STUDIES ON MICROPROPAGATION EFFICIENCY IN ORNAMENTAL STRAWBERRY VARIETIES (*FRAGARIA X POTENTILLA*)

STUDII PRIVIND EFICIENȚA MICROPROPAGĂRII VARIETĂȚILOR DE CAPŞUN ORNAMENTAL (*FRAGARIA X POTENTILLA*)

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Abstract. *Although the culture media currently used for the in vitro micropropagation of the octoploid strawberry should offer also the posibility of mass propagation of ornamental strawberry, the particularities of the varieties of Fragaria x Potentilla (deriving mainly from their different genetic origin) could result in significant differences in their response to in vitro culture. In order to develop a protocol for high efficiency in vitro propagation of ornamental strawberry, two varieties with different origin were investigated (Pink Panda and Serenata). In both ornamental strawberry varieties, the mean number of shoots formed per explant (original shoot, derived from meristem culture) was slightly higher when subcultured on Murashige and Skoog medium, as compared to Lee and Fossard medium (currently used for micropropagation of commercial strawberry), irrespective of the combination of growth regulators. After the first subculture, the highest rate of shoot proliferation was achieved on either medium supplemented with 1.0 mg/l BA, 0.2 mg/l IBA and 0.1 mg/l GA, or 1.0 mg/l BA, 1.0 mg/l IAA and 0.1 mg/l GA. This increased in subsequent subculture in Serenata variety to an average of over 20 shoots per explant, while in Pink Panda variety did not exceed 11 shoots per explant.*

Rezumat. *Deşi mediile de cultură folosite în mod curent pentru micropropagarea in vitro a căpşunului octoploid ar trebui să ofere şi posibilitatea înmulțirii pe scară largă a căpşunului ornamental, particularitățile varietăților de Fragaria x Potentilla (derivând în primul rând din originea lor genetică diferită) pot determina diferențe semnificative în răspunsul lor la cultura in vitro. In scopul elaborării unui protocol pentru înmulțirea in vitro cu eficiență ridicată a căpşunului ornamental, au fost investigate două varietăți cu origine diferită (Pink Panda şi Serenata). La ambele, numărul mediu de lăstari formați per explant (lăstar inițial, format din meristem) a fost uşor mai ridicat când au fost subcultivate pe mediul Murashige-Skoog, comparativ cu mediul Lee-Fossard (folosit în mod curent pentru micropropagarea căpşunului comercial), indiferent de combinația de regulatori de creştere. După prima subcultură, cea mai ridicată rată de proliferare a lăstarilor s-a înregistrat fie pe mediul suplimentat cu 1.0 mg/l BA, 0.2 mg/l IBA şi 0.1 mg/l GA, fie pe cel cu 1.0 mg/l BA, 1.0 mg/l IAA şi 0.1 mg/l GA. In subcultura următoare, aceasta a crescut la soiul Serenata la o medie de peste 20 lăstari per explant, în timp ce la soiul Pink Panda nu a depăşit 11 lăstari per explant.*

Sexual compatibility of *Potentilla palustris* with some *Fragaria* species (Niemirowicz-Szcytt, 1984; Sayegh and Hennerty, 1993), allowed the occurence 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. As the *Fragaria* x *Potentilla* hybrids, such as "Serenata", "Pink Panda", "Lipstick", "Red Ruby"and "Vivarosa", 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. Therefore, taking into consideration that they resemble octoploid cultivated strawberries, the *in vitro* micropropagation is the first choice.

The comparison of responses on different culture media and chosing the most appropriate for obtention of a high efficiency of shoot multiplication in *Fragaria x Potentilla* hybrids is not a simple task , primarily due to their different genetic origin. Knowing the fact that the efficiency of micropropagation depends to a great extent of the culture media used for the initiation of shoot cultures and maintenance of subcultures, we initiated a study aiming at the elaboration of an reliable protocol for the high rate *in vitro* propagation of the ornamental strawberry.

MATERIAL AND METHODS

Two varieties of ornamental strawberry (*Fragaria x Potentilla*), named "Pink Panda" and "Serenata", respectively, were established in vitro culture starting from meristems and then subcultured succesively on Murashige and Skoog (MS) and respectively Lee and Fossard (LF) media supplemented with various combinations of growth regulators (Table 1).

For the initiation of shoot cultures, meristems with 2-3 leaf primordia, of 0.1-0.3 mm in size, excised from runners formed by field plants of varieties "Pink Panda" (with pink flowers), and "Serenata" (with red flowers), were used.

Six treatments with different combinations and concentration of benzylaminopurine (BAP), kinetin (Kin), 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 (Table 1). 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. To avoid major statistical errors, at least 6 culture flasks with 5 shoots per flask were used as repetitions in each of the experimental treatment investigated.

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 3000 lux.

The observations were carried out at every 4 weeks, 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. Statistical analysis of the data obtained with "Pink Panda" and "Serenata" varieties respectively on the MS and LF media

supplemented with various combinations of growth regulators were performed using Windows SPSS 16.0 program (SPSS, 2007) at $p < 0.05$.

Table 1.

The combinations and concentration of growth regulators added to MS and LF media respectively, tested in order to establish an efficient protocol for the micropropagation of *Fragaria x Potentilla* varieties

Culture medium code	Basic medium	Growth regulators used and their concentration in the culture medium (mg/l)				
		BAP	IBA	IAA	GA_3	Kin
V1	MS, or LF	0.5	0.1	-	0.1	-
V2	MS, or LF	1.0	0.2	-	0.1	-
V3	MS, or LF	0.5	-	0.5	0.1	-
V4	MS, or LF	1.0	-	1.0	0.1	-
V5	MS, or LF	2.0	-	1.0	-	-
V6	MS, or LF	1.0	-	-	2.0	0.5

RESULTS AND DISCUSSIONS

After the first subculture, the highest multiplication rate calculated for "Pink Panda" variety (10.8 shoots/primary explant) was obtained with explants cultured on MS medium supplemented with 1 mg/l BAP, 0.2 mg/l IBA and 0.1 mg/l GA_3 (V2). A very closed rate of multiplication (10.15) was induced also by the LF medium supplemented with 2 mg/l BAP and 1 mg/l IAA (V5). Moreover, as shown by the Duncan's multiple range test, similar response was obtained in treatments with many different combinations of growth regulators added to either MS or LF media (Fig. 1).

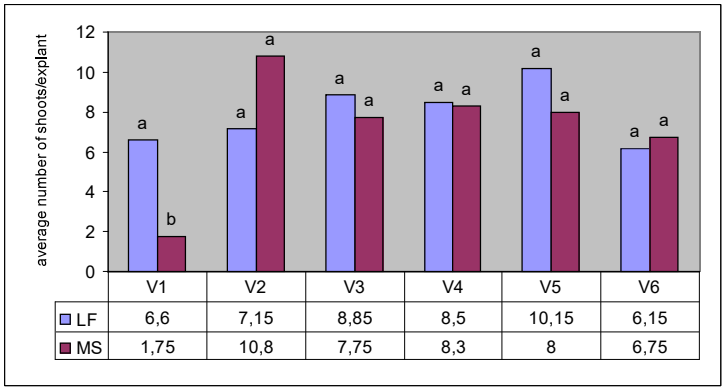
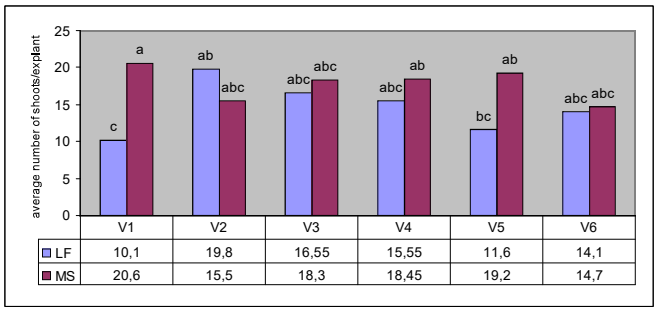


Fig. 1. The micropropagation rate of "Pink Panda" variety during the first subculture on either LF or MS media.

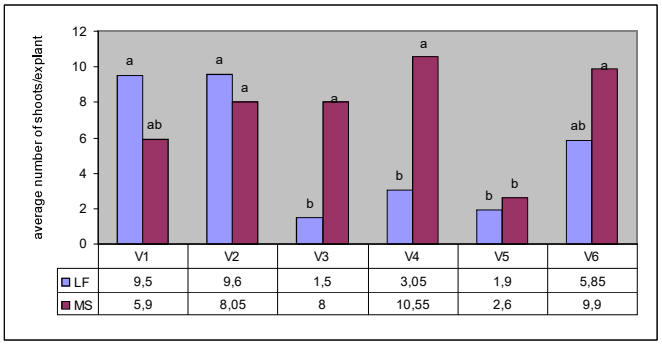
As compared to the "Pink Panda", the "Serenata" variety of *Fragaria x Potentilla* responded by a better rate of micropropagation during the first subculture on al the six variants of culture media, irrespective of the basic medium. Thus, an average number of 20.6 shoots formed per primary explant was calculated for the treatment with 0.5 mg/l BAP, 0.1 mg/l IBA and 0.1 mg/l GA_3 added to the MS (Fig. 2).



	V1	V2	V3	V4	V5	V6
LF	10,1	19,8	16,55	15,55	11,6	14,1
MS	20,6	15,5	18,3	18,45	19,2	14,7

Fig. 2. The micropropagation rate of the "Serenata" variety during the first subculture on either LF or MS media.

A significantly lower rate of micropropagation was obtained with this *Fragaria x Potentilla* variety when the same combination of growth regulators was added to the LF medium. Excepting the treatment with 0.5 mg/l BAP, 0.1 mg/l IBA and 0.1 mg/l GA_3, no other combinations of growth regulators resulted in significantly different rate of shoot micropropagation on the two basic culture media tested. The overall results obtained with "Serenata" variety have shown that even the lowest micropropagation rate calculated for the first subculture on LF medium (10.1 shoots/primary explant), was closed or even exceeded the best micropropagation rates induced in "Pink Panda"variety (Fig. 2).



	V1	V2	V3	V4	V5	V6
LF	9,5	9,6	1,5	3,05	1,9	5,85
MS	5,9	8,05	8	10,55	2,6	9,9

Fig. 3. The micropropagation rate of "Pink Panda" variety during the second subculture on either LF or MS media.

As shown by the Duncan's multiple range test, in "Pink Panda" variety, the number of shoots formed per primary explant on LF medium decreased in the second subculture for treatments which promoted the highest rates of shoot micropropagation at the end of the first subculture, and increased for those which previously resulted in the lowest rates of micropropagation (Fig. 3). The average number of shoots formed per primary explant when subcultured on this basic medium was highest in treatments with either 0.5 mg/l BAP, 0.1 mg/l IBA and 0.1 mg/l GA_3 (V1) or 1.0 mg/l BAP, 0.2 mg/l IBA, and 0.1 mg/l GA_3 (V2).

The same variety responded by slightly higher rates of micropropagation on MS medium, the highest number of shoots regenerated per primary explant (10.55) being found for explants subcultured on V4 variant of medium (Fig. 3). The statistical analysis revealed that similar rates of shoot micropropagation can be obtained with many other treatments.

In “Serenata” variety, the micropropagation rate maintained higher over the subsequent subculture as compared to “Pink Panda”, on any of the culture media tested, reflecting its superior genetic potential of *in vitro* multiplication. It is relevant the fact that at the end of the second subculture, on the LF medium, a rate of micropropagation as high as 24.3 was calculated for V2, respectively in treatment with 1.0 mg/l BAP, 0.2 mg/l IBA, and 0.1 mg/l GA_3 (Fig. 4).

A very closed value of the average number of shoots formed per primary explant (23.65) was calculated also for the treatment with 0.5 mg/l BAP, 0.5 mg/l IAA, and 0.1 mg/l GA_3. On the same variant of medium, a significantly lower value of the micropropagation rate was calculated for this intergeneric variety when the shoots were subcultured on LF medium, respectively 9.45 shoots/primary explant. However, the obtained results have shown that a very good rate of micropropagation can be induced in this variety also by the combination of 1.0 mg/l BAP, 0.5 mg/l Kin, and 2.0 mg/l GA_3 (which during the second subculture gave an average number of shoots formed per primary explant of 18.55).

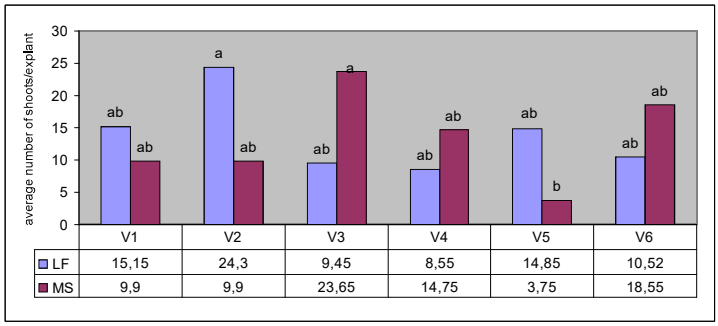


Fig. 4. The micropropagation rate of the “Serenata” variety during the second subculture on either LF or MS media.

An interesting response was observed during the third subculture, as the micropropagation rate decreased again in “Pink Panda” variety on all the variants of media consisting of combinations of growth regulators added to the LF basic medium which in previous subcultures promoted good rates of shoot multiplication, and increased for the explants subcultivated on variants which previously resulted in lower rates of shoot multiplication. Similarly, on the MS medium, the micropropagation rate decreases in all treatments excepting that which during the previous subculture resulted in the lowest average number of shoots formed per primary explant (V5). The overall results indicates that the highest values of the micropropagation rate were induced when 2.0 mg/l BAP and 1.0 mg/l IAA have been added to the basic culture media.

The statistical analysis have revealed that, similarly with the “Pink Panda”, the “Serenata” variety responded by a decreased ability of shoot micropropagation during the third subculture (Fig. 5). Thus, irrespective of the basic culture medium, the best rates of micropropagation does not exceeds 16 shoots formed per primary explant. However, good rates of shoot micropropagation were promoted in treatments with 1.0 mg/l BAP, combined with either 0.2 mg/l IBA or 1.0 mg/l IAA.

Fig. 5. *In vitro* micropropagated shoots in *Fragaria x Potentilla* varieties "Pink Panda" (left) and "Serenata" (right)

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CONCLUSIONS

(1) In both "Pink Panda" and "Serenata" varieties of *Fragaria x Potentilla*, the average number of shoots formed per primary explant was higher when the explants were subcultivated on the MS medium, rather than on LF medium (currently used for the micropropagation of the octoploid cultivated strawberry), indicating a more adequate composition of nutrients to the *in vitro* growth requirements of these intergeneric hybrids.

(2) Irrespective of the basic culture media, the micropropagation rate of both "Pink Panda" and "Serenata" varieties was demonstrated to be generally higher when combinations of 1.0 mg/l BAP with either 0.2 mg/l IBA or 1.0 mg/l IAA are used.

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