 mg/l IBA + 0.1 mg/l $GA_3$; M2/L2 - 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l $GA_3$; M3/L3 - 2.0 mg/l BAP + 1.0 mg/l IAA (bars represents the standard deviation; a, b, c, d, e, f: assessment of the significance of differences, by Duncan' Multiple Range Test, $p<0.05$).

The basal medium have had also a strong influence on the above mentioned trait, MS [14] medium allowing the development of the most vigorous plantlets in both 'Pink Panda' and 'Serenata' ornamental strawberry varieties, irrespective of the season. The comparison of the length of shoots formed on culture media supplemented with different combinations of growth regulators have shown the favourable effect of M2 medium (MS [14] basal medium + 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l

$GA_3$), on the length of shoots, which led to significantly higher values for this trait, as compared to any other variant of culture medium. For both varieties, the absence of giberellic acid from the culture medium (M3/L3 variant) was associated with the lower values of shoot length.

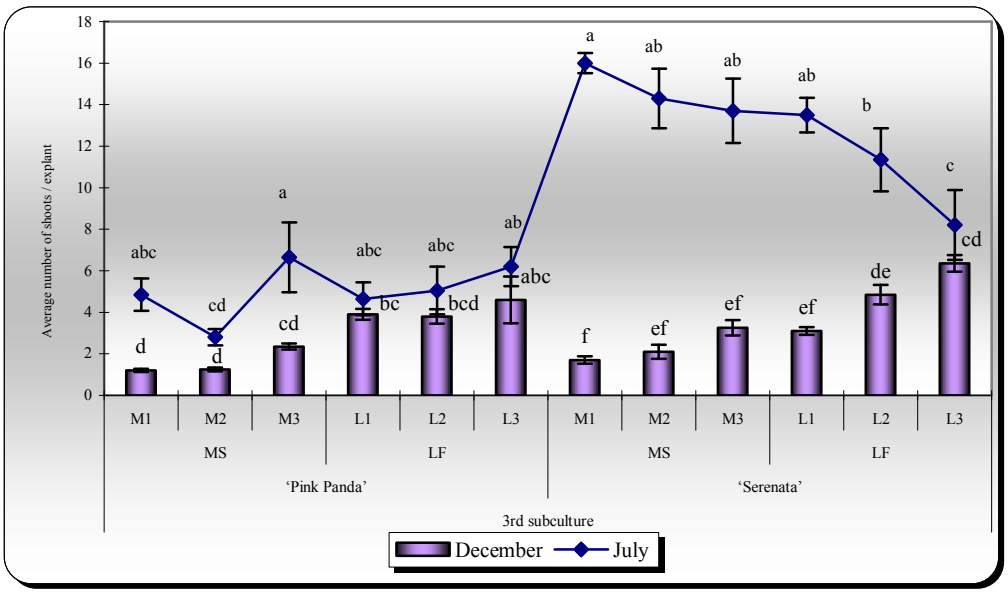


**Figure 3.** The influence of season on the *in vitro* multiplication capacity through axilary shoot formation in the *Fragaria x Potentilla* intergeneric hybrids; comparison between July and December; M1/L1 - 1.0 mg/l BAP + 0.2 mg/l IBA + 0.1 mg/l $GA_3$; M2/L2 - 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l $GA_3$; M3/L3 - 2.0 mg/l BAP + 1.0 mg/l IAA (bars represents the standard deviation; a, b, c, d, e, f: assessment of the significance of differences, by Duncan's Multiple Range Test, $p<0.05$).

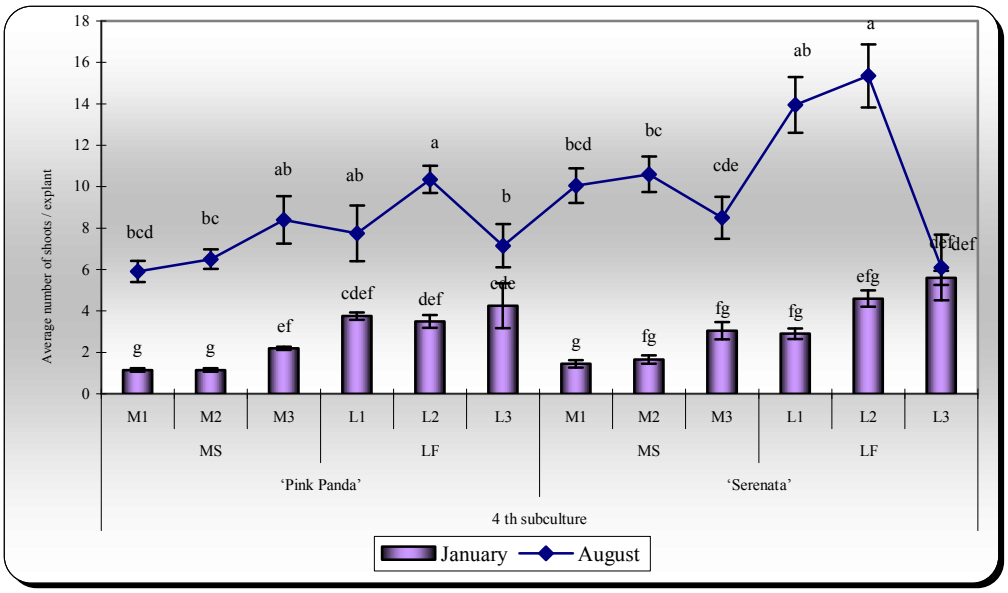


**Figure 4.** The influence of season on the *in vitro* multiplication capacity through axilary shoot formation in the *Fragaria x Potentilla* intergeneric hybrids; comparison between August and January; M1/L1 - 1.0 mg/l BAP + 0.2 mg/l IBA + 0.1 mg/l $GA_3$; M2/L2 - 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l $GA_3$; M3/L3 - 2.0 mg/l BAP + 1.0 mg/l IAA (bars represents the standard deviation; a, b, c, d, e, f: assessment of the significance of differences, by Duncan's Multiple Range Test, $p<0.05$).

**Table 1**. The influence of season and culture medium on the length of shoots obtained by *in vitro* multiplication in 'Pink Panda' and 'Serenata 'varieties **(**M1/L1 - 1.0 mg/l BAP + 0.2 mg/l IBA + 0.1 mg/l $GA_3$; M2/L2 - 1.0 mg/l BAP + 1.0 mg/l IAA + 0.1 mg/l $GA_3$; M3/L3 - 2.0 mg/l BAP + 1.0 mg/l IAA).

| Culture medium | | The length of regenerated shoots (mm) | | | |
|---|---|---|---|---|---|
| | | 'Pink Panda' | | 'Serenata' | |
| | | August | January | August | January |
| MS [14] | M1 | 1.55 ± 0.12 ef | 2.6 ± 0.12 bc | 1.85 ± 0.1 def | 2.24 ± 0.17 bcd |
| | M2 | 1.65 ± 0.35 def | 3.0 ± 0.35 a | 2.0 ± 0.17 cdef | 2.64 ± 0.22 ab |
| | M3 | 0.7 ± 0.09 g | 2.0 ± 0.35 cdef | 0.71 ± 0.06 g | 1.86 ± 0.25 def |
| LF [11] | L1 | 1.46 ± 0.07 f | 2.75 ± 0.37 ab | 0.9 ± 0.12 g | 1.64 ± 0.09 def |
| | L2 | 1.62 ± 0.05 def | 3.1 ± 0.18 a | 1.65 ± 0.1 def | 1.8 ± 0.17 def |
| | L3 | 0.75 ± 0.18 g | 2.2 ± 0.28 bcde | 0.8 ± 0.12 g | 1.6 ± 0.07 def |

Note: *Each value represents the average ± standard deviation (SD). Within each column, differences between any two variants followed by at least a common letter are not significant at $p<0.05$, according to Duncan's Multiple Range Test.

## DISCUSSION

The stage of physiological development of explants is considered a critical factor for the expression of morphogenetic response, in close correlation with the content of endogenous hormones - stimulators or inhibitors of the regeneration process. This dependence explains actually the variation in time of the intensity of regeneration process, correlated most likely with the seasonal variation of the concentration of essential growth regulators (auxins and cytokins), as was conclusively revealed by the results of the study carried out by us.

In this respect, have been demonstrated that for numerous plant species (including *Fragaria x ananassa*) there is an optimum stage of development for the expression of the whole regeneration potential of the various types of explants when cultured *in vitro*. Predieri *et al*. [18] observed the obvious effect of the season on the regeneration potential of the calli derived from *in vitro* cultured ovaries of some day-neutral strawberry varieties. The influence of season on the shoot regeneration ability of explants was reported in many other species, such as *Mamillaria elongata* [16], *Gloxinia* sp. [5], *Dendrobium* sp. [4], *Cymbidium hybridum* [3], *Ceratonia siliqua* [20] or *Quercus euboica* [9].

The use of explants in stages "ante" or "post" to that proved to be optimum, led often to a low rate of regeneration and proliferation.

Based on the data presented could be concluded that the *in vitro* multiplication capacity of meristem-derived shoots in the intergeneric hybrids *Fragaria x Potentilla* is significantly influenced by the season, but can be enhanced by the use of a culture medium with an adequate composition. Thus, if during the active growing period the highest rates of multiplication were induced when cultured on MS [14] basal medium, during the months of autumn and winter is recommended the inoculation of shoots on LF [11] basal medium for inducing similar rates of shoot multiplication. Also, depending on the season, the genotypes of ornamental strawberry showed differentiated requirements for the cytokinin/auxin ratio, the increase of average number of shoots formed per initial explant being favorized by a higher value of the cytokinin/auxin ratio. The influence of gibberelins should be also considered as important, as their influence on the length of shoots is generally recognized in the *in vitro* culture.

Similar results have been reported by other researchers, who suggested the following combinations of growth regulators for the micropropagation of ornamental strawberry: 1.0 mg/l BAP + 1.0 mg/l IAA + 0.05 mg/l $GA_3$; 0.5 mg/l BAP + 0.1 mg/l IBA + 0.1 mg/l $GA_3$ [6, 13]; 0.5 mg/l BAP + 0.1 mg/l IBA [7], and 0.2 mg/l BAP +0.01 mg/l IBA + 0.1 mg/l $GA_3$ [12]. Also, other authors reported that the highest rate of shoot proliferation is induced, generally, by the MS basal medium, supplemented with BAP and IBA [1, 17].

The overall observations carried out on the two investigated genotypes of ornamental strawberry *Fragaria x Potentilla*, have shown conclusively the influence of interaction between season and culture medium on the proliferation potential of explants in successive subcultures.

## REFERENCES


[1] Barcélo, M., El Mansouri, I., Mercado, J.A., Quesada, M.A., Alfaro, F.P. (1998): Regeneration and transformation via *Agrobacterium tumefaciens* of the strawberry cultivar Chandler. Plant Cell, Tissue and Organ Culture, 54: 29-36.

[2] Bentvelsen, G., Bouw, B., 2006. Breeding ornamental strawberries. Acta Horticulturae, 708: 455-458.

[3] Blidar, C.F., (2004): Evoluţia protocormilor de *Cymbidium hybridum* cultivaţi „in vitro" pe medii lichide, pe punţi din hârtie de filtru, în funcţie de sezonul de inoculare. pp. 213-227. In Cachiţă-Cosma, D., Ardelean, A., Fati, V.: Lucrările celui de al XII-lea Simpozion Naţional de Culturi de Ţesuturi şi Celule Vegetale „Fitopatologia celulei vegetale în regim de vitrocultură", 5 iunie 2003 Jibou, Daya Press, Satu-Mare.

[4] Blidar, C.F., Petruş-Vancea, A., Darvas, H., (2003): Influenţa sezonului asupra evoluţiei vitroculturilor de protocormi de *Dendrobium sp.*, subcultivaţi, pe punte de hârtie de filtru. Analele Universităţii din Oradea - Fascicula Biologie, 10: 351-358.

[5] Blidar, C.F., Tripon, I.M., 2002. Reactivitatea explantelor foliare de *Gloxinia* sp. în vitrocultură, în funcţie de sezon. Analele Universităţii din Oradea - Fascicula Biologie, 9: 253-262.

[6] Boxus, P., (1999): Micropropagation of strawberry via axillary shoot proliferation. pp: 103-114. In: Hall, R. D. (Ed.): Plant Cell Culture Protocols. Methods in Molecular Biology. Part III. Plant Propagation *In Vitro*, vol. 111, Humana Press Inc., Totowa NJ.

[7] Bozena, B., (2001): Morphological and physiological characteristics of micropropagated strawberry plants rooted *in vitro* or *ex vitro*. Scientia Horticulturae, 89: 195-206.

[8] Khanizadeh, S., Cousineau, J., Deschênes, M., Levasseur, A., Carisse, O., (2002): Roseberry and Rosalyne: two new hardy, day-neutral, red flowering strawberry cultivars. Acta Horticulturae, 567(1): 173-174.

[9] Kartsonas, E., Papafotiou, M., (2007): Mother plant age and seasonal influence on *in vitro* propagation of *Quercus euboica* Pap., an endemic, rare and endangered oak species of Greece. Plant Cell Tissue and Organ Culture, 90: 111-116.

[10] Kuznetsova, L., Grout, B., Baturin, S.O., Zilke, R., (2009): Improving double-usage (ornamental and fruiting) strawberry (*Fragaria x ananassa* Duch.) in Western Siberia. International Conference on FoodOmics, Abstract Book, University of Bologna, Cesena, Italy.

[11] Lee, E.C.M., de Fossard, R.A., (1977): Some factors affecting multiple bud formation of strawberry *(Fragaria x ananassa* Duchesne*) in vitro*. Acta Horticulturae, 78: 187-196.

[12] Li, H., Zhang, Z., Huang, F., Chang, L., Ma, Y. (2009): MicroRNA expression profiles in conventional and micropropagated strawberry (*Fragaria x ananassa* Duch.) plants. Plant Cell Reports, 28: 891-902.

[13] Litwińczuk, W., (2004): Field performance of "Senga Sengana" strawberry plants (*Fragaria x ananassa* Duch.) obtained by runners and *in vitro* through axillary and adventitious shoots. Electronic Journal of Polish Agricultural Universities, Horticulture 7(1): 3.

[14] Murashige, T., Skoog, F., (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologya Plantarum, 15(3): 473-497.
[15] Niemirowicz-Szczytt, K., (1984): The result of intergeneric pollination of *Fragaria x ananassa* Duch. and *Fragaria virginiana* Duch. by *Potentilla* species. Acta Societatis Botanicorum Polonie, 53: 443-454.
[16] Papafotiou, M., Balotis, G., Louka, P., Chronopoulos. J., (2001): *In vitro* plant regeneration of *Mammillaria elongata* normal and cristate forms. Plant Cell Tissue and Organ Culture, 65: 163–167.
[17] Passey, A.J., Barrett, K.J., James, D.J. (2003): Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria x ananassa* Duch.) using a range of explant types. Plant Cell Reports, 21: 397-401.
[18] Predieri, S., Fasolo Fabbri Malavasi, F., Ancherani, M. (1989): Regeneration of plants from strawberry (*Fragaria x ananassa* Duch.) unpollinated ovaries and petals. Acta Horticulturae, 265: 335-338.
[19] Sayegh, A.J., Hennerty, M.J. (1993): Intergeneric hybrids of *Fragaria* and *Potentilla*. Acta Horticulturae, 348: 151-154.
[20] Romano. A., Barros, S., Martins-Loucao, M. (2002): Micropropagation of the Mediterranean tree *Ceratonia siliqua*. Plant Cell Tissue and Organ Culture, 68: 35–41.
[21] http://www.abz-strawberry.nl/product.php?product_id=35&language=en (Accessed in 12 April 2010).

Submitted: 17 April 2010
Accepted: 29 April 2010

Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433