



# Effect of Salicylic Acid on Changes in Superoxide Dismutase Enzyme Activity, Protein, Proline, and Some Photosynthetic Pigments in Grape (*Vitis vinifera* L.) Bidane Ghermez and Bidane Sefid Cultivars at Two Growth Stages

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## Abstract

Salicylic acid (SA) is an endogenous growth regulator that participates in the regulation of physiologic processes in plants. It plays an important role in plant response to adverse environmental conditions. In this study, SA in three concentrations—0, 0.1 mM, and 1 mM—was sprayed on the leaves and fruits of two grape cultivars (Bidane Ghermez and Bidane Sefid) at two growth stages: unripening and ripening. The SA 0.1-mM concentration effectively increased leaf chlorophyll *a* of the Bidane Sefid cultivar at both growth stages. Chlorophyll *b* and proline of the leaves and berry skin of both cultivars increased at both growth stages under SA treatment, especially with the 0.1-mM concentration. The SA 0.1-mM concentration increased carotenoids in leaves and berry skin of Bidane Ghermez cultivar at both growth stages and of Bidane Sefid cultivar in the unripening stage. The SA 0.1-mM concentration was also associated with increased soluble sugars of the leaves and berry skin of both cultivars at both growth stages. However, the effect of SA on insoluble sugars was different. At the unripening stage, the SA 1-mM concentration significantly increased the total protein of the berry flesh and leaves of Bidane Sefid. The results show that in both growth stages, the SA treatment, especially the 1-mM concentration, effectively increased the superoxide dismutase (SOD) enzyme activity of leaves, skin, and flesh of berries of both cultivars. The results indicate that SA treatment in grapes may stimulate the synthesis of some photosynthetic pigments, carbohydrates, proline, protein, and SOD enzymes.

**Keywords** Ripening · Unripening · Proline · Protein · Superoxide dismutase

## Introduction

The most important sugars in fresh grapes are sucrose, glucose, and dextrose (Combe 1992; Zhang et al. 2021). Laronde et al. (1998) showed that high levels of sucrose and glucose are necessary to accelerate the anthocyanin biosynthesis, and insoluble sugars do not double these results. A high concentration of carbohydrates reduces the oxida-

tive damage and protects the protein structure during water shortages (Fazelian and Asrar 2011; Li and Wang 2021).

Proline is the most abundant amino acid in grapes (Nassar and Kliewer 1966), and proline accumulation has been reported to activate the antioxidant mechanism (Ben Ahmed et al. 2009). In fact, proline increases the tolerance and resistance to stresses by several mechanisms, including inhibiting hydroxyl radicals, regulating osmosis, preventing denaturation of enzymes, and preserving and synthesizing proteins (Ashraf and Foolad 2007). Studies have shown that when plants are confronted with different stressors, they have osmotic adjustment mechanisms to maintain their water condition by the accumulation of compounds such as proline, soluble sugars, and some ions. Under stress conditions such as salinity stress, soluble sugars and proline can act as osmotic proteins (Bartles and Sunkar 2005).

Sugars and amino acids that accumulate in grapes during ripening are imported via the phloem, while many sec-

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ondary metabolites are synthesized within the berry itself. Grapes import sucrose but accumulate hexoses. Conversion of sucrose to hexoses is most likely catalyzed by invertase. Expression of genes and an increase in invertase activity occur before veraison, so it seems unlikely that synthesis of this enzyme is a controlling factor for sugar accumulation during ripening. A number of proteins are newly synthesized in grapes during ripening, and several of these proteins have now been identified. Studies indicate that a dramatic change in gene expression occurs in grape berries at veraison, suggesting that ripening involves a coordinated increase in transcription of a number of different genes (Robinson and Davies 2000).

One of the effects of environmental stresses is oxidative damage and free radical production of reactive oxygen species (ROS) (Mittler et al. 2004). Antioxidant enzymes cooperate to scavenge free radicals. The most important antioxidant enzymes include ascorbate peroxidase (APZ), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). Nonenzymatic antioxidants such as glutathione (GSH), carotenoids, and tocopherols are antioxidant defense systems (Darvizheh et al. 2019). The enzyme SOD converts  $O_2^-$  into  $H_2O_2$  and  $O_2$ , then peroxidase (POD) and CAT,  $H_2O_2$  to  $H_2O$  (Wang et al. 2009).

Salicylic acid (SA) is an important plant hormone that influences various physiologic processes and biochemical properties of the plant. Studies show that when SA is applied at appropriate concentrations, this hormone increases the abilities of the antioxidant system of plant tissues by activating antioxidant enzymes and increasing some nonenzymatic substances such as anthocyanins, flavonoids, and carotenoids (Luo et al. 2012).

It seems that SA spray with increasing antioxidant capacity of chamomile, including carotenoids, reduces  $H_2O_2$  and lipid peroxidation and also protects more cell membranes and photosynthetic and photosynthesis pigments, as well as prevents chlorophyll catabolism (Costa et al. 2005). Changes in the chlorophyll pigments under SA treatment are highly dependent on the species and the treated concentration. In many observations, there was a significant increase in chlorophyll pigments with treatment of low concentrations of SA (Pancheva et al. 1996). Other studies have shown that SA increased chlorophyll and carotenoid pigments in corn (Khodary 2004) and that it increased the photosynthetic pigments, total carbohydrates, proline, and protein in basil (Abdel and Gharib 2006; Haghshenas et al. 2020). Other studies have shown that SA increased soluble and insoluble sugars in tomato (Poor et al. 2011) and wheat (El Tayeb and Ahmed 2010). In apple fruit, SA significantly increased the fruit weight, chlorophylls, carotenoids, proline, protein, and SOD (Turkyilmaz Unal et al. 2015). It has also been shown that SA, having an effect on antioxidant enzymes, causes plants to be resistant to environ-

mental stress. Plants produce some proteins in response to biotic and abiotic stresses, some of which are produced by phytohormones such as SA (Hussein et al. 2007). There are many reports of increasing and decreasing protein with SA stress. The study of corn showed that all amino acids were enhanced by SA; increasing the amino acids in tissue under stress was associated with protein portions (El-Tayeb 2005). In research on two beans and tomato under stress, the use of SA increased the activity of antioxidant enzymes, including SOD and APX (Senaratna et al. 2000). Some researchers have shown that SA (0.1-mM concentration) increased the protein and antioxidant enzyme activity compared with control (Chakraborty and Tongden 2005). Studies in parsley also showed that SA increased the activity of the chloroplast SOD bands and cytosol, but SA was more effective on SOD chloroplasts (Antonova Ananieva et al. 2004).

Therefore, this study was done to evaluate the effect of SA in different concentrations on photosynthetic pigments, soluble and insoluble sugars, proline, total protein, and SOD activity in leaf and different tissues of grapevine fruits of the Bidane Ghermez and Bidane Sefid cultivars in two stages, unripening and ripening. Variations in these compounds in different tissues of both cultivars were also compared in both growth stages.

## Materials and Methods

In this study, two cultivars of grapevine, Bidane Sefid (BS; white berries) and Bidane Ghermez (BG; red berries), grown in the garden of the Malayer Grape Research Center affiliated with the Ministry of Jihad Agricultural (Iran) were used. The grapevines are 10 years old, and their breeding system was row cropping and drip irrigation. The index for determining the unripening stage of grapevine fruit varieties was the berry size and the acidity, and for the ripening stage it was juiciness and sweetness of the berries. The fruits and leaves of BG and BS cultivars were treated in two growth stages (unripening and ripening) with three concentrations of SA: 0 (control), 0.1 mM, and 1 mM.

### Determination of Carotenoids and Chlorophyll *a* and *b*

Carotenoids and chlorophyll *a* and *b* were measured based on the Lichtenthaler (1987) method: 0.2 g of tissue was homogenized in 5 ml of 80% acetone and filtered through Whatman no. 2 filter paper. Then the volume of the extract obtained with acetone reached 10 ml. The intensity of the light absorption of the extract was read at 646.8 nm, 65.15 nm, and 470 nm using a spectrophotometer (Jenus model UV-12000, USA).

## Determination of Sugars

The reducing sugar was estimated using the method of Nelson (1944). The sulfuric acid phenol method (Dubois et al. 1956) was used to measure insoluble sugars (oligosaccharides and polysaccharides).

## Determination of Proline

Free proline was determined by the method of Bates et al. (1973). Approximately 0.5 g of fresh or frozen plant material was homogenized in 10 ml of 3% sulfosalicylic acid and filtered through Whatman no. 2 filter paper. Then 2 ml of the filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene, and the chromophore containing toluene was aspirated and cooled to room temperature. The absorbance was measured at 520 nm with a spectrometer (Jenus model UV-12000).

## Determination of Total Soluble Protein

For determination of total soluble protein and enzyme activity, 1 g of plant material was homogenized in a chilled (4 °C) mortar using a 50 mM Tris-HCl buffer (pH 7.0) containing 10 mM EDTA, 2 mM MgSO<sub>4</sub>, 20 mM cysteine, 10% (v/v) glycerol, and 2% (w/v) PVPP (Jasska 1996). The homogenate was centrifuged in a refrigerated centrifuge, and the supernatant obtained was used for protein determination and enzyme assay. The protein of the extracts was determined according to the method of Bradford (1976), using bovine serum albumin as the standard.

## Determination of Superoxide Dismutase Enzyme Activity

Superoxide dismutase enzyme (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of thiazolyl blue (MTT) as described by Gianopolitis and Ries (1977). The reaction mixture consisted of 0–100 µl enzyme extract, 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM MTT, 0.1 mM EDTA, and 75 µM riboflavin.

Test tubes were shaken and placed 30 cm from three 30-W fluorescent lamps. The reduction in MTT was measured by reading absorbance at 565 nm. Blanks and controls were run in the same manner but without illumination and enzyme, respectively. One unit of SOD activity was defined as the number of enzymes that produced a 50% inhibition of MTT reduction.

## Results and Discussion

### Effect of Salicylic Acid on Chlorophyll *a*

The results showed that chlorophyll *a* in leaves and berry skin of both cultivars at the unripening stage was greater than at the ripening stage. The use of SA in both concentrations did not have a significant effect on chlorophyll *a* of the leaves and berry skin of BG cultivar in either growth stage (Table 1). However, the SA in both concentrations had a significant effect on chlorophyll *a* of the leaves of BS cultivar at the unripening stage compared to control, with the 1-mM concentrations having a significantly greater effect. At the ripening stage, SA in the 1-mM concentration effectively increased the chlorophyll *a* of leaves compared to control. In addition, in the berry skin of BS cultivar, SA had an increasing effect in the 0.1-mM concentration at the unripening stage and a decreasing effect on chlorophyll *a* in both concentrations at the ripening stage. Studies have shown that SA increased chlorophyll *a* and *b* of strawberry fruit (Karlidag et al. 2009). In this study, the chlorophyll *a* of leaves and berry skin of BG cultivar at both growth stages was greater than the chlorophyll *a* of leaves and berry skin of BS cultivar. Meanwhile, except for the berry skin of BS cultivar in the ripening stage, in which the amount of chlorophyll *a* was higher than the berry skin of BG cultivars. In accordance with these results, chlorophyll *a* and *b* in red cultivars have been observed as greater than in white cultivars (Abbaspour et al. 2017). The chlorophyll *a* in both cultivars and at both growth stages was higher in the leaves than in berry skin. On the other hand, according to the results, chlorophyll *a* was higher in the control leaves of BG cultivar than in the control leaves of BS cultivar.

### Effect of Salicylic Acid on Chlorophyll *b*

The results of comparing the two growth stages showed that chlorophyll *b* of leaves and berry skin of both cultivars at the unripening stage was greater than at the ripening stage. In the leaves and berry skin of BS cultivar at both growth stages and the leaves of BG cultivar at the unripening stage, the SA 0.1-mM concentration effectively increased chlorophyll *b* compared to control (Table 1). But in berry skin of BG cultivar, the SA 0.1-mM concentration at the unripening stage and the 1-mM concentration at the ripening stage effectively increased chlorophyll *b* compared to control. In accordance with these results, SA has been shown to increase chlorophyll *a* and *b* SA the strawberry (Tohma and Esitken 2011). In this study, chlorophyll *b* was decreased at the ripening stage. Meanwhile, the use of SA could increase chlorophyll *b*. In addition, the chlorophyll *b* in leaves and berry skin of BS cultivar at both growth stages was greater than in leaves and berry skin of BG cultivar. Also, at the

unripening stage for both cultivars, chlorophyll *b* in berry skin was higher than in leaves, but at the unripening stage, chlorophyll *b* in leaves was higher than in berry skin.

The effect of SA at the unripening stage was a little better than at the ripening stage, as its effect on the leaves and berry skin of BS cultivar was greater than for BG cultivar. The highest amount of chlorophyll *b* was in berry skin of BS cultivar treated with SA in the 0.1-mM concentration at the unripening stage. The study showed that the SA, depending on the concentration, time, and plant used, had dual effects, but in optimum concentrations contributed by reducing pigments and increasing antioxidant capacity.

### Effect of Salicylic Acid on Carotenoids

The carotenoids of leaves and berry skin of both cultivars at the unripening stage were greater than at the ripening stage (Table 1). The use of SA 0.1-mM concentration in both cultivars at the unripening stage and in both concentrations at the ripening stage significantly increased the carotenoids of leaves and berry skin compared to control. But in leaves and berry skin of BS cultivar at the ripening stage, the SA in

both concentrations significantly decreased the carotenoids. In accordance with these results, in strawberry fruits SA increased the carotenoids (Karlidag et al. 2009). Also, the results showed that at both growth stages, the carotenoids of leaves and berry skin were higher in the BG cultivar than in the BS cultivar. Except at the unripening stage, the leaf carotenoids of the BS cultivar were greater than in the BG cultivar. In accordance with these results, Abbaspour et al. (2017), in their studies of grape, showed that carotenoids of red cultivars were greater than in white cultivars. The carotenoids of both cultivars at the unripening stage and the BG cultivar at the ripening stage were greater in berry skin than in leaves.

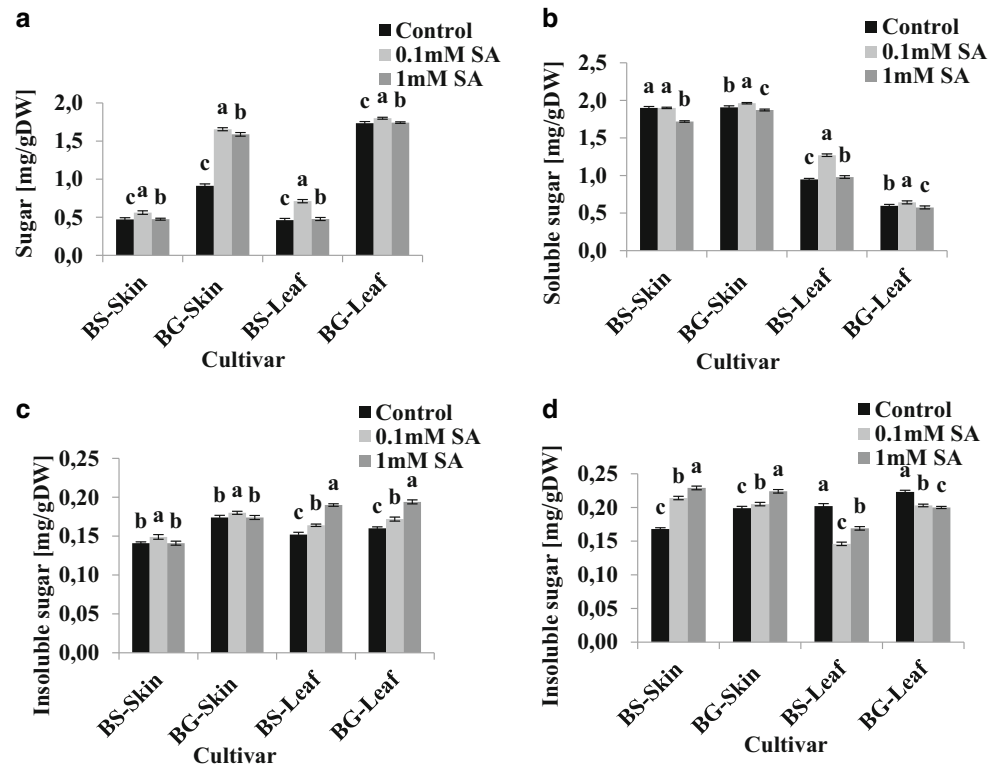
But in contrast, in the BS cultivar at the ripening stage, the carotenoids of leaves were greater than in berry skin. The SA effect at the unripening stage was greater than at the ripening stage. Also, at the unripening stage the effect of this treatment was the same for carotenoids of both cultivars. But at the ripening stage, the SA effect on carotenoids of leaves and berry skin in the BG cultivar was greater than in the BS cultivar. Therefore, the highest amount of carotenoid was observed in berry skin of the BG cultivar

**Table 1** Effect of salicylic acid (SA) in three concentrations of 0 (control), 0.1 mM, and 1 mM on chlorophyll *a*, chlorophyll *b*, and carotenoid content of leaves and berry skin of Bidane Sefid (BS) and Bidane Ghermez (BG) grape cultivars in unripening and ripening stages

Characteristic (mg/g FW <sup>-1</sup> )	Cultivar	Growth stage	Organ	Control	0.1-mM SA treatment	1-mM SA treatment		
Chlorophyll <i>a</i>	BG	Unripening	Leaf	88.57 <sup>a</sup>	77.28 <sup>a</sup>	79.58 <sup>a</sup>		
			Skin	46.96 <sup>a</sup>	41.28 <sup>a</sup>	51.48 <sup>a</sup>		
		Ripening	Leaf	26.57 <sup>a</sup>	28.14 <sup>a</sup>	25.10 <sup>a</sup>		
			Skin	2.49 <sup>a</sup>	1.82 <sup>a</sup>	2.21 <sup>a</sup>		
		BS	Unripening	Leaf	54.52 <sup>c</sup>	78.21 <sup>b</sup>	88.70 <sup>a</sup>	
				Skin	44.83 <sup>b</sup>	70.17 <sup>a</sup>	37.15 <sup>c</sup>	
	Chlorophyll <i>b</i>	BG	Unripening	Leaf	19.31 <sup>b</sup>	20.26 <sup>a</sup>	14.63 <sup>c</sup>	
				Skin	27.23 <sup>b</sup>	44.17 <sup>a</sup>	38.01 <sup>b</sup>	
			Ripening	Leaf	6.336 <sup>b</sup>	8.16 <sup>a</sup>	5.52 <sup>b</sup>	
				Skin	1.49 <sup>b</sup>	1.13 <sup>b</sup>	2.12 <sup>a</sup>	
			BS	Unripening	Leaf	35.22 <sup>b</sup>	54.24 <sup>a</sup>	55.19 <sup>a</sup>
					Skin	35.82 <sup>c</sup>	84.12 <sup>a</sup>	56.96 <sup>b</sup>
Carotenoids	BG	Unripening	Leaf	43.59 <sup>ab</sup>	47.80 <sup>a</sup>	40.37 <sup>b</sup>		
			Skin	63.73 <sup>a</sup>	67.35 <sup>a</sup>	63.31 <sup>a</sup>		
		Ripening	Leaf	11.68 <sup>b</sup>	13.78 <sup>ab</sup>	16.47 <sup>a</sup>		
			Skin	13.12 <sup>a</sup>	13.61 <sup>ab</sup>	14.38 <sup>a</sup>		
		BS	Unripening	Leaf	39.41 <sup>b</sup>	64.086 <sup>a</sup>	54.9 <sup>a</sup>	
				Skin	56.69 <sup>b</sup>	66.36 <sup>a</sup>	55.13 <sup>b</sup>	
	Ripening	Leaf	15.67 <sup>a</sup>	10.57 <sup>b</sup>	9.36 <sup>b</sup>			
		Skin	11.33 <sup>a</sup>	10.88 <sup>a</sup>	10.56 <sup>a</sup>			

Different letters indicate significant differences at the level of  $p \leq 0.05$   
FW fresh weight

**Fig. 1** Effect of salicylic acid (SA) in three concentrations—0 (control), 0.1 mM, and 1 mM—on soluble sugar (a at unripening stage and b at ripening stage) and insoluble sugar (c at unripening stage and d at ripening stage) of leaves and berry skin of grape Bidane Sefid (BS) and Bidane Ghermez (BG) cultivars. Different letters indicate significant differences at the level of  $p \leq 0.05$ . DW dry weight



treated with the 0.1-mM concentration at the unripening stage.

### Effect of Salicylic Acid on Soluble Sugar

The soluble sugar of berry skin in both cultivars at the ripening stage was greater than at the unripening stage (Fig. 1). As observed, the soluble sugar of BS cultivar leaves in the ripening stage was greater than at the unripening stage, but in leaves of the BG cultivar, the soluble sugar at the unripening stage was more than at the ripening stage. The SA in both concentrations at the unripening stage had the effect of significantly increasing the soluble sugar of leaves and berry skin of both cultivars compared to control, and the 0.1-mM concentration was more effective.

As at the ripening stage, the SA 0.1-mM concentration had an increasing effect on the soluble sugar of leaves and berry skin in both cultivars compared with control (Fig. 1). In accordance with these results, SA was observed to increase soluble sugar in apple (Mo et al. 2008). Also, the results of the comparison between the two growth stages show that the soluble sugar of berry skin of the BG cultivar was more than that of the BS cultivar at both stages. In addition, at the ripening stage the soluble sugar of leaves of the BS cultivar was greater than in the BG cultivar; in contrast, at the unripening stage it was greater in the BG cultivar. However, the soluble sugar in the studied tissues of both cultivars at the unripening stage was less than at

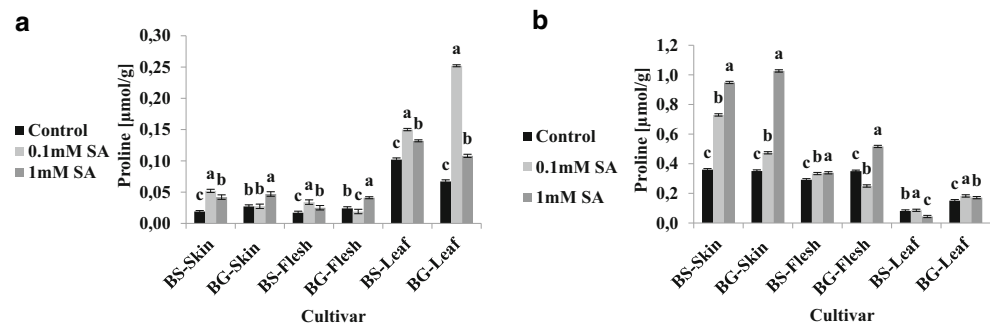
the ripening stage. But the effect of SA in both concentrations on the soluble sugar of leaves and berry skin of both cultivars at the unripening stage was greater than at the ripening stage. The effect of SA in both concentrations on the soluble sugar of berry skin of the BS cultivar compared to the BG cultivar was quite significant. The highest amount of soluble sugar was seen at the ripening stage in berry skin (both cultivars) treated with SA in the 0.1-mM concentration.

### Effect of Salicylic Acid on Insoluble Sugar

The insoluble sugar of leaves and berry skin of both cultivars at the ripening stage was greater than at the unripening stage (Fig. 1). The use of SA in both concentrations significantly increased the insoluble sugar of berry skin of both cultivars compared to control; at the unripening stage, the 0.1-mM concentration had more effect, and at the ripening stage the 1-mM concentration had more effect. Also, in the leaves of both cultivars at the unripening stage, SA in both concentrations significantly increased the insoluble sugar of the leaves of both cultivars, with the 1-mM concentration having a greater effect. But at the ripening stage, SA in both concentrations significantly decreased the insoluble sugar of the leaves in both cultivars compared to control (Fig. 1). In accordance with these results, SA was observed to increase insoluble sugar in the tomato (Wasti et al. 2012). In this study, the insoluble sugar at both stages



**Fig. 2** Effect of salicylic acid (SA) in three concentrations—0 (control) 0.1 mM, and 1 mM—on proline content of leaves, skin, and flesh of berries of Bidane Sefid (BS) and Bidane Ghermez (BG) cultivars in unripening stage (a) and ripening stage (b). Different letters indicate significant differences at the level of  $p \leq 0.05$



in leaves and berry skin of the BG cultivar was greater than that of the BS cultivar. Also, at both stages and in both cultivars, the insoluble sugar of leaves was greater than that of berry skin (except for leaves of the BG cultivar, in which insoluble sugar was less than that of berry skin at the unripening stage). The effect of SA on the insoluble sugar of the leaves of both cultivars at the unripening stage was greater than at the ripening stage, but in berry skin of both cultivars, the amount at the ripening stage was greater than at the unripening stage. The effect of SA on the insoluble sugar of berry skin of the BS cultivar at the ripening stage compared to another tissue was higher. In some plants such as pepper, SA ( $10^{-6}$  mM) increased the invertase enzyme of leaves and fruit and increased the insoluble sugar (Elwan and El-Hamahmy 2009). Also, the results indicate that the SA increased the sugar and antioxidant activity, so it helped to decrease environmental stresses (Elwan and El-Hamahmy 2009). Some studies have shown that SA increased the amount of photosynthetic pigment; decreased oxidative stress; protected chloroplast and cell membranes; protected macromolecules, such as proteins; increased the amount of sugar in plants; and also helped plants with regard to osmotic regulation (Khodary 2004). The highest amount of insoluble sugar was observed at the ripening stage in berry skin (both cultivars) treated with SA in the 0.1-mM concentration.

### Effect of Salicylic Acid on Proline

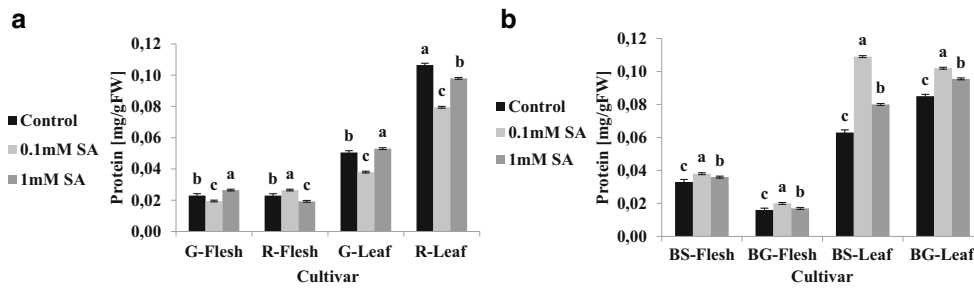
Proline in the skin and flesh of berries for both cultivars and in the leaves of the BG cultivar at the ripening stage was greater than at the unripening stage. But in leaves of the BS cultivar, proline was greater at the unripening stage than at the ripening stage (Fig. 2). The Drawert (1963) studies showed that proline increased during the maturity process in grape fruit. At the unripening stage, significant increases compared with control were observed for proline. In the skin and flesh of berries of the BS cultivar treated with the SA 0.1-mM concentration and in BG cultivar with the 1-mM concentration. At the ripening stage, SA in both concentrations significantly increased the proline in the skin and flesh of berries in both cultivars compared to control;

the 1-mM concentration was more effective. Also, the SA 0.1-mM concentration increased proline in the leaves of both cultivars at both growth stages compared to control.

In accordance with these results, in several fruits including tomato (Wasti et al. 2012), SA increased the proline. In this study, the proline of skin and flesh of berries at the unripening stage and the leaves of BG cultivar at the ripening stage were more than for the BS cultivar. Also, the proline of leaves at the unripening stage and of skin and flesh of berries at ripening stage were greater for the BS cultivar than the BG cultivar. Studies have shown that red cultivars such as Rasheh have more proline than white cultivars such as BS (Abbaspour et al. 2017). The comparison of the proline of different tissues at two growth stages also showed that the proline of leaves was more than for berry tissues in both cultivars at the unripening stage. But at the ripening stage, the proline of berry tissues was more than for leaves. The effect of the SA on the flesh and skin of berries at the ripening stage was greater than at the unripening stage. But in the leaves of both cultivars, the effect was great at the unripening stage. The studies showed that use of SA can neutralize the effect of stressors by increasing the concentration of organic solutions, including proline, to stabilize the essential proteins (Tayeb et al. 2006). Indeed, the highest amount of proline was observed at the ripening stage and in berry skin (both cultivars) treated with the SA 1-mM concentration.

### Effect of Salicylic Acid on Total Protein

The total protein differs in various tissues and stages. The total protein of the leaves and berry flesh of the BS cultivar at the ripening stage was greater than at the unripening stage and than in the BG cultivar contrast (Fig. 3). In accordance with the obtained results for BS cultivar in this research, in a study on grape the total protein increased during ripening (Giribaldi et al. 2007). At both growth stages, the total protein in leaves was more than in berry flesh (Fig. 3). At the unripening stage, total protein was significantly increased compared to control for the SA 1-mM concentration in leaves and berry flesh of the BS cultivar, in berry flesh of the BS cultivar for the SA 1-Mm concentration, and in



**Fig. 3** Effect of salicylic acid (SA) in three concentrations—0 (control), 0.1 mM, and 1 mM—on total protein content of leaves and berry flesh of Bidane Sefid (BS) and Bidane Ghermez (BG) grape cultivars in unripening stage (a) and ripening stage (b). Different letters indicate significant differences at the level of  $p \leq 0.05$

berry flesh of the BG cultivar for the SA 0.1 mM concentration. However, SA had a decreasing effect on the leaves of the BG cultivar at this stage.

At the ripening stage, the SA 0.1-mM concentration significantly increased the total protein of leaves and berry flesh of both cultivars. In accordance with these results, SA has been shown to increase the total protein in other grape cultivars (Renault et al. 1996). In a study of the grape leaves of BS cultivar, SA in a 2-mM concentration decreased the total protein, but concentrations of 0.5 mM and 1 mM did not have a significant effect on the total protein of leaves (Ershadi and Taheri 2013). The results in this investigation showed that the 0.1-mM concentration was effective in increasing the total protein of leaves of the BS and BG cultivars at the ripening stage. Studies have shown that SA played an effective role in regulating apoplastic proteins and antioxidant enzymes associated with stress resistance (Tasgin et al. 2003). Decrease in production, increase in protein degeneration, or both can be attributed to the increase and decrease of antioxidant activity (El-Tayeb 2005).

The total protein concentrations at both growth stages in the leaves of BG were greater than for the BS cultivar; in contrast, the total protein in berry flesh of BS was more than for BG cultivar (Fig. 3). The SA effect on the total protein for both cultivars at the ripening stage was greater than in the unripening stage, especially in the leaves and berry flesh of the BG cultivar, because in this cultivar the total protein of tissues decreased during ripening, but the use of the SA 0.1-mM concentration significantly increased the total protein in these tissues compared to control. Also, at both growth stages the total protein of leaves was (both cultivars) greater than that for berry flesh. The highest amount of total protein was at the ripening stage in leaves of BG and BS cultivars treated with SA in the 0.1-mM concentration.

### Effect of Salicylic Acid on SOD Enzyme Activity

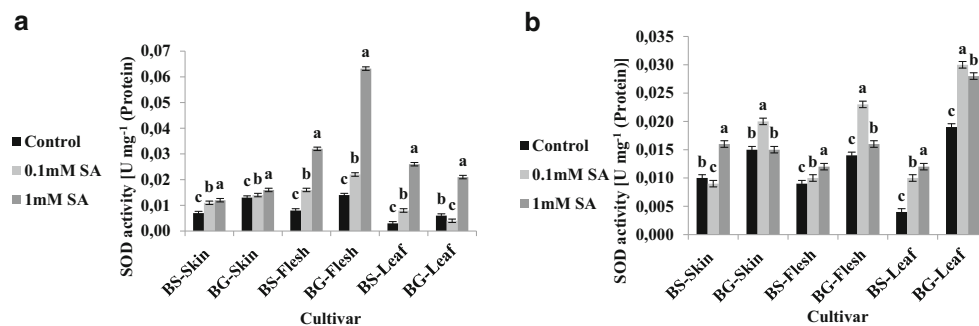
Salicylic acid has different effects on the SOD activity of different parts of the grape at both growth stages. In this study, the SOD activity in the leaves, skin, and flesh of

berries for both BS and BG cultivars was higher at the ripening stage than at the unripening stage (Fig. 4).

At the unripening stage, the use of SA in both concentration significantly increased the SOD activity of the leaves, skin, and berry flesh in both cultivars compared to control; the effect of the 1-mM concentration was greater than that of the 0.1-mM concentration. But at the ripening stage, the SA 0.1-mM concentration in the BG cultivar and the 1-mM concentration in the BS cultivar significantly increased the SOD activity of leaves, skin, and flesh of berries (Fig. 4). In accordance with these results, the effect of SA on the antioxidant system of several grape cultivars (Darcherin, Ghezal Osum, and Sahebi) was such that when SA was applied at appropriate concentrations (2 mM), this hormone enhanced the antioxidant system of the plant's tissues through the activation of SOD and CAT oxidant enzymes (Habibi 2012). Research on the tomato showed that SA increased the activity of antioxidant enzymes POD, SOD, and CAT; this activity may be due to the regulating role of SA on transcriptional or translational regulation (Hayat et al. 2005).

Salicylic acid directly and indirectly activates the antioxidant enzymes by increasing their activity due to ROS scavenging created by stress (Doulatbadian et al. 2008; Li and Wang 2021). In the present study, the activity of the SOD enzyme in berry flesh of both cultivars was higher than in the leaves and berry skin at the unripening stage, but this was higher in berry skin than in leaves and berry flesh in both cultivars at the ripening stage. The activity of the SOD enzyme of leaves, skin, and berry flesh of the BG cultivar was higher than for the BS cultivar (Fig. 4). On the other hand, at both growth stages, the effect of SA on the activity of the SOD enzyme of leaves, skin, and berry flesh of the BG cultivar was greater than for the BS cultivar. The highest activity of the SOD enzyme was in the unripening stage in the berry flesh treated with the 1-mM concentration in both cultivars and at the ripening stage in leaves and berry flesh of the BG cultivar treated with the 0.1-mM concentrations. In accordance with these results, studies have shown that SA stimulates extraordinarily sen-

**Fig. 4** Effect of salicylic acid (SA) in three concentrations—0 (control), 0.1 mM, and 1 mM—on superoxide dismutase enzyme (SOD) activity content of leaves, skin, and berry flesh of Bidane Sefid (BS) and Bidane Ghermez (BG) grape cultivars in unripening stage (a) and ripening stage (b). Different letters indicate significant differences at the level of  $p \leq 0.05$



sitive reactions during ripening of the grape, suggesting that there are active oxygen-scavenging systems such as SOD and CAT enzymes in grapes, which are stimulated at this stage (Kraeva et al. 1998; Zhou et al. 2019).

The results show a direct correlation between soluble and insoluble sugar and SOD activity. In fact, the amount of both of these compounds increased at the ripening stage. On the other hand, there was a positive correlation between chlorophyll *a*, carotenoids, insoluble sugar, and the activity of the SOD enzyme; that is, the content of all of these compounds in the tissues of the BG cultivar was greater than for the BS cultivar. Also, there was a direct correlation between proline and protein at the ripening stage, such that the proline and protein in the berry flesh of the BS cultivar were higher than in the BG cultivar, and in leaves, both of these compounds were higher in the BG cultivar.

In fact, the results show that the SA 0.1-mM concentration in most cases was more effective than the 1-mM concentration. Even in some cases in which the compound content decreased at the ripening stage, SA increased the compounds studied. It was also observed that in some cases, although the amount of these compounds in the BG cultivar was higher than in BS, the effect of SA on the BS tissues was greater.

## Conclusion

It has always been underestimated that due to modulating effect of SA in biotic and abiotic stresses on the direct role of this pseudohormone alone and in isolation from stress, which plays a very positive role in nutritional factors. In this study, comparing two growth stages of grape cultivars under SA treatment provided the most effective concentration of this treatment and the most effective stage of the treatment effect. In fact, the results showed that the effect of SA was different in different tissues and in two different stages of growth (unripening and ripening). However, in most tissues at lower concentrations, 0.1 mM was more effective. In fact, the results of this study show that chlorophylls *a* and *b* and carotenoids decreased in the fruit ripening stage (compared

with the unripening stage). But SA in both concentrations of optimal concentrations (0.1 mM) in both BG and BS cultivars increased chlorophyll *a* and *b* and carotenoids. Therefore, although chlorophyll *a* and *b* and carotenoids decreased in the ripening stage, SA at this stage was able to slightly compensate for this reduction. The results also show that soluble and insoluble sugar, proline, protein, and SOD enzyme activity in most of the tissues of both BG and BS cultivars at the ripening stage were greater than at the unripening stage. On the other hand, the effect of SA on these compounds in the ripening stage was greater.

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**Conflict of interest** F. Nazari, M. Maleki, and M. Rasouli declare that they have no competing interests.

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