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Iranian Grapevine Rootstocks and Hormonal Effects on Graft Union, Growth and Antioxidant Responses of Asgari Seedless Grape

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A B S T R A C T

This research investigated physiological and biochemical changes at rootstock–scion union of graft combinations between Iranian rootstocks and Asgari scion in response to NAA and BAP application. Grafting cuts of scion and rootstock were dipped into NAA (0, 200 and 400 mg·L^{-1}) and BAP (0, 250 and 500 mg·L^{-1}) solutions for 30 min, immediately after the cuttings were grafted using the wedge grafting technique. The highest successful grafting rate (82%) was recorded with Asgari–Asgari combination treated with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP. Treating the graft cut–surfaces with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP caused the lowest H_2O_2 and MDA contents. Among all the graft combinations, the highest increase in chlorophyll content with a high photosynthesis efficiency occurred in Asgari–Shahani when treated with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP. The graft union formation in the presence of 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP application enhanced CAT and POX activities. In conclusion, treatment of graft zone with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP enhanced antioxidant activities and reduced lipid peroxidation in scion leaves. However, several grapevine rootstocks tested in our research were also impacting the scion performance.

Keywords: antioxidant activity; auxin; cytokinin; graft union formation; grapevine; photosynthesis efficiency

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most important perennial crops in the world (Fatahi et al., 2003). Due to the diversified climate of Iran, the grape germplasm is rich and complex and consists of a large number of grapevine cultivars and wild populations (Tafazoli et al., 1993). Grafting is a viticultural technique used worldwide because rootstocks impart resistance to soil-borne pests and diseases as well as enhance tolerance to abiotic stresses (Cookson et al., 2013). Therefore, the grafting process is very important and comprises complex biochemical and structural changes during the adhesion of the two grafted partners, followed by callus formation and the establishment of a functional vascular system (Nickell, 1984; Hartmann et al., 1990; Cookson et al., 2013). Grafting success is influenced by plant genetics, growth characteristics, physiological and biochemical factors (Youqun, 2011). Differentiation of callus into vascular tissue (xylem and phloem vessels) is a result of a complex developmental process involving structural and physiological changes, aiming to restore the transport system. During this process, lignin is synthesized in cells to become part of the transport system. Several antioxidant enzymes such as peroxidase and catalase activity, are concentrated at the level of the graft union, and are related to the lignification process (Jeffree and Yeoman, 1983; Quiroga et al., 2000; Fernandez-Garcia et al., 2004).

However, graft union formation is influenced by several factors, among them the most important are the rootstock–scion combinations, propagation techniques, environmental conditions, pest and disease pressure, the activity of the rootstock and the use of plant growth regulators (Hartmann et al., 1990).

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The effect of some plant growth regulators such as auxins and cytokinins on callus development and formation of the new vascular tissue has been previously reported (Hartmann et al., 1990; Salisbury and Ross, 1992; Preece and Read, 1993). Kose and Guleryuz (2006) used auxins and cytokinins on grafted cuttings of grapevine cultivars to increase callus formation and successful grafting.

However, there have been no attempts to investigate the changes of antioxidant activities in the scion variety caused by cytokinins and auxins applied at the level of the graft union. Therefore, the objective of this study was to evaluate the impact of auxin, 1-naphthaleneacetic acid (NAA) and cytokinin, and 6-benzyl aminopurine (BAP) treatments when applied to the grafting union. We aim to evaluate the formation of the grafting union as well as the impact on antioxidant enzyme activities of the scion caused by hormone application and the use of different rootstocks.

2. Materials and methods

2.1. *Plant materials, grafting technique and hormones application*

Dormant hardwood cuttings of *Vitis vinifera* (cv. Asgari) and several rootstocks (Shahani, Keshmeshi and Yaghuti) were collected from the vineyard of Isfahan University of Technology, Department of Horticultural Sciences (Iran) in the month of March 2014. Vine canes were cut into 30 cm cuttings for the rootstocks and single-bud cuttings for scions. The single-bud scions of Asgari were grafted onto four rootstocks (Shahani, Keshmeshi, Yaghuti and Asgari), using the wedge grafting technique (Fig. 1). For grafting procedure a French grafting device was used, as described by Hartmann et al. (1990). The surface of the grafting cuts of both scion and rootstock material was dipped into a combination of NAA (0, 200 and 400 $mg \cdot L^{-1}$) and BAP (0, 250 and 500 $mg \cdot L^{-1}$) for 30 min before the grafting procedure. After the dipping procedures, cuttings were air-dried for 5 min and the grafting unions were wrapped in a white adhesive tape (Fig. 1).

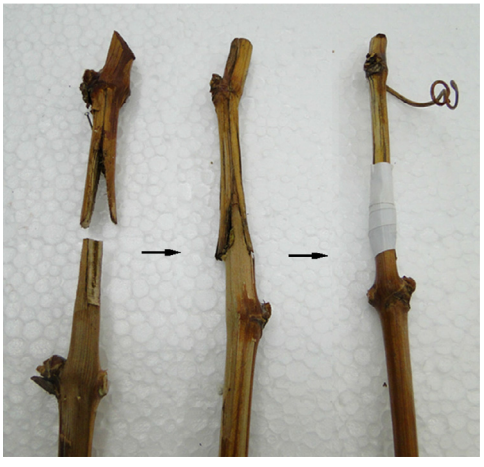

Fig. 1 The manual wedge grafting units in hardwood cuttings of grapevine made by a French grafting device
Arrows show the grafting process.

Fig. 2 Graft union formation of grafted cuttings of grapevine
Arrows show the proliferation of callus at the grafting point.

The grafted material was then placed in pots with rooting soil consisting of sand and perlite at a ratio of 1:1. A completely randomized design with three replications and 10 grafted cuttings per replicate was used for the experiment.

2.2. *Analysis of growth parameters*

Sixty days after grafting, the grafted cuttings were evaluated for growth (fresh weight) of the scion leaves. After, the leaves were dried in an oven at 80 °C for 48 h to determine the dry weight. Length of main scion shoot also was measured. The diameter of the main scion shoot was recorded 1 cm above the second node according to Celik (2000). The rate of graft take was expressed as the percentage of grafted grapevines that have an adequate callus formation at the level of the graft union (Fig. 2) as reported by Celik (2000). The rate of bud burst was also recorded as the percentage of grafted grapevines with adequate shoot length and diameter as suggested by Celik (2000).

2.3. *Analysis of physiological and biochemical parameters*

2.3.1. *Photosynthesis efficiency and chlorophyll measurement*

Chlorophyll fluorescence was measured using a fluorometer (Walz, Effeltrich, Germany) and photochemical efficiency of PS II was calculated as the ratio Fv/Fm. The relative chlorophyll content was measured with a portable leaf chlorophyll meter (SPAD 502, Minolta Co., Osaka, Japan).

2.3.2. *Antioxidant enzyme measurement*

Sixty days after treatment, the main scion leaves were harvested and frozen in liquid nitrogen and kept on −80 °C until used for biochemical measurements. Antioxidant enzyme assay of the main scion (Asgari) leaves was measured.

Frozen-leaf samples (0.1 g) were grounded in liquid nitrogen with an ice-cooled mortar and pestle and transferred into a buffer containing 25 $mmol \cdot L^{-1}$ of potassium phosphate (K_3PO_4) (pH 7.0), 0.4 $mmol \cdot L^{-1}$ of ethylenediamine tetra-acetic acid (EDTA), and of

Tris-HCl, 2% (w/v) polyvinylpolypyrrolidone. The mixture was homogenized at 12 000 r·min^{-1} for 20 min at 4 °C. The obtained enzyme extract was used for enzyme assays of catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX).

Ascorbate peroxidase activity was determined according to Nakano and Asada (1981). The reaction mixture (3 mL) contained 0.75 mL of 50 mmol·L^{-1} K_3PO_4 buffer (pH 7.0), 0.75 mL of 1 mmol·L^{-1} ascorbic acid, 0.75 mL of 0.4 mmol·L^{-1} EDTA, 0.03 mL of 10 mmol·L^{-1} H_2O_2, 0.57 mL of distilled water, and the enzyme extract. The oxidation of ascorbate was started by adding H_2O_2. The decrease in absorbance at 290 nm due to the oxidation of ascorbate was monitored. One unit of enzyme activity was defined as that which oxidized 1 mmol of ascorbate per min.

Catalase activity was assayed by measuring the initial rate of hydrogen peroxide oxidation using the method of Velikova et al. (2000). Three milliliter of assay reaction mixture contained 10 mmol·L^{-1} potassium phosphate buffer (pH 7.0), with an appropriate aliquot of enzyme extract and 33 mmol·L^{-1} hydrogen peroxide. The absorbance was assayed at 240 nm with a spectrophotometer (Unico S 2100 SUV, United Products and Instruments Inc., China).

Peroxidase was determined using the method of Srinivas et al. (1999) by following the formation of tetraguaiacol by measuring the absorbance at 470 nm, 3 mL reaction mixture contained 20 mmol·L^{-1} phosphate buffer (pH 7.0), 5 mmol·L^{-1} 2-methoxy phenol (guaiacol), and 1 mmol·L^{-1} hydrogen peroxide with enzyme extract. One unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 μmol of guaiacolin for 1 min.

2.3.3. *Malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and electrolyte leakage (EL) measurement*

The concentration of malondialdehyde (MDA) which is a product of lipid peroxidation was assessed by the thiobarbituric acid (TBA) according to Wang et al. (2009), as 1 g fresh leaves were detached from growing shoots, placed in a mortar containing 5 mL 0.6% TBA in 10% trichloroacetic acid (TCA) and ground with a pestle. The mixture was heated at 100 °C for 15 min. These samples were cooled on ice for 5 min. Subsequently, the mixtures were centrifuged at 5 000 r·min^{-1} for 10 min. The absorbance of the supernatant at 450, 532 and 600 nm wavelengths were recorded and MDA content was cal-

Table 1 Effect of different concentrations of NAA and BAP on length and diameter of main scion shoots, and fresh weight and dry weight of main scion leaves in Asgari–Shahani, Asgari–Keshmeshi, Asgari–Yaghuti and Asgari–Asgari graft combinations of grapevine (*Vitis vinifera*)

Rootstock–scion combination	Hormonal treatment		Main scion shoots		Main scion leaves	
	NAA/(mg·L^{-1})	BAP/(mg·L^{-1})	Length/cm	Diameter/mm	Fresh weight/g	Dry weight/g
Asgari–Shahani	0	0	11.78 h–l	10.23 g–k	1.77 f–k	0.47 i–l
	0	250	11.07 m–q	10.17 h–l	1.85 e–i	0.56 g–i
	0	500	10.85 o–r	10.63 d–h	1.83 e–j	0.53 g–j
	200	0	11.11 l–q	9.33 no	1.76 f–k	0.48 i–k
	200	250	12.25 e–i	10.24 g–k	1.95 c–h	0.59 g–i
	200	500	12.91 a–e	11.60 ab	2.13 c	0.83 de
	400	0	11.12 l–q	9.07 op	1.63 i–k	0.33 k–n
	400	250	12.63 c–g	10.39 f–j	1.87 e–h	0.57 g–i
	400	500	12.36 d–h	10.82 c–g	1.82 e–j	0.52 h–j
Asgari–Keshmeshi	0	0	10.92 o–q	9.65 k–o	1.74 g–k	0.44 i–l
	0	250	10.72 qr	10.02 i–m	1.77 f–k	0.48 i–k
	0	500	10.15 r	10.96 c–f	1.81 f–j	0.53 g–j
	200	0	11.46 j–o	9.61 l–o	1.61 jk	0.31 l–n
	200	250	12.18 f–i	11.00 b–e	1.82 e–j	0.54 g–j
	200	500	12.63 c–g	11.30 a–c	2.05 c–e	0.79 d–f
	400	0	10.95 n–q	8.08 qr	1.57 k	0.27 mn
	400	250	11.44 j–p	9.39 no	1.82 e–j	0.52 h–j
	400	500	11.93 g–j	9.56 m–o	1.85 e–i	0.56 g–i
Asgari–Yaghuti	0	0	11.21 k–q	8.02 qr	1.56 k	0.24 n
	0	250	11.16 l–q	9.33 no	1.72 h–k	0.39 i–j
	0	500	10.18 r	9.83 j–n	1.73 h–k	0.43 i–m
	200	0	11.28 j–q	7.91 r	1.63 i–k	0.31 l–n
	200	250	12.26 f–i	10.43 e–j	1.74 g–k	0.44 i–l
	200	500	12.24 e–i	10.60 d–i	1.97 c–g	0.66 f–h
	400	0	10.74 p–r	8.03 qr	1.57 k	0.23 n
	400	250	11.70 h–m	8.52 pq	1.82 e–j	0.49 i–k
	400	500	11.65 i–n	9.06 op	1.79 f–k	0.49 i–k
Asgari–Asgari	0	0	12.89 a–e	10.04 h–m	1.82 e–j	0.47 i–l
	0	250	12.67 b–f	10.93 c–f	1.89 d–g	0.54 g–j
	0	500	11.90 h–k	11.18 a–d	1.99 c–f	0.69 e–g
	200	0	12.88 a–f	10.37 f–j	1.86 e i	0.54 g–j
	200	250	13.45 a	11.59 ab	2.17 bc	0.95 cd
	200	500	13.36 ab	11.69 a	2.44 a	1.09 bc
	400	0	13.05 a–d	9.72 k–n	2.11 cd	1.09 bc
	400	250	13.13 a–c	11.20 a–d	2.38 ab	1.22 b
	400	500	13.39 a	11.39 a–c	2.45 a	1.45 a

Note: Within each section means displayed with different letters are significantly different from each other ($P \leq 0.05$).

culated on a fresh weight by the formula: MDA ($\mu mol \cdot g^{-1}$ FW) = $[6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}] \times 1\,000$.

Hydrogen peroxide (H_2O_2) was assessed spectrophotometrically after the reaction with potassium iodide (KI), according to the method presented in Velikova and Loreto (2005). Leaf tissues (1 g) were ground and homogenized in a mortar containing 10 mL 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 13 371 $r \cdot min^{-1}$ for 15 min. Afterwards, 0.5 mL of the supernatant was added to 0.5 mL of 10 $mmol \cdot L^{-1}$ potassium phosphate buffer (pH 7.0) and 1 mL reagent (1 $mol \cdot L^{-1}$ KI in fresh double distilled water) and then the absorbance of the supernatant was read at 390 nm. The blank probe was prepared using 0.1% TCA in the absence of leaf extract. The content of H_2O_2 was calculated applying a standard curve prepared by identifying concentrations of hydrogen peroxide.

Electrolyte leakage (EL) was measured by using a conductivity meter according to Ozden et al. (2009). Samples were cut into equal sized pieces (0.3 g per treatment) and placed in culture vessels containing 15 mL of distilled water and were left for 24 h at room temperature. The initial conductance of the solution was measured using a conductivity meter. The tubes were then autoclaved at 115 °C for 10 min and final readings were taken following autoclaving and additional 24 h incubation at room temperature. Electrolyte leakage (%) was calculated as initial measurements/final measurements ×100.

2.4. Statistical analysis

Experiments were performed using a completely randomized block design. All statistical analyses were carried out with SAS and MSTAT-C computer programs. The data were analyzed by One–Way Analysis of Variance (ANOVA). Mean separations were performed by Least Significant Difference (*LSD*) test. Differences at $P \leq 0.05$ were considered as significant.

3. Results

3.1. Growth parameters

In all the graft combinations, 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP significantly enhanced main scion shoot length (Table 1). Asgari–Asgari combination showed vigorous shoot growth, the longest shoots in the experiment (13.45 and 13.36 cm) and with the thickest diameter (11.59 and 11.69 mm) with 200 $mg \cdot L^{-1}$ NAA + 250 $mg \cdot L^{-1}$ BAP and 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP

Table 2 Effect of different concentrations of NAA and BAP on the rate of graft take and the rate of bud burst in Asgari–Shahani, Asgari–Keshmeshi, Asgari–Yaghuti and Asgari–Asgari graft combinations of grapevine (*Vitis vinifera*)

Rootstock–scion combination	Hormonal treatment		Rate of graft take/%	Rate of bud burst/%
	NAA/($mg \cdot L^{-1}$)	BAP/($mg \cdot L^{-1}$)		
Asgari–Shahani	0	0	33.33 no	31.10 mn
	0	250	48.90 i–k	40.00 kl
	0	500	57.77 f–h	48.90 h–j
	200	0	51.10 h–j	42.23 j–l
	200	250	64.43 d–f	55.53 e–h
	200	500	77.77 ab	68.90 b
	400	0	42.23 k–m	37.77 k–m
	400	250	62.23 e–g	53.33 f–h
	400	500	66.67 c–e	60.00 c–f
Asgari–Keshmeshi	0	0	28.90 o	28.90 n
	0	250	51.10 h–j	44.47 i–k
	0	500	55.53 g–i	51.10 g–i
	200	0	33.33 no	31.10 mn
	200	250	73.33 bc	62.23 b–e
	200	500	75.53 ab	68.90 b
	400	0	44.43 j–l	44.43 i–k
	400	250	55.53 g–i	51.10 g–i
	400	500	59.97 e–g	55.53 e–h
Asgari–Yaghuti	0	0	28.90 o	28.90 n
	0	250	35.53 m–o	31.10 mn
	0	500	37.77 l–n	35.53 l–n
	200	0	31.10 no	28.90 n
	200	250	64.47 d–f	57.77 d–g
	200	500	71.10 b–d	66.67 bc
	400	0	42.23 k–m	35.53 l–n
	400	250	62.23 e–g	57.77 d–g
	400	500	73.33 bc	64.43 b–d
Asgari–Asgari	0	0	48.90 i–k	48.90 h–j
	0	250	51.10 h–j	51.40 g–i
	0	500	60.00 e–g	57.77 d–g
	200	0	48.87 i–k	48.77 h–j
	200	250	73.33 bc	68.90 b
	200	500	82.23 a	77.90 a
	400	0	55.53 g–i	55.53 e–h
	400	250	64.47 d–f	62.23 b–e
	400	500	62.23 e–g	62.23 b–e

Note: Within each section means displayed with different letters are significantly different from each other ($P \leq 0.05$).

applications, respectively. Among all the tested graft combinations, except Asgari–Asgari combination, the thickest diameter of the main scion shoot (11.60 mm) occurred in Asgari–Shahani combination treated with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP application. Application of 200 mg·L^{-1} NAA + 0 mg·L^{-1} BAP and 400 mg·L^{-1} NAA + 0 mg·L^{-1} BAP decreased the diameter of main scion shoot of all graft combinations, whereas the lowest main scion shoot length was recorded with 0 mg·L^{-1} NAA + 500 mg·L^{-1} BAP application.

In all the graft combinations, except Asgari–Asgari, the highest fresh and dry weight of main scion leaves were obtained from the application of 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP, whereas the highest fresh and dry weight of main scion leaves of Asgari–Asgari combination (2.45 and 1.45 g, respectively) was recorded with 400 mg·L^{-1} NAA + 500 mg·L^{-1} BAP application. However, the highest concentration of NAA (400 mg·L^{-1}) without BAP application caused a decrease in the dry weight of main scion leaves of all graft combinations, except Asgari–Asgari combination in which 0 mg·L^{-1} NAA + 0 mg·L^{-1} BAP resulted in the lowest dry weight (0.47 g) of main scion leaves.

Based on morphological observations, the highest graft take success (82.23%) was recorded with Asgari–Asgari combination treated with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP. The results revealed that 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP applications significantly enhanced the graft take success and bud burst rate. However, the lowest rate of graft take success and bud burst were recorded with 0 mg·L^{-1} NAA + 0 mg·L^{-1} BAP in all graft combinations (Table 2).

3.2. *Physiological parameters*

200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP application produced the higher chlorophyll content and photosynthesis efficiency of all graft combinations compared with the other treatments. Among all the tested graft combinations, the highest increase in chlorophyll content (11.11) and photosynthesis efficiency (0.046) occurred in Asgari–Shahani and Asgari–Asgari grafting combinations respectively, treated with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP (Table 3). Application of 0 mg·L^{-1} NAA + 0 mg·L^{-1} BAP reduced chlorophyll content in most of the grafted combinations. The lowest H_2O_2 content was recorded with 200 mg·L^{-1} NAA + 500 mg·L^{-1} BAP application, while 0 mg·L^{-1} NAA + 0 mg·L^{-1} BAP gave the enhanced amount of H_2O_2 for all the graft combinations.

Table 3 Effect of different concentrations of NAA and BAP on chlorophyll content, photosynthesis efficiency, the content of H_2O_2 and electrolyte leakage in Asgari–Shahani, Asgari–Keshmeshi, Asgari–Yaghuti and Asgari–Asgari graft combinations of grapevine (*Vitis vinifera*)

Rootstock–scion combination	Hormonal treatment		Chlorophyll content	Photosynthesis efficiency	H_2O_2/(μmol·g^{-1} FW)	Electrolyte leakage/%
	NAA/(mg·L^{-1})	BAP/(mg·L^{-1})				
Asgari–Shahani	0	0	8.91 k–n	0.028 kl	6.46 e–k	1.13 e–i
	0	250	9.61 f–j	0.030 i–l	6.22 i–n	1.10 f–i
	0	500	9.98 d–h	0.031 h–k	5.99 l–p	1.04 g–j
	200	0	8.85 k–n	0.029 j–l	6.42 f–l	1.05 f–j
	200	250	10.90 ab	0.033 g–i	5.69 pq	0.99 ij
	200	500	11.11 a	0.042 b	5.34 q	0.93 j
	400	0	9.10 j–m	0.030 i–l	6.01 l–p	1.07 f–j
	400	250	9.84 d–i	0.039 b–d	5.86 n–p	1.03 g–j
	400	500	10.31 b–e	0.040 b–d	5.98 m–p	1.01 h–j
Asgari–Keshmeshi	0	0	8.73 l–o	0.027 lm	6.32 g–m	1.16 e–h
	0	250	10.27 b–e	0.031 h–k	6.02 l–p	1.14 e–i
	0	500	10.21 c–g	0.031 h–k	5.98 m–p	1.04 g–j
	200	0	10.47 b–d	0.029 j–l	6.71 d–g	1.13 e–i
	200	250	10.24 c–f	0.034 f–h	6.30 g–m	1.01 h–j
	200	500	10.83 a–c	0.041 bc	5.94 m–p	0.98 ij
	400	0	8.58 m–o	0.030 i–l	6.29 g–n	1.11 f–i
	400	250	10.23 c–f	0.038 c–e	5.74 o–q	1.11 f–i
	400	500	10.10 d–g	0.040 j–l	6.04 k–p	0.99 ij
Asgari–Yaghuti	0	0	8.31 no	0.020 j–l	7.02 cd	1.41 cd
	0	250	9.33 i–l	0.032 g–j	6.48 e–j	1.29 de
	0	500	9.43 h–k	0.041 bc	6.22 i–n	1.21 ef
	200	0	8.98 j–m	0.031 h–k	6.85 c–f	1.28 de
	200	250	9.79 e–i	0.042 b	6.08 j–p	1.18 e–g
	200	500	10.29 b–e	0.042 b	5.73 pq	1.14 e–i
	400	0	8.69 m–o	0.030 i–l	6.69 d- h	1.28 de
	400	250	9.58 g–j	0.035 e–g	6.47 e–k	1.17 e–h
	400	500	10.11 d–g	0.037 d–f	6.17 j–o	1.13 e–i
Asgari–Asgari	0	0	6.68 p	0.024 m	7.78 a	1.81 a
	0	250	8.32 no	0.027 lm	7.20 bc	1.82 a
	0	500	8.99 j–m	0.035 e–g	6.63 d–i	1.67 ab
	200	0	8.13 o	0.032 g–j	7.57 ab	1.70 ab
	200	250	9.06 j–m	0.040 b–d	6.87 c–e	1.61 b
	200	500	9.77 e–i	0.046 a	6.27 h–n	1.54 bc
	400	0	8.20 o	0.041 bc	7.73 a	1.69 ab
	400	250	8.84 k–n	0.040b–d	6.99 cd	1.64 ab
	400	500	8.86 k–n	0.041 bc	6.84 c–f	1.57 bc

Note: Within each section means displayed with different letters are significantly different from each other ($P \leq 0.05$).

Table 4 Effect of different concentrations of NAA and BAP on the concentration of MDA, activity of CAT, POX and APX in Asgari–Shahani, Asgari–Keshmeshi, Asgari–Yaghuti and Asgari–Asgari graft combinations of grapevine (*Vitis vinifera*)

Rootstock–scion combination	Hormonal treatment		MDA/ ($\mu mol \cdot g^{-1}$ FW)	CAT/ ($U \cdot mg^{-1}$ protein)	POX/ ($U \cdot mg^{-1}$ protein)	APX/ ($U \cdot mg^{-1}$ protein)
	NAA/($mg \cdot L^{-1}$)	BAP/($mg \cdot L^{-1}$)				
Asgari–Shahani	0	0	0.70 g–k	5.18 g–j	3.53 hi	0.77 b–g
	0	250	0.66 j–k	5.04 i–l	3.77 e–h	0.79 a–e
	0	500	0.66 j–k	5.97 a–d	3.86 e–g	0.82 ab
	200	0	0.78 de	5.54 d–g	3.41 ij	0.76 c–h
	200	250	0.47 pq	5.97 a–d	3.74 e–h	0.81 a–c
	200	500	0.42 qr	6.26 a	3.93 d–f	0.82 ab
	400	0	0.76 d–f	5.48 e–h	3.39 i–k	0.74 e–j
	400	250	0.68 h–l	6.03 abc	3.97 c–f	0.77 b–g
	400	500	0.51 op	6.30 a	3.99 c–f	0.78 b–f
Asgari–Keshmeshi	0	0	0.71 f–j	5.27 e–i	3.99 c–f	0.72 g–j
	0	250	0.63 lm	5.37 e–i	4.02 c–e	0.72 g–j
	0	500	0.56 no	5.62 c–f	4.23 bc	0.74 e–j
	200	0	0.72 f–i	5.21 f–j	3.99 c–f	0.70 ij
	200	250	0.47 pq	5.64 c–f	4.25 a–c	0.80 a–d
	200	500	0.41 r	6.12 ab	4.53 a	0.84 a
	400	0	0.73 e–h	5.07 h–l	4.15 b–d	0.74 e–j
	400	250	0.55 no	5.64 c–f	4.33 ab	0.80 a–d
	400	500	0.47 pq	5.70 b–e	4.41 ab	0.81 a–c
Asgari–Yaghuti	0	0	0.79 cd	4.29 n–q	3.09 lm	0.71 h–j
	0	250	0.73 e–h	4.68 l–n	3.22 j–l	0.69 j
	0	500	0.70 g–k	5.07 h–l	3.58 g–i	0.70 ij
	200	0	0.74 d–g	4.41 m–p	2.89 m–o	0.69 j
	200	250	0.65 kl	5.25 f–i	3.55 hi	0.73 f–j
	200	500	0.57 n	5.34 e–i	3.88 d–f	0.75 d–i
	400	0	0.71 f–j	4.51 m–o	3.05 l–n	0.75 d–i
	400	250	0.65 kl	4.78 j–m	3.71 f–h	0.70 ij
	400	500	0.59 mn	5.03 i–l	3.91 d–f	0.70 ij
Asgari–Asgari	0	0	0.92 a	3.57 s	2.63 op	0.32 n
	0	250	0.85 b	4.33 n–q	2.79 n–p	0.36 n
	0	500	0.69 g–k	4.23 o–q	2.69 op	0.56 k
	200	0	0.84 bc	3.78 rs	2.57 p	0.34 n
	200	250	0.67 i–l	4.69 k–n	3.02 l–n	0.47 lm
	200	500	0.70 g–k	5.12 g–k	3.12 k–m	0.61 k
	400	0	0.74 d–g	3.91 q–s	2.56 p	0.34 n
	400	250	0.66 j–l	4.02 p–r	2.90 m–o	0.42 m
	400	500	0.70 g–k	4.06 p–r	2.91 m–o	0.50 l

Note: Within each section means displayed with different letters are significantly different from each other ($P \leq 0.05$).

Applying NAA and BAP to the graft point also resulted in the decreased MDA concentration of main scion leaves (Table 4). As seen in Table 4, the amount of MDA of main scion leaves was the lowest when 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP was applied for all graft combinations. Results on membrane permeability, estimated as electrolyte leakage is presented in Table 3. Although the highest electrolyte leakage was observed with the application of 0 $mg \cdot L^{-1}$ NAA + 0 $mg \cdot L^{-1}$ BAP, there were no significant differences among all hormonal treatments for all the grafting combinations.

3.3. *Biochemical parameters*

The activities of CAT, APX, and POX were also changed depending on graft combinations and hormonal treatments (Table 4). Most of the hormonal treatments induced a continuous increase in CAT and POX activities in the main scion leaves of all graft combinations (Table 4). The graft union formation in the presence of 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP application resulted in the enhanced CAT and POX activities, although the graft combination interaction was much pronounced and no significant difference among treatments containing 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP, 400 $mg \cdot L^{-1}$ NAA + 250 $mg \cdot L^{-1}$ BAP and 400 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP were detected. Asgari–Shahani and Asgari–Keshmeshi were characteristic by considerably enhanced CAT and POX activities more than the other graft combinations (Table 4). No differences in the activity of APX were observed among hormonal treatments in all graft combinations, except for Asgari–Asgari in which significant enhancement of the APX activity was recorded with 0 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP and 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP. Table 5 indicates a positive correlation between the rate of grafting success and chlorophyll content, photosynthesis efficiency, CAT and POX activities. However, there was a negative correlation between the rate of grafting success and electrolyte leakage, MDA and H_2O_2 contents. The activity of APX did not show a significant correlation with the rate of graft take success (Table 5).

Although there could be differences in the time of graft union formation for different scion–rootstock combinations, the callus tissue at the grafting point was visible from about 30 d after grafting (Fig. 2). Cookson et al. (2013) similarly reported that in scion–rootstock combinations of grapevines, the callus tissue was visible from 14 d after grafting and was well developed by 28 d after grafting.

Table 5 Correlation of studied parameters in grafted Asgari scions on different Iranian rootstocks of grapevine as affected by NAA and BAP applied on graft zone

	Chlorophyll content	Photosynthesis efficiency	Content of H_2O_2	Concentration of MDA	Electrolyte leakage	Activity of CAT	Activity of POX	Activity of APX
Rate of graft take	0.46**	0.65**	−0.29**	−0.55**	−0.08	0.29**	0.20*	0.03
Chlorophyll content		0.42**	−0.74**	−0.79**	−0.67**	0.74**	0.69**	0.67**
Photosynthesis efficiency			−0.27**	−0.47**	−0.03	0.20*	0.12	0.40
Content of H_2O_2				0.67**	0.76**	−0.83**	−0.73**	−0.79**
Concentration of MDA					0.61**	−0.80**	−0.80**	−0.90**
Electrolyte leakage						−0.70**	−0.69**	−0.61**
Activity of CAT							0.80**	0.79**
Activity of POX								0.78**

Note: * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$.

4. Discussion

Our results indicated that growth of grafted grapevine could be different due to graft combination and different concentrations of NAA and BAP. A previous report by Stino et al. (2011) indicated that the variability in scion vegetative growth response is related to different rootstocks in grapevine. Endogenous growth regulators have been also assumed to play an important role in grafting (Dumanoglu et al., 2014). According to Celik (2000), scion shoot length determines the quality of final grafting process and the quality of the grafted grapevines. Our data showed that hormonal treatment at the level of the graft union enhanced the scion shoot length. Kose and Guleryuz (2006) reported that in grapevines application of auxins decreased graft success rate, while treatment with cytokinin enhanced callus formation of the graft union. Our current study was conducted to determine the potential of using auxin–cytokinin combination to improve the grafting process. Kose and Guleryuz (2006) reported that BAP stimulated rapid proliferation of callus between scion and rootstock in grapevine. The callus formation on graft union is a key factor determining whether there is a good compatibility between scion and rootstock (Celik, 2000), which subsequently, results in the grafting success.

Plant mineral nutrition from the rootstock and allocation of photosynthetates produced by the scion are pivotal for the formation of graft union (Sabir, 2011). He et al. (2009) reported that grafting in tomato improved photosynthesis by enhancing the activities of antioxidant enzymes. In the current study, the enhanced chlorophyll content and photosynthetic efficiency of Asgari scion could be attributed to the positive effect of BAP on antioxidant activities, although the graft combination was also significant. These results are in agreement with Martinez-Ballesta et al. (2010) who demonstrated that grafting reduced the damages and improved the photosynthetic performance of the scion variety.

The cellular content of MDA, H_2O_2 and level of electrolyte leakage reflect the cellular damages, resulting from oxidative stress (Dhindsa et al., 1981). Our results demonstrated that the hormonal treatments of the graft union induced a decrease in endogenous H_2O_2 concentration of the main leaves of the scion. Callus growth inhibition may be the cause of H_2O_2 (Ozden and Karaaslan, 2011) and the presence of BAP resulting in enhanced callus production at the level of the graft union, could be due to the reduction of H_2O_2 caused by BAP application. A decreased amount of H_2O_2 and MDA in response to NAA and BAP application, could be related and the cause of an enhanced antioxidant activity of the scion and a subsequent decrease of its oxidative stress. Pasternak et al. (2007) reported the existence of a strong auxin-mediated link between the activation of oxidative stress and subsequent progression of cell division. These results suggest that NAA and BAP may exert some actions on the cell surface, reducing the diffusion of H_2O_2 into the grape leaf cells. A similar phenomenon regarding exogenous proline application was observed in grapevine (Ozden et al., 2009). Ozden and Karaaslan (2011) asserted that BAP in high concentrations resulted in an increase of electrolyte leakage in *Vitis vinifera*. Moreover, the physiological responses induced by poor vascular union at the graft union may lead to growth inhibition of the scion, due to restricted communication between the above and below portion of the cutting (Martinez-Ballesta et al., 2010).

Hormonal treatments of the graft union associated with rootstock effects in grapevine are pivotal for the success of the grafting process. The activities of some antioxidants such as CAT, APX and POX were analyzed in the upper part of the graft union (scion) to determine the involvement of each in graft union formation in response to hormonal treatment application. In our study, BAP caused an increase in the activity of POX which has been reported to be located mainly in the graft union, responsible for the lignifications and resulting in enhanced graft compatibility (Wettern et al., 1998; Fernandez-Garcia et al., 2004; Pederson, 2006). The effect of exogenous cytokinin application of antioxidant protection has been reported by several researchers (Clarke et al., 2002; Mytinova et al., 2011; Ozden and Karaaslan, 2011). BAP may have an important role on callus growth by regulating antioxidant enzyme activities and acting as an effective free radical scavenger. CAT and APX activities were found to be increased gradually with increasing concentration of BAP (Ozden and Karaaslan, 2011). The antioxidant responses of scion caused by hormonal treatments on the graft point in grapevine are important as we were able to prove in our experiments, however, additional studies are needed to better determine the specific role during the grafting process. In particular, it is important to clarify whether the hormone applications on the grafting cuts, by modifying antioxidant activity in scion, can affect the compatibility and incompatibility of different grafting combinations. Kose and Guleryuz (2006) suggested that further studies were necessary to find the potential auxin–cytokinin combination for improving grafting success in grapevines. In the current investigation, 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP application produced better results than the other hormone treatments for several mor-

phological and biochemical traits of Asgari scions. The use of auxins and cytokinins in combination was more effective than the single application of each compound.

5. Conclusion

In our study, 200 $mg \cdot L^{-1}$ NAA + 500 $mg \cdot L^{-1}$ BAP application produced better results than the other hormonal treatments for all morphological and biochemical characteristics that were measured for Asgari. This work has also demonstrated the importance of hormonal application in order to ensure a significant success rate of the graft union. The use of mixture of auxins and cytokinins is more effective than the use of each single chemical compound alone. In conclusion, antioxidant activities during the graft union formation have contributed significantly to the success of the graft union. However, our study included the changes in antioxidant enzyme activities caused by contributions of auxins and cytokinins at various ratios to examine effects on stimulating or suppressing the graft union formation efficiency.

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