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Comparative study of the effect of culture media and hormonal treatment on callus formation and regeneration of some native Sistan grapes

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ABSTRACT

Grapevins have Bronze ages archive in Sistan area of Iran.a comparative study on the effect of different culture media (MS, MS/2 , Chee and Pool, WPM) and 3 levels of BAP (0, 1,2 mg/l) on callus production of 2 native cultivars tissue was carried out. Explants from shoot and leaf tissue were transferred onto different culture media. results showed MS culture medium had maximum effect on callus production in stem apical meristem tissue and effective hormone treatment was 2 mg/l BAP and 4-6 weeks in darkness. 0.5 mg of callus transferred to culture media with NAA 10 µm and BAP 5 µm. shoot proliferation was obtained by subculturing the micro cutting on same media as used for shoot formation. Maximum rooting occurred on ½ MS culture medium without hormone.

Key Words: Regeneration, Callus, Kinetin, NAA.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops grown in the world today in terms of both total acreage and dollar value (Gallettaand Himerlic 1989). Grapes belong to the Vitaceae family, which comprises of about 60 species. There are over 100 species reported in the literature, 65 of which are thought to be genuine and another 44 are questionable, probably they are inter specific hybrids. Preliminary observation trail conducted at BARI concluded that grape can be grown in Bangladesh as a fruit crop (Biswas and Nazrul 1997). The use of *in vitro* techniques for propagation of various *V. vinifera* cultivars has been well documented (Chee and Pool 1982; Singh et al. 2000; Mhatre et al. 2000; Singh et al. 2004).

Protocols have also been reported for muscadine grape (Lee and Wetzstein 1990; Gray and Benton 1991; Sudarsono and Goldy 1991; Thies and Graves 1992; Torregrosa and Bouquet 1995) and some wild grapes (Poudel et al. 2005). It is evident that there is little empirical data on the *in vitro* cloning and performance of several new grape rootstocks. The first report of *in vitro* culture of grapevines was by Morel (1944). Muscariarmeniacum is commonly known as grape hyacinth owing to its clusters of small, bell-shaped, cobalt-blue flowers that look like clusters of upside-down grapes (Grey-Wilson et al. 1981). M. armeniacum, belonging to the family Asparagaceae, is cultivated in pots and gardens in

the temperate regions. Traditional methods of propagation of Muscari species are rather slow, since the bulblet production from the mother bulbs is extremely low (Uzun et al. 2014). Tissue culture has helped to develop new strain of food crops, cereals, vegetables flowers, oil seeds and plantation crops such as spices, coffee, tea and rubber. The present investigation was undertaken to identify the best hormonal combination for callusing of Fakhri and Red Yaghooti on different media and investigate the shoot regeneration and root induction ability of different explants of grape, during spring, 2014, University of zabol, Iran.

MATERIAL AND METHODS

Young actively growing shoot tips were harvested from 5-to 7 –year –old at the Zabol Research Center experimental vineyard and placed between layers of moist paper towels.in the laboratory, shoot tips were further dissected to remove all extraneous leaves and tendril. Fully expanded leaves were sterilized for 15 minutes in 0.5% sodium hypochlorite containing a few drops Of Tween 20 and rinsed three times in sterile distilled water. Shoot tips and stems with 1.0 cm in length were individually placed in culture tubes. Leaf disks, without midribs, were excised with a cork borer (5ram diameter) and placed abaxial side up on the culture medium. Average of the initial fresh weight of leaf disk was about 3.8mg. Culture media were prepared to analysis the effect of the combination of basal media (MS, MS/2, Chee and Pool, WPM) and 3 levels of BAP (0, 1, 2 mg/l) on callus production of shoot tip, stem and leaf VitisVinifera. After explant transfer, culture tubes were sealed with Parafilm, then placed upright and maintained at 20˚C under darkness.the primary explants were cultured 4 to 6 weeks, then callus were excised and cultured on fresh medium for another 5 weeks. Then the growth rate of the callus calculated with formula of Banthorpe (1984).

$G = m_1 - m_0 / m_1 \times t$
G = growth rate
$m_1 =$ weightprimery of callus production
$m_0 =$ weight of callus production after 5 weeks
t = time / day

After about 40 days of culture, the induced primary callus was transferred to vitamin-, inositol- and MS medium supplemented with NAA 10 µm and BAP 5 µm. Some of the callus was transferred to MS or 1/2 MS with no plant growth regulators. The experiment was repeated 3 times from each previous culture cycle and the data were pooled as above. , subsequently, regeneration of *Vitis vinifera* was compared using different media with hormonal treatment. The three replications of a 3*3*4*6 factorial, completely randomized design were used in the analysis of interaction between different explants, levels of hormone, culture media and cultivars on callus formation (table 1). The effects of different levels of NAA and BAP on the callus regeneration were analysed using three replications of a 3*3 factorial completely randomized.

RESULTS AND DISCUSSION

The presence of plant growth regulators in the culture medium is often considered to be the major factor for callus development. Almost all original explants produced callus. All of the samples had a large basal callus, which was excised and discarded with subsequent subculture. Shoot tips on MS medium with BAP 2 mg/l of Fakhri cultivar produced the most fresh and dry weight and volume of callus. Stem on WPM media with BAP 2 mg/l of Red Yaghooti cultivar produced the least fresh and dry weight of callus. Whereas the cultures on different media without BA did not respond. Aerial root formation was also observed in case of induced callus. Shoot tips and stem cultures at ½ MS without hormone produced more aerial

Table 1. Grape ANOVA statistical analysis.

Source	df	Weight
A	2	0.01**
B	3	0.002**
C	2	0.001**
D	1	0.013**
A*B	6	0.00079**
A*C	4	0.001**
A*D	2	0.0009**
B*C	6	0.003**
B*D	3	0.002**
C*D	2	0.001**
A*B*C	12	0.001**
A*B*D	6	0.001**
B*C*D	6	0.002**
A*B*C*D	16	0.001**
Error	144	0.00008
CV	---	15.63

A=level B= culture media C=explant D=cultivar
**=Significant at 1%

roots as compared with other treatments. Number and length of roots in these cultures increased with passage of time. The test cultivars differed in callus production rates with Red Yaghooti significantly performing the poorest (p>0.01) on all media. However Fakhri was significantly better (p> 0.01) here. A cytokinin * cultivar interaction is shown in (Table1) this interaction was due to differential responses of cultivars to cytokinin

concentration treatments .therefore optimization of cytokinin concentration for each cultivar was needed to maximize callus production. 0.5 mg of callus transferred to different culture media with NAA and BAP hormone .MS supplemented with NAA 10 μm and BAP 5 μm was found to be the most effective for maturation and conversion to whole plantlets. Approximately 30% of this callus

MS) BAP*NAA (5+10) **1/2MS) BAP*NAA (5+10)**

Figure 1. Plant regeneration of grape by BAPand NAA hormones.

germinated within 20-40 d after transfer to the above medium. Plantlets were successfully established in pots. A critical step is the maturation process, specifically the ability to complete callus formation leading to complete plantlets. In other species the importance of ABA and marmitol for differentiation and further germination has been demonstrated. However, in grapevine ABA or mannitol did not favour germination, whereas NAA 10 μm and BAP 5 μm were found to be effective. This is similar to results in *Pharbitisnil* (Jia and Chua 1992) and watermelon (Compton and Grey 1993).By comparison , optimal shoot multiplication occurred on MS medium with NAA 10 μm and BAP 5 μm. 1/2 MS was more effective in inducing rooting than MS. only 1/2 MS without hormone treatment significantly increased percent rooting. Some of the callus that was transferred to MS or 1/2 MS with no plant growth regulators only produced root.

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