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INFLUENCE OF THE CONTAINER SIZE ON THE RATE OF *PRUNUS AVIUM* SHOOT MULTIPLICATION

BRANKA PEVALEK-KOZLINA

(Department of Molecular Biology, Faculty of Science, University of Zagreb)

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Experiments were carried out to investigate the growth response of wild cherry shoots inoculated into two container types (test tubes, 30 × 160 mm and Erlenmeyer flasks, 100 ml), possessing identical quality and quantity of medium (mod. WPM medium supplemented with BA, IBA and GA_3) per shoot, the same opening size, glass and closure type and external environment, but different air volume and surface area. The shoot multiplication of all the six genotypes tested was significantly higher ($P < 0.01$) in Erlenmeyer flasks in comparison with test tubes.

Introduction

The effect of vessel type and size on plant cultures is not often recognized and is certainly not understood, although the shape and size of containers can have important consequences.

In general, the fact is that cultures in larger flasks grow and develop better than in small flasks. Meier-Dinkel (1987) obtained a higher number of shoots in *Quercus robur* and *Q. petraea* cultures when they were cultured in bottling jars (250 ml) than in test tubes (25 × 150 mm). Similar results were observed, but not documented, with some other woody species (Bilkey and McCown 1978). The experiments with *Saintpaulia* (Bilkey and McCown 1978) confirmed this observation. Start and Cummings (1976) demonstrated that shoot growth of *Saintpaulia* was greater in 120 ml jars than in 60 ml ones. Wozniak at al. (1982) also found that the quantity and quality of shoots in *Gerbera* shoot tip cultures were better in 125 ml jars than in 25 × 150 mm test tubes. It is not yet clear why vessel volume has such a strong effect on culture growth. The hypothesis is that larger air volume of vessels permits adequate air exchange and toxic gases evolved (CO_2, ethylene, etc.) are more diluted. The closure type has also a strong influence on culture growth, for example on the occurrence of vitreous plants. The way in which a container is closed influences also the exchange of the inner gas phase with the surrounding atmosphere. However, many of the responses to vessel characteristics are probably highly tissue- and genotype-specific and it is very imporant to recognize them.

In these experiments I tested the shoot growth, i.e. the multiplication rate of wild cherry shoots inoculated in different types of containers.

Material and Methods

Material

Multiple shoot cultures of 6 genotypes of *Prunus avium* were established from juvenile plants, i.e. one-year-old plants (genotype 1 and 3), five-year-old tree (genotype 5/11) and also from adult trees, i.e. suckers initiated on tree roots (genotype P0, P3 and P5). The buds from actively growing shoots were used as initial explants. They were successfully disinfected in a chlorine product (Izosan-G, »Pliva«, Zagreb) and mercuric chloride (Pevalek-Kozlina and Jelaska 1987).

Methods

Wild cherry shoots were subcultured on modified Woody Plant Medium, WPM (McCown and Lloyd 1981) without chlorine ions and with 60 mgl^{-1} Na FeEDTA, containing 2% sucrose, 0.9% Bacto-agar, 2.2 μM 6-benzylaminopurine (BA), 2.5 μM indole-3-butyric acid (IBA) and 0.3 μM gibberellic acid (GA_3).

The explants were inoculated in two types of culture containers by: (1) single node in test tubes (30 × 160 mm) filled with 15 ml of nutrient medium and (2) by three inocula of a node in Erlenmeyer flasks (100 ml) filled with 45 ml of medium. Both test tubes and flasks contained the same volume of medium per explant, medium depth and had the same size of opening. Only the air volume and surface area varied (Table 1). After inoculation, containers were capped with cotton plugs and aluminium foil.

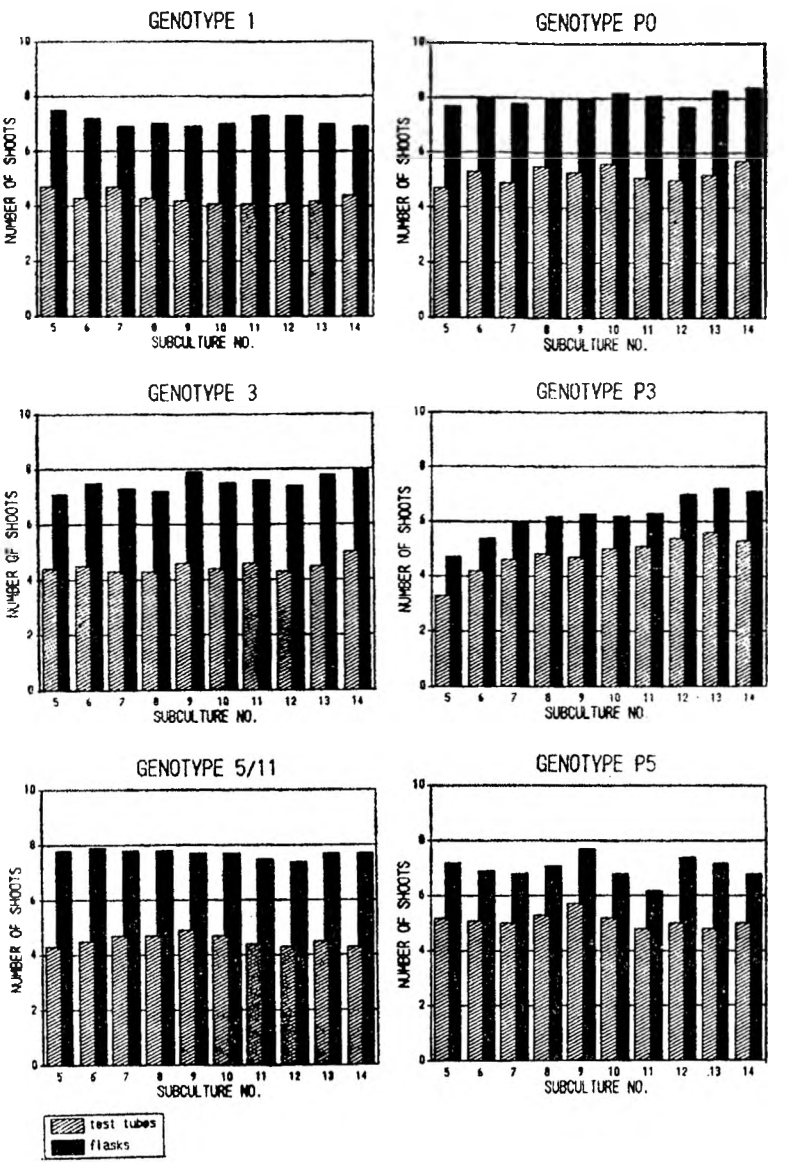

Fig. 1. Effect of container size on multiplication rate in wild cherry culture. Basal medium: mod. WPM, 2% sucrose, 0.9% agar, 2.2 μM BA, 2.5 μM IBA and 0.3 μM GA_3 (estimated after 4 weeks in culture).

Table 1. Culture conditions in two types of culture containers used.

CULTURE CONDITIONS	CULTURE CONTAINER TYPE	
	Test tubes	Erlenmeyer flasks
Medium capacity (ml)		
Total	15	45
Per explant	15	15
Air capacity (ml)		
Total	80	130
Per explant	80	43.3
Medium depth (mm)	28	23
Surface area (cm^2)		
Total	6.2	28.2
Per explant	6.2	9.4

The pH of the medium was adjusted to 5.6 before autoclaving (15 min at 115°C and 1.2 $kgcm^{-2}$). The cultures were incubated in a growth room at 24° ± 2°C under a 16 hr photoperiod and day-light fluorescent tubes illumination (TEŽ — Zagreb, 40 W, with a special range of 40—700 nm, 17 MW^{-2}).

The results were analyzed using ANOVA ($P < 0.01$).

Results

The number of developed shoots (over 0.5 cm in height), i.e. multiplication rate was estimated after a 4 week incubation period through 10 successive subcultures (5th to 14th subculture). Every subculture comprised 20 replicate cultures at least.

The multiplication rate of all the genotypes tested was significantly higher when grown in Erlenmeyer flasks than in test tubes. The quantity of medium per explant was the same in both container types used, as well as the medium layer depth, but air capacity and surface area varied. The air capacity was about 50% greater in test tubes than in flasks while the surface area was greater in flasks (Table 1). Some differences in growth rate between individual genotypes were evident. The multiplication rate, i.e. mean number of shoots after 4 weeks in culture through 10 successive subcultures differed slightly from genotype to genotype; it was from 4.3 to 5.2 when shoots were grown in test tubes, and from 6.2 to 8.0 in Erlenmeyer flasks. The fact is that all shoots grown in flasks multiplied better. All shoots obtained were vigorous, green and had normally developed leaves and vitrification was not noticed.

Discussion

The experiment on wild cherry cultures has shown that the type of glassware has an effect on culture growth, as indicated by other authors (McCown and Sellmer 1987). Meier-Dinkel (1987) also noticed a better development of *Quercus robur* and *Q. petraea* cultures when cultured in jars than in test tubes but did not explain it. McCown and Sellmer (1987) observed similar results with some other woody species, but the results were not documented. A similar phenomenon was observed in herbaceous plants cultures. Bilkey and McCown (1978) obtained better growth in *Saintpaulia* cultures when cultivated in 125 ml Erlenmeyer flasks than in 30 ml glass jars. Wozniak at al. (1982) reported that the type of culture vessel used influenced the quantity and quality of shoots in *Gerbera* shoot tip cultures with 125 ml jars giving better results than 25 × 150 mm test tubes.

In my experiments with *Prunus avium* cultures I also noticed a significantly higher multiplication rate when shoots were grown in Erlenmeyer flasks than in test tubes, although both container types tested possessed an identical quality and quantity of medium per shoot, medium depth, opening size and external environment. I must emphasize that some genotype-specific influences were also present, but they had not such a strong effect on shoot multiplication as the vessel type did.

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SAŽETAK

UTJECAJ TIPA POSUDE NA BRZINU MIKRORAZMNOŽAVANJA DIVLJE TREŠNJE

Branka Pevalek-Kozlina

(Zavod za molekularnu biologiju Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Istražen je utjecaj dva tipa posuda za kultiviranje na rast izdanaka divlje trešnje (*Prunus avium* L.) u kulturi *in vitro*. Praćena je stopa multiplikacije u epruvetama veličine 30 × 160 mm i tikvicama po Erlenmeyeru od 100 ml na mod. WPM mediju uz dodatak 2,2 μM BA, 2,5 μM IBA i 0,3 μM GA_3. Sastav i sadržaj medija po eksplantatu te vrsta stakla, promjer otvora i tip zatvaranja bili su identični. Volumen zraka po eksplantatu bio je veći u epruvetama, dok je površina medija po eksplantatu bila veća u tikvicama. Kod svih šest istraženih genotipova uočena je signifikantno veća stopa umnožavanja ($P < 0,01$) izdanaka uzgajanih u Erlenmeyerovim tikvicama u usporedbi s epruvetama.

Dr. Branka Pevalek-Kozlina
Department of Molecular Biology
Faculty of Science
University of Zagreb
Rooseveltov trg 6, P. O. Box 933
41001 Zagreb, Yugoslavia