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Full Length Research Paper

Effect of cutting position and indole butyric acid (auxin) concentration on rooting response of *Araucaria heterophylla*

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The effects of cutting position (tip, middle and basal) and concentration of indole butyric acid (IBA) (0, 5, 7.5 and 11 g/L) on root and shoot growth of *Araucaria heterophylla* were evaluated to develop a method for vegetative propagation for this tree species. Leaf number, number of adventitious roots, root length and survival rates were measured. These parameters were significantly influenced by the interactive effect of cutting position and hormonal concentration. Tip cuttings with the11 g/L IBA treatment showed higher root number, leaf number and root length whereas the other treatment combinations showed no root or shoot growth. Further, plant death was observed for the 5 and 7.5 g/L IBA treatments.

Key words: *Araucaria heterophylla,* cutting position, hormonal concentration, vegetative propagation.

INTRODUCTION

Araucaria heterophylla is a coniferous tree species with economic, social and environmental importance (Hazrat et al., 2006) in Africa. According to Bengoa (2000) and Azocar et al., (2005), *A. heterophylla* forests are a primary source of firewood, livestock shelter, construction materials and income for the Mapuche Pewenche community of southern Chile. *A. heterophylla* is also an important landscape tree species and is a dominant ornamental plant in urban areas of Ethiopia.

Despite these benefits, limited numbers of *A. heterophylla* seedlings are available in the market for gardeners with seedlings ranging in price from $43-195 USD as a result of few mature trees available and low seed production. A potential solution is the development of a vegetative propagation method (Pijut et al., 2011). Vegetative propagation can also be used to conserve superior genotypes, maintain valuable traits, reduce the high risk period when the tree is small and fragile, as well as reduce juvenile period (Hartmann et al., 2011; Gehlot et al., 2014). A successful system of vegetative production will allow producers to propagate plants throughout the year (Assis et al., 2004; Xavier et al., 2009).

An effective system of vegetative propagation is lacking for *A. heterophylla*. Propagules rooting is also influenced by endogenous and exogenous factors, such as

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Table 1. Comparison among cutting position (tip, middle and basal parts).

Dependent variable	Cutting position	Mean ± Std. Error
Root length	Tip	2.000±0.300 [a]
	Middle	2.220E-16±0.300 [b]
	Bottom	2.220E-16±0.300 [b]
Root number	Tip	1.333±0.173 [a]
	Middle	-4.626E-17 ±0.173 [b]
	Bottom	-1.943E-16±0.173 [b]
Leaf number	Tip	1.167±0.127 [b]
	Middle	6.476E-17±0.127 [b]
	Bottom	-8.327E-17±0.127 [b]

Means with the same letter are not statistically different ($P \leq 0.05$). The values represent mean ± S.E.

ontogenetic and physiological state, cutting position, humidity, temperature, light incidence, substrate, nutrition, hormonal balance and genetics (Li et al., 2009; Pijut et al., 2011). Furthermore, according to Ibironke (2017), rooting and shooting performance of cuttings are directly influenced by the types of growing media, thus, the selection and preparation of the medium is extremely important in terms of plant growth and quality because rooting performance depends on the type of medium used in propagation. One of the most effective and widely used auxins is indole-3-butyric acid (IBA), which has low toxicity, low mobility and high chemical stability (Hartmann et al., 2011). Thus, this research investigated the appropriate cutting position and auxin concentration (IBA) for rooting response of *A. heterophylla.* Thus, the research attempted to fill the gap by investigating the appropriate cutting position and auxin concentration (IBA) for rooting response of *A. heterophylla.*

MATERIALS AND METHODS

Study area

The experimental evaluation was conducted at the Teaching Nursery of Wondo Genet College of Forestry and Natural Resource (WGCF-NR), Ethiopia. The research site is located 263 km south of Addis Ababa and 13 km Southwest of Shashemene town. The campus is located on the eastern escarpment of the Ethiopian Rift Valley in the Southern Nation Nationalities and Peoples Regional State at 7° 6' N latitude and 38° 7' E longitudes with an altitude of 1700 m above sea level (Belaynesh, 2002). This region of Ethiopia is characterized by bimodal rainfall distribution with 1247 mm annual precipitation. The short rainy season ranges from March to May and the long rainy season lasts for five months from June to October. The mean monthly temperature is 19.5°C, with mean monthly maximum temperature of 26.3°C and mean monthly minimum temperature of 12.4°C (Amare et al., 2014).

Approach

The experiment included 12 treatment combinations. Three concentrations of IBA (5, 7.5 and 11 g/L) were evaluated for root induction with no auxin treatment used as a control, along with three cutting positions upper, middle and lower segments of *A. heterophylla* stems. Each treatment combination has three cuttings totaling 36 cuttings. Cuttings were harvested early in the morning from 6-year old *A. heterophylla* trees form (WGCF-NR). Cuttings were 30 cm long and each cutting had 38 leaves.

Cuttings were maintained under moist condition to prevent desiccation before treatment. Hormonal treatments were conducted by placing the 4 to 5 cm distal portion of each cutting in one of three IBA solutions for five minutes. Cuttings were placed in water for the control treatment and all cuttings were planted at the same time and date in 25 cm × 30 cm polyethylene tunnel. Polyethylene tunnels were placed under the lat-house in the nursery site of WGCF-NR to reduce direct sunlight. Treatments were arranged in a completely randomized design with three replications. Cuttings were watered regularly to maintain the humid environment needed for rooting. Growing medium consisted of a mixture of three soil types (forest, sand and clay soils) using a 3:2:1 ratio based on volume.

Newly developed number of leaves**,** number of roots, root length and visual quality of auxiliary shoots were recorded for each explant 50 days after planting. Data were analyzed using two factorial ANOVA test at 5% level of significance. The statistical analysis was done using SPSS version 16.0.

RESULTS

Effect of cutting position on root and shoot performance

The number of roots, root length and leave number varied for stem cutting positions (Table 1). The tip cuttings appeared green in color as compared to cuttings from the middle and basal part of the stem (Figure 1).

Effect of different IBA treatments on root and shoot performance

Cuttings treated with 11 g/L IBA showed significantly greater root lengths (Table 2). Control cuttings with no

(A) (B)

Figure 1. Effect of cutting position on rooting and shoot performance of *A. heterophylla:* (A) Basal cuttings position treated with 11g/l IBA. (B) Tip cuttings position with no IBA treated.

Table 2. Comparison among different concentration of IBA.

Dependent variable	IBA concentration (g/L)	Mean±Std.Error
Root length	Control	1.975E-16±0.347^{b}
	5	2.715E-16^{b}±0.347^{b}
	7.5	-9.861E-17^{b}±0.347^{b}
	11	2.667^{a}±0.347^{b}
Root Number	Control	-1.563E-16±0.200^{b}
	5	4.048E-18±0.200^{b}
	7.5	-7.406E-17±0.200^{b}
	11	1.778±0.200^{b}
Leaf Number	Control	7.615E-17±0.147^{b}
	5	-1.637E-17±0.147^{b}
	7.5	-1.849E-16±0.147^{b}
	11	1.556±0.147^{a}

Means with the same letter are not statistically different ($P \leq 0.05$). The values represent mean ± S.E

IBA treatment were alive and remained green without the production of root systems. Cuttings treated with 5 and 7.5 g/L IBA wilted and perished (Figure 2). The highest numbers of dead and wilted cuttings were recorded for the 7.5 g/L IBA treatment. The highest root number was recorded for 11 g/L treatment, whereas root systems failed to develop for the other treatments. Additionally, the 11 g/L showed a significantly higher leaf number.

Comparative effect of cutting position and different IBA concentration

The comparisons between cutting position and IBA treatment are presented in Table 3. Root development was only observed for the tip cuttings with the 11 g/L IBA treatment, which resulted in significantly higher mean root number, root length, and leaf number for this treatment

Figure 2. Effect different of IBA treatments on rooting and shoot performance of *A. heterophylla*. (A) Tip cuttings position with 11 g/l IBA treated. (B) Tip cuttings position with no IBA treated. (C and D) Middle and lower segments position treated with 11 g/L.

compared to other treatments.

DISCUSSION

Vegetative propagation has been an excellent method to support genetic improvement of forest species, allowing the reproduction of genetically superior individuals and providing greater uniformity of the plants (Sutton, 2002). The main aim of the study was to know whether growth regulators (IBA) would have any better response than the untreated control on root and shoot initiation of *A. heterophylla* tree species. This tree species were successfully propagated using tip cuttings with 11 g/L IBA (Tworkoski and Takeda, 2007), whereas the other cuttings did not respond to root even though they were treated with IBA treatments. Plant deaths were recorded during data collection from cuttings treated with auxin. Effect of auxin treatments on initiation and promotion of roots and shoots were found inconsistent in the cuttings. Different cutting positions had different response for root and shoot initiation. The rooting percentages observed in this study were low when compared to other ornamental species with established vegetative propagation protocols (Almeida et al., 2007; Negishi et al., 2014). However, performance of hormonal concentration has a positive correlation in promoting root and shoots initiation of the cuttings (Kala et al., 2017; Singh, 2017; Rambabu et al., 2017). In contrast to Eganathan et al., (2002) finding, no significant variation was observed in plant height and leave number per plant among the different treatments which might be attributed to the slow growth rate of the plant.

Conclusion and recommendation

This study is the first description of vegetative propagation in *A. heterophylla* using cutting positions and auxin concentrations. The study clearly indicated the feasibility of developing an *in vivo* propagation protocol for the plant from tip cutting as explants. The present established

Table 3. Comparison of the interaction effect between cutting position and different IBA concentration.

Dependent variable	Cutting position	Auxin concentration (g/L)	Mean±Std.Error
Root length	Tip	0 (Control)	1.483E-16±0.601[b]
		5	3.704E-16±0.601[b]
		7.5	-1.184E-15±0.601[b]
		11	8.000±0.601[a]
	Middle	0	2.961E-16±0.601[b]
		5	7.401E-17±0.601[b]
		7.5	2.961E-16±0.601[b]
		11	2.220E-16±0.601[b]
	Bottom	0 (Control)	1.480E-16±0.601[b]
		5	3.701E-16±0.601[b]
		7.5	5.921E-16±0.601[b]
		11	-2.220E-16±0.601[b]
Root number	Tip	0 (Control)	-1.726E-16±0.347[b]
		5	8.630E-17±0.347[b]
		7.5	-1.232E-16±0.347[b]
		11	5.333a±0.347[b]
	Middle	0 (Control)	-1.234E-16±0.347[b]
		5	-1.604E-16±0.347[b]
		7.5	2.465E-17±0.347[b]
		11	7.404E-17±0.347[b]
	Bottom	0 (Control)	-1.727E-16±0.347[b]
		5	8.635E-17±0.347[b]
		7.5	-1.234E-16±0.347[b]
		11	-5.674E-16±0.347[b]
Leaf number	Tip	0 (Control)	7.980E-17±0.255[b]
		5	2.429E-17±0.255[b]
		7.5	-7.282E-16±0.255[b]
		11	4.667±0.255[a]
	Middle	0 (Control)	6.785E-17±0.255[b]
		5	-9.869E-1±0.255[b]
		7.5	1.236E-17±0.255[b]
		11	2.775E-16±0.255[b]
	Bottom	0 (Control)	8.018E-17±0.255[b]
		5	2.467E-17±0.255[b]
		7.5	1.604E-16±0.255[b]
		11	-5.983E-16±0.255[b]

Means with the same letter are not statistically different (P≤0.05). The values represent mean ± S.E

vegetative propagation protocol for *A. heterophylla* has a considerable practical significance and the process has to be successfully exploited for large scale production of cloned plants for sustainable utilization and supply of this valuable ornamental plant. Shoot tip stem cuttings showed better performance and survival rate than other stem cutting. Therefore, vegetative propagation of *A. heterophylla* using other types of auxin, either alone or in combination, should be studied so as to identify the most suitable auxin type and/or combination for successful *in vivo* propagation of the plant. Further, the experiment should be tested on different soil mixtures to determine the best soil mixture for better root ability. The experiment should be further studied for extended period to detect the effects of the different auxin concentrations on the different stem cuttings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Almeida FD, Xavier A, Dias JM, Paiva HN (2007). Efficiency of auxins (AIB and ANA) non-rooted miniatures of clones of Eucalyptus cloeziana F. Muell. Journal of Revista Árvore 31:455-463.

Amare S, Nega C, Zenebe G, Goitom T, Alemayoh T (2014). Landscape–scale soil erosion modeling and risk mapping of mountainous areas in eastern escarpment of Wondo Genet watershed, Ethiopia. International Research Journal of Agricultural Science and Soil Science 4(6):107-116

Assis TF, Fett-Neto AG, Alfenas AC (2004). Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. In: Walter C Carson M. (Ed.). Journal of Plantation Forest Biotechnology pp. 303-333.

Azocar G, Sanhueza R, Aguayo M, Romero H, and Muñoz M, (2005). Conflicts for control of Mapuche-Pehuenche land and natural resources in the Biobío highlands. Journal of Latin American Geography 4:57-76.

Belaynesh Z (2002). Perception on forest resource changes in and around Wondo Genet catchment and its near future impacts. Ethiopian Msc in forestry programme thesis, report number 2002:65, Wondo Genet Collage of Forestry.

Bengoa J (2000). Historia del pueblo Mapuche: siglo XIX y XX. Editorial Lom. Santiago, Chile 423 p.

Eganathan P, Srinivasa C, Anand A, Swaminathan MS (2002). Vegetative propagation of three mangrove tree species by cuttings and air layering Research Foundation. Journal of Wetlands Ecology and management 8:281-286.

Gehlot A, Gupta RK, Tripathi A, Arya I, Arya S (2014). Vegetative propagation of Azadirachta indica: effect of auxin and rooting media on adventitious root induction in mini-cuttings. Journal of Advance in Forestry Science 1(1):106-115.

Hartmann HT, Kester DE, Davies Jr. FT, Geneve RL (2011). Plant propagation: principles and practices. 8. ed. São Paulo: Prentice-Hall 915 p.

Hazrat G, Abdul Mateen K, Noorul A (2006). Accelerating the Growth of Araucaria Heterophylla Seedlings through Different Gibberellic Acid Concentrations and Nitrogen Levels. Journal of Agricultural and Biological Science 1:1990-6145.

Ibironke OA (2017). Response of Selected Ornamentals to Rooting Hormone in Different Propagating Media. Journal of Botany Research 1(1):22-28.

Kala S, Reeja S, Kumaran K (2017). First Report on Success of Stem Cuttings on Simarouba glauca, Dc – An Easy Method for Mass Multiplication of Superior Mother Trees. International Journal of Current Microbiology and Applied Sciences 4(6):2646-2653.

Li SW, Xue L, Xu S, Feng H, An L (2009). Mediators, genes and signaling in adventitious rooting. The Botanical Review 75:230-247.

Negishi N, Nakahama K, Urata N, Kojima M, Sakakibara H, Kawaoka A (2014). Hormone Level Analysis on Adventitious Root Formation in *Eucalyptus Globulus*. Journal of New Forests 45:577-587.

Pijut PM, Woeste KE, Michler CH (2011). Promotion of adventitious root formation of difficult-to-root hardwood tree species. Horticultural Reviews 38:213-251.

Rambabu M, Ujjwala D, Ramaswamy N (2014). Effect of plant growth regulators on callus induction of an endangered forest tree Givotia Rottleriformis grif. World Journal of Pharmacy and Pharmaceutical Sciences 6(6):1808-1819.

Singh KS (2017). Multiplication of Phalsa (*Grewia asetica* L.) Cv. Dwarf Type through Hardwood Stem Cutting Under Srinagar Garhwal Himalayas. International Journal of Current Microbiology and Applied Sciences 6(2):1173-1178.

Sutton B (2002). Commercial Delivery of Genetic Improvement to Conifer Plantations Using Somatic Embryogenesis. Journal of Annals of Forest Science 59:657-661.

Tworkoski T, Takeda F (2007). Rooting response of shoot cuttings from three peach growth habits. Journal of Scientia Horticulturae 115:98-100.

Xavier A, Wendling I, Silva RL (2009). Silvicultura clonal: princípios etécnicas. Viçosa, MG: Universidade Federal de Viçosa 1:272.

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Full Length Research Paper

In vitro regeneration of two grapevine *(Vitis vinifera* L.*)* varieties from leaf explants

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The traditional way of grapevine (*Vitis vinifera* L.) propagation is time consuming and allows disease transmission from generation to generation. Moreover, it is difficult to improve this crop through conventional plant breeding methods. Therefore, the objective of this study was to develop efficient *in vitro* regeneration protocol for 'Canonannon' and 'Chenin Blanc' varieties of grapevine using leaf explants. MS medium supplemented with different concentrations of thidiazuron (TDZ) alone or in combination with α-naphthalene acetic acid (NAA), and 6-benzyl aminopurine (BAP) alone or in combination with indole-3-butyric acid (IBA) were used for regeneration of shoots from leaves. The regenerated shoots were transferred to shoot multiplication medium and subsequently to rooting medium and the plantlets were acclimatized after rooting. The rooting medium consisted of MS medium containing different concentrations of IBA or indole-3-acetic acid (IAA). The highest number of shoots per leaf explant was obtained from both 'Chenin Blanc' (2.3 ± 0.3) and 'Canonannon' (2.2 ± 0.2) on medium supplemented with 2.0 mg/L BAP. Among 16 different combinations of TDZ and NAA, the maximum number of shoots per explant (1.5 ± 0.2) was obtained from 'Canonannon' on medium containing 1.0 mg/L TDZ and 0.1 mg/L NAA. However, when these shoots were transferred to shoot multiplication medium, 10 ± 0.51 shoots per explant were obtained from 'Chenin blanc' on MS medium supplemented with 2.0 mg/L BAP. The highest number of roots per explant (8.3 ± 0.30) was obtained on medium containing 2.0 mg/L IBA. The survival rate of 'Chenin Blanc' and 'Canonannon' was 83.3 and 75 %, respectively after one month of acclimatization.

Key words: Callus induction, growth regulators, hyperhydricity, organogenesis.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most widely distributed fruit crop in the world. Although most grapevines are produced in areas with temperate climate, some cultivars have cultivation potential under high-temperature of tropical and sub-tropical conditions. According to Patrice et al. (2006), *V. vinifera* is highly distributed and constituted over 90% of the world's grapes. In case of Ethiopia, wineries are importing about 300 tons of grapes annually in the form of dried raisin, grape juice concentrates, natural wine extracts and citric

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acid (Kinfe et al., 2017). Grapevine is grown worldwide for a variety of purposes including wine, fresh fruit, juice, jams, jellies, raisins and other processed products (Ferreira et al., 2004). It is also a major horticultural crop with great applications in food and pharmaceutical industries. Regardless of its enormous uses, grapevine cultivation is affected by different biotic and abiotic stresses. Although it is the third most important fruit crop in the world after banana and citrus, the demand for grapevine fruit is increasing because of increase in the number of wine industries and more demand for fresh and dried fruits (Fayek et al., 2009). According to Aazami (2010), genetic improvement of the classic cultivars in order to obtain high quality wine and table grape varieties through conventional hybridization methods does not appear to be enough. Moreover, genetic improvement of grapevine through conventional breeding is severely limited because of its polyploidy nature and the existing cultivars are highly heterozygous (Gray and Fisher, 1985). The non-conventional methods such as *in vitro* screening and genetic engineering have enormous potential for genetic improvement of plants including grapevine. However, for this purpose, development of *in vitro* regeneration protocol is a pre-requisite (Fikadu, 2016). As response of explants to culture conditions is dependent on genotype, each cultivar of a species requires its own *in vitro* regeneration protocol. The application of these modern genetic improvement techniques in different parts of the world is limited to a few outstanding regional cultivars (Fikadu, 2016). Therefore, the objective of the present study is to develop *in vitro* regeneration protocol for 'Canonannon' and 'Chenin Blanc' varieties of grape vine that were introduced from abroad and being cultivated in Ethiopia.

MATERIALS AND METHODS

Plant material

In vitro cultured grapevine varieties, 'Chenin Blanc' and 'Canonannon', were maintained by sub-culturing of shoots and nodes at four-week intervals on MS (Murashige and Skoog, 1962) shoot multiplication medium supplemented with 1.0 mg/L BAP in combination with 0.1 mg/L IBA and 30 g/L sucrose at Addis Ababa University. The pH of the medium was adjusted to 5.8 and 7.0 g/L agar was added. The medium was then autoclaved at 121°C for 15 min and 40 ml was dispensed into each sterile Magenta GA-7 culture vessels. The cultures were maintained at temperature of 27 ± 2°C and light intensity of 40 µmol $m^{-2}s^{-1}$ at 16 h photoperiod. Unless and otherwise indicated, all cultures were maintained at these culture conditions.

Shoot regeneration from leaf explants

Upper most expanding young leaves from the four-week-old *in vitro* propagated two varieties of grapevine shoots were excised aseptically and cultured on shoot regeneration medium. The shoot regeneration medium is MS medium containing different concentrations of BAP alone (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 mg/L) or BAP (0.0, 0.5, 1.5, 2.0 and 3.0 mg/L) in combination with IBA (0.0, 0.1, 0.5 and 1.0 mg/L), or TDZ alone (0.0, 0.1, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/L) or TDZ (0.0, 0.1, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/L) in combination with NAA (0.0, 0.01, 0.1 and 0.5 mg/L). All leaves were wounded by scalpel blade across main vein and cultured on 90 mm diameter Petri dishes containing 20 ml medium with adaxial side of the leaves contacting the medium. The regenerated shoots were transferred to the same fresh medium after four weeks and all Petri dishes containing the shoots were covered with transparent (thin) cloth for two weeks. The cloth was used for light reduction. The number of leaf explants that produced shoots and induced callus, and the number of shoots per explant were recorded.

Multiplication of regenerated shoots

The shoots that were regenerated from leaf explants were excised and cultured on shoot multiplication medium in Magenta GA-7 culture vessels that contained 40 ml medium. The shoot multiplication medium was MS medium consisted of 2.0 mg/l BAP. When problem of hyperhydricity was encountered, culture vessels were ventilated aseptically under laminar air flow cabinet, the agar concentration was increased from 7 to 8%, and most of the leaves were trimmed. The number of shoot per explant were recorded and compared with the number of shoots per explant that were produced by the stock plants that were maintained on shoot multiplication medium from which leaf explants for regeneration experiment were obtained.

Rooting and acclimatization

One-month-old shoots from shoot multiplication medium were cultured on rooting medium. The rooting medium was full strength MS medium containing different concentrations of IBA (1.0, 2.0, 3.0 and 4.0 mg/L) or IAA (2.0 and 4.0 mg/L). The number of roots, length of roots and plantlets were recorded after 30 days. The plantlets having sufficient root and shoot systems were taken out from the culture vessels and the roots were washed under running tap water to remove the agar and sucrose. These plantlets were then transferred to glasshouse and planted in 12 cm diameter plastic pots containing sterilized red soil, sand and cow dung manure at the ratio of 1:2:1 respectively. The plantlets were covered with transparent polythene bags and watered every other day. The polythene bags were gradually removed after two weeks and the number of survived plants was recorded after a month.

Statistical analyses

Completely Randomized Design (CRD) was used. Six explants per Petri dish were used for the whole experiments of shoot regeneration from leaf explants and each experiment had five replications. The one-way analysis of variance (ANOVA) was used to compute the percentage and mean number of regenerated shoots per-explant, the number and length of roots and their survival rate in glasshouse. All data were analyzed at p ($\alpha < 0.05$) using SPSS 16 version statistical software.

RESULTS

Shoot regeneration from leaf explants

Shoots were regenerated directly from leaf explants after

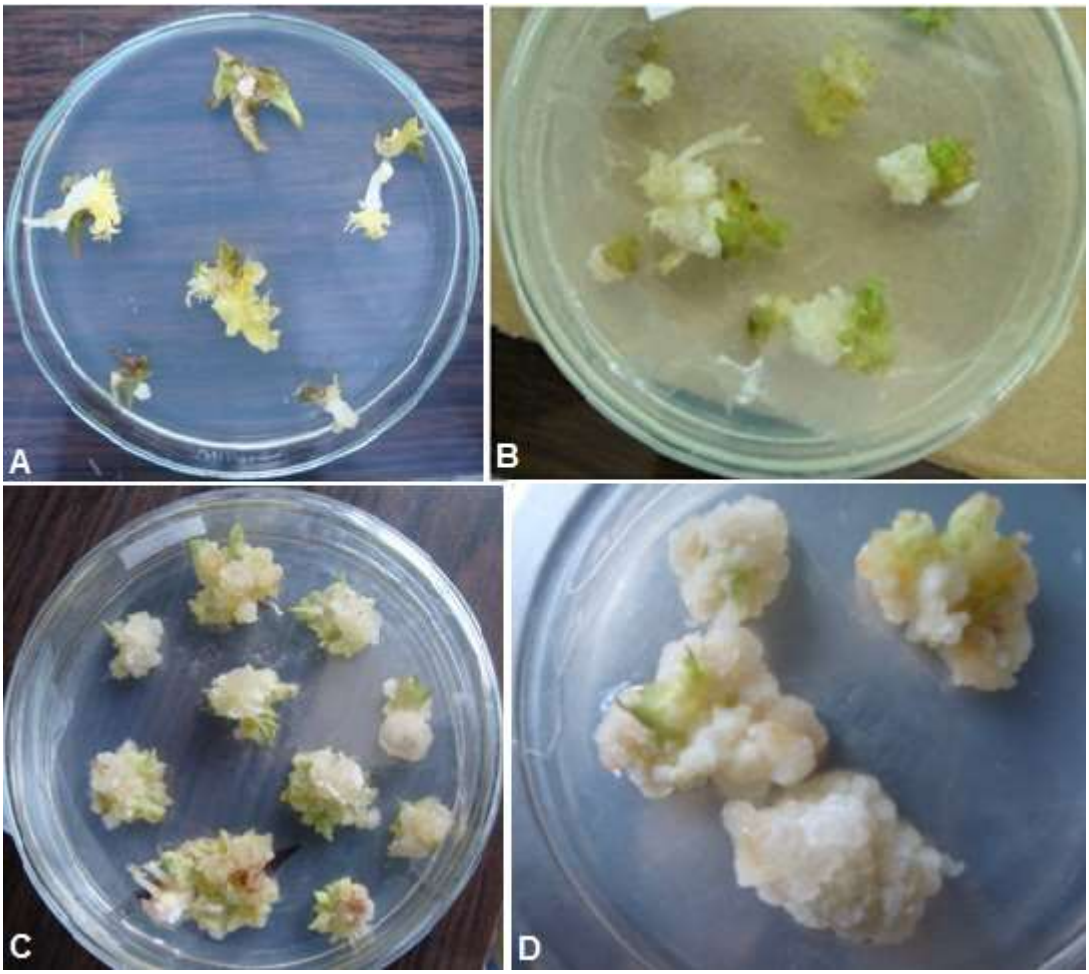

Figure 1. Direct shoot regeneration and callus induction from leaf explants after 30 days of dark incubation: Direct shoot regeneration from 'Canonannon' (A) and 'Chenin Blanc' (B) on MS medium containing 2.0 mg/l BAP and 0.1 mg/L IBA. Callus induction of 'Canonannon' on MS medium containing 1.5 mg/L BAP and 1.0 mg/L IBA (C) and 2.0 mg/L BAP and 0.1 mg/L IBA (D).

four weeks of culture (Figure 1A and B) and there was significant difference in percentage of shoot regeneration and number of shoots per explant among different concentrations of BAP and TDZ. The highest number of shoots per leaf explant were obtained on the medium containing 2.0 mg/L BAP from both 'Chenin Blanc' (2.3 ± 0.3) and 'Canonannon' (2.2 ± 0.2) varieties. However, the number of shoots regenerated from leaf explants of both cultivars was reduced when the concentration of BAP was reduced or increased from 2.0 mg/L (Table 1). Different concentrations of BAP or TDZ alone triggered similar responses on explants of both varieties. Callus induction was significantly low at all concentrations of TDZ used in this experiment, but explants of both cultivars that were cultured on medium containing 0.5, 2.0, and 4.0 mg/L BAP produced callus though the size and percentage was low. Shoots were regenerated from calli on medium containing 3.0 mg/L BAP. However, shoots were not regenerated from leaf explants that were cultured on medium containing TDZ alone at all tested concentrations, and on medium containing 0.5, 4.0 and 5.0 mg/L BAP.

Effect of TDZ and NAA on shoot regeneration

Among sixteen different combinations of TDZ and NAA, 45% of 'Canonannon' and 29.8% of 'Chenin Blanc' leaf explants exhibited direct regeneration on medium containing 1.0 mg/L TDZ in combination with 0.1 mg/L NAA (Table 2). There was significant difference in percentage of shoot regeneration, number of shoots per explant and percentage of callus induction among different concentrations of TDZ in combination with NAA. The maximum mean number of shoots per explant was also obtained on this medium. Leaf explants cultured on medium containing 0.5 mg/L TDZ combination with 0.01

Table 1. Effect of different concentrations of BAP and TDZ on *in vitro* shoot regeneration of 'Canonannon' and 'Chenin Blanc' cultivars of grapevine from leaf explants.

Growth regulators Concentrations (mg/L)		'Canonannon'			'Chenin Blanc'		
		Callus induction (%)	Regeneration (%)	No. of shoots per explant	Callus induction (%)	Regeneration (%)	No. of shoots per explant
Control	0.0	0.0^{d}	0.0^{c}	0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}
BAP	0.5	8.3^{bd}	0.0^{c}	0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}
	1 .0	0.0^{d}	0.0^{c}	0 ± 0.0^{c}	21.0^{bc}	3.0^{b}	1 ± 0.1^{b}
	1.5	0.0^{d}	4.3^{b}	1 ± 0.0^{b}	4.2^{cd}	8.0^{b}	1.2 ± 0.2^{ab}
	2 .0	0.0^{d}	88.4^{a}	2.2 ± 0.2^{a}	0.0^{d}	86.0^{a}	2.3 ± 0.3^{a}
	2.5	25.0^{a}	5.8^{b}	1 ± 0.1^{b}	33.0^{a}	0.0^{c}	1.3 ± 0.1^{ab}
	3 .0	12.5^{b}	1.4^{b}	1 ± 0.1^{b}	4.2^{cd}	3.0^{b}	1 ± 0.0^{b}
	4 .0	0.0^{d}	0.0^{c}	0 ±0. 0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}
	5 .0	0.0^{d}	0.0^{c}	0 ±0. 0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}
$T^{D}Z$	0.1	0.0^{d}	0.0^{c}	0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}
	0.5	0.0^{d}	0.0^{c}	0 ±0. 0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}
	1.0	21.0^{ab}	0.0^{c}	0 ± 0.0^{c}	8.3^{cd}	0.0^{c}	0 ± 0.0^{c}
	2.0	17.0^{ab}	0.0^{c}	0 ± 0.0^{c}	13.0^{cd}	0.0^{c}	0 ± 0.0^{c}
	3.0	17.0^{ab}	0.0^{c}	0 ± 0.0^{c}	17.0^{c}	0.0^{c}	0 ± 0.0^{c}
	4.0	0.0^{d}	0.0^{c}	0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0 ± 0.0^{c}

Means followed by the same letters in the same column are not significantly different at 5 % level of probability. Data are represented as mean ± SD.

and 0.1 mg/L NAA exhibited callus induction and shoot regeneration in 'Canonannon' cultivar. However, leaf explants of both cultivars cultured on medium containing 0.5 mg/L TDZ in combination with 0.5 mg/L NAA induced calli (14.6% of 'Canonannon' and 50% of 'Chenin Blanc') and no shoots were regenerated from these calli.

Effect of BAP and IBA on shoot regeneration

Among different concentrations of BAP in combination with IBA, 90% of leaf explants of 'Chenin Blanc' and 71.7% of 'Canonannon' exhibited direct regeneration on medium supplemented with 2.0 mg/L BAP in combination with 0.1 mg/L IBA (Table 3). Similarly, the highest number of shoots per explant was obtained on this medium for both cultivars. At lower concentrations of BAP (0.5 and 1.5 mg/L), 'Chenin Blanc' did not show any response of regeneration while 'Canonannon' exhibited 1.0 ±0.0 shoots per explant on medium containing 0.5 mg/L BAP combined with 0.1 mg/L IBA and 1.5 mg/L BAP combined with 0.5 mg/L IBA. The highest percentage of callus induction, 40% of 'Chenin Blanc' and 31.3% of 'Canonannon' was exhibited by the leaf explants cultured on 1.5 mg/L BAP in combination with 1.0 mg/L IBA (Table 3 and Figure 1C and D). However, shoots were not regenerated from these calli of 'both cultivars.

Shoot multiplication

When shoots obtained from regeneration experiment were cultured on shoot multiplication medium (Figure 2A and B), the highest number of shoots per explant (10 ± 0.51) from 'Chenin Blanc' and 4.7 ± 0.3 for 'Canonannon' were obtained on medium containing 2.0 mg/L BAP. However, when shoots from *in vitro* maintained stock plants were cultured on the above same medium, the highest number of shoots per explant obtained from 'Chenin Blanc' and 'Canonannon' were only 3.3 ± 0.3) and 4.3 ± 0.3 respectively (Figure 3).

Hyperhydricity (vitrification) was a serious problem observed during this work. As a result of hyperhydricity, some regenerated shoots of both cultivars that were cultured on shoot multiplication medium started to lose leaves after three weeks of culture. This problem was observed more frequently on 'Chenin Balnc' cultivar than 'Canonnanon'. However, the percentage of hyperhydric shoots was reduced when the concentration of agar was increased from 7 to 8%, when the cultures were ventilated under laminar airflow cabinet twice a week and when the shoots were subcultured every three weeks instead of four weeks.

Rooting and acclimatization

Shoots started to produce roots in the first 10 days after

Table 2. Effect of TDZ and NAA combinations on *in vitro* shoot regeneration of 'Canonannon' and 'Chenin Blanc' cultivars of grapevine from leaf explants.

Growth regulators concentrations (mg/L)		'Canonannon'			'Chenin Blanc'		
TDZ	NAA	Callus induction (%)	Regeneration (%)	No. of shoots per explant	Callus induction (%)	Regeneration (%)	No. of shoots per explant
0.0	0.0	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{c}	0.0^{c}	0 .0 ± 0.0^{c}
0.1	0.01	0.0^{c}	8.5^{b}	1.3 ± 0.3^{ab}	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}
0.1	0.1	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{c}	0.0^{c}	0.0 ±0. 0^{c}
0.5	0.01	2.4^{b}	6.4^{b}	1.0 ± 0.0^{b}	2.8^{b}	0.0^{c}	0.0 ± 0.0^{c}
0.5	0.1	7.3^{b}	6.4^{b}	1.0 ± 0.0^{b}	8.3^{b}	0.0^{c}	0.0 ± 0.0^{c}
0.5	0.5	14.6^{b}	0.0^{c}	0.0 ± 0.0^{c}	50.0^{a}	0.0^{c}	0.0 ± 0.0^{c}
1.0	0.01	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}
1.0	0.1	18.2^{b}	29.8^{a}	1.5 ± 0.2^{a}	18.4^{b}	45.0^{a}	1.4 ± 0.2^{a}
1.0	0.5	7.3^{b}	0.0^{b}	0.0 ± 0.0^{c}	8.3^{b}	0.0^{c}	0.0 ± 0.0^{c}
1.5	0.01	9.8^{b}	19.0^{a}	1.0 ± 0.0^{b}	16.7^{b}	0.0^{c}	0.0 ± 0.0^{c}
1.5	0.1	0.0^{c}	7.0^{b}	1.0 ± 0.0^{b}	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}
1.5	0.5	24.4^{a}	6.4^{b}	1.3 ± 0.3^{a}	8.3^{b}	25.0^{a}	1.3 ± 0.3^{a}
2.0	0.01	7.3^{b}	6.0^{b}	1.0 ± 0.0^{b}	0.0^{c}	15.0^{b}	1.0 ± 0.0^{b}
2 .0	0.1	34.1^{a}	6.0^{b}	1.0 ± 0.0^{b}	8.3^{b}	15.0^{b}	1.0 ± 0.0^{b}
2.0	0.5	0.0^{c}	0.0^{c}	0.0 ±0. 0^{c}	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}
3.0	0.01	0.0^{c}	0.0^{c}	0.0 ±0. 0^{c}	0.0^{c}	0.0^{c}	0.0 ± 0.0^{c}
3.0	0.1	0.0c	0.0^{c}	0.0 ±0. 0^{c}	0.0^{c}	0.0c	0.0 ± 0.0^{c}

Means followed by the same letters in the same column are not significantly different at 5 % level of probability

Table 3. Effect of BAP and IBA combinations on *in vitro* shoot regeneration of 'Canonannon' and 'Chenin Blanc' cultivars of grapevine from leaf explants.

Growth regulators concentrations (mg/L)		'Canonannon'			'Chenin Blanc'		
BAP	IBA	Callus induction (%)	Regeneration (%)	No. of shoots per explant	Callus induction (%)	Regeneration (%)	No. of shoots per explant
0.0	0.0	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}
0.5	0.1	12.5^{c}	6.5^{b}	1.0 ± 0.0^{b}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}
0.5	0.5	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}
0.5	1.0	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}
1.5	0.1	31.3^{a}	0.0^{c}	0.0 ± 0.0^{c}	16.0^{b}	0.0^{c}	0.0 ± 0.0^{c}
1.5	0.5	3.1^{cd}	8.7^{b}	1.0 ± 0.0^{b}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}
1.5	1.0	31.3^{a}	0.0^{c}	0.0 ± 0.0^{c}	40.0^{a}	10^{b}	1.0 ± 0.0^{b}
2.0	0.1	0.0^{d}	71.7^{a}	1.4 ± 0.1^{a}	0.0^{d}	90.0^{a}	1.3 ± 0.1^{a}
2.0	0.5	0.0^{d}	8.7^{b}	1.3 ± 0.0^{a}	0.0^{d}	10.0^{b}	1.0 ± 0.1^{b}
2.0	1.0	21.9^{b}	4.3^{b}	1.0 ± 0.0^{b}	24.0^{b}	0.0^{c}	0.0 ± 0.0^{c}
3.0	0.1	16.9^{c}	13.5^{b}	1.0 ± 0.0^{b}	0.0^{d}	7.9^{b}	1.0 ± 0.0^{b}
3.0	0.5	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}
3.0	1.0	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}	0.0^{d}	0.0^{c}	0.0 ± 0.0^{c}

Means followed by the same letters in the same column are not significantly different at 5 % level of probability.

Figure 2. Shoot multiplication, rooting and acclimatization: Multiplication of shoots of leaf explant origin of 'Chenin Blanc' (A) and 'Canonannon' (B) on MS medium containing 2.0 mg/L BAP after 30 days. Rooted shoots of 'Canonannon' (C and D) and 'Chenin Blanc' (E and F) on MS basal salt medium supplemented with 2.0 mg/l IBA after 30 days of culture. Acclimatized plantlets of 'Chenin Blanc' (G) and 'Canonannon' (H) after 30 days.

culture on rooting medium. The highest mean number of roots per plantlet produced by 'Canonnannon' was 7.0 ± 0.92 whereas that of 'Chenin Blanc' was 6.7 ± 0.73 on medium containing 2.0 mg/L IBA (Table 4). The best mean root length produced by 'Canonnanon' and 'Chenin Blanc' were 5.5 ± 0.63 and 5.4 ± 0.50 on the above same medium, respectively.

There was no significant difference in mean shoot length per plantlet among the control and medium containing 1.0 and 2.0 mg/L IBA in both cultivars. When the performances of IBA and IAA were compared in the number and length of roots as well as length of shoot per plantlet, IBA performed much better than IAA. After one month acclimatization of the plantlets in glasshouse, 83.3% of 'Chenin Blanc' and 75% of 'Canonannon' survived and no aberrant plants were observed (Figure 2C to H).

DISCUSSION

Different types and concentrations of growth regulators significantly affected frequency of shoot regeneration, number of shoots per explant and percentage of callus induction of the two cultivars, 'Chenin Blanc' and 'Canonannon' in our study. In many woody plant species,

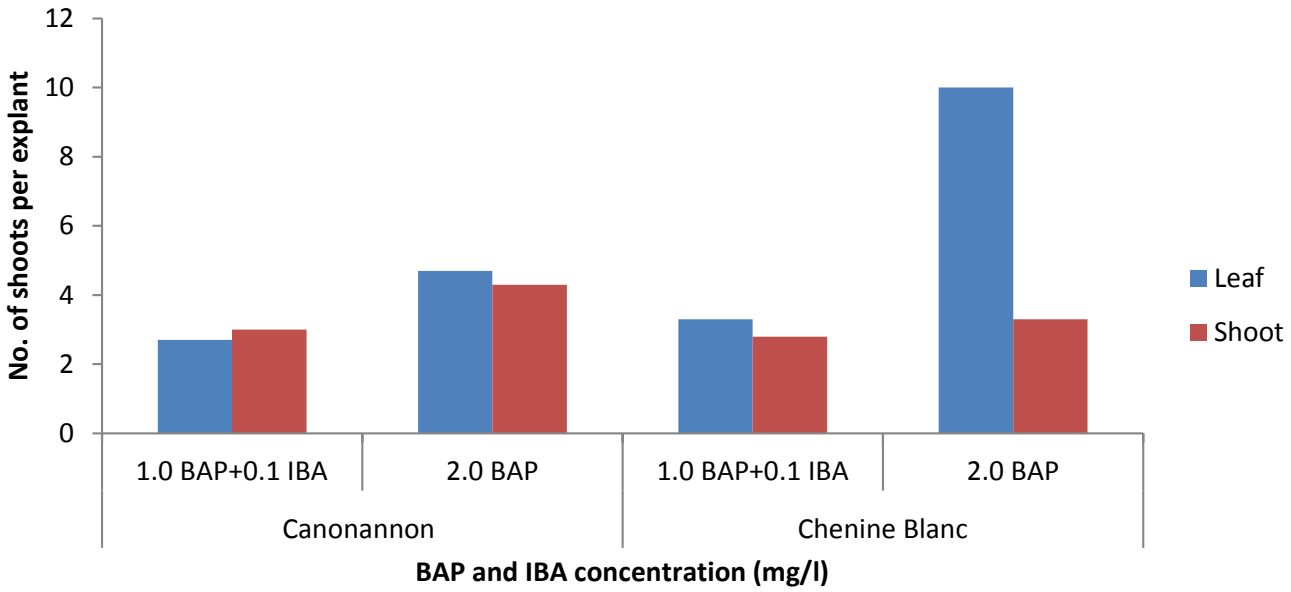

Figure 3. Number of shoots per explant that were produced by shoots of leaf explant origin and shoots that were maintained *in vitro* as stock on MS medium containing 2.0 mg/L BAP or 1.0 mg/ BAP in combination with 0.1 mg/L IBA.

Table 4. Effect of different concentrations of IBA and IAA on rooting of 'Canonannon' and 'Chenin Blanc' cultivars of grapevine.

Type of *GRs	GRs conc. (mg/L)	'Canonannon'			'Chenin Blanc'		
		Number of roots	Length of roots	Length of shoots	Number of roots	Length of roots	Length of shoots
Control	0.0	3.9 ± 0.23^{b}	4.7 ± 0.42^{ba}	7.5 ± 0 .23^{a}	3.6 ± 0.16^{b}	3.6 ± 0.22^{b}	7.2 ± 0.47^{a}
IBA	1.0	4.3 ± 0.54^{b}	4.3 ± 0.63^{ba}	7.4 ± 0.37^{a}	4.5 ± 0.42^{bc}	4.9 ± 0.60^{a}	7.3 ± 0.30^{a}
	2.0	7.0 ± 0.92^{a}	5.5 ± 0.63^{a}	8.3 ± 0.30^{a}	6.7 ± 0.73^{a}	5.4 ± 0.50^{a}	7.9 ± 0.40^{a}
	3.0	3.2 ± 0.29^{b}	2.9 ± 0.53^{b}	6.8 ± 0.34^{a}	3.7 ± 0.6^{b}	2.8 ± 0.40^{cb}	5.2 ± 0.55^{c}
	4.0	2.3 ± 0.31^{bc}	2.9 ± 0.23^{b}	3.9 ± 0.34^{b}	2.4 ± 0.26^{b}	2.1 ± 0.40^{c}	3.1 ± 0.58^{d}
IAA	2.0	2.4 ± 0.51^{bc}	3.4 ± 0.51^{ba}	3.8 ± 0.49^{b}	2.2 ± 0.37^{b}	3.6 ± 0.60^{bc}	2.8 ± 0.29^{d}
	4.0	3.14 ± 0.55^{b}	2.3 ± 0.47^{b}	3.43 ± 0.48^{b}	5.4 ± 0.92^{ac}	2.4 ± 0.20^{bc}	5.6 ± 0.29^{c}

*GRs = Growth regulators.
Means followed by the same letters in the same column are not significantly different at 5 % level of probability.

callus induction and plant regeneration have been achieved using TDZ (Huetteman and Preece, 1993). In addition, this cytokinin promotes efficient micro-propagation of many recalcitrant woody species at relatively low concentration (< 1.0 µM). However, in our study, although significant shoot induction was obtained on medium supplemented with TDZ combined with different concentrations of NAA, the highest percentage of shoot regeneration was exhibited by a medium supplemented with BAP in combination with IBA. Aazami (2010) also reported that BAP was the most effective among other cytokinins in promoting plant regeneration of *V. vinifera* cultivars 'Soltanin' and 'Sahebi' from shoot apical meristem. During *in vitro* culture, presence of cytokinin in the medium promotes shoot regeneration. However, in the present study, it also promoted callus induction, which is in agreement with the work of Baker and Bhatia (1993) who worked on shoot regeneration from leaf explants of quince (*Cydonia oblonga*).

The highest percentage of shoot regeneration was obtained on medium supplemented with 2.0 mg/L BAP in combination with 0.1 mg/L IBA in both varieties. Such high number of shoot formation per explant can be used for a variety of purposes, including plant improvement through *in vitro* selection and as a prerequisite for genetic engineering if the regeneration of shoots is from callus. Similarly, if regeneration is directly from the explants without passing through callus phase, that can be used for mass propagation of true-to- type clones. Although *in vitro* regeneration experiments were done on grapevine

varieties of ‘Cabernet Sauvignon’, ‘French Colombard’, ‘Grenache’, ‘Thompson Seedless’, ‘White Riesling’, *V. vinifera x rupestris* and *V. rupestris* using leaf explants (Stamp et al., 1990), this is the first report of regenerating shoots from leaves of ‘Canonannon’ and ‘Chenin Blanc’ on *in vitro* regeneration using leaf explants as each cultivar requires its own regeneration protocol.

The effect of light and type of leaf explant on *in vitro* regeneration was also studied and the explants cultured in light and dark conditions responded differently. In our study, leaves without petiole and petioles were cultured on different concentrations of TDZ, BAP or TDZ in combination with NAA, and BAP in combination with IBA. However, these explants did not regenerate shoots. First regenerated shoots were observed on the 25th day, sometimes at the wounded edges and mostly from swollen petiole tip. On the 30th day, the number of regenerated shoots increased and could be easily identified. Such response of leaf explants was observed in the previous work on other grapevine cultivars (Pe´ros, 1998). This time of regeneration is shorter when compared to the work of Aazami (2010) and Stamp et al., (1990). Thus, our results indicated that culture age of four weeks in the dark is necessary for shoot regeneration of grapevine varieties of ‘Canonannon’ and ‘Chenin Blanc’ using leaf explants.

The number of shoots produced per explant on shoot multiplication medium showed significant difference between shoots used from *in vitro* stock plants and the shoots used from regenerated leaf explants. There was also significant difference in the number of shoots per explant between the two cultivars. The shoots that were obtained from leaf explants through regeneration and cultured on shoot multiplication medium produced 10 ± 51 mean number of shoots per explant in ‘Chenin Blanc’ cultivar whereas the same explants produced 4.7 ± 0.29 shoots per explant in ‘Canonannon’ cultivar. Contrary to this, the highest mean number of shoots produced per explant from *in vitro* maintained stock shoots was 4.3 ± 0.3 for ‘Canonannon’ and 3.3 ± 0.3 for ‘Chenin Blanc’ cultivar. This could be probably due to the shoots obtained through regeneration from leaf explants are more juvenile than the shoots that were maintained on shoot multiplication medium. Generally, higher mean root number, root length and shoot length per plantlet were exhibited by shoots cultured on medium containing different concentrations of IBA than shoots cultured on different concentrations of IAA. The highest mean number of roots per plantlet produced by ‘Canonannon’ was 7.0 ± 0.92 whereas that of ‘Chenin Blanc’ was 6.7 ± 0.73 on medium containing 2.0 mg/L IBA. Kinfe et al., 2017) reported that among different concentrations of IAA used for rooting, 5.2 ± 1.0^{a} and 3.5 ± 0.6^{a} roots per plantlet were obtained from ‘Canonannon’ and ‘Chenin Blanc’ respectively on MS medium containing 4.0 mg/L IAA. However, she did not use IBA for rooting experiments. In our study, the shoots that exhibited highest number of shoots per explant showed better survival percentage during acclimatization.

Conclusion

Different types and concentrations of growth regulators significantly affected frequency of shoot regeneration, number of shoots per explant and percentage of callus induction of the two cultivars, ‘Chenin Blanc’ and ‘Canonannon’ in our study. Even though significant shoot induction was obtained on medium supplemented with TDZ combined with different concentrations of NAA, the highest percentage of shoot regeneration was exhibited by a medium supplemented with BAP in combination with IBA. The highest percentage of shoot regeneration was obtained on medium supplemented with 2.0 mg/L BAP in combination with 0.1 mg/L IBA in both varieties. The number of shoots produced per explant on shoot multiplication medium showed significant difference between shoots used from *in vitro* stock plants and the shoots used from regenerated leaf explants. There was also significant difference in the number of shoots per explant between the two cultivars. The shoots that were obtained from leaf explants through regeneration and cultured on shoot multiplication medium produced 10 ± 51 mean number of shoots per explant in ‘Chenin Blanc’ cultivar whereas the same explants produced 4.7 ± 0.29 shoots per explant in ‘Canonannon’ cultivar. Generally, higher mean root number, root length and shoot length per plantlet were exhibited by shoots cultured on medium containing different concentrations of IBA than shoots cultured on different concentrations of IAA.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Aazami MA (2010). Effect of some growth regulators on “*in vitro*” culture of two *Vitis vinifera* L. cultivars. Romanian Biotechnological Letters 15(3):5229-5232.

Baker BS, Bhatia SK (1993). Factors effecting adventitious shoot regeneration from leaf explants of quince (*Cydonia oblonga*). Plant Cell, Tissue and Organ Culture 35(3):273-277.

Fayek MA, Jomaa AH, Shalaby AB, Al-Dhaher AM (2009). Meristem tip culture for *in vitro* eradication of grapevine leaf roll associated virus-1 (GLRaV-1) and grapevine fan leaf virus (GFLV) from infected flame seedless grapevine plantlets. Iniciación a la Investigación 4:1-11.

Ferreira RB, Monteiro SS, Pic MA, Pereira A, Teixeira AR (2004). Engineering grapevine for increased resistance to fungal pathogens without compromising wine stability. Trends in Biotechnology 22(4):168-173.

Fikadu K (2016). Review on *in vitro* regeneration of some selected grapevines (*Vitis vinífera* L) cultivars from shoot and leaf culture. Journal of Natural Sciences Research 6(23):46-51.

Gray DJ, Fisher LC (1985). *In vitro* shoot propagation of grape species, hybrids and cultivars. Proceedings of the Florida State Horticultural Society 98:172-174.

Huetteman CA, Preece JE (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture 33:105-119.

Kinfe B, Feyssa T, Bedada G (2017). *In vitro* micropropagation of grape vine (*Vitis vinifera* L.) from nodal culture. African Journal of Biotechnology 16(43):2083-2091.

Kumsa F (2017). Effect of growth regulators on indirect organogenesis of two grapevines (*Vitis vinifera* L.) cultivars. African Journal of Biotechnology 16(16):852-859.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology **15**:473-497.

Patrice T, Thierry L, Mark RT (2006). Historical origins and genetic diversity of wine grapes. Trends in Genetics 22(9):511-513.

Stamp JA, Colby SM, Meredith CP (1990). Direct shoot organogenesis and plant regeneration from leaves of grape (*Vitis* spp.). Plant Cell, Tissue and Organ Culture 22(2):127-133.

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