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Improvement in Apex Culture in an Iranian Grapevine (*Vitis vinifera* L. 'Bidaneh Sefid') through Fragmented Shoot Apices

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ABSTRACT

Virus-free materials of an important Iranian grapevine (*Vitis vinifera* L.' *Bidaneh Sefid* '), propagated with simple and rapid *in vitro* propagation method, was grown through fragmented shoot apices. The growth of shoot apex fragments on liquid culture medium of Murashige and Skoog (1962) supplemented with 2.0 mg BAP/L resulted in leaf-like structures after 4 weeks. The leaf-like structures increased in size to 20-25 mm in length after transferred on solid medium and during 3 weeks shoots start to proliferated from basal swelling of these leaf-like structures which were sub-cultured regularly and obtained plantlets were rooted easily in a hormone-free medium of Murashige and Skoog in half concentration contained 20g/L sucrose. Root initiation started within 12 days. Then, plants were acclimatized and 90% of plants were transferred to greenhouse successfully. This *in vitro* propagation method showed potential to produce a large number of healthy plants from virus-free materials in a short period of time and also had a potential value in commercial clonal grapevine propagation. About 6000 plants were produced during 20 weeks just by only two subculturing from one single apex.

Key Words: Grapevine; Bidaneh Sefid; *In vitro*; Fragmented shoot apices

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 (Galletta & Himerlic, 1989). Every year, a variety of virus diseases occur on vines that effect on production and quality. Avoidance the viruses is the best way to protect the vineyards from virus diseases. Using certified materials ensure that the materials have been tasted for known strains of viruses and viruses have been eliminated. So vine plantation must be from certified virus-free sources. Since only a few plants are usually certified virus-free, it takes several years to propagate enough vines by conventional methods (Harris & Stevenson, 1979). One basic drawback of conventional methods, however, is that they don't allow a rapid buildup of grape material that is in limited supply. More and more often today as a result of clonal selection programs, as unique virus-free clones are identified by grape virologist, or as new varieties are produced by grape breeders, there is a need for the rapid buildup of unique techniques so that vines may be available in sufficient quantities for commercial production (Raymond *et al*., 1984). Tissue culture results in rapid clonal multiplication and uniformity in obtained clones, vigorous growth, normal yield and healthy plants (Blazina *et al*., 1991). In the event that a valuable clone is not available as certified material, it is possible to produce virus-free plants from infected vines, using heat-treatment and meristem culture. Many *in vitro* techniques can be used for mass clonal propagation of vitis species. Some reports concerning mass propagation of vitis species by shoot apices (Barlass & Skene, 1978; Chee & Pool, 1982; Fanizza *et al*., 1984; Goussard, 1981; Harris & Stevenson, 1979; Li & Eaton, 1984; Monette, 1983; Morini, *et al*., 1985), axillary buds (Jona & Webb, 1978; Lee & Wetzstein, 1990; Novak & Juvova, 1982; Pool & Powell, 1975), and some reports demonstrate the feasibility of producing vines via somatic embryogenesis (Krul & Worley, 1977; Krul & Myerson, 1980; Mullins & Srinivasan, 1976; Srinivasan & Mullins, 1980). From the economic point of view, after producing healthy plants, it needs a method that can potentially produce large number of healthy vines in a short period. To achieve this, in this study we described a simple and rapid method by which large scale of adventitious buds were proliferated from singe fragmented shoot apex of an Iranian grapevine cultivar Bidaneh Sefid. Apart from brief references (Barlass & Skene, 1978, 1980) formation of adventitious buds from single fragmented shoot apex of grapevine are still rare and has not been reported with Iranian cultivars yet. This cultivar is same as Thompson seedless cultivar and is used extensively for producing raisins. Raisins produce from this cultivar is considered unique in the world in kind and quality.

MATERIALS AND METHODS

Apical shoot-tips with 5-10 mm in length from virus-free plants of an important Iranian grapevine cultivar Bidaneh Sefid were obtained from meristem culture of grape rootstocks in Tehran University grapevines collection. Shoot-tips were surface sterilized in 70% EtOH for 30 sec. Subsequently the shoot-tips sterilized in 0.1% AgCl

containing 0.1% Tween 20 for 3 min constant stirring. After surface sterilization, shoot-tips were rinsed constantly three times in sterile distilled water for 5, 10, 15 min. Outer leaves of the apical buds removed in a laminar air flow cabinet, and shoot apices containing 2-3 leaf primordia were then excised. Individual apices were cut in to several fragments with scalpel on dry and pre-sterilized petri-dishes and then further teased apart in 10 mL liquid culture medium of Murashige and Skoog (1962) supplemented with 2.0 mg BA/L and 30 g sucrose/L.

Petri-dishes then were sealed with Parafilm and incubated in growth chamber at 28°C during a 16 h light period and 24°C during 8 h dark period. The light source was white fluorescent tubes providing 40 μE m^{-2} sec^{-1} at the culture level.

RESULTS AND DISCUSSION

Leaf-like structures with average 10-15 mm in length with basal swelling of the central vein obtained from the differentiation of cell clumps after 4 weeks on liquid medium and transferred to the MS medium supplemented with 2.0 mg BA/L and solidified with 6.5 g agar/L. The leaf-like structures increased in size to 20-25 mm in length on solid medium and during 3 weeks shoots start to proliferate from basal swelling of leaf-like structures (Fig. 1). After 6 weeks, 18-24 visible buds could be seen in each cluster (Fig. 2). Plantlets obtained in the first step after 6 weeks, are individually separated from the clump and sub-cultured to the same basal medium. After 3 weeks from each sub-culturing, 3-4 adventitious buds appeared (Fig. 3). Shoots allowed to growth from these adventitious buds eventually for 6 weeks (Fig. 4). Ideally shoots that are 1.5 cm long are selected for further culture. They should be trimmed 2 mm below the basal node and placed in to the medium taking care that their cut ends are submerged. Multiple shoot formation was occasionally accompanied by basal callus development during second sub-culture (Novak & Juvova, 1982). Shoots of the type suitable with at least 20 mm in length included 3-4 expanded leaves are trimmed 2 mm below their basal node, and planted vertically in to the clear and sterilized tube containing hormone-free basal medium of MS for root formation. Root initiation starts within 12 days. After 3 weeks, rooted plantlets which showing vigorous shoots and root elongation (Fig. 5) transferred to Jiffy pots containing Perlite and Peatmoss in proportion of 1:1. Plantlets maintained continuously under mist system in greenhouse condition and in order to moisture maintenance, pots were covered by plastic cover. After ten days, plants were transferred to another greenhouse without any mist system. At the end of second ten days, the covers removed and plants allowed to growth under greenhouse condition in bigger pots (Fig. 6). Rooted plants fertigatted by diluted nutrient of MS solution. once during 3 day for a month. 90% of plants transferred to greenhouse successfully.

Fig. 1. Proliferation of shoots from basal swelling of leaf-like structures 3 weeks after transfer to the solid medium

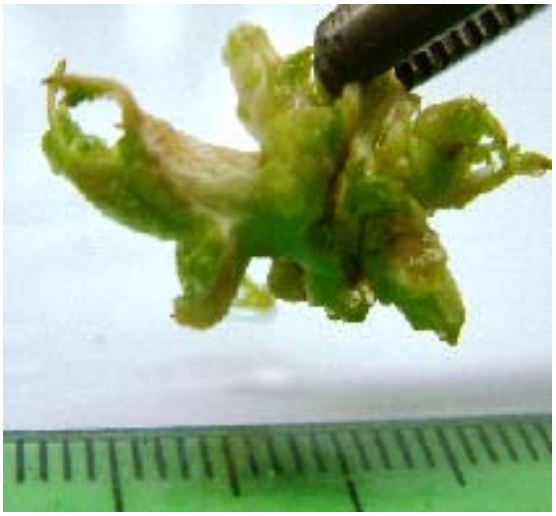

Fig. 2. Proliferation of shoots from leaf-like structure after 5 weeks

The formation of adventitious buds from fragmented shoot apices, for commercial clonal propagation of virus-free materials in about Iranian grapevine cultivars has not been reported yet. There are a few references about formation of adventitious buds from single fragmented shoot apex of grapevine (Barlass & Skene, 1978, 1980). In this study we reported a useful technique for induction of adventitious buds from fragmented shoot apices of an important Iranian grapevine cultivar Bidaneh Sefid obtained from shoot-tip meristem.

The described method is simple, rapid, involving only two media include proliferation and rooting media and has potential to produce about 6000 healthy single rooted plantlets during 4-5 month just by only two sub-culturing

Fig. 3. Adventitious buds 3 weeks after first subculture

Fig. 4. Developing the growth of shoots from adventitious buds and Shoots with expanded leaves 6 weeks after first subculture

Fig. 5. Root formation of single shoot were excised from cultures after transfer to hormone-free basal medium

Fig. 6. A plantlet of 'Bidaneh Sefid' grapevine 20 weeks after fragmentation of shoot apex

from one single apex. Barlass and Skene (1978) in their study on *in vitro* propagation through this method, could produced 8000 plantlets during 4 month. Also, root formation was commenced during 7 days in their study. These differences could be related to difference response of cultivars to *in vitro* condition. So, this method depends on cultivars and culture conditions, having the potential to produce more plantlets during the same period. Another advantage of this method is high rate of shoot and root formation, although further studies need on plantlets obtain from this method from point of chromosome abnormality and also ploidy levels of plantlets obtained after subculture. Barlass and Skene (1978) showed that chromosome counting has not revealed abnormal genetic condition in the plantlets of Cabernet Sauvignon cultivar. This *in vitro* propagation method has commercial potential to produce healthy clones of grapevine cultivars in a short time.

Although other *in vitro* micro-propagation techniques can be used in order to produce and propagation of virus-free plants obtained from *in vitro* thermotherapy and shoot-tip meristem culture, however large scale propagation of healthy plantlets by fragmented shoot-tip apices when there are a few number of virus-free plantlets, is more useful and has potential to produce large number of healthy plant materials in shorter time.

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