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Microclonal Propagation of Grapevine *(Vitis vinifera* L.) cv. Al-Bayadi. Grown in Taif, KSA

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Abstract

A protocol for rapid multiplication of Grapevine (Vitis vinifera L.) cv. Al-Bayadi. Grown in Taif, Saudi Arabia was developed using shoot tips and internodes segments explants excised from mature plants in filed. Explants were cultured on Murashige and Skoog (MS) medium with different concentrations of BAP (6-Benzylaminopurine) (0.0, 0.5, 1.0, 1.5 and 2 mg/l) to investigate in vitro stages shoot initiation, multiplication and elongation. Effect of different concentrations of IBA (Indole-3-butyric acid) (0.0, 0.1, 1.0 and 2 mg/l) and 0.1 mg/l of NAA (Naphthalene acetic acid) on root formation of shoots were studied. The highest percentage of shoot initiation (90%), maximum average number of multiplied shoots (3.7) and highest average number of elongated shoots (3.6) were observed on MS medium supplemented with 2 mg/l BAP. For rooting, highest percentage (100%) of rooted shoots was obtained on MS medium supplemented with 2 mg/l IBA+0.1 mg/l NAA. Plantlets with 4 to 5 roots of 3 to 5 cm length were transferred to pots containing sterile peat moss for acclimatization in greenhouse.

Keywords: Grapevine, Shoot tips, Internodes segments, *In vitro* propagation, Shoot initiation, Multiplication, Rooting, Acclimatization.

Introduction

Grapevine (*Vitis* sp.) belongs to the family Vitaceae and several known types of grapes are named after their place of origin, European grapevine has been grown in cultivation throughout recorded history[1]. Grapevine *(Vitis vinifera* L.) is considering as one of the most important crops cultivated worldwide, The world's grapevine area totals reached 7.437.142 hectares producing 66.935.198 tons, while area totals reached 11.676 hectares producing 144.431 tons in Saudi Arabia[2].

Grapevines are propagated by seeds, cutting, layering, budding, or grafting. Propagation methods of grapevine have been modernized by using of virus free planting stock, mist propagation procedures for leafy cutting, and rapid machine-grafting techniques. Most commercial propagation is by dormant hard wood cuttings[3].

In vitro propagation is an alternative method to propagate grapevines. Numerous methods for grapevine *in vitro* propagation have been described[4]. The general principles in micropropagation are the same for all plants, details of the techniques for plants often differ[5]. The first report of *in vitro* culture of grapevines was by Morel[6] and many studies achieved such as callus culture, somatic embryos, protoplasts culture, organogenesis, and propagation *in vitro* using shoot tips and nodal culture have been published[7,8]. Grapevines tissue culture using nodal cuttings containing a single axillary bud can propagate elite varieties faster than traditional methods[8]. Rapid multiplication of grapevine plantlets has been achieved through nodal cuttings[9]. This method yields true-to-type plants by avoiding potential somaclonal variation associated with vitrification caused in part by cytokinins in shoot multiplication medium[10]. High rates of multiplication have also been associated with increased incidence of somaclonal variation[11].

The main objective of this study was to establish a micropropagation protocol for large scale production of Taify Grapevine (Al-Bayadi cultivar) *via* shoot tips and internodes segments.

Materials and Methods

Preparation of explants: Shoot tips and internodes segments buds were selected and excised from grapevine (*Vitis vinifera* L.) plant grown in Taif governorate., after that, it were rinsed using tap water to remove the superficial dust followed by a detergent for 3 min and surface sterilized by dipping in 70% ethanol for 1 min, then it were incubated in 20% Clorox (Sodium hypochlorite 5.25%) for 5 min and subsequently rinsed three times with sterile distilled water (s.d. H_2O).

Media preparation: Murashige and Skoog (MS) medium supplemented with 3% sucrose (w/v) and different

concentrations of BAP (0.0, 0.5, 1.0, 1.5 and 2 mg/l) were used for shoot initiation, shoot multiplication and shoot elongation stages, while different concentrations of IBA (0.0, 0.1, 1.0 and 2 mg/l) and 0.1 mg/l NAA were used for root formation. The pH of all media was adjusted to 5.7 using 1.0 N potassium hydroxide (KOH) and 1.0 N hydrochloric acid (HCl) before adding 0.8% (w/v) phytoagar. Media were autoclaved at 121°C, for 20 min and 1.5 k/cm^2 pressure.

***In vitro* culture:** Shoot tips and internodes segments explants were cultured for three to four weeks on MS medium with various concentrations of BAP (0.0, 0.5, 1.0, 1.5 and 2 mg/l) and 3% (w/v) sucrose for shoot initiation, multiplication and elongation. Induced shoots were subcultured on the same media for another three to four weeks.

For rooting stage, elongated shoots (3 to 4 cm length) were transferred on MS medium with different concentrations of IBA (0.0, 0.1, 1.0 and 2 mg/l) in combination with 0.1 mg/l of NAA for a further four weeks. MS medium without growth regulators was used as a control for all the *in vitro* cultures. All the *in vitro* cultures were incubated at 26 ± 2°C in a growth room on a 16/8 hour dark /light and 3000 lux light intensity provided by cool-white fluorescent light. After successful rooting, the rooted plantlets with 5 to 6 roots of length 3 to 5 cm were carefully washed with warm H_2O to remove agar and traces of medium, then they were transplanted to plastic pots containing sterile peat moss. The top of the pots was covered with transparent plastic.

Experimental design: For shoot initiation, multiplication and elongation experiments, every treatment contained of 40 shoot tips and internodes segments containing axillary bud explants. For rooting experiments, every treatment contained of 30 elongated shoots. All experiments were carried out in three replicates.

The results were recorded from different experiments four weeks after culture. For statistical analysis of data, analysis of variance (ANOVA) and mean separation were carried out using Duncan's multiple range test and significance was determined at the ($p < 0.01$) level. Data analysis was performed using ASSISTAT version 7.7 beta (2016) computer package.

Results and Discussion

Effects of different concentrations of BAP on shoot initiation, multiplication and shoot elongation are presented in (Table-1). According to using of different concentrations of BAP a different significant effects were noticed on shoot initiation, average number of multiplied shoots and average number of elongated shoots.

The highest percentage of shoot initiation (90%), maximum average number of multiplied shoots (3.7) and highest average number of elongated shoots (3.6) were observed on MS medium containing 2 mg/l BAP, this treatment was more significant at the ($p < 0.01$) level than other treatments. While the lowest percentage of shoot initiation (10%), minimum average number of multiplied shoots (0.20) and minimum average number of elongated shoots (0.25) were observed on MS medium without growth regulators (Figure-1 A, B, C and D). After successful shoot multiplication, the elongated shoots (4 to 5 cm) length were excised and transferred to MS medium supplemented with different concentrations of IBA and NAA for root formation. No rooted shoots were observed on MS medium that lacked auxins. Highest percentage (100%) of rooted shoots were obtained from MS medium supplemented with 2 mg/l IBA+ 0.1 mg/l NAA however; lowest rooting percentage (40%) was on MS medium with 0.1 mg/l IBA+ 0.1 mg/l NAA, this treatment was more significant at the ($p < 0.01$) level than other treatments. (Table-2 and Figure-1E). Plantlets with 4 to 5 roots of 3 to 5 cm length were successfully transferred to pots containing sterile peat moss for adaptation (Figure-1F).

Discussion: The main functions of cytokinins in tissue culture are induction of adventitious shoots. The results of this study suggest that higher levels of BAP as cytokinin (2.0 mg/l) were suitable for shoot initiation (Table-1). This result is in an agreement with Bumagina *et al*[12] who noticed that higher levels of BAP induction shoot initiation in "Agadi" grapevine variety.

As shown in (Table-1) branching shoot need to BAP and this Result is in an agreement with Kimon *et al*[13]. However, the results contradicting those of Hwang and Kim[14] they reported that addition of BAP at 2.2 or 4.4 µM increased branching shoots. Yae *et al*[15] obtained multiplication shoot on MS medium plus BAP. Gray and Benton[16] reported the effect of BAP and TDZ added as 5 and 20 µm BA and 5 µm TDZ produced the maximum average number of shoot per cultured apex of 3 muscadine grape cultivars.

Minal *et al*[17] reported that using of BAP was successfully initiated shoot formation in grape. Stimulation of grapevine multiplication *in vitro* by using BAP (Table-1), this result is consistent with Lee and Wetzstein[18] they reported the higher levels of BAP strongly stimulation shoot multiplication. However, Hamed *et al*[19] studied the effect of different growth regulators and different concentrations on shoot length of jack fruit and observed that BAP at 2.0 mg/l gave the highest significant shoot length. This was probably attributed to the genotypic differences.

The obtained results showed that using of auxins (IBA and NAA), in general, produced the best results in almost all studied traits. These results could be explained on the bases that IBA and NAA induced number of responses which involved cell division, protein and nucleic acids synthesis which are concomitants of auxin-induced growth and changes in wall plasticity of plant cell[20]. These results are close to previous studies[21-23] which reported that in *V. vinifera* cv. "Perlette" up to 94% rooting were obtained on MS medium supplemented with IBA and NAA.

Table-1
Effect of different concentrations of BAP on shoot initiation, multiplication and elongation of Taify Grapevine (Al-Bayadi cultivar).plant *via* shoot tips and internodes segments

Growth regulator (mg/l) BAP	Number of Explants	% Initiated shoots	Average number of shoots/explants	Average number of elongated shoots
0.0	40	10e	0.20d	0.25e
0.5	40	25d	0.64c	1.1c
1.0	40	37.5c	1.4b	0.86d
1.5	40	50b	1.2b	1.7b
2.0	40	90a	3.7a	3.6c

Means with the same letter are not significantly different ($p < 0.01$) according to Duncan's test.

Table-2
Effect of different concentrations and combinations of IBA and NAA on Root formation of Taify Grapevine (Al-Bayadi cultivar)

Growth regulator (mg/l) IBA+NAA	Number of shoots	Number of rooted shoots	% Rooted shoots
0.0+0.0	30	0d	0d
0.1+0.1	30	12c	40c
1.0+0.1	30	17b	56b
2.0+0.1	30	30a	100a

Means with the same letter are not significantly different ($p < 0.01$) according to Duncan's test.

Figure-1
Stages of *in vitro* propagation of Grapevine *(Vitis vinifera* L.) cv. Al-Bayadi. Grown in Taif, KSA. (A). Shoot tips and internode segments explants. (B). Shoot initiation from shoot tips. (C,D), Multiplication and elongation of induced shoots on MS medium containing 2 mg/l BAP. (E). Rooted plantlets on MS medium with 2 mg/l IBA + 0.1 mg/l NAA. (F). Plantlets with six to eight roots of 6 to 8 cm length were successfully transferred to pots for acclimatization

Conclusion

In this study, a protocol for *in vitro* propagation of micropropagation of Taify grapevine (Al-Bayadi cultivar) through shoot tips and internodes segments were established. This protocol has three culture stages consisting of shoot initiation, shoot multiplication and shoot elongation in the presence of 2 mg/l BAP, finally a rooting stage with 2 mg/l IBA+ 0.1 mg/l NAA. This protocol will help for large scale propagation of grapevine (Al-Bayadi cultivar) for horticulture, pharmaceutical industries and *in vitro* germplasm conservation of Taify grapevine (Al-Bayadi cultivar).

References

1. H Ahmad (2013). Introduction To horticulture. New York. Tornto London.

2. FAO STAT (2012). FAOSTAT: Statistical Database. 10 February http://faostat.fao.org.

3. Hartmann H.T. and Kester D.E. (1975). Plant Propagation: Principles and Practices, 4th edition. Englewood Cliffs, New Jersey: Prentice-Hall, Inc., 727.

4. Gray D.J. and Fisher L.C. (1986). *In vitro* shoot propagation of grape species, hybrids and cultivars. *Proceedings of the Florida State Horticulture Society*, 98, 172-174.

5. Vasil I.K. and Thorpe T.A. (2013). Plant cell and tissue culture. Kluwer Acad. Pub., Netherlands. 293-312.

6. Morel G. (1944). Le développement du mildiou sur des tissus de vigne cultives in vitro. C.R. Hebd Seances Académie des sciences, 218, 50-52.

7. Read P.E. (2004). Micropropagation: Past Present and Future. *Acta Horticulturae*, 748, 17-27.

8. Torregrosa L., Bouquet A. and Goussard P.G. (2001). *In vitro* culture and propagation of grapevine. *In*: Molecular Biology and Biotechnology of the Grapevine. Roubelakis-Angelakis, K. (ed.). Kluwer Academic Publishers, Amsterdam, pp195-240.

9. Nas M.N., Eskridge K.M. and Read P.E. (2005). Experimental designs suitable for testing many factors with limited number of explants in tissue culture. *Plant Cell Tiss Org.*, 81, 213–220.

10. Heloir M.C., Fournioux J.C., Oziol L. and Bessis R. (1997). An improved procedure for the propagation *in vitro* of grapevine (*Vitis vinifera* cv. Pinot noir) using axillary-bud microcuttings. *Plant Cell Tiss Org.,* 49:223-225.

11. Karp A. (1989). Can genetic instability be controlled in plant tissue cultures. International Association of Plant Tissue Culture Newsletter, 58, 2.

12. Bumagina S.I., Khachumova S.S., Shamova N.M. and Butenko R.G. (1988). *In vitro* Culture of avillary buds of grape. *Biologiya –Kul'tiviruemykh kleton I biotech-ologiya*, 1, 147.

13. KT Bird, JR Johnson, J Jewett-Smith (1998). *In vitro* culture of the sea grass *Halophila decipiens. Aquat Bot.*, 60(4), 377-387.

14. Hwang J.H. and Kim S.K. (1990). The effect of plant growth regulator on *in vitro* growth of differentially chilled grape shoots. *J Korean Soc Hortic Sci.,* 31(2), 142-149.

15. Yea B.W., Shin Y.U., Kang D.S., Lee D.K., Choo K.S., Monn Y.J. and Hwang J.S. (1990). Factors affecting lateral shoot proliferation in grapevine CV. Rizamat. *in vitro, Horticulture*, 32(3): 34-41.

16. Gray D.J. and Benton C.M. (1991). *In vitro* micro-propagation and plant establishment of muscadine grape cultivars (*Vitis rotundifolia*). *Plant Cell Tiss Org.,* 27 (1), 7-14.

17. Minal M., Salunkhe C.K., Rao P.S. and Mhatre M. (2000). Micropropagation of *Vitis vinifera* L: towards an improved protocol. *Scientia Horiculturae*, 84(3-4), 357-363.

18. Lee N. and Wetzstein H.Y. (1990). *In vitro* propagation of muscadine grape By axillary shoot proliferation. *J Amer Soc Hort Sci*., 115(2), 32-329.

19. Hamed A.M., Ali E.A.M. and Ehab B.S. (2007). *In vitro* propagation of jackfruit (*Artocarpus heterophyllus* L.). *J. appl. sci. res*., 3(3), 218-226.

20. MB Wilkins, VM Jiménez, E Guevara, SF Pascholati (1984). Advanced Plant Physiology. The Bath Press, Avon, 13-15.

21. Lewandowski V.T. (1991). Rooting and Acclimatization of Micropropagated Vitis labrusca Delaware. *Hortic Sci*., 26, 586-589.

22. MS Barreto, A Nookaraju - Indian Journal of Horticulture (2007). Effect of auxin types on in vitro and ex vitro rooting and acclimatization of grapevine as influenced by substrates. *Indian J Hortic.*, 64(1), 11-17.

23. Butiuc-keul A.L., A. Cotse, Bcraciunas A. Halmagyl, A. Deliu, C. Farago, M. Iliescu and Iuoras, R. (2008). *In vitro* clonal propagation of several grapevine cultivars. *Acta Hortculturae* (ISHS), 83, 151-156.