

THE REGENERATIVE CAPACITY OF THE CALLUS, IN TWO GENOTYPES OF ORNAMENTAL STRAWBERRY

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Abstract. Callus formation and shoot regeneration efficiency from “Pink Panda” and “Serenata” genotypes of ornamental strawberry, using liquid culture medium provided with filter paper bridges were investigated. The effects of different combinations and concentration of 3-indolil-acetic acid (IBA) and 6-benzylaminopurine (BAP) added in Murashige - Skoog (MS) basal medium were evaluated with leaf and petiole explants. It was found that the highest frequency of explants forming callus (100%) have been induced in both genotypes investigated on the culture medium containing 1.0 mg/l IBA and 3.0 mg/l BAP. The same hormonal balance promoted shoot regeneration with a frequency of 68% from leaf explants and 39.33% from petiole explants shoots in “Serenata” genotype. Similarly, in “Pink Panda” genotype shoot regeneration via callus was induced with a frequency of 46.66% from leaf explants and 36% from petiole explants.

Keywords: ornamental strawberry, callus, shoot, liquid culture.

Rezumat. Capacitatea regenerativă a calusului la două genotipuri de căpșun ornamental. Pentru determinarea potențialului de regenerare de lăstari via calus, utilizând medii de cultură lichide prevăzute cu punți din hârtie de filtru, fragmentele de frunză și segmentele de pețiol au fost vitrocultivate pe mediul de bază Murashige – Skoog (MS) suplimentat cu diferite combinații și concentrații ale acidului 3-indolil-butiric (AIB) și 6-benzilaminopurinei (BAP). Pentru ambele genotipuri cea mai înaltă rată de calusogeneză a fost obținută prin cultivarea explantelor somatice pe mediu conținând 1.0 mg/l AIB și 3.0 mg/l BAP. La genotipul “Serenata”, aceeași balanță hormonală a indus regenerarea de lăstari din calusul derivat din explante de frunză și de pețiol cu o frecvență de 68%, respective 39.33%. De asemenea, la genotipul “Pink Panda”, regenerarea de lăstari via calus a fost indusă cu o frecvență de 46.66% în cazul fragmentelor de frunză și de 36% în cazul segmentelor de pețiol.

Cuvinte cheie: căpșun ornamental, calus, organogeneză indirectă, mediu lichid.

INTRODUCTION

In the early twentieth century, especially as a result of amateur growers and not because of systematic improvement work, there were dozens of varieties of strawberry; currently the varieties selected or created throughout the history of two centuries and a half of cultivated strawberry is impressive and is harmoniously completed by ornamental strawberry varieties. Intergeneric hybrids *Fragaria* × *Potentilla* known as ornamental strawberry varieties, such as “Pink Panda” (with pink flowers) and “Serenata” genotypes (with red flowers) were noted by the beauty of flowers and prolonged blossoming season (May - October) combined with production of edible fruits.

Although there have been achieved numerous reports on adventitious bud and shoot regeneration from somatic explants (RUGINI & ORLANDO, 1992; LIS, 1993; SORVARI et al., 1993; PASSEY et al., 2003; DEBNATH, 2005, 2006) cultured on agar-gelled media, liquid culture has been recently considered as an alternative approach to strawberry micropropagation. Some modern techniques including shaken of liquid culture and bioreactor (TAKAYAMA & AKITA, 1998; ZIV et al., 2003; HANHINEVA et al., 2005; EDAHIRO & SEKI, 2006; DEBNATH, 2009), or liquid culture system combined with some kinds of substrate made of simple material (DUONG et al., 2004) have been successfully applied for strawberry micropropagation. In shake culture and bioreactor, there appears the problem of explant asphyxiation, which can be avoided by taking the advantage of the surface tension and floating explants (DILLEN & BUYSENS, 1989; MEHROTRA et al., 2007) or supporting of explants on different kinds of absorbent substrate.

In this study, the reactivity of leaf and petiole explants of “Pink Panda” and “Serenata” genotypes supported on the surface of the liquid culture through filter paper bridges was investigated, as method, which can reduce strawberry micropropagation cost.

MATERIAL AND METHODS

Plant material. “Pink Panda” and “Serenata” genotypes from the *Fragaria* Germplasm Collection of the Institute for Fruit Growing, Pitesti, Mărăcineni, Romania were cultured *in vitro* starting from meristems on LEE & FOSSARD (1977) basal medium, supplemented with 1.0 mg/l kinetin (Kin) and 2.70 mg/l indolil-acetic acid (AIA). Leaf and petiole explants collected from six weeks old *in vitro* plantlets, precultured for two weeks on LF basal medium containing 0.5 mg/l BAP, 0.5 mg/l IBA and 0.2 mg/l GA₃ (SORVARI et al., 1993), were used as explants.

Callus induction experiments. Callus induction media consisted of MURASHIGE & SKOOG (1962) basal medium modified (CaCl₂ being introduced at a concentration of 330.0 mg/l, instead of 440.0 mg/l as the original recipe) and supplemented with three different combinations of plant growth regulators, as following: 0.5 mg/l IBA + 3.0 mg/l BAP as M1 variant, 1.0 mg/l IBA + 3.0 mg/l BAP as M2 variant and 3.0 mg/l IBA + 5.0 mg/l BAP as M3 variant. Dextrose, at a concentration of 40 g/l was used as carbon source in all culture media.

Both the leaf explants (0.3-0.5 mm diameter) and petiole segments (0.3-0.5 mm) were supported on the surface of the liquid medium on filter paper bridges. Modeling of the filter paper bridges and applying this method have been completed according to the methodology proposed by BLIDAR (2004). The liquid culture medium was added to 25 cc Pyrex tubes after the introduction of filter paper bridges, such containers being sterilized by autoclaving. Into each tube on filter paper bridge was placed one somatic explant. The leaf explants were placed with their abaxial surface in contact with the filter paper bridges and petiole segments were placed horizontally on the filter paper bridges. Thus, nutrition of the somatic explants was done through a wick system. After 42 days of incubation in the dark, in the growth chamber at the temperature of 22-24°C, the cultures were maintained under a photoperiod of 16 hours light/8 hours darkness and at a relatively low intensity of light (starting from a light intensity of about $40 \mu\text{mol m}^{-2} \text{s}^{-1}$), obtained by covering culture vessels with white paper plates. The number of explants forming callus was scored after 42 days of culture for both varieties.

Shoot organogenesis. Callus cultures initiated from the leaf and petiole-derived calluses were maintained on the same medium without subculturing them, until the induction of the shoots. The number of shoots formed per callus was determined after 70 days in culture under a 16 hour light photoperiod and at a relatively low intensity of light.

Experimental design and statistical analysis. The rate of callogenesis (%) was determined as the ratio of the number of explants that developed callus to the total number of explants. Similarly, the frequency of shoots regeneration was determined as the ratio of the number of calluses that regenerated shoots. To avoid major statistical errors, all of the experimental treatment was performed with twenty-five replicates of one somatic explant. Statistical analysis of the data obtained with “Pink Panda” and “Serenata” varieties respectively, on basal media containing different concentrations of auxins for callus induction, were performed using Duncan’s Multiple Range test, at $p < 0.05$, working with Statistical Package for the Social Science (SPSS) statistical software (version. 16.0).

RESULTS AND DISCUSSION

The callogenic response was induced after 12 days of *in vitro* culture, in response to IBA and BAP added in MS basic medium. These calluses were formed all over the surface of the somatic explants and were predominantly initiated at the wound site of the petiole segments and leaf fragments (Fig. 1). It is known that plant hormones are present in higher quantities after wounding and are involved in cell proliferation at the wound site (KHAL, 1983).

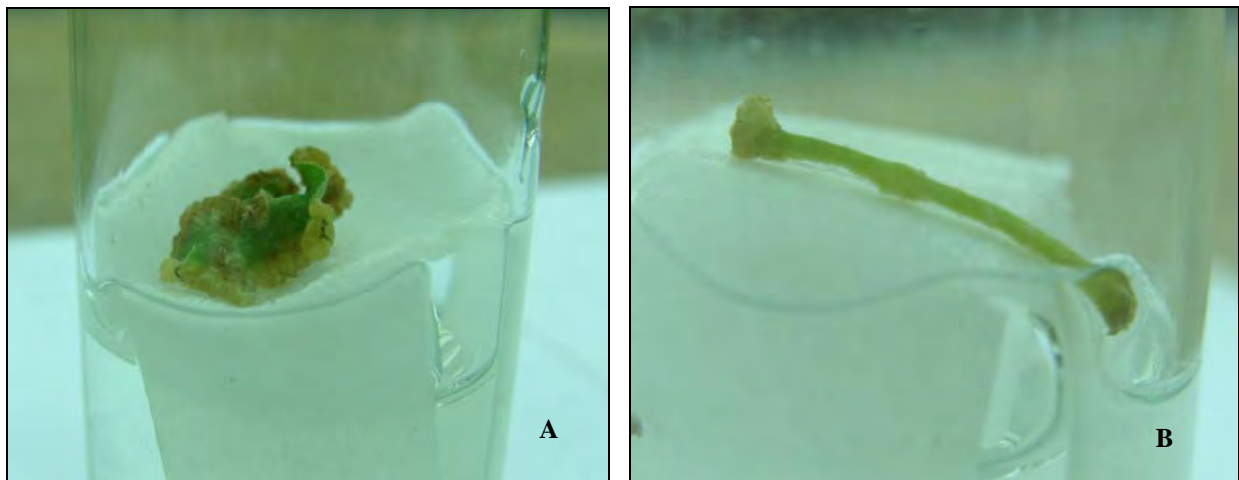


Figure 1. Initial stage of callus induction from leaf fragments (A) and petiole segments (B) supported on filter paper bridge on the surface of liquid culture medium. / Figura 1. Inițierea procesului de calusogeneză din explante de frunză (A) și explante de pețiol (B) susținute pe punte din hârtie de filtru la suprafața mediului de cultură lichid (original).

At the end of the dark incubation period, no significant differences were evident between the ability of somatic explants cell proliferation. Thus, in “Serenata” genotype, the rate of callogenesis was 96.2% for petiole explants and 96.8% for leaf explants in culture medium containing low concentration of IBA and BAP (0.5 mg/l IBA + 3.0 mg/l BAP). In “Pink Panda” genotype, the same combination of growth regulators promoted callus formation from the petiole explants and leaf explants with a frequency of 100% and 92.8%, respectively (Fig. 2).

For both ornamental strawberry genotypes, the highest overall percentage of somatic explants forming callus (100%) was induced in experimental variant M2, characterized by the hormonal balance 1.0 mg/l IBA + 3.0 mg/l BAP. Statistical analysis revealed significant differences in callus induction frequency between the experimental variants M1 and M2 on the one hand and M3 experimental variant, on the other hand. Thus, an inhibition of callogenesis occurred for both types of explants on media with an increased BAP concentration (5.0 mg/l instead of 3.0 mg/l). In “Pink Panda” genotype, the lowest percentage of leaf explants (40%) and petiole segments (64.2%) was induced in culture medium supplemented with 1.0 mg/l IBA and 5.0 mg/l BAP. However, in “Serenata” genotype the same combination of growth regulators promoted callus formation from the petiole and leaf explants, with a frequency of 54.4% and 38.8%,

respectively (Fig. 2). These observations were similar to those noted in previous experiments focused on determining the callogenetic response of somatic explants grown on solidified culture medium (SUTAN et al., 2010) Trebuie corectat numele. Similar results have also been reported by other authors, such as NEHRA et al. (1989); BARCELÓ et al. (1998); GRUCHALA et al. (2004).

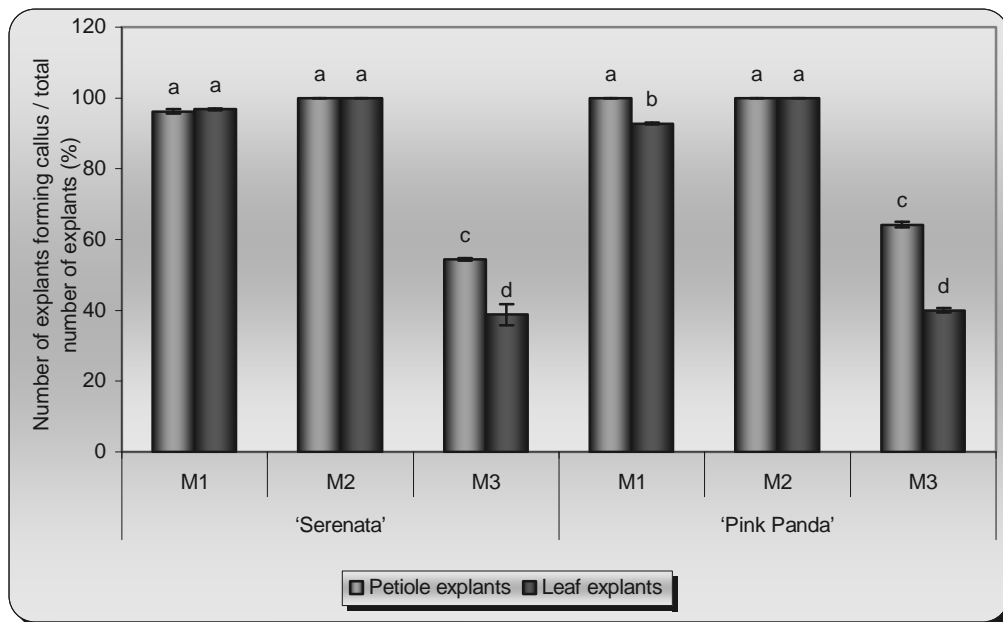


Figure 2. The ability to form callus of leaf and petiole explants cultured on liquid medium provided with filter paper bridge (bars represent standard deviation; a, b, c, d: indicate percentages significantly different from each other, using Duncan's Multiple Range Test, $p < 0.05$).

Figura 2. Capacitatea de calusogeneză a explantelor de frunză și de pețiol susținute pe punți din hârtie de filtru la suprafața mediului de cultură lichid (barele reprezintă abaterea standard; a, b, c, d: interpretarea semnificației diferențelor cu ajutorul testului Duncan, $p < 0.05$).

In "Serenata" genotype, shoot regeneration was observed after 27 days of culture for petiole explants, and after 30 days in the case of leaf explants. In "Pink Panda" intergeneric hybrid first adventitious buds were formed from pieces of leaf, in the absence of light, after approximately 38 days of *in vitro* culture. For the petiole explants, the time elapsed until the first adventitious bud formation was approximately 50 days. This study showed that, irrespective of the genotype, adventitious shoots were regenerated from callus formed at the end of petiole segments, while in case of leaf explants regenerative processes are more intense in the callus formed in the median region of leaf fragments (Fig. 3).

Statistical analysis revealed that depending on their origin, calluses derived from diverse somatic explants may have totally different regenerative potential, so that selection of explant type may be important in improving culture system for micropropagation of these two intergeneric hybrids *Fragaria* × *Potentilla*. Thus, in "Serenata" genotype the frequency of shoot regeneration from leaf – derived calluses was significantly higher compared to that from petiole – derived calluses. Also, in "Pink Panda" genotype leaf - derived calluses regenerated shoots at a relatively higher percentage, compared with calluses formed from petiole explants, even though statistical analysis revealed no significant differences between the two types of explant (Fig. 4).

Differences in efficiency of shoot regeneration between petiole and leaf explants may be primarily due to the differences of endogenous growth regulators level in the explant. Also, the better regenerative ability of leaf explants can be attributed to the size of explants. In this respect, PIERIK (1998) reported that larger explants sometimes regenerate easier than smaller ones, and that the larger explants produce more shoots *in vitro*.

This study showed that in both ornamental strawberry genotypes regenerative potential of calluses originating from somatic explants was strongly dependent on the hormonal balance in the culture medium, the average percentage of calluses that formed shoots in the experimental variant M3 being significantly lower, compared with M1 and M2 experimental variants. In this context it is worth mentioning that in "Serenata" genotype shoot regeneration from leaf-derived calluses cultured on media supplemented with 0.5 mg/l IBA + 3.0 mg/l BAP (M1 variant) or 1.0 mg/l IBA + 3.0 mg/l BAP (M2 variant) was induced with a frequency of 65.33% and 68%, respectively. Shoot regeneration was induced also, but at a low frequency (5.33% from leaf – derived calluses and 2.76% from petiole – derived calluses), when somatic explants were cultured on medium supplemented with 1.0 mg/l IBA + 5.0 mg/l BAP (Fig. 4).

Similarly, in "Pink Panda" genotype the results reported in figure 4, indicate a higher regenerative potential of calluses derived from leaf and petiole explants cultured in the presence of 0.5 mg/l IBA + 3.0 mg/l BAP (M1 variant) or 1.0 mg/l IBA + 3.0 mg/l BAP (M2 variant), but introduction of BAP at a concentration of 5.0 mg/l in culture medium resulted in poor regeneration of calluses. For example, while the incidence of shoots regeneration from leaf derived – calluses was 48% in medium supplemented with 1.0 mg/l IBA + 3.0 mg/l BAP, in the M3 experimental variant increases of BAP concentration at 5.0 mg/l shoot regeneration was induced with a frequency of only 4% (Fig. 4).

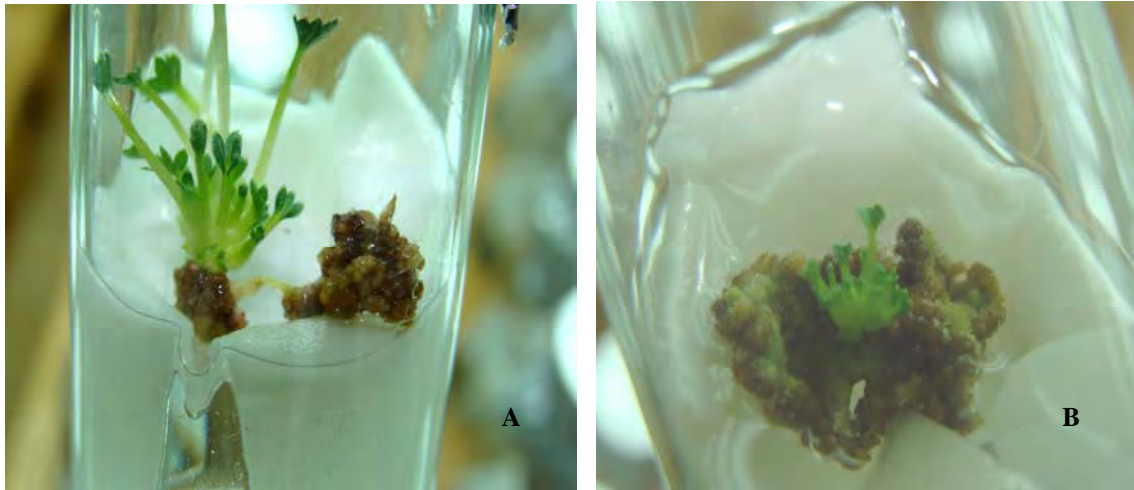


Figure 3. Shoot regeneration from petiole – derived callus (A) and leaf – derived callus (B) supported on filter paper bridges on the surfaces of liquid culture medium in “Serenata” genotype. / Figura 3. Regenerare de lăstari via calus din explante de petiol (A) și de frunză (B) susținute pe punți din hârtie de filtru la suprafața mediului de cultură lichid la genotipul “Serenata” (original).

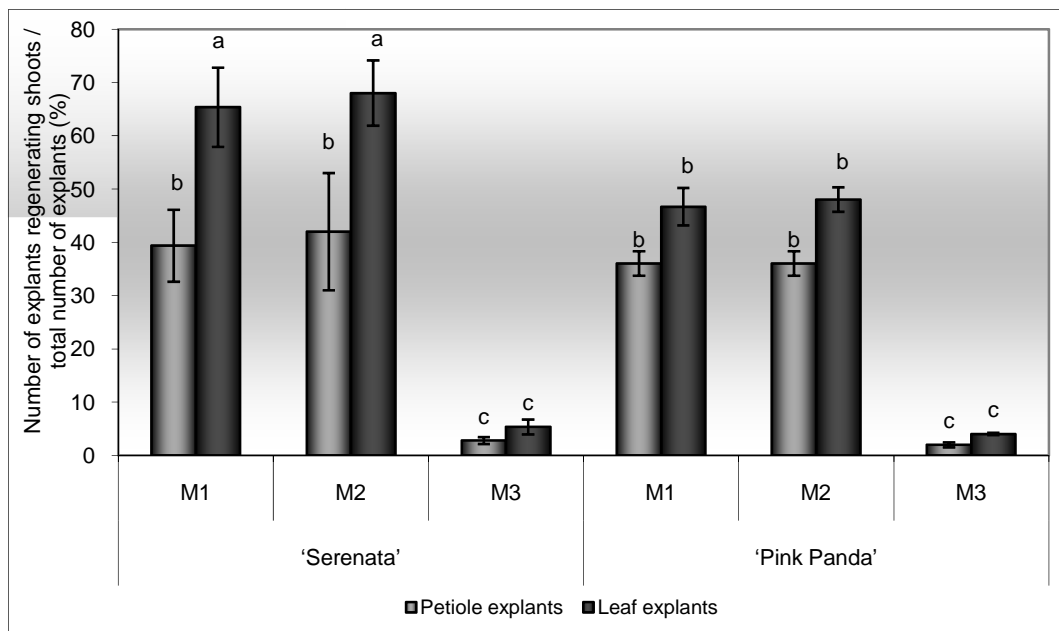


Figure 4. Percentages of leaf and petiole- derived calluses showing shoot formation in ‘Serenata’ and ‘Pink panda’ genotypes of intergeneric hybrids *Fragaria x Potentilla* (bars represent standard deviation; a, b, c: indicate percentages significantly different from each other, using Duncan’s Multiple Range Test, $p < 0.05$). / Figura 4. Frecvența de regenerare de lăstari via calus din explante de frunză și pețiol la genotipurile de căpșun ornamental ‘Serenata’ and ‘Pink panda’ (barele reprezintă abaterea standard; a, b, c: interpretarea semnificației diferențelor, cu ajutorul testului Duncan, $p < 0.05$).

In this study, the number of adventitious shoots regenerated per callus ranged from 5 to 12, with no significant differences between the mean values calculated for each experimental variant. Also, have been noted explant polarity effect, expressed by relatively high number of shoots formed at one end of the petiole segments, while adventitious buds formed at opposite end (in a small number) could not complete their evolution. *In vitro* regenerated shoots were light green and it turned dark green on exposure to high light intensity.

CONCLUSIONS

1. In this study, combining advantages of both liquid culture medium provided with filter paper bridges and maintenance of tissue-derived calluses in culture without their transfer to the fresh medium associated with relatively high frequency of shoot regeneration in a short time, period of culture and micropropagation cost of ‘Serenata’ and ‘Pink Panda’ genotypes of intergeneric hybrids *Fragaria x Potentilla* were reduced.

2. In “Serenata” and “Pink Panda” genotypes of ornamental strawberry, hormonal balance defined by 1.0 mg/l IBA and 3.0 mg/l BAP promoted callus formation from the highest percentage of petiole and leaf explants and induced shoot proliferation from the highest percentage of somatic explants derived calluses.

3. Leaf fragments exhibited a higher regeneration potential than petiole segments.

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