

Investigation of different media content on meristem organization of raspberry (*Rubus idaeus*) in conditions *in vitro*

Istraživanje medija različitog sadržaja na organiziranje meristema *in vitro*

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SAŽETAK

Istraživani su optimalni uvjeti za razmanžanje i ukorjenjivanje kultivara Willamette *in vitro*. Visok stupanj proliferacije i razmanžanja nakon 90 dana kulture na Murashige i Skoog (MS) mediju sa 100 mg·l⁻¹ mio inositola, 2 mg·l⁻¹ tijamina-HCl, 1 mg·l⁻¹ piridoksina-HCl, 1 mg·l⁻¹ nikotinske kiseline, 30 g·l⁻¹ saharoze i 8 g·l⁻¹ agara razmatran je s dvije koncentracije BA (0,2 i 2,0 mg·l⁻¹). Primijenjen je i medij MS pola koncentracije. Značajno je bilo odstupanje proliferiranih izboja, ovisno o mediju. Dobili smo visoku razinu ukorjenjenja te smo istražili prilagodbu ponovno naraslih izboja na sterilnom supstratu u uvjetima obilne vlage.

Ključne riječi: malina, razmnožavanje *in vitro*, sastav medija

ABSTRACT

Optimal conditions for *in vitro* multiplication and rooting of cv. Willamette were investigated. High level of proliferation and multiplication after 90 days of culture on Murashige and Skoog (MS) medium with 100 mg·l⁻¹ myo-inositol, 2 mg·l⁻¹ thiamine-HCl, 1 mg·l⁻¹ pyridoxine-HCl, 1 mg·l⁻¹ nicotinic acid and 30 g·l⁻¹ sucrose and 8 g·l⁻¹ agar was considered together with two concentrations of BA (0,2 and 2 mg·l⁻¹). Half strength MS medium was also used. There was a significant variation of the proliferated shoots, depending on media. We got high level of rooting and we have investigated acclimatization of regenerants grown on sterile substrate in conditions of high humidity.

Key words: raspberry, micropropagation *in vitro*, MS medium

INTRODUCTION

In the last ten years there have been many research papers dealing with investigations into the effects of many combinations of cytokinins and auxins on the multiplication of raspberry *Rubus idaeus* in conditions *in vitro*. Willamette is one of the worldwide leading cultivars, introduced into Macedonia and its propagation is very actual. In this paper we investigate its regenerative ability in conditions *in vitro*.

Ferradini et al. (1977) investigated micro propagation of five raspberry cultivars (*Rubus idaeus*) (Canby, Selezione 1401, Selezione 1415, Skeena i Veten) and two cultivars of *Rubus ulmifolius* (Black Satin i Jumbo). According to the ecotype 48.2 to 93.7% of meristems developed to the 'rosette' stage (5-10 mm long) after 30 days of culture in a growth chamber, on Murashige and Skoog (MS) modified medium enriched with 0.5 mg BA/liter. For the proliferation phase, 5 different media were compared for 4 subsequent cultivates of 30 days each. Average multiplication rate ranged from 2.5 to 9.2 for *R. ulmifolius* cultivars compared with 1.0 to 3.8 among the raspberry cultivars. The *R. ulmifolius* cultivars showed good proliferation in most of the tested media compared with only 1 or 2 the raspberry cultivars.

From the cited reference it is obvious that combinations of phytohormones in media, and also a choice of the genotype have a role in the regenerative ability. In some cultivars the level of proliferation depends on concentrations of the cytokinine benzilaminopurine BA.

The aim of this paper is to investigate the effect of concentration of cytokinines and different mineral solutions on different phase of regeneration of cultivars Willamette in conditions *in vitro*.

MATERIALS AND METHODS

Apical buds from plants of the cultivar Vilamet were removed by cutting the shoot axis. Isolated parts of the tissue, 2 cm long were rinsed two times in tap water for one hour and sterilized for ten minutes in 5% calcium hipocloride in aseptic conditions (laminar air flow cabinet), and rinsed in sterilized water. All media, solutions and glassware were previously autoclaved at 125°C for 20 min. All operations were made under sterile conditions. Cuttings were inoculated on sterile media.

Stabilization was achieved in test tubes containing MS media (Murashige i Skoog, 1962); 3% sucrose, 0,8% agar, but modified by reducing the concentrations of macro and micronutrients and vitamins to one half. After 2 monthly subcultures in the same conditions, the explants were proliferated in 100 ml full strength media, with monthly subcultures in a growth chamber, at 22 ±2°C, with 16 hour light / 8 hour dark photoperiod and light intensity of 750-1250 cd sr/m².

After regenerants had formed a leaf rosette, they were transferred on MS media as described above with or without adding BA in concentration of 0.2 and 2 mg·l⁻¹.

Regenerated plants rooted in sterile substrate in conditions of high humidity.

Table 1 Influence of mineral solution and different BA concentrations on meristem organization into leaf rosette

Tablica 1. Utjecaj mineralne otopine i različitih koncentracija BA na organizaciju meristema u lisnu rozetu

Media Medij	Number of isolated meristem Broj izoliranih meristema	Number of roliferated meristem Broj roliferirani meristema	Meristems formed leaf rosete meristemi formirani u lisnu rozetu		Necrotized isolates Nekrotizirani izolati	
			No	%	No	%
MS	50	10.7	9.3	87.19	1.0	9.35
MS + 0.2 mg/l BA	50	11	8	72.73	4.0	36.36
MS + 2 mg/l BA	50	6	6	100.00	0.0	0.00
½ MS	50	8	5	62.50	4.0	50.00
½ MS + 0.2mg/l BA	50	9.3	1.3	13.93	6.0	64.52
½ MS + 2 mg/l BA	50	20	5	25.00	15.0	75.00

RESULTS AND DISCUSSION

Investigated genotype showed high level of regeneration on MS medium. Meristems were cultivated on fresh medium every two weeks. After eight weeks of cultivation some leaves were formed and multiplication began. We used MS medium (Murashige and Skoog, 1962), 3% sucrose, 0.8% agar, but modified by reducing the concentration of macro and micronutrients and vitamins to one half.

After eight weeks of cultivation regenerants were 0.5 to 1 cm big. Such formed leaf rosette we cultivated on media for multiplication with addition of BA in concentrations of 0.2mg and 2mg/l. Proliferation of shoots varied significantly depending on concentrations of BA in media. Auxiliary buds were formed in the beginning very slowly (1 axillary buds) and after the third cultivate the number of buds was 9.

As we can see in table 1 the combination of two different concentrations of BA (0.2mg and 2mg/l) had a positive influence on of axillary buds forming. Besides good multiplication, shoots had a good growth, compared to control plants. Delayed growing of shoots on media with addition of phytohormones gave the effect of elongation of regenerates.

We got 92% of rooting, and we investigated acclimatization of regenerates grown on a sterile substrate in conditions of high humidity. Karakullukcu et al. (1994) had similar percentage rooting. When solid MS medium was used without growth regulators they got 87% of shoots rooted, compared with 92.3% in the liquid MS medium and rock wool.

CONCLUSIONS

According to the data obtained these conclusions were made:

- High level of proliferation and multiplication of 90 days of culturing on Murashige and Skoog (MS) medium with 100 mg/l mio inositol, 2mg/l tiamin-HCl, 1mg/l piridoxin-HCl, 1mg/ml nicotinic acid and 30 g/l sucrose and 8g/l agar was considered;
- High regenerative capacity with significant variation of the proliferated shoots was obtained depending on the media content;
- The cultivar Willamette showed high rooting potential and regenerated plants were transferred on the sterile substrate in conditions of high humidity.

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Primljeno – Received: 20.12.2006.