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Effects of Plant-derived Smoke, Karrikin, and Salinity Stress on *Prunus armeniaca* cv. Şalak seeds and seedlings: A Morphological, Biochemical, and Molecular Approach

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ABSTRACT

The effects of plant-derived smoke on seed germination and plant growth, depending on concentration and time, are widely known. However, there are very few studies demonstrating that it provides tolerance to abiotic stresses. This study comprehensively compares the effects of SW and KAR1 on seed germination and morphological, biochemical, and molecular changes observable in the examined seeds. Moreover, the study shows that it regulates the expression of some genes encoding antioxidant enzymes in apricot seedlings (*Prunus armeniaca* L.) exposed to salinity stress (100 mM NaCl). The highest germination rate was 1:1000 DS with 60% and 1 µM KAR1 with 72%. In terms of shoot development, root and stem length, 1:100 concentration in the DS group and 1 µM concentration in the KAR1 group gave the best results. The shoot development rates were 95.83% and 87.50% in the DS and KAR1 groups, respectively. While the root length was 137.68 and 141.92 mm in the DS and KAR1 groups, respectively, the stem length was 103.78 and 102.67 mm, respectively. The data revealed that SW (1:1000 v/v) and KAR1 (1µM) increased the expression levels of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) genes in the samples taken from the apricot seedlings treated with salt at hours 3, 6 and 9. This increase varies in SW and KAR1 depending on time. When the biochemical results were examined, it was seen that the application of SW and KAR1 to the seedlings under salinity stress led to a significant decrease in the thiobarbituric acid reactive substances (TBARS) content. We can assert that SW is more effective than KAR1 on TBARS content. Morphological, molecular, and biochemical results revealed enhanced germination, growth, gene expression, and TBARS content in apricot seeds and seedlings exposed to SW and KAR1. This data may be applicable to more comprehensive trials.

Keywords: Antioxidant enzyme, Smoke water, TBARS, Salt stress, Karrikin, Gene expression

1. Introduction

Salinity, an abiotic stress, is among the main environmental factors limiting plant growth. Arid and semi-arid areas account for approximately 46% of the earth's surface. About 50% of these areas encounter salinity problems at different levels. The FAO/UNESCO World Soil Map data reports that 954 million hectares of land worldwide are affected by salt, and productivity on this land is restricted. Salinity in soil occurs in both natural and artificial ways. The former is observed in arid and semi-arid areas, in extremely hot conditions, in flat basins where soil drainage is insufficient, and when salts carried by precipitation are brought to the soil's surface. The latter occurs as a result of reckless irrigation and excessive use of fertilizers (Özbek et al. 1999; Sönmez & Sönmez 2007). Nowadays, many agricultural lands suffer from gradual degradation due to salinity. Desertification may be inevitable unless precautions are taken. According to a number of studies, of the apricot is among the group of plants sensitive to salinity stress (Bernstein et al. 1956; Bernstein 1965; Maas 1984; William 1986; Gucci & Tattini 1997; Karaoğlu & Yalçın 2018). The effects of salinity stress on plants occur as osmotic and ionic stress. Salt that becomes accumulated in the root zone of the plants and in the soil restricts water uptake by roots, which results in osmotic stress. Osmotic stress causes oxidative damage in plants as a result of the deterioration of the nutrient balance and membrane properties, and a decrease in the photosynthetic activity and stomatal conductance, and an increase in the formation of reactive oxygen species (ROS) due to decreased photosynthetic activity (Munns & Tester 2008; Rahnama et al. 2010). Ion stress occurs with the accumulation of Na^+ and Cl^- ions in plants. While the Na^+ ion prevents the uptake of K^+ and Ca^{2+} ions and the regulation of stomatal conductance, Cl^- causes chlorophyll degradation, leading to a deterioration in photosynthetic activity. Ion toxicity causes deteriorated ion balance and physiological disorders through excessive ion uptake in the roots and leaves (Tavakkoli et al. 2011). Plants have developed various adaptation mechanisms to combat the increase in ROS due to environmental stress. Plants possess enzymatic and non-

enzymatic antioxidant defense systems that protect their cells from oxidative damage. Enzymatic antioxidant systems include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), etc.; while non-enzymatic antioxidant systems include ascorbic acid (ASH), glutathione (GSH), alkaloids, phenolic compounds, protein (such as proline) and non-protein amino acids, lipid peroxidation (TBARS), and α-tocopherols (Gill & Tuteja 2010).

A large body of research is available on many plant species grown in different environments where plant-derived smoke increases seed germination and seedling, shoot, and root development. These studies have shown that smoke has a stimulating or inhibitory effect on germination and plant growth in plants, depending on the concentration used and the exposure time (Baxter & Van Staden 1994; Van Staden et al. 2006; Kulkarni et al. 2008; Çatav et al. 2018; Khatoon et al. 2020). The germination-stimulating property of smoke is known to be due to KARs (KAR1-KAR6), a special type of lactone known as butenolide (3-metil-2H-furo [2,3-c] pyran-2-one) fused to a pyran ring (Flematti et al. 2015; Kemeç Hürkan 2023). In addition, nitrogen oxides and cyanohydrins have also been found to promote germination (Nelson et al., 2012). However, it is known that smoke tends to have a "bidirectional regulating" effect on germination, as higher concentrations of smoke inhibit germination, while lower concentrations have a germination-promoting property (Light et al. 2002). In addition, nitrogen oxides and cyanohydrins have been shown to promote germination (Nelson et al. 2012). Nevertheless, it is noted that smoke tends to have a "dual regulatory" effect on germination as higher concentrations of smoke inhibit germination and lower concentrations promote germination (Light et al. 2002). It has been revealed that phenolic compounds, such as cresols, dihydroxybenzenes and 2-furoic acid, naphthalene, 3,4,5-Trimethylfuran-2(5H)-one (2,3,4-trimethylbut-2-enolide), in the smoke are responsible for the germination-inhibiting activity (Light et al. 2010; Kemeç Hürkan 2023).

KARs are thought to be used by plants to protect themselves against different combinations of abiotic factors, such as oxidative stress, drought, low light intensities, high temperature, salinity, low osmotic potential (Ghebrehiwot et al. 2008; Jamil et al. 2014; Banerjee et al. 2019). Although many studies have shown that plant-derived smoke and KARs stimulate germination, there is very little research that investigates the various abiotic stress conditions. These studies suggest that the application of plant-derived smoke or KAR to plants under salt stress further increases proline accumulation while decreasing the MDA accumulation and production of H_2O_2 (Sharma et al. 2012; Vardhini & Anjum 2015). In addition, the effects of plant-derived smoke and KARs on the transcription factors and various antioxidant enzymes (CAT, SOD, and GPX) against salinity stress have been investigated (Sharifi & Shirani Bidabadi (2020); Çatav et al. 2021; Hayat et al. 2022). Previous research has indicated that plant-derived smoke and KAR increase the expression of CAT, SOD, and GPX genes in plants under salt stress (Shah et al. 2020; Sharifi & Shirani Bidabadi 2020; Çatav et al. 2021; Shah et al. 2021; Hayat et al. 2022).

The apricot (*Prunus armeniaca* L.) is in the stone fruit group belonging to the Rosaceae family. Apricots are divided into 6-8 ecological groups and 13 regional subgroups due to their of propagation by seed and growth in very different ecological conditions (Layne et al. 1996; Ledbetter 2008; Asma 2011). The apricot is considered to be an important fruit for human health as it is rich in sugar, potassium, phosphorus, calcium, iron, dietary fiber, and vitamin A (β-carotene) (Açkurt 1999). There are approximately 58 apricot varieties in Turkey. Twenty-eight of these are registered apricot varieties. Şalak is an edible apricot variety grown in the Iğdır and Kağızman regions. The fruits are oblong shaped and very large, and the average fruit weight is 50-65 g. The fruits are sweet, the skin and flesh color are yellow, the shape is symmetrical, and the abdominal line is very prominent. The Şalak apricot ripens in the last week of June under the ecological conditions of Iğdır and Malatya (Akbaba et al. 2023; Asma 2011; Aydoğdu 2016). In terms of soil requirements, apricot trees prefer deep permeable, warm, rich in organic matter and nutrients, good drainage without high ground water, pH: between 6.5-7.5, loamy or loamy-calcareous, without salinity problems (Korkmaz 2007; Asma 2011).

It is important to develop various methods for the propagation of the apricot, both for human health and because it is the most exported fruit for Türkiye. The cheapest and easiest method for apricot sapling production is graft propagation. In the graft propagation method, the seeds of cultivated apricot varieties and apricot seedling rootstocks are widely used. Additionally, almond, peach and plum rootstocks are used. Hacıhaliloğlu, Şalak, Şekerpare, Alyanak etc. are the most common apricot varieties produced by grafting on seedling rootstocks (Yıldırım 2006; Asma 2011). The present study was conducted with the aim of producing seedlings to be grafted with seeds.

Seeds of most fruit trees, especially those belonging to the Rosaceae family, either do not germinate or show an incredibly low germination rate unless they are pre-treated to break dormancy (Kaşka 1970). Apricot seeds have two different types of dormancies, both endogenous and exogenous, caused by the components of the seed itself and the endocarp, respectively. Apricot seeds require cold stratification to break dormancy. For this, it is necessary to keep the apricot seeds at 4-7 ºC degree for 90-105 days (Fadl et al. 1978; Polat 2007; Szymajda et al. 2013).

The encouraging findings in the above-mentioned research have led to the question of whether plant-derived smoke and KAR may promote plant growth more effectively under stressful circumstances. In order to answer this question, the present study aims to determine whether SW and synthetic KAR1 provide protection against salt stress and changes in the antioxidant defense system in apricots (*P. armeniaca*, cv. Şalak). The study saw the first use of SW and KAR1 in stone fruit seeds (*Prunus armeniaca*). This constitutes the originality of the study. Apricots are sensitive to salinity stress and to find a solution to this salinity stress, the apricot seeds of the Şalak cultivar were grown *in vivo* with SW and KAR1 to determine the germination rate,

shoot development rate, root and stem lengths, stem diameters, number of leaves, leaf area, and lipid peroxidation (TBARS) activity. The changes in the expression of genes belonging to enzymes [catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX)] were investigated by qPCR. This study sheds light on the morphological, biochemical, and molecular responses related to smoke and KAR in plants exposed to salt stress.

2. Material and Methods

2.1. Plant material, growth conditions, and treatments

In the study, mature seeds (tree age 10-12) of *P. armeniaca* (cv. Şalak), belonging to the Rosaceae family, were used as material. According to the literature review, apricot seeds need cold stratification to break their dormancy. By optimizing the cold stratification process, it was decided that keeping it at 4 ºC±1 degree for 90 days was suitable for seed germination. The sterilization process was performed adopting the method described by Kemeç Hürkan & Akı (2022). After the testa parts of the sterilized seeds were peeled, they were transferred to Petri dishes with filter paper inside. The experiment in which the seeds were sown was repeated ten times (5x10), with five seeds in each Petri dish. In order to obtain SW, 1 kg of *Medicago sativa* L. straw was burned, and the smoke was dissolved in water (1L) in the filtering flask. The *Medicago sativa* L. straw was burned at 275 ºC for 60 min. until it turned to ash and was then filtered through a sterile syringe filter with a 0.22 µm pore size (Kemeç Hürkan & Akı 2023). Afterwards, according to the treatment groups (Control: sterile distilled water, SW: 1:100; 1:500; 1:1000 (v,v), KAR1: 0.01; 0.1; 1 µM), each of petri dish containing seeds was individually wetted with the previously prepared solutions. The KAR1 material was obtained from Toronto Research Chemicals Canada. The Petri dishes were wrapped with cling film to prevent the moist filter papers from drying out. The seeds were stored under dark conditions at 4 ºC±1 (wet stratification in cold) until germination. Since apricot seeds need cold weather to germinate, the seeds in the petri dishes were kept at 4 ºC±1. The seeds were germinated after one week and transferred to vials (24 compartments) containing a mixture of perlite and peat, and the experiment was repeated four times. The plants were grown in a climatic chamber with a long day photoperiod of 16 h light/eight h dark at 24 ºC±1. The vials were watered regularly every two-three days with the solutions used in Hoagland (100%-Himedia TS1117) + treatment groups according to the dryness of the soil.

2.2. Growth measurements

One month after germination, the measurements were performed for the statistical analyses of the morphological properties of the seedling. The root and stem lengths of the developing shoots were measured with a digital caliper and recorded by calculating the average lengths. The diameters of the stems were measured with a digital caliper at 2 cm above the axis where the soil touches the stem. The leaf area was measured using ImageJ 1.53k software. We chose three mature leaves from the middle position of the stem. The measurements were done as quadruplicates.

2.3. Salt stress treatment

Each vial containing one-month-old plants was irrigated with a NaCl (Bioshop SOD004) solution prepared at a concentration of 100 mM (2000 µS cm^{-1}). The sampling was performed at hours 0, 3, 6, and 9; after the leaves were placed in 1.5 mL tubes, they were dipped in liquid nitrogen and stored at -20 ºC until examined for gene expression.

2.4. Lipid peroxidation contents

The lipid peroxidation content was determined according to the method specified by Madhava Rao & Sresty (2000). The obtained supernatants were measured at absorbances of 532 nm and 600 nm wavelengths, and the calculations were made using the extinction coefficient of 155 mM^{-1} cm^{-1}.

2.5. Gene expression

The gene expression analysis was carried out with 1:1000 v/v for SW and 1 µM for KAR1 concentrations, which were morphologically more effective than the treatment groups. The DNA extraction was performed using TRIzol (Thermo, USA) according to the manufacturer's protocol, and the leaf tissues of 100 mg of the apricot plants were used in the study. The RNA concentration and quality were checked using Qubit 2 (Invitrogen, USA) and NanoDrop (Maestrogen, Taiwan) instruments, and the integrity of RNA with 2% agarose gel electrophoresis.

Before the cDNA synthesis, the RNA isolates were treated with the DNase 1 enzyme (Thermo, USA) against genomic DNA (gDNA) contamination. One microgram of RNA was used for the cDNA synthesis, which was performed according to the protocol provided by the manufacturer using the High Capacity cDNA Reverse Transcription Kit (Thermo, USA) (PCR profile set for cDNA synthesis: 25 ºC: 10 min; 37 ºC: 120 min; 85 ºC: 5 min). Actin (ACT) gene was used to confirm the success of the cDNA synthesis. The sequences, melting temperatures (T_m), and amplicon size (bp) of the gene-specific primers are given in Table 1.

Table 1- Primer specifications designed for qRT-PCR

Target enzymes	*Primer sequences (Forward/Reverse, 5′→3′)*	T_m	*Expected amplicon size (bp)*
Actin	[1]CCCTAAGGCTAACAGAGAAAAGA	59.20	212
	[1]CAGCAAGGTCCAGACGAAGAAT	57.40	
Katalase	CTCATACTGGTCTCAGGCAGA	58.62	116
	CCCACTGCTGGGAACTCAAA	60.18	
	TCTCATACTGGTCTCAGGCAG	58.62	117
	CCCACTGCTGGGAACTCAA	59.54	
	TCATACTGGTCTCAGGCAGATAAA	59.04	118
	TTGCCCACTGCTGGGAACT	61.77	
Superoxide dismutase	CCACATCGGCATAACATCCG	59.13	119
	TGGTTCAACGTGATCTCAGAA	57.26	
	GGAGATGGCCCAACTACTGT	59.10	120
	GAAATGCGGTCCAGTTGACA	58.80	
	AACCAACGGTTGCTTGTCAA	58.80	120
	CCATCGTCCCCAACAGTGAT	59.70	
Glutathione peroxidase	ACGTGGCTTCAAAATGTGGATTGA	61.76	128
	AGGCTCTTGGCCCCCAAA	61.19	
	ACGGGTTCTCTTTGAAATCACC	58.85	129
	CCTTTCCGTCAATATCCTTGACAC	59.67	
	AGGTGACCTTGTCAAGTGGAA	59.16	119
	CCAGTTTCTGGATATCCCTCTCAA	59.59	

[1] (Wang et al. 2014); T_m: Melting temperature in ºC; bp: base pair.

The quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed using SYBR Green dye to determine the expression of genes encoding CAT, SOD, and GPX enzymes in the apricot plant using Rotor-Gene-Q 5 Plex HRM (Qiagen, USA) with 72-well carousel. Each qRT-PCR tube (Qiagen Cat. No. 981103) included 5 µL RealQ Plus 2x Master Mix Green (Ampliqon Cat. No. A323402), 0.5 µL of 10 picomoles of each primer, 10 ng of cDNA and the reaction was completed to 10 µL with nuclease-free water. Three independent biological replicates and three technical replicates were used for each treatment group for the qRT-PCR studies. The qRT-PCR conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 45 cycles at 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 30 s. In the study, -RT control (without reverse transcriptase enzyme) and negative control (without template) reactions were also established for the control. The ACT gene was also used for normalization (Wang et al. 2014). The $2^{-\Delta\Delta CT}$ method was employed to evaluate gene expression (Livak & Schmittgen 2001). The data retrieved were analyzed using the Rotor-Gene Q Series Software 2.3.5 software.

2.6. Data analysis

According to the randomized plots trial design, all the data gathered for the purpose of this study were assessed using ANOVA on XLSTAT 2021, a statistical software. The Duncan's test was run to identify the differences between the averages once the statistically significant transactions were identified at a significance level of 5%. The obtained data were presented as mean ± standard deviation in a table.

3. Results

3.1. Morphological Growth parameters

The highest rate of germination was 1:1000 SW at 60% and 1 µM KAR1 at 72% (Table 2). It was observed that the germination increased as the concentration decreased in the SW treatment and increased as the concentration increased in the KAR1 treatment. The shoot development was observed in the sown seeds after two weeks. The shoot growth rates were assessed one month after sowing and before the salt stress application. In terms of the shoot development, a 95.83% ratio with 1:100 concentration yielded the best result in the SW treatment and an 87.50% ratio with 1 µM concentration in the KAR1 treatment. Shoot development analysis showed that there was no statistically significance observed among the control and the treatment groups of 1:500 SW, 1:1000 SW, 0.01 µM KAR1 and 1 µM KAR1.

Table 2- The effects of the SW and KAR on the growth parameters of *Prunus armeniaca* (cv. Şalak) seeds are provided in Table 2. The results are presented as mean ± SE. The values with different superscripts in the same row are significantly different from each other (P<0.05; Duncan's test) [SW: smoke water (1:100, 1:500, 1:1000 v/v), KAR1: karrikin (0.01, 0.1, 1 µM)].

Treatment	*Seed germination (%)*	*Shoot development (%)*	*Root length (mm)*	*Stem length (mm)*	*Stem diameter (mm)*	*Number of leaves*	*Leaf area (cm^2)*
Control	34.00±0.229^{ef}	77.27±0.400^{bc}	129.06±0.830^{abc}	90.90±0.889^{c}	2.17±0.220^{abc}	14.00±2.080^{a}	3.96±0.627^{ab}
1:100 SW	40.00±0.387^{cdef}	95.83±0.186^{a}	137.68±0.701^{a}	103.78±0.559^{a}	2.01±0.182^{c}	14.05±1.564^{a}	4.09±1.257^{a}
1:500 SW	50.00±0.403^{bcd}	79.17±0.380^{bc}	113.77±0.625^{bcd}	81.90±0.561^{cd}	2.26±0.283^{a}	11.35±1.797^{c}	3.09±0.924^{bc}
1:1000 SW	60.00±0.387^{ab}	85.42±0.400^{ab}	110.39±0.194^{cd}	78.87±0.341^{d}	2.10±0.215^{abc}	12.85±2.197^{ab}	3.37±0.834^{abc}
0.01 µM KAR1	46.00±0.229^{bcde}	83.33±0.312^{abc}	104.31±0.488^{d}	83.99±0.226^{cd}	2.05±0.234^{c}	13.35±1.590^{ab}	2.57±1.143^{c}
0.1 µM KAR1	54.00±0.391^{bc}	68.75±0.462^{c}	136.30±0.862^{ab}	92.08±0.200^{bc}	2.08±0.291^{bc}	12.35±1.424^{bc}	3.54±1.015^{ab}
1 µM KAR1	72.00±0.332^{a}	87.50±0.250^{ab}	141.92±0.143^{a}	102.67±0.254^{ab}	2.26±0.288^{ab}	14.10±1.670^{a}	3.97±0.981^{ab}
P	**P<0.002**	**P<0.025**	**P<0.002**	**P<0.0001**	**P<0.009**	**P<0.0001**	**P<0.005**

Values with different superscripts in the same row are significantly different from each other (P<0.05; Duncan's test). All morphological measurements were made before salt stress application.

The root lengths were observed to have decreased as the concentration decreased in the SW treatment. In the KAR1 treatment, on the other hand, as the concentration increased, an increase in root length occurred (Figure 1). Compared to the control, the 1:100 concentration with 137.68 mm yielded the best result in the SW treatment, while the 1 µM concentration with 141.92 mm did so in the KAR1 treatment.

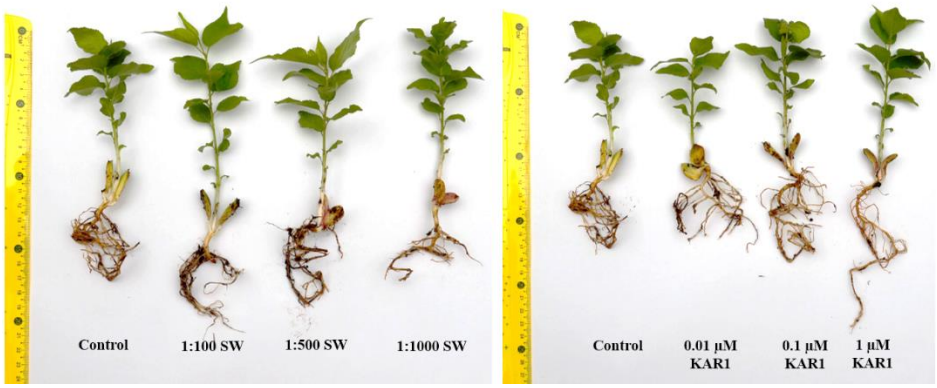

Figure 1- Effects of smoke water and karrikin on root growth

In the treatment groups after one month, a decrease in the stem length was observed in the SW treatment as the concentration decreased, and increased stem lengths were detected in the KAR1 treatment as the concentration increased (Figure 2). The 1:100 concentration with 103.78 mm in the SW treatment and the 1 µM concentration with 102.67 mm in the KAR1 treatment yielded the best results.

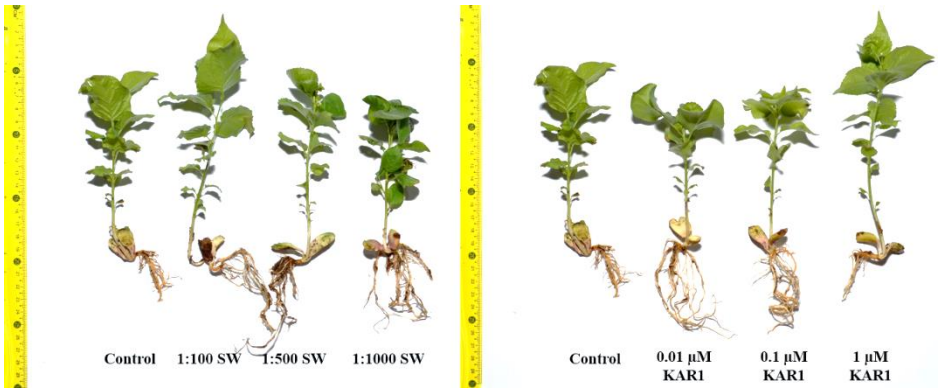

Figure 2- Effects of smoke water and karrikin on stem length

In terms of the stem diameter, 2.26 mm with 1:500 concentration yielded the best result in the SW treatment and 2.26 with 1 µM concentration in the KAR1 treatment. In terms of the number of leaves, the SW application gave the best result with 14.05 and 1:100 concentration, while the KAR1 application gave the best result with 1 µM concentration and 14.10. In terms of the number of leaves, the SW application gave the best result with 14.09 cm^2 and 1:100 concentration, while the KAR1 application

gave the best result with 1 µM concentration and 3.97 cm^2. The SW and KAR1 applications gave similar results in terms of stem diameter, number of leaves and leaf area, depending on concentration.

3.2. Lipid peroxidation parameters

The effect of the treatment groups on the amount of lipid peroxidation was found to be statistically significant (P<0.05). In the TBARS level, there was a 2.31% decrease in the SW treatment and a 0.77% increase in the KAR1 treatment compared to the control (Figure 3).

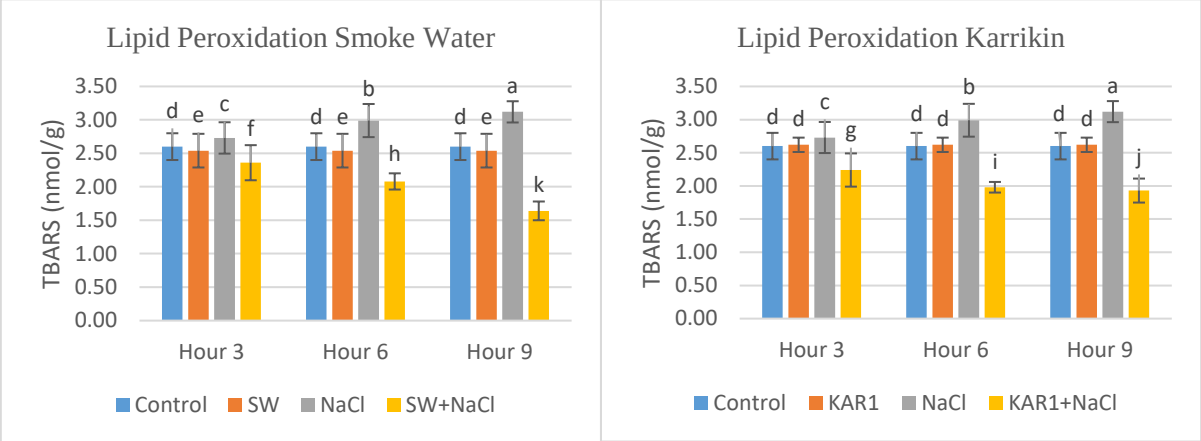

Figure 3- Effects of smoke water and karrikin on lipid peroxidation. SW: smoke water (1:1000 v/v), KAR1: karrikin (1 µM), NaCl: salt stress (100 mM). Results are presented as mean ± SE. Values with different superscripts in the same row are significantly different from each other (P<0.05; Duncan's test).

At hours 3, 6, and 9 of the salt treatment, the TBARS levels increased by 5%, 15%, and 20%, respectively, in cells compared to the control, indicating that cellular membrane damage occurred a result of salt stress. It was determined that there was a decrease in the TBARS levels when salt was applied in the SW and KAR1 treatments. The TBARS levels were found to have decreased by 9.23%, 20%, and 36.92% in the SW treatment compared to the control at hours 3, 6, and 9, respectively. In the KAR1 treatment, there was a 13.85%, 23.85%, and 25.77% decrease in the TBARS levels at hours 3, 6 and 9, respectively, compared to the control. These results suggest that SW and KAR1 may mitigate salt stress-induced cellular membrane damage. However, it was concluded that SW provided a more effective improvement than KAR1.

3.3. Gene expression

3.3.1CAT gene expression parameters

The effects of the treatment groups on CAT gene expression were found to be statistically significant (P<0.05). The results concerning the CAT gene expression showed that the SW treatment increased gene expression by 11% compared to the control (Figure 4).

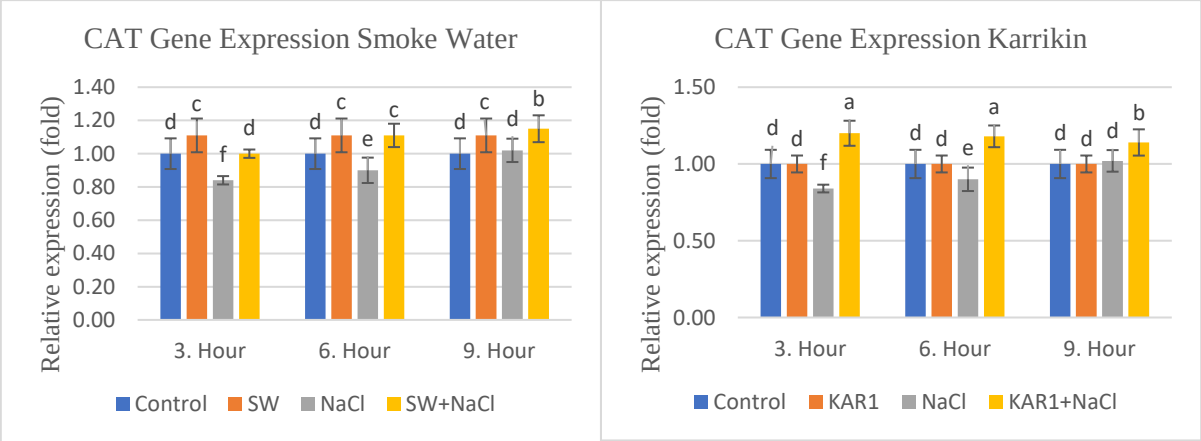

Figure 4- CAT qRT-PCR expression results. SW: smoke water (1:1000 v/v), KAR1: karrikin (1 µM), NaCl: salt stress (100 mM). Results are presented as mean ± SE. Values with different superscripts in the same row are significantly different from each other (P<0.05; Duncan's test).

It was determined that there was a decrease of 16% and 10% in gene expression compared to the control at hours 3 and 6 of salt treatment, respectively and an increase by 2% at hour 9. It was found that there was an increase in the gene expression when the salt was applied in the SW and KAR1 treatments. An 11% and 15% increase was detected in the gene expression in the SW treatment compared to the control at hours 6 and 9, respectively. In the KAR1 treatment, there was an increase of 20%, 18% and 14% in gene expression at hours 3, 6 and 9, respectively, compared to the control.

The SW treatment alone was more effective in increasing gene expression than KAR1. When salt was applied in the SW and KAR1 treatments, it was understood that the KAR1 treatment was more effective in gene expression than the SW treatment. While the SW treatment showed more effects at hour 9, the KAR1 treatment started to show its effect from hour 3, which was the first step of stress. As a result, the KAR1 treatment was found to be more effective than the SW treatment when salt was applied in the SW and KAR1 treatments in CAT gene expression.

3.3.4. SOD gene expression parameters

The effects of experimental groups on SOD gene expression were found to be statistically significant ($P<0.05$). The results regarding SOD gene expression showed that the SW treatment increased gene expression by 5% compared to the control (Figure 5).

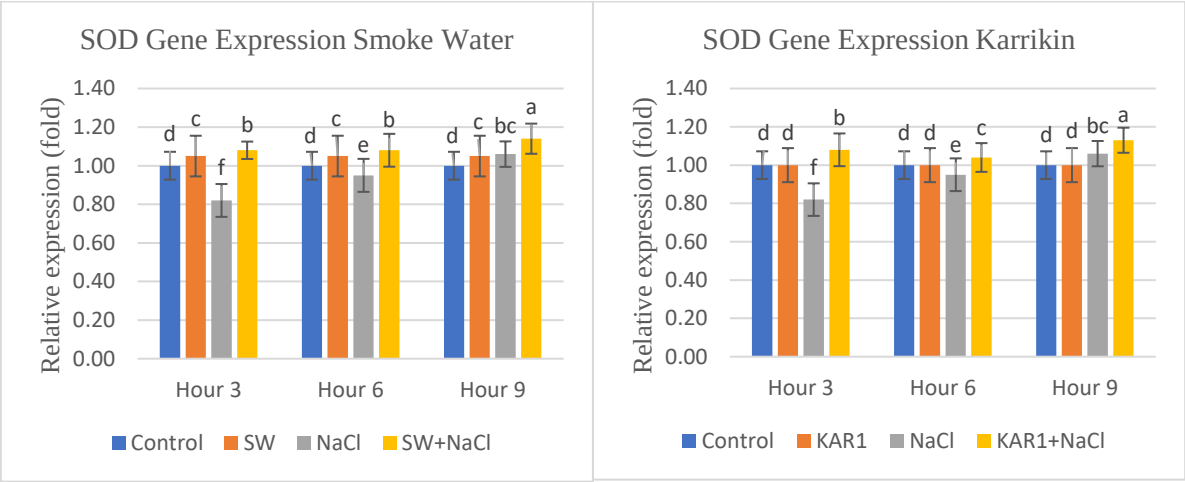

Figure 5- SOD qRT-PCR expression results. SW: smoke water (1:1000 v/v), KAR1: karrikin (1 µM), NaCl: salt stress (100 mM). Results are presented as mean ± SE. Values with different superscripts in the same row are significantly different from each other ($P<0.05$; Duncan's test)

It was determined that there was a decrease of 18% and 5% in gene expression compared to the control at hours 3 and 6 of salt treatment, respectively and an increase by 6% at hour 9. An increase was identified in gene expression when salt was applied in the SW and KAR1 treatments. It was revealed that there was an 8%, 8% and 14% increase in gene expression in the SW treatment compared to the control at hours 3, 6 and 9, respectively. In the KAR1 treatment, an 8%, 4%, and 13% increase occurred in gene expression at hours 3, 6 and 9, respectively, compared to the control.

It was concluded that the SW and KAR1 treatments alone were not considerably effective in gene expression. When salt was applied in the SW and KAR1 treatments, both showed similar results and appeared to be more effective at hour 9 of application. As a result, SW and KAR1 treatments have similar effects on SOD gene expression.

3.3.5. GPX gene expression parameters

The effects of the experimental groups on GPX gene expression were found to be statistically significant ($P<0.05$). The results regarding GPX gene expression showed that the SW treatment increased gene expression by 3% compared to the control (Figure 6).

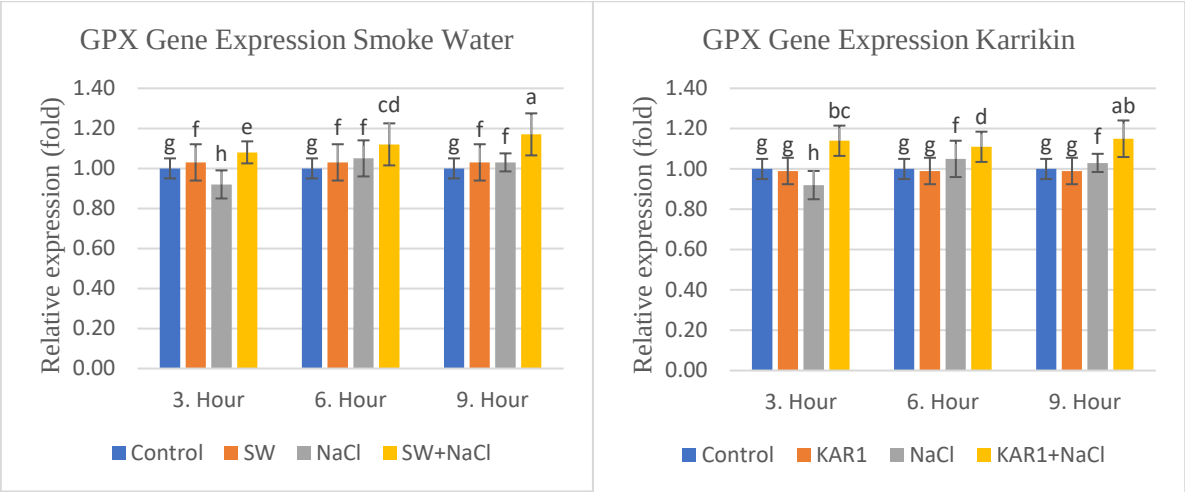

Figure 6- GPX qRT-PCR expression results. SW: smoke water (1:1000 v/v), KAR1: karrikin (1 µM), NaCl: salt stress (100 mM). Results are presented as mean ± SE. Values with different superscripts in the same row are significantly different from each other (P<0.05; Duncan's test).

It was determined that there was an 8% decrease in hour 3 of the salt treatment compared to the control, and a 5% and 3% increase in gene expression compared to the control at hours 6 and 9, respectively. It was observed that there was an increase in gene expression when salt was applied in the SW and KAR1 treatments. There was an 8%, 12%, and 17% increase in gene expression in the SW treatment compared to the control at hours 3, 6 and 9, respectively. In the KAR1 treatment, there was an increase of 14%, 11%, and 15% in gene expression compared to the control at hours 3, 6 and 9, respectively.

It was determined that the KAR1 treatment alone was more effective in gene expression than the SW treatment. When salt was applied in the SW and KAR1 treatments, both seemed to be more effective at hour 9. The SW and KAR1 treatments in GPX gene expression showed similar results when salt was applied.

4. Discussion

There are many studies suggesting the use of SW and KARs when germinating seeds as these substances increase the germination rate (Baxter & Van Staden 1994; Çatav et al. 2015; Flematti et al. 2015; Kochanek et al. 2016; Tavşanoğlu et al. 2017). KARs are water-soluble substances that have seed germination-promoting activity at incredibly low concentrations, usually below 10^{-9} mol/L (Light et al. 2009; Nelson et al. 2012). However, higher concentrations of SW inhibit germination, while lower concentrations are known to exhibit a germination-promoting property (Light et al., 2002). Therefore, in order to maximize its stimulant biological activity, SW should be diluted with water before use, usually at ratios of 1:250, 1:500, 1:1000, 1:1500, and 1:2000 (v/v), depending on the plant species (Van Staden et al. 2004). At high concentrations (1:100 or less dilution), SW inhibits germination. However, lower concentrations (1:1000 dilution) significantly increase germination compared to the control (Light et al. 2002). Consistent with the literature, this study observed that the germination of apricot seeds increased as the SW concentration decreased, whereas the germination increased as the concentration increased in the KAR1 treatment. The best germination optimization for SW was obtained at a concentration of 1:1000 (v/v) and for KAR1 at a concentration of 1µM.

There are hypotheses that both SW and KAR1 interact with other plant growth regulators and frequently exhibit cytokinin and auxin-like activities (Chiwocha et al. 2009). Since it is thought to exhibit activities, as these phytohormones do, it has been shown that KAR and SW provide a significant increase in shoot and root length, seedling development and lengths, number of leaves, leaf area, root and shoot dry and fresh weights, stem diameters and number of fruits after germination (Baxter & Van Staden 1994; Kulkarni et al. 2006; Van Staden et al. 2006; Kulkarni et al. 2008; Chumpookam et al. 2012; Çatav et al. 2018). In a study on tomato, okra, bean, and corn seeds, SW was found to significantly increase seedling growth by increasing both root and shoot lengths. The results show that the smoke-derived compound also exhibits stimulating effects after germination and can be used as a plant growth regulator (Van Staden et al. 2006). The treatment of okra seedlings with SW has shown a significant increase in shoot/root length, shoot fresh/dry weight, number of leaves, total leaf area, and stem diameter. Thus, the application of SW to leaves is thought to be a useful and inexpensive technique to increase the seedling growth of vegetable crops (Kulkarni et al. 2007). The present study revealed that KAR1 and SW caused an increase in the shoot, root, and stem lengths. However, when compared with the available literature, no remarkable results were found in the number of leaves, stem diameters, and leaf area. Contrary to the germination results, the best concentration for SW in the morphological data was 1:100. This may be due to the decrease in the SW concentration in the soil when the soils in the vials were irrigated with the Hoagland solution, and, consequently, the decrease in the KAR concentration in its content. Because KAR in nature can be washed away by rain and decompose relatively quickly in sandy soils, their concentration is constantly decreasing (Flematti et al., 2015).

Salinity is one of the abiotic stress factors that delimit the water uptake ability of plants and cause ion toxicity (Munns & Tester 2008). It is known that these two conditions caused by salt stress negatively affect cell growth rate, leaf development, stomatal conductance, photosynthesis, ion balance, and oxidative homeostasis (Jiang & Deyholos 2006; Shabala & Munns 2012; AbdElgawad et al. 2016). Exposure to salt stress is associated with increased electrolyte leakage, proline content, H_2O_2 production, and MDA accumulation. Studies show that SW or KAR application to a plant under salt stress decreases MDA accumulation and H_2O_2 production while further increasing proline accumulation (Shah et al. 2020; Sharifi & Shirani Bidabadi 2020; Çatav et al. 2021; Shah et al. 2021; Hayat et al. 2022). In this study, it was determined that the accumulation of MDA increased only in the plants exposed to salt stress, and a decrease in MDA accumulation was observed when SW and KAR were applied to the plant under salt stress. These results suggest that SW and KAR can minimize the toxic effects of ROS in apricot plants under salt stress. Antioxidant enzymes are essential for shielding cells and macromolecules from oxidative damage brought on by ROS. It is well known that various plant species' responses to severe environmental factors and plant growth regulators alter the expression of these genes and the activity of these enzymes (Sharma et al. 2012; Vardhini & Anjum 2015). The present study investigated the independent impacts of SW and KAR on the transcription levels of several antioxidant enzymes (CAT, SOD, and GPX) under salt stress. Consistent with previous studies, our results indicated that SW and KAR increased the expression of CAT, SOD, and GPX genes under salt stress. According to a study by Sharifi & Shirani Bidabadi (2020), it was observed that KAR treatment increases the activities of CAT and SOD enzymes with salinity stress, while decreasing the activity of the GPX enzyme. Thus, it has been revealed that KAR activates the adaptation mechanism against salinity stress. Hayat et al. (2022) report that SW applied to wheat plants increases the activities of SOD, APX, and POD enzymes. In the SW treatment used in our study, an increase was observed in the SOD activity compared to the control, and it activated the protection mechanism of the plant against the damage caused by salt stress. As the study by Çatav et al. (2021) notes, salt stress with SW treatment increases the expression of Cu/Zn-SOD, Fe-SOD, and Mn-SOD genes. This finding coincides with our study and that of Hayat et al. (2022). The study also has claimed that the SOD and APX activities were greater in the treated seedlings than in the control seedlings and proposed that overexpression of the genes encoding these enzymes was crucial in enhancing salt tolerance (Hayat et al. 2022). Additionally, the above study demonstrated that while salt decreased the expression of the CAT gene in the treatment group, it had no effect on the function of the CAT gene. This decrease in the expression of the CAT gene in the above study coincides with our study. Consistent with the data of our study and of Shah et al. (2020, 2021), gene expressions of SOD, CAT, POD, and APX were found to be significantly higher in wheat seedlings treated with KAR1 under salinity stress. The related literature confirms that SW and KAR increase the activity of antioxidant enzymes and gene expression against salinity stress. It appears that KARs can control endogenous H_2O_2 production, MDA accumulation, and proline accumulation, prevent electrolyte leakage, and improve membrane integrity under abiotic stresses.

5. Conclusions

The apricot plant is a commercially important crop plants that can be used both for edible and dried purposes. Although the use of SW and KAR have been studied on many plants, there has yet to be any study of its use on fruit trees; this study will be a first in the literature. In the present research, the seeds of Şalak, were germinated with SW and KAR1, and the germinated seeds were transferred to the soil and their morphological development was evaluated. The germination results showed that SW and KAR1 have similar results with the literature by stimulating the germination with the determined concentration in this study. Our study found that germination increased as SW concentration decreased, and germination increased as KAR1 concentration increased. Literature data has shown that SW and KAR1 contribute significantly to the development of the plant after germination. In the present study, KAR1 and SW caused an increase in shoot, root and stem length.

The apricot is one of a group of plants sensitive to salinity stress and the Iğdır Plain is in the saline soil category in terms of its characteristics. While many studies have shown that SW and KAR1 contribute to plant growth in terms of germination and morphology, there are few studies showing that they increase tolerance in abiotic stress factors. In this research, it has been proven that SW and KAR1 protect the plant against the harmful effects of ROS by increasing the transcription levels of antioxidant enzymes (CAT, SOD and GPX) and reducing the amount of lipid peroxidation (MDA).

Smoke water is a cheap and simple substance that has the potential to be used in agriculture, horticulture, and laboratory studies, and can be stored and used for many years. Since it acts as a plant growth regulator on plants in many studies, it is an economic substance that supports seed germination, shoot, root and plant growth even at very low concentrations in contrast to commercially available plant growth regulators (gibberellic acid, auxin, cytokinin, abscisic acid, strigolactone, etc.), which are very expensive, cannot be stored for long, and are sensitive to heat.

We believe that the obtained data make important contributions relating to the interaction of plants with their environment, germination physiology, and discovery of new and more effective molecules in the protection of plants against abiotic stress.

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