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In Vitro Micro-Grafting of Some Iranian Grapevine Cultivars

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Abstract

Shoot-tip micro-grafting of some Iranian grapevine cultivars were evaluated under in vitro conditions. Shoot-tip explants of four grapevine cultivars ('Soltanin', 'Fakhri', 'Sahebi' and 'Ghizil-Uzum') were grafted on in vitro derived shoots of 41B cultivar. The results showed that, both in vivo and in vitro derived shoot-tips had satisfactory growth characteristics. In vitro culture derived shoot-tips had higher (40.3- 60.9) successful graft union compared to in vivo grown shoot-tips. There was significant difference between cultivars regarding successful graft union and subsequent growth related traits. Shoot and root related growth characteristics of in vivo grown grafted shoot-tips had some advantages in contrast with in vitro grown explants. In conclusion, micro-grafting is an alternative suitable propagation method leading to higher growth potential of grafted populations.

Key words: Grapevine, Micro-grafting, Shoot-tip, In vitro, 'Soltanin'

Introduction

Grapevine is a major horticultural crop with great applications in food and pharmaceutical industries. The higher proportion of grapevine production is recorded in temperate regions of the world. However, some cultivars have cultivation potential under high-temperature tropical and sub-tropical conditions. Food and Agriculture Organization (FAO) reported that annual worldwide production of grapevine was 60.9 million tons [1]. Some of grapevine orchards are un-grafted own-rooted plants. However, these are highly sensitive to insects, fungal diseases, nematodes and especially phylloxera, leading to heavy yield and quality losses. Grafting of disease-free explants is an appropriate alternative for propagation of grapevine cultivars [2,3]. Micro-grafting is a well recognized propagation method capable of use in most plant species with promising results. In this method, shoot-tips of 0.1- 0.8 mm in length are accurately grafted on seed born or in vitro culture derived plantlets. Micro-grafting is a commercial practice for production of virus free plant materials, especially in test tubes, since 1970s [4,5,6]. Micro-grafting as a clonal propagation method for production of virus and other disease-free plant materials hold some advantage against thermotherapy, meristem culture or integrated thermo-treated meristem culture. The main advantages of this method over other methods have been described as virus elimination as well as improved growth and productivity of resulting grafted plants [2]. This procedure was able to overcome some physiological and anatomical problems encountered in some species and cultivar combinations [7,8,9]. Soot-tip origin and preparation are main factors in successful micro-grafting operation. These factors are highly dependant on species and cultivars involved in grafting as well as propagator ability. The diverse sources of shoot-tip explants are cuttings from new shoots of thermo-treated plants, shoots from single nodes of in vitro cultures or easily formed new shoots of in vivo grown plants [10,11]. The aim of the present experiment was to evaluate the micro-grafting responses of some important Iranian cultivars under in vitro conditions.

Material and Methods

This experiment was conducted in the Tissue Culture Laboratory of Horticultural Sciences Department, University of Maragheh, Iran during 2008- 2009.

Plant material: Four highly consumed and well recognized Iranian grapevine cultivars: 'Soltanin', 'Fakhri', 'Sahebi' and 'Ghizil-Uzum' were employed as shoot-tip explant donors. 41B was utilized as rootstock for micro-grafted plants. Cuttings of rootstock were cultured in common rooting media (perlite + vermiculite as 8:2). Single node explants were taken from 10 cm new shoots. Explants were surface sterilized in 15% sodium hypochlorite for ten minutes. Thereafter, the nodal cuttings were three times rinsed with sterilized distilled water. Explants were cultured in MS [12] medium solidified with $6gL^{-1}$ agar and enriched with $0.1mgL^{-1}$ TDZ, $0.5mgL^{-1}$ IBA and $30gL^{-1}$ sucrose. Cultures were maintained in incubator for 3 weeks under 16/8 photoperiod and 24±1˚C. Consequently, shoots were transferred to MS medium enriched with $0.5mgL^{-1}$ BAP and $0.5mgL^{-1}$ GA_3 for shoots elongation. Three weeks old shoots were selected as rootstock for micro-grafting operation.

In vivo shoot-tip preparation: Shoot-tip explants were derived from in vivo and in vitro culture grown plants. Shoot-tip explants of in vivo grown plants were afforded from selected, well-nourished disease-free trellis trained plants during mid June. 2-3 cm distal shoots were rinsed with tap water for 2 hours followed by surface sterilization with 15% sodium hypochlorite (1-2 drops of tween 20) for 15 minutes and three times rinsing with sterilized distilled water.

In vitro shoot-tip preparation: 2-3 cm distal end of in vivo grown plants were transferred to the laboratory. Plant materials were sterilized as the method described above. Explants were transferred to MS medium solidified with $6gL^{-1}$ agar and enriched with $0.5mgL^{-1}$ GA_3, $2mgL^{-1}$ BAP and $30gL^{-1}$ sucrose. They were incubated at 16/8 photoperiod and 24±1˚C. Thereafter, explants were cultured in MS medium enriched with $1mgL^{-1}$ BAP and $0.5mgL^{-1}$ IBA. Three weeks later, shoots were sub-cultured in a new medium. Shoot-tip explants with two leaf primordia (0.3-0.8 mm) were excised under stereo-microscope.

Micro-grafting: Rootstock shoots were cut off at 1 cm height and shoot-tip explants were placed on rootstocks cut off surface trying made to maximum connection of the two pieces. Grafted assemblies were cultured on MS medium solidified with $6gL^{-1}$ agar and containing $30gL^{-1}$ sucrose. Two month later, successful graft union percentage, shoot growth, leaf number, shoots and roots fresh and dry weight and root length were determined.

Data analysis: Data were subjected to variance analysis by SAS (version 8.02) software. Mean comparisons were carried out by Duncan's multiple range test at $p\leq0.05$ and $p\leq0.01$.

Results

Shoot-tip micro-grafting induced appropriate growth in grafted unions. Meanwhile, there were problems in graft union conjunction. There was significant difference ($p\leq0.01$) between shoot-tip origins regarding successful graft union percentage (table 1). In vitro culture derived shoot-tips had higher connection percentage compared with in vivo grown explants. Meaningful differences were observed for studied traits between in vivo and in vitro derived explants for all of studied cultivars (table 2). The highest percentage for successful graft union belonged to 'Sahebi'. In contrast, the least amount of this trait belonged to in vitro derived shoot-tips of 'Ghizil-Uzum' and in vivo derived shoot-tips of 'Fakhri' respectively (Table 1).

Table 1. Successful graft union percentage of some Iranian grapevine cultivars micrografted on 41B rootstock.

Cultivars	Shoot-tip origin	Successful graft union (%)
'Soltanin'	In vitro	51.6
	In vivo	13.8
'Fakhri'	In vitro	50.1
	In vivo	9.1
'Sahebi'	In vitro	60.6
	In vivo	17.3
'Gizil-Uzum'	In vitro	40.3
	In vivo	11.5
Cultivar		*
Shoot-tip		**
Cultivar * shoot-tip		ns

Ns, * and **: non significant, and significant at P≤0.05 and P≤0.01 based on Duncan's multiple range test.

Table 2 shows that grafted assemblies had significant differences concerning shoot length, leaf number and shoot fresh and dry weight. At the same time, no significant difference was recorded between in vitro and in vivo derived shoot-tips. However, in vivo derived scions had some higher shoot characteristics compared to in vitro ones. 'Soltanin' had the highest amounts for shoot length, leaf number and shoot fresh and dry weight. Contrarily, 'Sahebi' and 'Fakhri' received the best (?) quantities for shoot related traits.

There were considerable differences between cultivars considering root related traits. The highest and the lowest root length belonged to 'Sahebi' and 'Soltanin' respectively. 'Soltanin' attained the greatest amounts for root fresh and dry weight while the lowest amount was recorded in 'Ghizil-Uzum' (Table 2).

Table 2. Shoot and root related traits of some Iranian grapevine cultivars micro-grafted on 41B rootstock.

Cultivars	Shoot-tip origin	Shoot length (cm)	Leaf number	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (Cm)	Root fresh weight (g)	Root dry weight (g)
'Soltanin'	In vitro	17.77	11	0.94	0.19	3.25	0.51	0.05
	In vivo	17.91	11	1.1	0.22	4.13	0.52	0.05
'Fakhri'	In vitro	10.23	6	0.51	0.06	4.36	0.41	0.04
	In vivo	11.58	7	0.63	0.07	4.11	0.33	0.04
'Sahebi'	In vitro	10.62	6	0.59	0.07	5.18	0.48	0.03
	In vivo	10.51	6	0.51	0.07	5.22	0.49	0.03
'Gizil-Uzum'	In vitro	15.45	8	0.71	0.07	4.03	0.33	0.04
	In vivo	15.62	9	0.73	0.08	4.33	0.31	0.03
Cultivar		**	**	**	*	*	*	*
Shoot-tip		ns	ns	*	ns	ns	ns	ns
Cultivar * shoot-tip		*	ns	*	ns	ns	ns	ns

Ns, * and **: non significant, and significant at P≤0.05 and P≤0.01 based on Duncan's multiple range test.

Discussion

Origin and type of shoot-tip are of main determinants in successful micro-grafting operation. Shoot-tips of four different cultivars were employed for micro-grafting. In vitro culture derived shoot-tips had better response compared to in vivo derived counterparts. These findings are consistent with the results in apple [13], peach, plum and apricot [8,14].

Meanwhile, in vivo grown shoot-tips of citrus fruits had improved micro-grafting attributes compared to in vitro ones [6]. One of the main aims of micro-grafting is to provide a solution for some physiological and anatomical problems between related species and cultivars. This has been answered in citrus fruits [6,15], almond [16] and plum [17]. Another common problem is the low successful graft union percentage. In most cases and for the majority of plants this is due to the small size of shoot-tip organ, making problematic the excision, handling, grafting and subsequent maintenance of grafted assembly and leading to the drying of shoot-tips and low graft integration.

Despite that, in vivo derived shoot-tips are larger in size than in vitro derived ones, making handling of them easier [11]. However, they contain more phenolic compounds and hormonal concentrations results in higher polyphenol oxidases and peroxidases activity and hence higher browning and drying of fresh tissue just before and beyond grafting member's integration [18]. This troublesome was overcome in peach [19] and apricot [14] by sampling shoot-tips from mother in vivo grown plants in different times during growing season. In vitro culture condition has the advantage that it is not dependent upon growing season. Furthermore, it is possible to perform micro-grafting in any desired time. Another principal benefit of in vitro culture condition over in vivo grown plants is attaining the high number of shoot-tip explants owing to possible frequent subcultures of individual shoots under controlled conditions [2]. Unlike other asexual propagation methods, micro-grafting produces disease-free, especially virus-free plants, with possible benefits of scion rootstock combinations [20,21]. In grapevine, micro-grafting is a high-tech method for production of disease free material for cultivation as well as in breeding programs for detection of virus infections [20,22]. This method of propagation yields a homogenous clonal disease free population of plants, capable of high establishment and performance potential in field conditions.

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