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Combined Application of Embryo Culture and *in vitro* Micropropagation in Early plum Cultivars propagation (*Prunus domestica* L.)

Dj. Ruzic, R. Cerovic

ARI 'Serbia', Fruit and Grape Research Centre, Cacak, Yugoslavia

Abstract: The paper presents the possibility of combined application of embryo culture and *in vitro* propagation of early domestic plum cultivars.

Under sterile conditions, embryos were placed on the medium containing sucrose, agar and mineral salts according to Murashige and Skoog (1962). After germination, seedling tips were collected and further multiplied by the method of micropropagation. Multiplication index ranged from 1:2.41 to 1:4.27, and rooting percentage from 87.5-100%.

Key words: early plum cultivars, embryo culture, micropropagation

Introduction

Embryo culture is widely used in breeding work for overcoming the phenomenon of immature embryo, as well as embryo abortion occurring at crossing distant species and genera. Crossing of early cultivars of stone fruit species also results in seeds with morpho-physiologically immature embryos. To obtain hybrid seedlings from such crosses, embryo culture method is used.

Hänning (1904) was first to carry out the successful embryo culture in *Cruciferae* and Tukey (1933) in fruit species. In the field of embryo culture, the papers on the following fruit crops have been reported: peach (Kester and Hesse, 1955; Zagaja and Hough, 1960; Fideghelli, 1968; Damiano, 1976), plum (Bini and Belini, 1973), as well as in grapevine (Gosal and Bajaj, 1983; Goldy et al., 1989; Burger and Trautmann, 1994). In some cases, abnormal development of

primary roots and stem was noticed in embryo culture (Ivanička and Pretova, 1980; Ascunia and de Assis, 1983) or the embyos developed into small and short plantlet (Yeung and Thorpe, 1981).

To overcome possible malformations, frequent infections in embryo development, poor vitality and slow growth, the combined application of embryo culture and micropropagation of seedlings obtained *in vitro* was studied.

Materials and method

As an initial material, fruits obtained by crossing early plum cv. Čačanska Rana with cvs. Ruth Gerstetter and California Blue were used.

The stones from which the mesocarp had been previously removed were kept for 24 hr in water, followed by 48 hr in Difocap - 0.25% fungicide. Isolated embryos were surface sterilized for 10 sec in 70% ethanol supplemented with Tween (a few drops), and then for 5 min in 10 % sodiumhypochlorite. Embryos were subsequently rinsed three times in sterile water and after removal of the seed coat placed on the medium.

Prior to sterilisation, the pH value of the medium was adjusted to 5.7 with 0.1N KOH, and sterilisation was carried out in autoclave at 120°C for 20 min.

The embryos were cultivated on Murashige and Skoog (MS) medium (1962) containing 20 gl^{-1} sucrose and 7 gl^{-1} agar. The test tubes with embryos were kept in cold chamber at 4°C in dark for a month, and then transferred to a chamber with the temperature of 25°C ± 1°C with 16/8 hr photoperiod, light/dark.

Terminal parts were collected from germinated hybrid seedlings and further subcultured on MS medium with (in) mgl^{-1}: N_6-benzylaminopurine (BAP), 1; indolyl-3-butyric acid (IBA), 0.1-1; gibberellic acid (GA_3), 0.1. Rooting medium contained MS half-strength mineral salts with1 and 2 mgl^{-1} IBA and 0.1 mgl^{-1} GA_3.

Rooted plantlets were potted in Jiffy pots and acclimatized under mist.

Results and discussion

Frequent infections occurred during embryo germination. Infections most commonly appeared at the onset of embryo germination. However, in some cases they also occurred later on the already developed seedlings, which is particularly unfavourable (Mišić and Vinterhalter, 1980).

The terminal part, which was taken from germinated embryos, was cultivated on two standard media for multiplication of stone fruit species (Tab. 1). To accelerate the process of multiplying obtained hybrid seedlings, only two media were used, which gave best results with plum cv. Požegača (Ružić, 1982; Ružić and Cerović, 1985). The medium with 1 mgl^{-1} IBA produced a higher multiplication index and longer shoots (Fig. 1)

Table 1. Multiplication parameters of plum hybrid seedlings obtained by embryo culture

Medium	Multiplication index	Plant length (in cm)
MS* + BAP : IBA = 1 : 1	1 : 4.27	0.80
MS + BAP : IBA = 1 : 0.1	1 : 2.60	0.75

* Media tested contain 0.1 mgl^{-1} GA_3

At the base of shoots which multiplied, there was very little callus. Newly-formed shoots had relatively less growth and development on both media, compared to other stone fruit crops (Ružić, 1982). However, individual shoots were sometimes up to 2 cm in length. The medium with 0.1 mgl^{-1} IBA produced shoots with short internodes, i.e. with rosette appearance.

The two standard rooting media gave a very high rooting percentage (Ružić and Cerović, 1985) (Tab. 2).

Table 2. Rooting of plum hybrid seedlings obtained by embryo culture

Medium	No days after appearance of first roots	Rooting (%)	Average length of roots (cm)	Average number of roots	Average length of lateral roots (cm)
MS 1/2 + IBA : GA_3 = 2 : 0.1	19	100	1.17	4.5	0.5
MS 1/2 + IBA : GA_3 = 1 : 0.1	12	100	1.00	4.0	0.3

The root number and length were slightly higher on the medium with 2 mgl^{-1} IBA, although roots faster initiated on the medium with 1 mgl^{-1} IBA (as early as after 12 days).

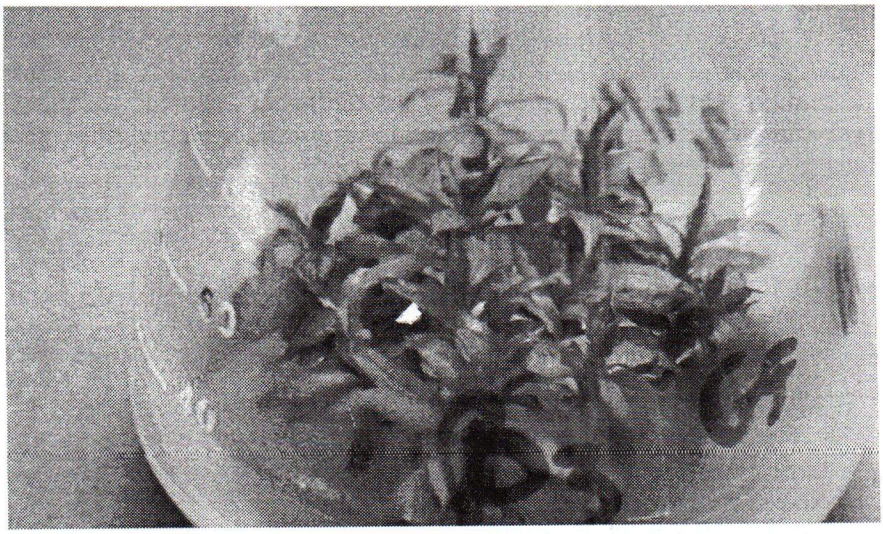

Fig. 1. Hybrid plum seedlings on multiplication medium

The rooted plantlets were well acclimatized under mist. After 2 months of acclimatization, they could be transferred outdoors (Fig. 2).

Fig. 2. Acclimatized hybrid plum seedlings after 2 months

Conclusion

Embryo culture is an important method that could be successfully used for obtaining hybrid seedlings from seed of early plum cultivars with insufficiently developed embryos.

However, the combined application of embryo culture and micropropagation was proved as highly efficient for overcoming the losses occurring in the embryo culture procedure. Thus, micropropagation over a short time interval can yield a large number of uniform plants from hybrid seedlings obtained by embryo culture.

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KOMBINOVANA PRIMENA EMBRIO KULTURE I METODE MIKROPROPAGACIJE *in vitro* U RAZMNOŽAVANJU RANIH SORTI ŠLJIVE (*Prunus domestica* L)

-originalan naučni rad-

Đ. Ružić, R. Cerović
Institut "Serbija", Centar za voćarstvo i vinogradarstvo, Čačak

Rezime

U radu je ispitivana mogucnost kombinovane primene embrio kulture i mikropropagacije *in vitro* ranih sorti domace šljive.

Embrioni su u sterilnim uslovima postavljeni na medijum koji je sadržavao saharozu, agar i mineralne soli po Murashige i Skoogu (1962). Posle klijanja uzimani su vrhovi sejanaca koji su dalje umnoženi metodom mikropropagacije. Index multiplikacije se kretao od 1 : 2.41 do 1 : 4.27, a procenat ožiljavanja od 87.5-100%.

Ožiljene biljcice dobro su se aklimatizovale pod mist izmaglicom i posle 2 meseca mogle su se preneti u spoljne uslove odgajivanja.