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Comparative Studies on the Proliferatıon of Lateral Buds of *Vitis vinifera* L.cv. Cardinal During Different Periods of Six Months of the Year at *in Vitro* Condition

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ABSTRACT

In this study, we aimed using the lateral buds of *Vitis vinifera* L.cv. Cardinal and with tissue culture method *in vitro* conditions on MS medium in a six months period to determine different monthly proliferation. We used for the lateral buds for the micropropagation period a ½ MS nutrition medium containing 2 mg/I BAP (6-benzyl amino purine). The seedlings, developed in this nutrition medium after few sub-cultures were put in a supported rooting solution 2 mg/I BAP + 1 mg/I NAA and we obtained rooted seedlings in a short period of time. The best buds efficiency was obtained, over 50%, during the months of June and July. As a conclusion from the fifth week of culture were obtained from each lateral bud 4 - 7 seedlings and in a period even shorter than one-year a successful quantity of healthy seedlings.

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

INTRODUCTION

The *Vitis vinifera* L.cv. Cardinal is a perennial semi-woody plant from *Vitaceae* family. It can be found in three general centers: Eurasia, Far Orient and America (Alleweldth, 1983). It was bought from California as an early grape variety and was producted in our country. The grape, which used a fruit type in our country in many different ways not only has a big importance for healt but also has an alimentary importance.

In vitro multiplication is the most common proliferation technique for many fruit species like pistachio (Tilkat *et al.,* 2005), actinidia (Adiyaman, 2003), almond (Işıklan, 2003) and vitis (Namli, 1995). Connected to the subject, Barlas and Scene, have a very successful research on using *Vitis vinifera* shoot tip cuts for regeneration and propagation at *in vitro* and on the factors that influence that (Barlas & Skene, 1979; Barlas & Skene, 1979; Barlas & Skene, 1980). Lé, used for propagation of grapevine shoots a 10 mm/I BAP modified MS nutrition solution (Lé, 1987). According to Chee and Pool, the 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 success of the micropropagation is connected to the genotype of the grapevine used. Because of that, the varieties cultivated will show different reactions *in vitro* culture conditions (Chee & Pool, 1983). Novak and Juvova emphases that shoot development were increasingly successful in buds taken from nodes at increasing distance from the apex *in vitro* micropropagation (Novak & Juvova, 1980). Goussard, got the maximum of the shoot *in vitro* production culture using a mixed BAP and Zeatin in 2 mg/I nutritive solition (Goussard, 1981). Choi *et al*. (1993) reported that for meristem *in vitro* grapevine culture the best month is May.

The purpose of this study is using that information to determine the best period for lateral buds micropropagation, to determine the best way for the production of rooted seedlings for *Vitis vinifera* L.cv. Cardinal and to create a protocol for other researchers in the domain.

MATERIALS AND METHODS

For this study we used the lateral buds of *Vitis vinifera* L.cv. Cardinal. The material was taken from the vineyard of Dicle University. We used the Gauthered *in vitro* laboratory techniques in our work (Gautheret, 1959).

Lateral buds of the Cardinal used as material were firstly washed with tapwater for 15 min; then, depending up on the development of the material, they were kept in 96% ethanol for 40 seconds, in 5% NaOCl (sodium hipochlorite) for 30 min. Later they were cleaned from NaOCl by rinsing them in sterilized water 5 times for 5 min. The buds isolated in this way were separately transferred into 72 test-tubes containing the half strength MS medium (Murashige & Skoog, 1962) supplemented with 2 mg/I BA (Fig. 1). The basal MS medium contained 30 g/I sucrose and 8 g/I DIFCO-agar. The pH of medium was adjusted to 5.6 and the medium was sterilized outoclaving at 1 MPa for 20 min. Explants were left to grown in the culture room under 3000 lux 16/8 photoperiod at 25 ± 2ºC. After three consecutive replications the leafet seedlings were moved for rooting in a ½ MS + 1 mg/I NAA nutritive medium and after two weeks

we got rooted seedlings (Fig. 2).

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 humiditiy for 4 - 5 weeks 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 successfully adapted *in vivo* condition (Fig. 3).

RESULTS AND DISCUSSION

The lateral buds were found to increase in volume approximately 10 days and formed the first leaves after 17 days after culturing (Fig. 4). Afterwards was noticed the formation of stem buds and a kologenez activity. From the 40th day to in every bud was obtained 4 - 7 seedlings. The seedling transferred into rooting medium after few sub-cultures. In approximate three weeks were determinate rooted seedlings putting the seedlings in rooting medium in aproximate three weeks were observed rooted seeds. We give the detailed development scale of lateral buds according to the year months in the Table I. According to that the Vitis vinifera L.cv. Cardinal that we used for culture lateral buds showed different development stages according to year months and the best months for starting this process when we can see a development rate of 50% are June and July.

The isolation period of lateral bud is very important.

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

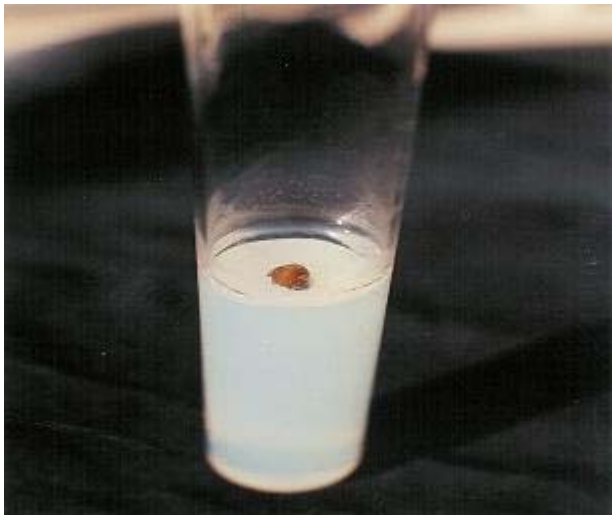

Fig. 2. Development seedlings from lateral buds cultured in June and July

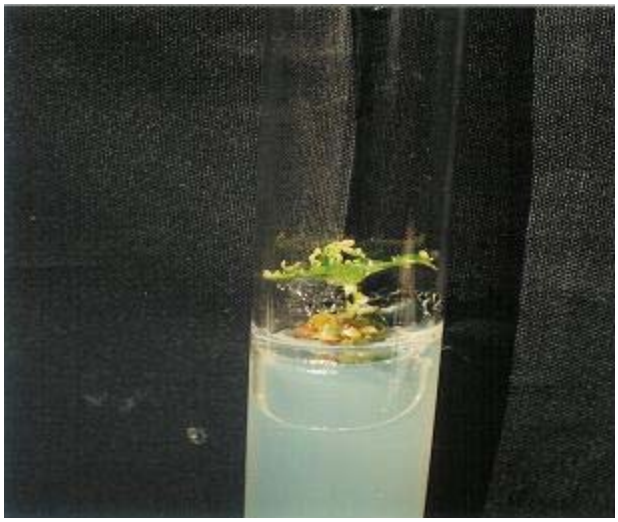

Fig. 3. The root formation on half strength MS medium with 1 mg/l NAA

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 Cardinal proliferation depend on monthly isolated during the year in 1995–1996

Months	Total tube number	Evaluation of the same shoot length of same tubes	Average of shoot length (cm)
		25 tubes	0,8 - 2,0
February	96	26 tubes	2,0 - 3,5
		45tubes	3,5 - 4,4
		25 tubes	0,8 - 1,7
March	96	26 tubes	1,7 - 3,0
		45tubes	3,0 - 4,6
		25 tubes	0,8 - 1,8
April	96	26 tubes	1,8 - 3,5
		45tubes	3,5 - 4,8
		25 tubes	0,9 - 2,0
May	96	26 tubes	2,0 - 3,2
		45tubes	3,2 - 5,0
		25 tubes	1,5 - 2,0
June	96	26 tubes	2,0 - 4,4
		45tubes	4,4 - 7,0
		25 tubes	1,5 - 2,5
July	96	26 tubes	2,5 - 5,5
		45tubes	5,5 - 7,0

Choi *et al*. S9110 and Kyoho grapevine strains in their research on different grape sorts they determined the best period for meristem culture as the month of June. Researches on *Vitis vinifera* L.cv. Alphonse lateral buds indicate that in the period of November and December the productivity reaches the most favorable point with efficiency of 80% to 85% (Işikalan *et al*., 1998). In paralel with this research, Adıyaman *et al*. (2004) worked on the culture of lateral buds of *Vitis vinifera* L.cv. Perle de Csaba, and reported that for an efficient productivity of 80% the best period is the month of December. The studies of Mellor and Stace-Smith on potatoes meristem showed as the best period for rooting is the spring time and the beginning moths of summer (Mellor & Stace-Smith, 1969). Rosati *et al*. (1980) on their study on *in vitro* propagation, they found that the populus tree meristem the best period of culture is the winter moths. The results of our research show accordance to the study of Choi *et al*. (1993) and Mellor and Stace-Smith (1969). The results obtained show that the culturing period of micropropagation of populus apex determined by Rosati *et al.* (1980) are in parallel with the studies on *Vitis vinifera* L.cv. Alphonse by Işıkalan *et al.* (1998) and on *Vitis vinifera* L.cv. Perle de Csaba by Adıyaman *et al.* (2004), whereas, shows rather different resultrs from our work.

As a conclusion we can say that for the culture of lateral buds every species and more every variety of species we have different periods of time for the best productivity. Also as a conclusion we can say that for Cardinal grapevine varietes a very basic solution of MS especially with the influence of BAP is enough to determine a process of micropropagation.

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