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Relationship Between the Use of Gallic Acid and Genetic Stability of Grapes

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THE effect of Gallic acid (GA) at 200, 300, and 350 mg L^{-1}, on the genetic stability of SO4 and Freedom micropropagated grape rootstocks was studied through two subcultures. Plantlets' vegetative parameters didn't differ significantly between both primary subcultures. An inverse correlation occurred between elevated GA concentration in culture medium and plantlets' vegetative growth, meanwhile, it was a promoter by a moderate concentration of 200 mg L^{-1}. Furthermore, when GA exceeded 350 mg L^{-1} caused plantlets' breakdown. Moreover, fingerprinting analysis, RAPD-PCR has been performed to investigate relationships among GA presences in culture media during subcultures and their genetic profile. It detected 93 monomorphic bands with a ratio of 73.8 % and 18 polymorphic bands with a ratio of 16.2 %, out of them, 11 in the 1st subculture, while 7 in the 2nd subculture were detected. Under GA concentrations with a total ratio of 16.2 %, the polymorphism ratio was 61.1 % in the 1st subculture and decreased to 38.9 % in the 2nd subculture using 350 mg L^{-1} of GA. Gallic acid concentrations, of 200, 300 and 350 mg L^{-1} in growth medium preserved both micro-propagated rootstocks' genetic stability through two subcultures.

Keywords: Grape, Gallic Acid, Mutations, RAPD, PCR.

Introduction

Grapevines are usually propagated by cuttings, thus, the resulting clones of a population are genetically identical to each other (except for somatic mutations) and to the mother plant (Rita Vignani et al., 2002). However, years ago plants micro-propagation *via* tissue culture technique became the main part of modern agriculture, but it is known that the repetitive *in vitro* subcultures alter plantlet's genetic structure due to several reasons such as the species or the tissue culture technique itself. This restricts the use of *in vitro* propagation, so preserving a growing plant from mutations has a great value, as the edible and economic importance of (*Vitis* sp.) production mitigation is well known (Ritschel et al., 2010).

Gallic acid is a natural secondary plant metabolite, it's a triphenole with low molecular weight and an auxin synergist that has been long proven as a cutting root growth promoter promoted *Eranthemum tricolor* cuttings root growth (Randhawa and Mukhopadhyay 1986). GA, its derivatives, and other phenolic acids affected seedling and early plant growth of rye, barley, oats, sorghum, corn, and wheat (Krogmeier and Bremner, 1989, Bhattacharya, 2005). Furthermore, GA has the potency to prevent cell DNA damage (Ferk et al., 2011). A study of gallic and pyrogallic acids impact on cucumber seeds at five concentrations pointed out that, they significantly decreased growth, fresh and dry weights, where the high concentrations like 10^{-3} M and 5 µM caused the maximum

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inhibition (Barket et al., 2012 and Liu et al., 2013). Also, GA was found to have an inhibitory effect on human cells' tumor growth (Ho et al., 2013, Locatelli et al., 2013). It can stabilize cells *α*- synuclein structure (Liu et al., 2014). Megan Sylvia et al. (2015) noticed that GA can play a role in *Rubus* callus plant growth developmental regulation. Moreover, GA mitigated toxin-induced injury tolerance, having a beneficial effect against salinity and osmotic stresses (Konakci et al., 2015a and Konakci et al., 2015b). Exogenous GA can be used as an effective growth promoter that affected plants' physiology and reduced free radicals Singh et al. (2017). Furthermore, it was proven that, GA act as a strong antioxidant can protect biological cells since it has the capability to restore antioxidants' statues to their normal levels through gene involving in cell oxidative mechanism pathway activation (Gao et al., 2019 and Radan et al., 2019).

Traditional methods for fruit characterization recognition depend on the morphological properties which are also affected by environmental and growth factors, and subsequently decrease markers efficiency **(**Ohmi et al., 1993). So, DNA molecular markers have been widely used to assess genetic diversity and germplasm characterization. The use of RAPD markers for studying genetic divergence was efficient, despite their lower reproducibility in comparison to other molecular markers (Herrera et al., 2002, Ulanovsky et al., 2002, PintoCarnide et al., 2003, Kocsis et al., 2005). In this respect, Modgil et al. (2005) and Lakshmanan et al. (2007) assessed the genetic stability of 10 micro-propagated apple and long-term micro-propagated banana plants using RAPD to analyze DNA genetic similarities and dissimilarities, if any, between mother plants and *in vitro* plants. However, Alizadeh et al. (2008) subjected three grape rootstocks and their 30 *in vitro* regenerates to ISSR to ascertain their genetic stability. The amplification size of each ranged from 100 to 1800 bp. When nodal segments were utilized as an *in vitro* culture initiation source, no variation was detected among the regenerated plantlets and their mother plants. Again, micro-propagated plantlets derived from three rootstocks were subjected to RAPD and ISSR analyses. RAPD had polymorphisms other than ISSR, and both techniques generated profiles that were highly uniform and monomorphic (Alizadeh and Singh, 2009). Singh et al. (2017) used ISSR as an efficient tool for the genetic stability assessment of varieties at mass multiplication protocols such as rootstock genotypes under *in vitro* conditions with a high-reliability degree.

Therefore, this study aimed to detect the genetic variability when two grape *in vitro* cultured lines, SO4 and Freedom, were cultured on GA-containing media over two subcultures. Fingerprint analysis via RAPD-PCR was used to investigate GA effects on both rootstocks.

Materials and Methods

Experimental steps

Culture establishment

Plant material

Shoot tips of two rootstocks' namely, SO4 (*V. berlandieri* x *V. riparia* Michx.) and Freedom (*V. champinii* Planch. x (*V. solonis* hort. Berol. ex Planch. x *V. othello*)) were collected and sterilized to start culture. The explants were cultured on various following media concentrations for the two subcultures*:*

Chemicals

Free MS (Morashige and Skoog 1962) medium, MS+ gallic acid (3, 4, 5- trihydroxybenzoic acid) were prepared with the concentrations of 200, 300, and 350 mg L^{-1}.

Subcultures

After a month of each culture, plantlets were sub-cultured on fresh medium with the same previous concentration reaching the last one (according to plantlet survival) and the following measurements' were taken:

Plant vegetative measurements

Plantlets height (cm), No. of leaves per plantlet, average leaf area (cm^2), average root length (cm), roots fresh weight (g), and roots dry weight (g) were measured at the end of each subculture for all treatments.

Molecular study

Mother plant samples were genetically analyzed through fingerprinting at the start. Then monthly samples were taken from different plantlets during each subculture to be compared to the mother plants for any alteration detection using the RAPD technique.

DNA: was extracted from the two rootstock leaves for both subcultures by bio basic kits protocol.

PCR- Amplification of RAPD: Amplification reaction was done in 25µl reaction mixture containing 2µl of genomic DNA, 3µl of the primer, 2.5µl of 10X Taq DNA polymerase

reaction buffer, 1.5 units of Taq DNA polymerase, and 200 mm of each dNTPs. The following PCR program was used in a DNA Thermocycler (PTC-100 PCR version 9.0-USA). Initial denaturation was detected at 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec., 42°C for 90 sec. for annealing temperature, 72°C for 90 sec. and finally extended at 72°C for 2 min.

RAPD- PCR products were separated on 1.5 % agarose gels in 1X TAE buffer and ethidium bromide (Sambrook et al., 1989). DNA ladder 100bp was used and PCR products were visualized by UV-transilluminator and photographed by gel documentation system, Biometra - Bio Documentations, the amplified bands were scored as (1) for the presence and (0) for the absence of all studied grape genotypes as gel analyzer protocol.

RAPD analysis: A set of 9 random 10-mer primers (Table 1) was used in polymorphism detection among the 2 grape accessions.

TABLE 1. Code and sequences of nine RAPD primers.

Primer code	Sequence (5`→3`)	Program analysis
OPA-02	CAGGCCCTTC	Gel analyzer3.
OPA-04	AATCGGGCTG	SPSS Version20.
OPA-07	GAAACGGGTG	PCR program.
OPB-07	GGTGACGCAG	
OPB-10	CTGCTGGGAC	
OPO-10	TCAGAGCGCC	
OPO-13	GTCAGAGTCC	
OPO-14	AGCATGGCTC	
OPO-19	CAATCGCCGT	

Statistical analysis

Collected data were arranged in a completely randomized design (CRD). Data statistical analysis was done according to Snedecor and Cochran (1980). Comparisons among the means of the treatments were held using the new L.S.D. values at a 5 % level.

Results

Vegetative measurements

Plantlets height

As illustrated in (Table 2), plantlets' heights didn't differ significantly in the two subcultures for both rootstocks, since SO4 height recorded 11.60 cm in the first one and 12.07 cm in the second subculture to. Also, Freedom heights were 9.34 and 9.66 cm in the first and second subcultures, respectively.

Regarding GA concentrations, there was a significant reverse relationship between the concentration and the height since the tallest SO4 plantlets (13.92 cm) were recorded with the control, whereas the shortest (10.34 cm) were measured with 350 mg L^{-1} of GA. Similarly, Freedom control plantlets were the highest (10.33 cm) while 350 mg L^{-1} of GA plantlets were the shortest (8.92 cm) with significant difference between them.

Gallic acid concentrations under the two subcultures significantly affected plantlets' height. The tallest SO4 plantlets (14.67 cm) were measured with the control in the second subculture, while the shortest ones (10.17 cm) were found with 350 mg L^{-1} of GA in the first subculture. Freedom' tallest plantlets (10.35 cm) were recorded with the control and 200 mg L^{-1} GA in the second subculture, and the shortest ones (8.67 cm) were found with 200 mg L^{-1} GA in the first subculture.

No. of leaves per Plantlet

It is clear from the results of Table 2 that subcultures didn't affect the leaves number significantly of both rootstocks.

Regarding GA concentrations effect, SO4 number of leaves did not differ significantly than the control. On the contrary, GA affected Freedom leaves number significantly as the highest number (10.50) was counted with 350 mg L^{-1} of GA, while the lowest (6.33) was recorded with the control.

Moreover, various GA concentrations during both subcultures had no significant effect on SO4 leaves number.−Whereas, Freedom showed the highest number of leaves (11) with 350 mg L^{-1} of GA plantlets that sub-cultured twice but recorded the lowest number of leaves (6) in the first subculture plantlets with the control and 300 mg L^{1} GA concentrations with significant differences among them.

TABLE 2. Plantlet height, No. of leaves per plantlet and average leaf area as affected by GA at different concentrations through two subcultures.

Plantlet height (cm)										
	SO4					Freedom				
GA (mg L^{-1})	Cont.	200	300	350	Mean A	Cont.	200	300	350	Mean A
Sub1	13.17	11.40	11.67	10.17	11.60	10.30	8.67	9.65	8.75	9.34
Sub2	14.67	11.88	11.23	10.51	12.07	10.35	10.35	8.86	9.08	9.66
Mean B	13.92	11.64	11.45	10.34		10.33	9.51	9.25	8.92	
LSD (A) = N.S LSD (B) = 1.39 LSD (AXB) = 1.96						LSD (A) = N.S LSD (B) = 1.06 LSD (AXB) = 1.50				
No. of leaves per Plantlet										
	SO4					Freedom				
GA (mg L^{-1})	Cont.	200	300	350	Mean A	Cont.	200	300	350	Mean A
Sub1	11.03	12.00	12.67	13.67	12.34	6.00	7.67	6.00	10.00	7.42
Sub2	10.67	10.67	11.67	11.00	11.00	6.67	8.33	7.00	11.00	8.25
Mean B	10.85	11.33	12.17	12.33		6.33	8.00	6.50	10.50	
LSD (A) = N.S LSD (B) = N.S LSD (AXB) = N.S						LSD (A) = LSD (B) = LSD (AXB) =		N.S 2.09 2.96		
Average leaf area (cm^2)										
	SO4					Freedom				
GA (mg L^{-1})	Cont.	200	300	350	Mean A	Cont.	200	300	350	Mean A
Sub1	5.10	3.88	3.68	1.49	3.54	4.32	3.80	2.80	2.20	3.28
Sub2	6.63	4.48	3.96	3.65	4.68	5.14	3.45	2.78	2.57	3.48
Mean B	5.87	4.18	3.82	2.57		4.73	3.63	2.79	2.38	
LSD (A) = 0.29 LSD (B) = 0.42 LSD (AXB) = 0.59						LSD (A) = N.S LSD (B) = 0.59 LSD (AXB) = 0.84				

Average leaf area

It could be seen in Table 2 that the average leaf area of SO4 plantlets differed significantly between subcultures where it was 3.54 cm^2 in the first subculture while it was large (4.68 cm^2) in the second one. On the contrary, Freedom leaves area lacked significance between the two subcultures and recorded 3.28 and 3.48 cm^2 in first and second subcultures, successively.

Considering GA concentrations' effect on both rootstocks, it was significant and inhibitor when increased in the medium. SO4 largest leaves (5.87 cm^2) were scored with the control and the smallest (2.57 cm^2) were recorded with 350 mg L^{-1} of GA, while Freedom's largest leaves (4.73 cm^2) were measured with the control but the smallest (2.38 cm^2) were obtained with 350 mg L^{-1} of GA.

The interaction between subcultures and GA concentrations was significant, the largest leaf area (6.63 cm^2) with SO4 and (5.14 cm^2) in Freedom was obtained with free MS medium in the second subculture. Whereas, the smallest leaf areas (1.49 cm^2) in SO4 and (2.20 cm^2) with Freedom were recorded with 350 mg L^{-1} GA containing medium in the first subculture.

Average root length

Results of Table 3 show that the average root length of both rootstocks did not differ significantly between the two subcultures.

Concerning the GA concentrations' effect on SO4 plantlets, the tallest roots (29.83 cm) were scored with 200 mg L^{-1}, while the shortest (24.25 cm) was significantly recorded with the control. Similarly, in Freedom significant differences were found among treatments since the tallest roots (28.50 cm) were measured with 200 mg L^{-1} of GA and the shortest (23.42 cm) were measured with the control.

Considering the interaction between both subcultures and GA concentrations on SO4 plantlets, it could be seen that there were significant differences among treatments. The tallest roots (30.33 cm) were measured with 200 mg L^{-1} of GA in the second subculture whereas the shortest (23.33 cm) were measured with the control in the second subculture. On the other hand, Freedom roots length was differed significantly among treatments since the tallest ones (29.67 cm) were measured with 200 mg L^{-1} of GA in the first subculture, while the shortest (23 cm) were measured with the control in the second subculture.

Roots Fresh weight

Table 3 illustrate that the subculture had no significant effect on SO4 or Freedom roots fresh weight.

As for GA concentration, it did not affect SO4 fresh weight significantly. However, its' presence significantly affected Freedom roots' fresh weight where GA at 200 mg L^{-1} gave the heaviest ones (7.82 g) while the control showed the lightest roots (4.09 g).

The interaction between the two subcultures and GA concentrations on SO4 rootstock didn't show significant differences among the treatments. However, it differed significantly in Freedom where the highest roots weight (8.63 g) was found with 200 mg L^{-1} GA in the second subculture, but the lowest weight (3. 74 g) was recorded with the control in the second subculture.

Roots Dry weight

As shown in Table 3 it is clear that both rootstocks roots' dry weight weren't affected significantly due to the subculture.

The two rootstocks show similar significant responses to GA concentrations, where SO4 and Freedom scored the heaviest dry roots weights (2.30 and 2.37 g) with GA at 200 mg L^{-1} while they had the finest weights (1.51 and 1.05 g), successively with the control.

Moreover, the interaction between the two subcultures and GA concentrations on root dry weight showed a significant effect as SO4 heaviest root (2.51 g) was recorded with 200 mg L^{-1} GA plantlets in the second subculture, meanwhile the heaviest weight in Freedom (2.39 g) was weighed with 200 mg L^{-1} GA in the first subculture. The finest weight of SO4 (1.42 g) was measured with the control plantlets sub-cultured twice, while was (1 g) for Freedom control plantlets sub-cultured once.

Molecular screening

Fig. 1 a-d and 2 and Table 4 clear that, out of the 12 RAPD primers tested, only nine produced reproducible and polymorphic bands. One hundred and eleven loci were identified, which represented 83.8 % of total monomorphic bands. The number of loci for each primer ranged from eight to 19 with an average of 12.3 per primer. The size of amplification products ranged from 300 to 1400 bp**.** The patterns obtained by RAPD analysis were sample-dependent (Luo et al., 2001).

The present study revealed 16.2 % polymorphism as a low ratio, while Tamhankar et al. (2001) showed that the polymorphism levels depend on the analyzed species, these authors obtained 94 % of polymorphism for wild vine species and rootstocks and more than 90 % for *V. vinifera* genotypes, however, almost all bands were monomorphic among *V. labrusca*. RAPD-PCR technique of nine primers revealed 111 different bands, 93 of them were monomorphic bands (83.8 %) and 18 polymorphic bands (16.2 % polymorphism) as shown in Table 4.

Results in Table 5 revealed 11 generic markers since three markers of them detected in the first subculture as two out of them were positive markers and one was a negative marker. While eight molecular markers were found with the second subculture, three of them were negative and five were positive markers by ratio over all markers reached 63.63%.

TABLE 3. Average root length, roots fresh weight, and roots dry weight as affected by GA at different concentrations through two subcultures.

Average root length (cm)

	SO4					Freedom				
GA (mg L^{-1})	**Cont.**	**200**	**300**	**350**	**Mean A**	**Cont.**	**200**	**300**	**350**	**Mean A**
Sub1	25.17	29.33	24.00	24.00	25.63	23.83	29.67	27.33	25.00	26.46
Sub2	23.33	30.33	28.67	27.97	27.58	23.00	27.33	27.00	25.00	25.58
Mean B	24.25	29.83	26.33	25.98		23.42	28.50	27.17	25.00	
LSD (A) = N.S LSD (B) = 2.88 LSD (AXB) = 4.07						LSD (A) = N.S LSD (B) = 4.32 LSD (AXB) = 6.11				

Roots fresh weight (g)

	SO4					Freedom				
GA (mg L^{-1})	**Cont.**	**200**	**300**	**350**	**Mean A**	**Cont.**	**200**	**300**	**350**	**Mean A**
Sub1	4.08	8.52	7.28	6.40	6.57	4.45	7.00	6.76	6.67	6.22
Sub2	5.68	6.00	6.08	6.38	6.04	3.74	8.63	7.60	6.91	6.72
Mean B	4.88	7.26	6.68	6.39		4.09	7.82	7.18	6.79	
LSD (A) = N.S LSD (B) = N.S LSD (AXB) = N.S						LSD (A) = N.S LSD (B) = 1.39 LSD (AXB) = 1.96				

Roots dry weight (g)

	SO4					Freedom				
GA (mg L^{-1})	**Cont.**	**200**	**300**	**350**	**Mean A**	**Cont.**	**200**	**300**	**350**	**Mean A**
Sub1	1.60	2.09	1.96	2.04	1.92	1.00	2.39	2.10	1.96	1.86
Sub2	1.42	2.51	2.02	1.89	1.96	1.11	2.35	2.33	2.20	2.00
Mean B	1.51	2.30	1.99	1.96		1.05	2.37	2.22	2.08	
LSD (A) = N.S LSD (B) = 0.42 LSD (AXB) = 0.59						LSD (A) = N.S LSD (B) = 0.40 LSD (AXB) = 0.56				

TABLE 4. Total number, monomorphic, polymorphic, unique bands in sub culture1, and unique bands in sub culture2 and polymorphism % as revealed fewer than 3 concentrations of GA using 9 RAPD primers on SO4 and Freedom grape rootstocks.

Primer code	Total bands	Monomorphic bands	Polymorphic bands	Unique bands (Sub culture1)	Unique bands (Sub culture2)	polymorphism%
OPA-02	19	16	3	2	1	15.8%
OPA-04	15	13	2	1	1	13.3%
OPA-07	10	8	2	1	1	20%
OPB-07	13	11	2	1	1	15.4%
OPB-10	11	9	2	1	1	18.2%
OPO-10	15	13	2	2	0	13.3%
OPO-13	10	8	2	1	1	20%
OPO-14	10	9	1	1	0	10%
OPO-19	8	6	2	1	1	25%
Total bands	111 (100%)	93 (83.8%)	18 (16.2%)	11 (9.9%)	7 (6.3%)	16.2%

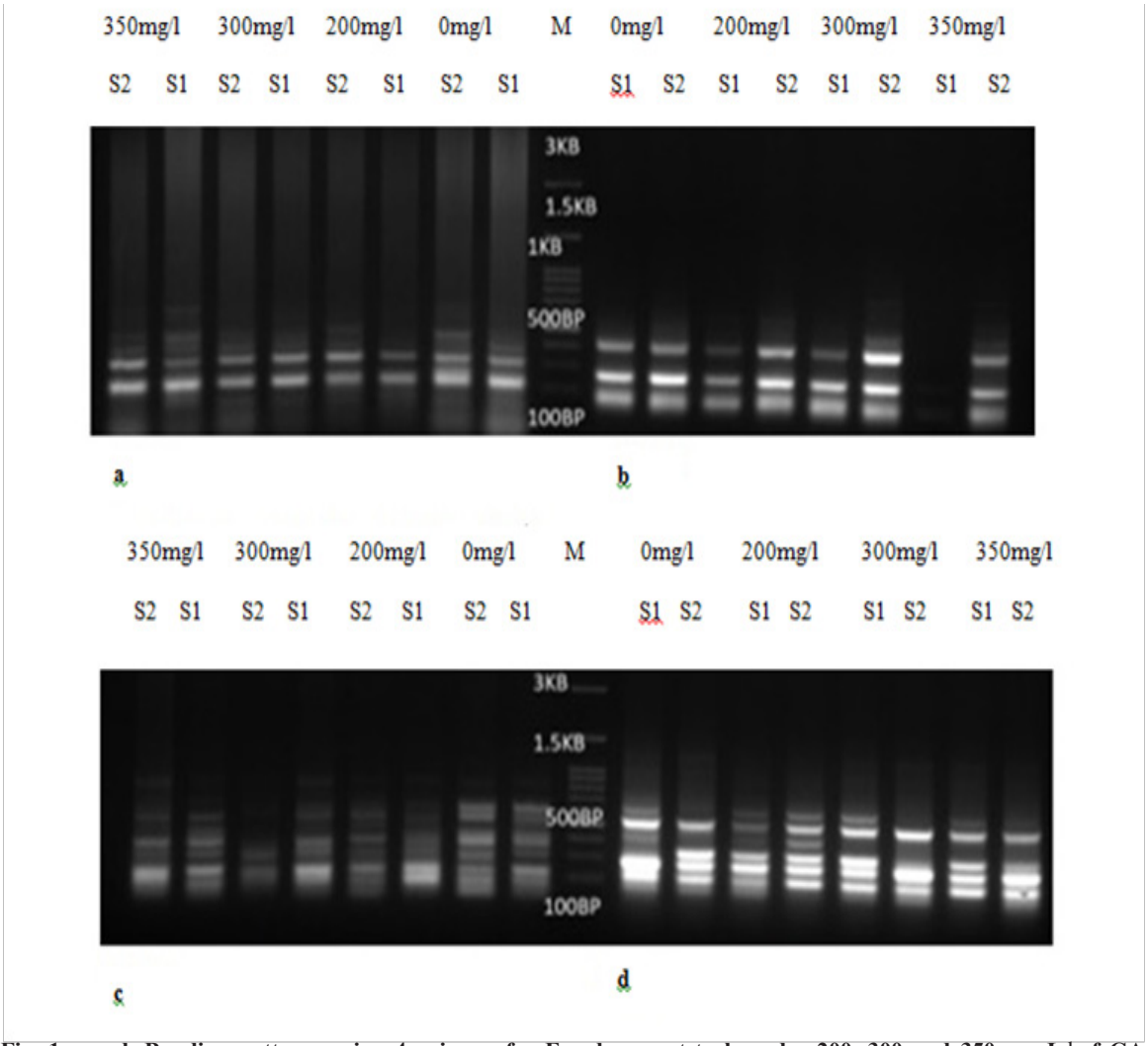

Fig. 1. a- d. Banding patterns using 4 primers for Freedom rootstock under 200, 300 and 350 mg L^{-1} of GA concentrations during subculture 1 (S1) and subculture 2 (S2)- a. OPA-02, b. OPA-04, c. OPA-07, d. OPB-07.

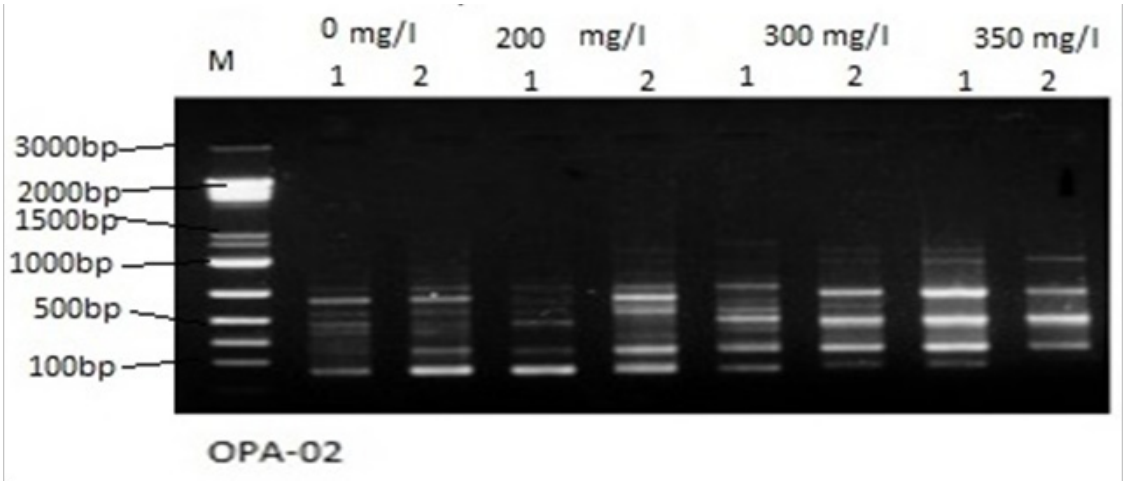

Fig. 2. DNA fingerprinting using RAPD markers with OPA-02 primer for SO4 rootstock under 200, 300 and 350 mg L^{-1} of GA concentrations during subculture 1 (S1) and subculture 2 (S2).

TABLE 5. Markers assisted selection with GA using 9 RAPD primers.

Primer code	No. of unique band	Molecular size (MS)	Subculture 1	Subculture2	N or P Markers and P %
OPA-02	2	710 and 350bp	1	1	P
OPA-04	1	510bp	0	1	P
OPA-07	1	910	0	1	N
OPB-07	1	560	0	1	P
OPB-10	1	230	0	1	N
OPO-10	2	620 and 170bp	1	1	N
OPO-13	1	310bp	0	1	P
OPO-14	1	680bp	1	0	P
OPO-19	1	430bp	0	1	N
Total			3= (2p+1n)	8= (5p+ 3n)	63.63%

Discussion

The two rootstocks under this study showed similar patterns of responses to various tested treatments, but SO4 plantlets seemed more sensitive, or in another word, less stable genetically than Freedom plantlets which may be due to the genetic structure itself of each one of them. Those results go with Alizadeh et al. (2008) and Alizadeh and Singh **(**2009) findings.

Non-significant differences in vegetative characters among plantlets under various treatments reflect the beneficial role of GA in promoting *in vitro* growth and its ability to suppress some genetic variations that may occur through micro- propagation. This may be due to GA's contribution in preventing DNA oxidative damage depending on its ability to restore cell DNA methyltransferase. These results are in harmony with those of (Kam et al., 2014, Konakci et al., 2015a, Konakci et al., 2015b, Gao et al., 2019, Radan et al., 2019). Meanwhile, the high GA concentrations (400- 1000 mg L^{-1}) were growth inhibitors and later lethal for the plantlets because they are a type of phenolic acid (Barket et al., 2012, Kam et al., 2014, Singh et al., 2017). A moderate dose of it can stimulate rapid tissue proliferation and cell cycle changes, while a high dose will damage the tissue, GA monitored gene regulation in fact (Megan Sylvia et al., 2015).

Moreover, RAPD-PCR is useful in genetic variation examination, if occurred, as it cleared the homomorphism and polymorphism percentages (Alizadeh and Singh, 2009, Herrera et al., 2002, Ulanovsky et al., 2002, PintoCarnide et al., 2003 and Kocsis et al., 2005).

Conclusion

This trial depended on GA as a cryopreserving agent for micro propagated grapes namely, SO4 and Freedom sub-cultured twice on MS medium. Gallic acid presence at moderate concentrations such as 200 and 300 mg L^{-1} in micro propagation culture medium of SO4 and Freedom rootstocks has a positive influence since it demands acceptable vegetative growth parallel with genetic stability during two subcultures.

In addition, molecular markers as efficient breeding approaches were successfully used to estimate the genetic variability between the studied samples. Moreover, RAPD was a successful technique and very important in explaining genetic variability. In this study, the best GA concentration was 300 mg L^{-1} that helps with genetic stability in different isolations.

Abbreviations

GA: gallic acid, RAPD: Random amplification of polymorphic DNA, PCR: polymerase chain reaction, SSC: soluble solids content, PGRs: plant growth regulators.

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Conflict of interests

No conflict of interests appeared among authors during this work.

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العلاقة بين استخدام حمض الجاليك والثبات الوراثي لاثنين من أصول العنب الشائعة

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أجري هذا البحث لدراسة تأثير إضافة التركيزات ٢٠٠، ٣٠٠ و 350 ملجم لتر$^{-1}$ من حمض الجاليك في بيئة الزراعة على الثبات الوراثي لأصلي العنب SO4 و فريدم الناميين بالمعمل. لوحظ أن النمو الخضري للنبيتات لم يتأثر معنوياً خلال المرتين الأوليين من النقل على بيئة نمو جديدة بذات التركيز بينما تدهورت بشدة بعد ذلك وصولاً للتلون البني والذبول الكامل. كما ان التركيز المتوسط ٢٠٠ ملجم لتر$^{-1}$ من حمض الجاليك كان له تأثير محفز على نمو النبيتات و ظهرت علاقة عكسية بين المستويات المتصاعدة منه و مقاييس النمو، فبتجاوز تركيزه في البيئة 350 ملجم لتر$^{-1}$ أدى لموت النبيتات. و بتحليل البصمة الوراثية للنبيتات بواسطة RAPD-PCR باستخدام 9 بادئات وجدت 111 حزمة وراثية منهم 93 متماثلة بنسبة 73.08 % و 18 متباينة بنسبة 16.2 %، ظهرت منهم 11 باند مختلفة بالنقل على البيئة لأول مرة و 7 باند بالنقل على البيئة في المرة الثانية. كما لوحظ ان وجود حمض الجاليك في البيئة بتركيز 350 ملجم لتر$^{-1}$ ، سُجل تباين وراثي بلغ ٦١,١ ٪ في أول تجديد للبيئة وانخفض إلى ٣٨,٩ ٪ عند تجديد البيئة للمرة الثانية. و خلصت الدراسة إلى أنه بإستخدام التركيزات ٢٠٠، 300 و 350 ملجم لتر$^{-1}$ من حمض الجاليك في بيئة النمو يمكن حفظ الثبات الوراثي لكلا الأصلين الناميين بالمعمل خلال النقل على البيئة لمرتين. و أخيراً فإن وجود حمض الجاليك بتركيز ٣٠٠ ملجم لتر$^{-1}$ في بيئة النمو من قد حقق نمواً مناسباً للنبيتات مع الحفاظ على التركيب الوراثي لها.

الكلمات الدالة: العنب، حمض الجاليك ، الطفرات، RAPD، PCR