

***In vitro* plant regeneration from somatic tissue of strawberry
Fragaria x ananassa Duch.**

***In vitro* regeneracija poganjkov v somatskem tkivu jagode
Fragaria x ananassa Duch.**

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Abstract. Successful shoot regeneration in somatic tissue is the basic requirement for *in vitro* induction of genetic variability as the new tool in plant breeding.

Somatic tissue excised from *in vitro* multiplied strawberry plants were tested on ability for plant regeneration. Leaves, petiole and stipules were inoculated on initial medium with BA and 2,4-D, or on medium with BA only after 1 hour pulse treatment with 2,4-D. Callus was induced on all sliced surfaces of explants inoculated on initial medium with growth regulators BA and 2,4-D during first 7 days of culture. Explants inoculated on initial medium with BA, after pulse treatment with 2,4-D did not develop callus but abundantly produced phenolic compounds, and turned necrotic in the first 24 hours.

Spontaneous plant regeneration was noticed on leaves explants with less developed callus tissue on initial medium with growth regulators during second week of culture. High percentage of explants with regenerated shoots were obtained after transfer on hormone-free medium.

The highest plant regeneration ability was in leaf tissue, less in petiole and stipules.

Callus induced in leaf tissue showed ability for constant plant regeneration during three months of culture and careful 4-week interval transfer on basal MS medium with 4.4/4M BA and 40 g/l sucrose.

Keywords: *in vitro*, somatic tissue, leaf explants, plant regeneration, strawberry, *Fragaria x ananassa* Duch., cv. Elsanta, cv. Marmolada

Izveček. Uspešna regeneracija poganjkov v somatskem tkivu je osnovni pogoj za *in vitro* indukcijo genetske raznolikosti kot novega pristopa v zlahtnjenju rastlin.

Somatsko tkivo, pridobljeno iz *in vitro* razmnoženih rastlinic jagod, smo testirali glede sposobnosti za regeneracijo rastlin. Del izsečkov iz listov, pecljev in stipul smo prenesli neposredno na začetno gojišče z BA in 2,4-D, del pa predhodno 1 uro tretirali z 2,4-D in nato prenesli na gojišče s samo BA. Kalus se je induciral v prvih 7 dneh rasti kulture na rezani površini vseh izsečkov na gojišču z rastlinskimi hormoni BA in 2,4-D. Izsečki, predhodno tretirani z 2,4-D in inokulirani na gojišče z BA, niso razvili kalusa, ampak so proizvedli obilo fenolnih sestavin ter po 24 urah propadli.

Tekom drugega tedna rasti kulture na začetnem gojišču z rastnimi regulatorji BA in 2,4-D smo opazili spontano regeneracijo poganjkov na listnih izsečkih s slabo razvitim kalusnim tkivom.

Visok odstotek izsečkov z regeneriranimi poganjki smo pridobili po prenosu na gojišče brez rastnih regulatorjev.

Največjo sposobnost za regeneracijo poganjkov smo ugotovili pri listnem tkivu, slabšo pa pri tkivu pecljev in stipul.

Kalus, induciran v listnem tkivu, je pokazal sposobnost za stalno regeneracijo poganjkov tekom treh mesecev rasti kulture in po previdnem 4-tedenskem predstavljanju na bazalno MS gojišče z 4.4 μ M BA in 40 g/l sladkorja.

Ključne besede: *in vitro*, somatsko tkivo, listni izseček, regeneracija poganjkov, jagoda, *Fragaria x ananassa* Duch., cv. Elsanta, cv. Marmolada

Introduction

Development and application of reliable protocols for plant regeneration from tissue culture become the new tools for the improvement of strawberry (*Fragaria x ananassa* Duch.) cultivars.

Strawberry regeneration has been obtained *in vitro* from different somatic tissue: cell suspension (DAMIANO et al. 1995), leaves (NEHRA et al. 1989; NEHRA et al. 1990), petioles (JONES et al. 1988), stipules (RUGINI and ORLANDO 1992, JEMMALI et al. 1992), cotyledons (MILLER and CHANDLER 1990), anthers (ROSATI et al., 1975).

Because regeneration efficiency is strongly dependent on the cultivar, regeneration methods for strawberry shoots from nonmeristematic tissue have not been well defined yet.

The purpose of the present work was the evaluation of ability for *in vitro* shoot regeneration in strawberry somatic tissue.

Material and methods

Donor strawberry plants (*Fragaria x ananassa* Duch.) cv. Elsanta and cv. Marmolada, were propagated *in vitro* by protocol described by Boxus (Boxus et al. 1977).

Leaf strips, petioles and stipules were inoculated on basal MS medium with thiamine 0.4 mg/l, agar 8 g/l, pH 5.8. Four treatments were used: 1) BA 4.4 μ M + 2,4-D 2.3 μ M + 40 g/l sucrose; 2) BA 4.4 μ M + 2,4-D 2.3 μ M + 80 g/l sucrose; 3) BA 4.4 μ M + 40 g/l sucrose; explants were under pulse treatment in MS liquid medium with 2,4-D 22.6 μ M 1 hour prior inoculation; 4) is as (2) and also with pulse treatment as mentioned above.

Hundred and twenty leaf explants, 100 petiole explants of length 0.5 mm, and 60 stipule explants were inoculated for each treatment.

Explants with regenerated shoots were cultivated on MS hormone-free medium which promoted shoot elongation. Shoots grew in dense clusters, but it was possible isolated well developed plantlets. It was successfully acclimatized 194 plantlets of strawberry cultivar Elsanta.

Results

Regenerative ability was strongly influenced by genotype for three type of explants tested. Shoot regeneration from leaf, petiole and stipule explants in the strawberry cultivars Elsanta and Marmolada is presented in Table 1. Cultivar Elsanta showed better ability for callus initiation and shoot

regeneration in both treatments used. High percentage of leaf explants developed callus in the presence of BA and 2,4-D, and sucrose 40 g/l. Presence of 80 g/l sucrose suppressed callus development. Shoots which were initiated and regenerated on leaf explants cultured on initial medium, continued to grow on medium with BA 4.4 μ M. It was possible to harvest regenerated plantlets from leaf explants during three months of culture, when explants with regenerative callus were transferring on fresh medium MS with BA every 4th week.

Petiole explants also developed callus and regenerated shoots, but in lower percentages than leaf explants.

In this experiment was not possible to obtain callus initiation and shoot regeneration in stipule explants of strawberry cv. Elsanta.

Induction of callus was very poor in leaf explants and petioles of cv. Marmolada. Stipule explants showed slightly better ability for shoot regeneration.

Pulse treatment with 22.6 μ M 2,4-D on all three explant types of strawberry cultivars Elsanta and Marmolada caused abundant production of fenolic compounds and browning of plant tissue.

Discussion

The results show that ability of strawberry cv. Elsanta and cv. Marmolada to regenerate in vitro from somatic tissue (leaf, petiole, stipule) depends on the genotype. This fact also confirmed the results of experiments on cv. Redcoat and cv. Honeoye carried out by NEHRA et al. (1988). ŽEBROWSKA et al. (2002) tested leaf and petiole of cv. Kama and clone B-302 on capacity for plant regeneration, and also confirmed influence of the genotype. In their results petiole explants showed higher ability for regeneration, but higher number of regenerated plantlets per explant was in leaf tissue. We obtained better capacity for plant regeneration in leaf tissue of strawberry cv. Elsanta, and no ability for shoot regeneration in leaf and petiole tissue of cv. Marmolada. Only low percentage of stipule explants of cv. Marmolada regenerate shoots in our experiment. But MONTICELLI et al. (1995) reported on very high competence to regeneration from stipules of strawberry cv. Teodora and cv. Clea. They claimed also importance of 2,4-D as inducing factor in initial medium for callus induction, and reported similar as JEMMALI et al. (1992) that BA is sufficient to induce shoot regeneration. We noticed that explants with callus induced on medium with BA 4.4 μ M and 2,4-D 2.3 μ M, continuously regenerate shoots in leaf explants subsequently subcultured on medium with BA alone. POPESCU et al. (1997) reported on the organogenetic potential of petiole-derived calli for a long period from at least 18 up to 29 weeks, but shoot formation was no longer recorded after 19 weeks.

In our experiment leaf explants of cv. Elsanta were able to regenerate shoots during 12 weeks.

Table 1: Shoot regeneration from leaf, petiole and stipule explants in two strawberry cultivars at two different treatments.

Cultivar/explant	Treatment	% callus regeneration explant ⁻¹	% shoot regeneration explant ⁻¹	Number of plantlets explant ⁻¹
Elsanta	Leaf	100	31.2	12*
		2	79.5	8*
	Petiole	1	61.3	3
		2	20.8	3
	Stipule	1	2.0	0
		2	0	0
Marmolada	Leaf	1	3.5	0
		2	1.8	0
	Petiole	1	1.2	0
		2	0	0
	Stipule	1	1	3
		2	0	0

*shoots were harvested in vitro during 3 months

Conclusion

Shoot regeneration from somatic tissue (leaves, petioles, stipules) of strawberry (*Fragaria x ananassa* Duch.) is highly dependent on genotype.

2,4-D was essential for callus induction, while BA alone was later sufficient to induce shoot regeneration.

Regenerative calli induced on leaf explants of strawberry (*Fragaria x ananassa* Duch.) cv. Elsanta showed shoot regeneration ability during next three months in the presence of 4.4 μ M BA.

Povzetek

Pri testiranju sposobnosti regeneracije jagod cvs. Elsanta in Marmolada iz različnih somatskih tkiv in vitro gojenih rastlin in učinkovitosti različnih načinov tretiranja s hormoni smo ugotovili:

- * neučinkovitost tretiranja izsečkov z 2,4-D za 1 uro pred prenosom na gojišče z BA,
- * velike razlike v sposobnosti regeneracije med proučevanimi kultivarjema,
- * zelo slabo sposobnost regeneracije cv. Marmolada, pri kateri smo regeneracijo opazili samo pri 1 % izsečkov iz stipul,
- * zelo dobro tvorbo kalusa in regeneracijo poganjkov cv. Elsanta iz izsečkov iz listov in nekoliko slabšo, a dobro regeneracijo iz izsečkov iz listnih pecljev
- * neučinkovitost regeneracije iz stipul na preskušanih gojiščih,
- * boljšo tvorbo kalusa oz. regeneracijo poganjkov na gojišču s 40 g/l saharoze v primerjavi z 80 g/l saharoze.

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