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The Comparison on the Proliferation of Lateral Buds of *Vitis vinifera* L. cv. Perle de Csaba during Different Periods of the Year in *in vitro* Conditions

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ABSTRACT

In this study, it was aimed to examine and compare the proliferation capacity of early ripening grape species of *Vitis vinifera* L.cv. Perle de Csaba's lateral buds in *in vitro* conditions according to the months by the micropropagation method. During micropropagation, Murashige and Skoog (MS, diluted as 1/2) medium containing 30 g L^{-1} sucrose, 2 mg L^{-1} 6-benzylaminopurine (BA) and 7 g L^{-1} agar were used. 1/2 MS medium supplemented with L mg L^{-1} α- Naphthaleneacetic acid (NAA) was used for rooting of cutting. In year time period rooted seedlings were obtained from cultured lateral buds and the healthiest and productive seedlings were obtained from materials cultured in November, December and January. In the experiment time period the most seedling development month is December (80%) in contrast to the months of June and July, as the development seedling is about 10-15%.

Key Words: *Vitis vinifera; In vitro;* Lateral buds; Micropropagation

INTRODUCTION

In vitro multiplication is the most common proliferation tecnique for many fruit species like almond (Işıkalan, 2003), actinidia (Adıyaman, 2003) and pistachio (Onay, 2000a; Tilkat, 2003; Onay *et al*., 2003). The grapevine (*vitis spp*.) is the world's widely grown grape fruit crop (Monette, 1988). Grapevine micropropagation is being carried out by axillary shoot development on shoot tips and by adventitious shoot production on either fragmented shoot apicies or leaf blade explants. The degree of success obtained at each stage depends on the genotype of the grapevine, as different *vitis* species, cultivars and hybrids respond differently to a given set of cultural conditions (Chee & Pool, 1983). Shoot tips from greenhouse, shadehouse and field-grown vines have been successfully used for micropropagation (Chee & Pool, 1982; Haris & Stevenson, 1982; Stevenson & Monette, 1983).

Shoot development was increasingly successful in buds taken from nodes at increasing distance from the apex (Novak & Juvova, 1980) and obtained a higher percent survival in explants axillary shoot tips than in those from terminal shoot tips (Yu & Meredith, 1986). It has been reported that, in their study on grapevine meristem culture, the most favourable period for cultivation of meristem was the month June (Choi *et al*., 1993).

Namli (1995) used MS containing 2 mg L^{-1} BAP starting from lateral buds of *Vitis vinifera* L.cv. Cardinal grapevine species for micropropagation and suggested that the best development period in *in vitro* conditions was that of June. While, Işıkalan *et al*. (1998) micropropagated *Vitis vinifera* L.cv. Alphonse by 2 mg L^{-1} BA and during rooting, 2 mg L^{-1} NAA was added into the 1/2 MS. They reported that rooted seedling were obtained from *Vitis vinifera* L.cv. Alphonse lateral buds, cultivated in yearly period of time, and ıt was determined that the success rate of seedlings obtained from materials cultivated in the months November and December was about 80-85%.

The aim of the present study was to determine the best isolation period for proliferation capacity of lateral buds of *Vitis vinifera* L.cv. Perle de Csaba.

MATERIALS AND METHODS

In this study, lateral buds of *Vitis vinifera* L.cv. Perle de Csaba was used as source of plant material. The material was provided from the vineyard in the campus of Dicle University, Diyarbakır province of south-east Turkey. Lateral buds of Perle de Csaba used as material were firstly washed with tapwater for 15 minutes; then, depending upon the development of the material,they were kept in 70 or 96% ethanol for 30 seconds, in 3 or 5% sodium hypochlorite (NaOCI) for 30 minutes. Later they were cleaned from NaOCI by rinsing them in sterilized water five times for five minutes. The buds isolated in this way were separetely transferred into 24 test-tubes containing the half strength MS medium (Murashıge & Skoog, 1962) supplemented with 2 mg L^{-1} 6-benzylaminopurine (BAP).

The basal MS medium contained 30 g L^{-1} sucrose and 7 g L^{-1} DIFCO-Bacto agar. The pH of the medium was adjusted to 5.8, and the medium was sterilized autoclaving at 1 MPa for 20 minutes. Explants were left to grow in the culture room under 3000 lux 16/8 photoperiod at 25 ± 2°C. Unless otherwise stated, all experiments were conducted with 24 explants per treatment; repeated three time in a months. The number of shoots and shoot length were recorded after six weeks of culture. Shoots obtained in *in vitro* conditions from lateral buds were rooted on the half strength MS medium containing 1.0 mg L^{-1} NAA. The rooting responce was scored after 30 days of culture.

In vitro rooted shoots were washed over night in running water before being potted in 1:1 mixture of sand and soil. Plantlets, were covered with a plastic beaker to maintain 90 ± 5% relative humidity for 4-5 week before transfer into the growth room. The growth room was illuminated by mercury fluorescent lamps (400 w). The plants were irrigated every 2-3 days with water and after 30 days were succesfully adapted at *in vivo* conditions

RESULTS AND DISCUSSION

The lateral buds were found to increase in volume approximately 15 days and formed the first leaves after 25 days after culturing (Fig. 1). This primary development and transforming period into plants varied according to the months in that lateral buds were isolated.

Fig. 1. First state of lateral buds on half strength MS medium with 2 mg L-1BA

Fig. 2. Development seedlings from lateral buds cultured in July

Fig. 3. The most favourable and productive seedlings from materials cultivated in December

Fig. 4. The adaptation at in vivo conditions of plantlet regenerated from lateral buds

Table I. Amean long and development rate of lateral buds of Perle de Csaba proliferation depend on monthly isolated during the year in 1996-1997

Months	Shoots per explants	Shoot length (cm)	Average rates of development (%)
June	3.66	1.5	15.25
July	4.66	1.5	19.41
August	7.33	2.5	30.54
September	10.66	3.0	44.41
October	12.00	3.3	50.00
November	17.66	4.5	73.58
December	19.33	6.0	80.54
January	15.66	4.0	65.25
February	14.33	3.5	59.70
March	12.00	3.0	50.00
April	9.66	2.8	40.25
May	7.33	2.0	30.54

Data recorded on the 40th day and presents an average of 24 replicates per treatment with three time repetitions of the experiment

The mean number of shoots and average lenght of shoots that grown up from cultured lateral buds were shown in the Table I. All tested treatments induced new axillary shoots from the cultured explants (lateral buds). 40 days after incubation 15, 19, 30, 44, 50, 73, 80, 65, 59, 50, 40 and 30% of cultured lateral gave bud-shıoot proliferation from the explants collected in June, July, August, September, October, November, December, January, February, March, April and May, respectively.

From this experiment it appears that, best results for proliferation capaticy was obtained from the lateral buds isolated in December at the rate of 19.33 shoot explant in 40 days of culture (Fig. 3), whereas this rate of 3-4 shoot per explant was observed from buds cultured in June, July (Fig. 2).

The isolation period of lateral bud is very important. Choi *et al*. (1993) determined that in the studies on S.9110 and Kyoho grapevine strains, the best time for culturing meristems was June. In a similar study on Cardinal grapevine, it was reported that more than 50% development was obtained in June and July, and the best period was found to be july (Namlı, 1995). Işıkalan *et al.* (1998) indicated that *in vitro* propagation studies, the isolation period of meristems were important and that the lateral buds of Alphonse grapevine must be cultured in November.

The results obtained show that the culturing period of micropropagation of Alphonse determined by Işıkalan *et al.* (1998), is in paralel with our work, whereas the differences obtained with culturing period of other grapevine varieties (Cardinal, Kyoho, S.9110) determined by Namlı (1995) and Choi *et al.* (1993).

As a results of this study, it may be concluded that the best time for culturing of lateral buds varies for each species and even for their varieties. The shoots developed in *in vitro* conditions were rooted on 1/2 MS medium with 1.0 mg L^{-1} NAA. The *in vitro* rooted shoots were succesfully adapted at *in vivo* conditions (Fig. 4).

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