

MICROPROPAGATION OF RASPBERRY CULTIVARS BY TERMINAL AND LATERAL BUD EXPLANTS

MICROPROPAGAREA UNOR SOIURI DE ZMEUR DIN EXPLANTE DE MUGURI TERMINALI ȘI LATERALI

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Abstract: The purpose of this study was to establish the optimal conditions for *in vitro* micropropagation of two raspberry cultivars (Opal and Cayuga). Axillary and terminal buds, nodal meristems and leaf discs were used as explants. After sterilization, the explants were placed on MS medium supplemented with different concentrations of cytokinins (BAP, TDZ and K) and auxins (2,4D and IAA) added alone or in combination with GA₃. For both cultivars, the greatest number of shoots, approximately 4.67 per explant, was induced on medium containing 0.5mg/l AIA+ 1mg/l BAP + 0.5mg/l GA₃ and the maximum shoot length, approximately 2.58 cm, was obtained on medium containing 0.5mg/l AIA+ 1mg/l BAP. We concluded that the organogenesis process can be efficiently induced for both raspberry cultivars from terminal buds on MS medium supplied with 0.5mg/l 2,4D+ 1mg/l TDZ or 1mg/l AIA+ 1mg/l BAP + 0.5mg/l GA₃

Key words: Raspberry, cytokinines, GA₃, organogenesis

Rezumat: Scopul acestui studiu este de a stabili condițiile optime de propagare *in vitro* a două soiuri de zmeur (Opal și Caiuga). Mugurii terminali și axilari decatafilizati, meristeme nodale și porțiuni de limb foliar au fost plasați, după sterilizare, pe mediul de cultura MS suplimentat cu diferite tipuri de citochinine (BAP, TDZ, K) și auxine (2,4D și AIA) adăugate singular sau în combinație cu giberelina (GA₃). Cel mai mare număr de lăstari, 4.67 per explant, s-a obținut pe mediul suplimentat cu 0.5mg/l AIA+ 1mg/l BAP + 0.5mg/l GA₃, iar cea mai mare lungime a lăstarilor, aproximativ 2.58 cm, pe mediul suplimentat cu 0.5mg/l AIA+ 1mg/l BAP. În concluzie, procesul de organogeneza poate fi indus eficient în ambele soiuri din muguri terminali pe mediu MS suplimentat cu 0.5mg/l 2,4D+ 1mg/l TDZ sau 1mg/l AIA+ 1mg/l BAP + 0.5mg/l GA₃

Cuvinte cheie: zmeur, citochinine, GA₃, organogeneza

INTRODUCTION

In vitro micropropagation of raspberry cultivars can be used for large-scale multiplication of selected genotypes, germplasm conservation and breeding of new cultivars better adapted to specific soil and climate conditions. Therefore, it is necessary to first determine the optimal conditions for *in vitro* micropropagation. Studies on raspberry micropropagation have been carried out since the 80's to augment or replace the common propagation methods and to

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produce virus free plants. Recently various aspects of raspberry tissue culture have been studied to develop a methodology. Ability to produce adventitious shoots varies from genotype to genotype in *Rubus* sp. (Cousineau M. and Donnelly, 1991; Turk et al., 1994; Graham et al., 1997). Genetic differences of raspberry cultivars require certain modifications of standard growth conditions to produce viable *in vitro* plants. Regeneration of adventitious shoots of *Rubus* spp. from different explants has been reported for leaves (Fiola et al., 1990; Swartz et al., 1990; Owens y de Novoa, Conner, 1992), petioles (Cousineau, Donnelly, 1991), leaf discs and internodal stem segments (McNicol, Graham, 1990), cotyledons (Fiola et al., 1990) and mature embryos (Fiola, Swartz, 1990). Many regeneration studies have focused on the optimal culture media composition. Early reports on *Rubus* species used the cytokinin N6-benzyladenine (BA) for initiating organogenesis process from different explants. Zeatin, another cytokinin, has been found to be more effective for shoot proliferation of some woody species, e.g., in *Vaccinium* (Debnath 2003). Thidiazuron, a cytokinin-like compound, was found to be very effective for stimulation of adventitious shoots in *Rosaceous* crops, including fruit trees (Korban et al., 1992). Similar results were reported for *Rubus* spp. (Fiola et al., 1990; Swartz et al., 1990; Cousineau, Donnelly, 1991). Studies made with raspberry leaf tissues (Cousineau, Donnelly, 1991), or cotyledons of blackberry-raspberry hybrids (Fiola et al., 1990) also illustrated that organogenesis may be affected by growth regulators, incubation temperature or photosynthetic radiation. The goal of this work was to develop a protocol for inducing a high regeneration rate by testing the response of different explants of two *Rubus* to different culture media.

MATERIAL AND METHOD

Plant material. Primocanes from *Opal* and *Cayuga* cultivars were collected from plants grown in the greenhouse using sterile razor blades, placed in plastic bags and stored at 4 C. Under a laminar flow hood, 3–5 cm long stem segments from terminal or medial part of primocane, with a healthy bud were immersed in 70% ethanol for 5 s, and then transferred to a solution containing 0.6% sodium hypochlorite with two drops of Tween-20 for 25 min. After being immersed in solution three times they were rinsed with sterile water and then kept submerged in sterile water, prior to being trimmed and placed on culture medium. The same sterilization process was applied to nodal meristems and 2–3 cm leaf discs. All explants were placed for regeneration on MS medium with different combination of growth regulators (tab 1). Cultures were checked daily for any sign of contamination and they were sub-cultured every 6 weeks. Proliferating explants were kept at 25C and 16-h photoperiod in the growth chamber at $70 \mu\text{mol m}^{-2} \text{s}^{-1}$. The number of responsive explants and shoots/explant was recorded after 21 days of culture. Individual shoots were excised and subcultured for shoot growth on the same media and shoot length was recorded after 45 days of culture.

RESULTS AND DISCUSSIONS

Analyzing morphogenetic reaction of different explants (table 1) we found that leaf discs responded only by cell proliferation, but the other explants

developed organogenesis or cell dedifferentiation depending on the hormonal composition of culture media.

The adventitious and terminal bud explants had a different reaction. The terminal buds developed shoots on all media variants excepting those supplied with kinetin where only cell proliferation occurred. Lateral buds developed shoots only on media supplied with TDZ and GA3. The nodal meristems had a similar reaction with lateral buds, except on medium supplied with TDZ it only produced callus.

Table 1

Morphogenetic reaction intensity of different explants of Opal and Cayuga varieties on MS basal medium supplemented with different plant growth regulators

MS medium supplemented with:	Explant type	Morphogenetic reaction	
		Opal	Cayuga
0.5mg/l 2,4D+ 1mg/l BAP	Terminal buds	Organogenesis 19%	Organogenesis 21%
	Lateral buds	Cell proliferation 2%	Cell proliferation 5%
	Nodal meristem	Cell proliferation 3%	Cell proliferation 17%
	Leaf disc	-	-
0.5mg/l 2,4D+ 1mg/l TDZ	Terminal buds	Organogenesis 61%	Organogenesis 67%
	Lateral buds	Organogenesis 11%	Organogenesis 17%
	Nodal meristem	Cell proliferation 27%	Cell proliferation 11%
	Leaf disc	Cell proliferation 3	Cell proliferation 3%
1mg/l AIA+ 2mg/l BAP	Terminal buds	Organogenesis 20%	Organogenesis 24%
	Lateral buds	Cell proliferation 36%	Cell proliferation 32%
	Nodal meristem	Cell proliferation 19%	Cell proliferation 21%
	Leaf disc	-	-
1mg/l AIA+ 1mg/l BAP	Terminal buds	Organogenesis 11%	Organogenesis 15%
	Lateral buds	Organogenesis 3%	Organogenesis 7%
	Nodal meristem	-	Organogenesis 3%
	Leaf disc	-	-
1mg/l AIA+ 1mg/l BAP + 0.5mg/l GA3	Terminal buds	Organogenesis 88%	Organogenesis 98%
	Lateral buds	Organogenesis 81%	Organogenesis 91%
	Nodal meristem	Organogenesis 84%	Organogenesis 94%
	Leaf disc	-	-
1mg/l 2,4D+ 5mg/l AIA + 1mg/l K	Terminal buds	Cell proliferation 81%	Cell proliferation 82%
	Lateral buds	-	-
	Nodal meristem	Cell proliferation 78%	Cell proliferation 88%
	Leaf disc	Cell proliferation 81%	Cell proliferation 86%

Different organs used as source of explants had different levels of carbohydrates, proteins and growth regulators in their tissues. The effect of organ source on explant regeneration is thought to be mostly due to the changes in endogenous levels of growth substances. Explants taken from young tissues generally undergo direct organogenesis better than those taken from older tissues. Takayama and Misawa (1982) showed that buds and roots were produced from young leaf explants of *Begonia*, whereas mature leaf explants usually died in culture.

The intensity of morphogenetic reaction in raspberry cultivars was dependent on both explant type and hormonal balance. Terminal buds were the

most responsive explants. In Cayuga 98% of the terminal buds showed morphogenetic reaction on medium supplied with GA3.

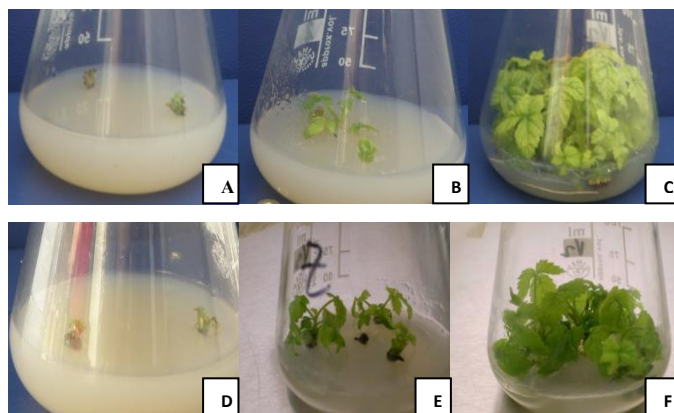


Fig. 1 - Multiple shoot development from terminal buds on Cayuga (a-c) and Opal (d-f) varieties

Very good results were obtained when culture medium was supplied with TDZ (61% of apical buds from Opal and 67% from Cayuga develop shoots). However, the supplementation of medium with BAP resulted in a weaker morphogenetic reaction regardless if it was combined with 2,4 D (maximum 19% at Opal and 21% at Cayuga) or IAA (maximum 20% at Opal and 24% at Cayuga).

Generally, stimulation of multiple shoot or bud formation is achieved by culturing explants on medium supplemented with relatively high levels of cytokinins. TDZ has been shown to promote shoot regeneration in woody plant species (Briggs et al., 1988; Preece et al., 1991; Baker and Bhatia, 1993), at a much lower concentrations than adenine-type of cytokinins. TDZ induced organogenesis via a reduced dominance of the apical meristem, resulting in formation of adventitious and/or axillary buds directly on the cultured shoot tips (Huetteman and Preece, 1993; Lu, 1993).

On the medium supplemented with BAP in combination with GA3 the morphogenesis was significantly enhanced. Independent on the explant type, more than 81% in Cayuga and 91% in Opal of the explants showed morphogenetic reaction.

The highest rate was obtained for cultures initiated from terminal bud of Cayuga where 98% of them gave shoots. The application of exogenous gibberellins and cytokinins (GA3 and BA) could induce the emergence of bud from dormancy which might explain the increase in the number of explants with organogenetic response. GA3 also improved plant regeneration via elongation of the embryo axis and by acceleration of the embryo maturation. Several previous studies provided clues for cross talk between GA and cytokinin.

Cytokinins act early during shoot initiation to control meristem activity (Schmulling, 2002), and GAs act at later stages, regulating cell division and expansion to control shoot elongation (Richards et al., 2001).

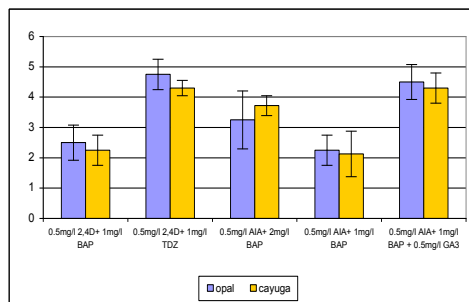


Fig. 2 - Effect of PGR type and concentration on number of shoots per explants from two *Rubus* genotypes

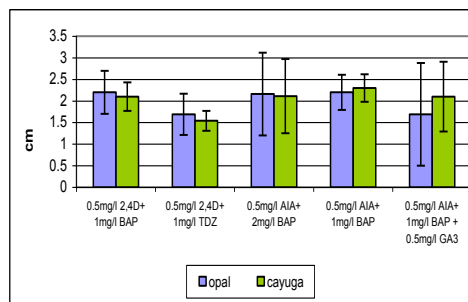


Fig. 3 - Effect of PGR type and concentration on shoots length produced from terminal buds of two *Rubus* genotypes

Multiple shoot development was common for both raspberry cultivars used in this study. Large numbers of shoots were produced on media supplemented with TDZ and BAP+GA3 (up to 4 shoots/explant). A good multiplication rate (more than 3 shoots/explant) was obtained on media with high level of BAP (2 mg/l).

On media supplemented with BAP the shoots obtained were longer than on the variants supplemented with TDZ. However, difficulties in the regeneration of plantlets from shoots, poor elongation of shoots and inadequate rooting (Huetteman and Preece, 1993; Lu, 1993) were reported for shoots obtained on TDZ-supplemented media. This impaired growth of TDZ-induced regenerates may result from the use of high TDZ concentrations in the medium or the prolonged exposure of the cultured tissue to this compound.

CONCLUSIONS

1. The organogenesis process can be efficiently induced for both raspberry cultivars (Opal and Cayuga) from terminal buds on MS medium supplied with 2,4D and TDZ or BAP and GA3
2. High auxine concentration in combination with kinetin induced calusogenesis in both cultivars
3. TDZ and GA3 improved the multiple shoot development, but BAP improved the shoot elongation

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