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Research article

Changes in physico-biochemical traits and antioxidant enzyme in *Vitis* rootstock treated with GR24 in arid conditions

Emine Sema Çetin*

University of Yozgat Bozok/Faculty of Agriculture – Dept. of Horticulture, Erdogan Akdag Campus – 66100 – Yozgat – Turkey.
*Corresponding author <esema.cetin@yobu.edu.tr>

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ABSTRACT: Plants play an essential role in the ecological cycle; however, they face numerous global biotic and abiotic stress factors. The capacity of plants to survive in adverse conditions is called stress tolerance. Drought stress is among the major stresses faced by plants, as arid conditions affect plant development and productivity, crop yield, relative water content, nutrient intake, photosynthesis, dry matter accumulation, and respiration. Hormones play an essential role in plant stress metabolism. Until recently, it was thought that plant hormones consisted of five groups: auxin, gibberellin, cytokinin, abscisic acid, and ethylene. However, other substances synthesized by plants, such as jasmonates, brassinosteroids, salicylic acid, and nitric oxide, have also shown critical vital functions in the plant, like the hormones. Another hormone in these substances is strigolactone (SL), which has been reported to perform essential functions in stress physiology. This research investigated the effects of SL applications on drought stress on 1103 Paulsen American Grapevine Rootstock. GR24, a synthetic analog of SLs, was applied to grapevine rootstock. Physical and biochemical changes were determined (chlorophyll, membrane injurity, prolin, lipid peroxidation, soluble protein, superoxide dismutase, ascorbate peroxidase activities, phenolic compounds, plant hormones, and mineral elements). The results show that GR24 applications effectively alleviate the harmful effects of drought stress in most features.
Keywords: drought, enzyme, grapevine, hormones, strigolactone

Introduction

As a result, plants are affected by harsh conditions and, they undergo stressful situations. Drought stress, which is an abiotic stress with a rate of 26 %, ranks at the top of increasing stress factors worldwide. Only 15 % of current agricultural areas are irrigable (World Bank, 2020), a serious sign that most plants are exposed to drought stress. Since it is impossible to eliminate the drought situation in most cases, approaches to provide stress endurance to plants bear a significant importance. Currently, strategies focus on genetic engineering to minimize crop losses; however, this method is costly and requires a large body of knowledge. Thus, finding natural, easy-to-use, practical, and non-harmful alternatives to human health is essential. Hormones provide conditions for the plant to cope with various types of stress (Christmann et al., 2006; Pérez-Torres et al., 2008; Evelin et al., 2009). Hormones play a key role in plant adaptation to the environment by modifying the source and sink interaction and the direction of nutrients to different plant parts (Wani et al., 2016). Plant hormones can increase tolerance to abiotic stresses by preventing electrolyte leakage from plant cells, neutralizing reactive oxygen species, or activating stress-related genes (Rachappanavar et al., 2022). Strigolactone (SL) is a plant hormone derived from carotenoids, and SLs are natural compounds with no negative impact on human health. Plant hormones are essential in developing stress response (Messing et al., 2010); therefore, the hypothesis is that SLs may also be effective in this direction. Researchers reported that SLs are involved in resistance to different stresses, such as low-light stress (Mayzlish-Gati et al., 2010; Tian et al., 2018; Zhou et al., 2022), high-light stress (Thula et al., 2023), cold stress, heat stress (Omoarelojie et al., 2020; Chi et al., 2021), heavy metal stress (Niu et al., 2021; Qiu et al., 2021), which have been the study subjects for investigating the effect of SLs in recent years. This work aimed to investigate the effect of SL applications on the resistance mechanism against drought stress in grapevine.

Materials and Methods

This study used 1103 Paulsen American grapevine rootstock as plant material. Cuttings of rootstock were obtained from Bursa Tarim Inc.& Co within the second week of April. The cuttings were prepared as five buds with 35-40 cm dimensions. All other buds were dulled during planting to ensure that only a single top bud was left. Afterward, the buds were planted in 2 L pots containing 1:1 perlite/turf and were taken to greenhouse conditions. For two months after planting (Figure 1), the cuttings were irrigated with Hoagland solution (No:2, basal salt mixture) twice a week (Hoagland and Arnon, 1950). Applications were started in third week of the month of June to determine the SL effects of drought stress. In research, SL applications are carried out by using GR24, which is an SL analog widely used in many biological events. In the present study, GR24 (Chiralix, A Symeres Company) was disolved in 3 % acetone and applied at three different concentrations [0 (control), 5 µM, and 10 µM] to the rootstock root area. The control application consisted of pure water containing 3 %

Figure 1 – View of grapevine rootstocks in the greenhouse.

acetone. GR24 was applied once every two days until the 14th day. One month after GR24 application, drought stress applications were started. Before the applications, the field capacity levels of the pots with equal amounts of turf and perlite were determined. Under drought stress, irrigations were arranged at 25 % of field capacity. Control plants were irrigated at field capacity level. As damage started to be seen in stress plants, stress applications were ended. The amount of chlorophyll was determined while the shoots were still on the plant. Then, the shoots were removed, and the physical properties were determined. Next, the shoots were kept in the freezer until the other analyses.

Shoot length, shoot weight, and average number of leaves per shoot

The shoot length of the rootstocks was measured with a ruler (cm), while the shoot weight of the rootstocks was measured with a scale, and the average number of leaves per shoot (ANLS) was determined as pieces.

Chlorophyll

The chlorophyll analyses were realized with the SPAD (Soil Plant Analysis Development) method by using chlorophyllmeter (measuring area of 2 mm × 3 mm between 9.9-199.9 SPAD units/SPAD-502 Plus, Konika Minolta Sensing, Inc.).

Membrane injurity index

The membrane injurity index (MII) was calculated by measuring the electrolyte emitted from the cell (Fan and Blake, 1994). Discs of 17 mm in diameter were taken from the third leaves of the shoots, and their electrical conductivity (EC) was measured after being kept in deionized water for 5 h. Discs were kept at 100 °C for 10 min, and afterward, the EC value of solution was measured again. The MII in leaf cells was determined in terms of percentage (%) by using the formula below:

$$MII = (Lt - Lc / 1 - Lc) \times 100$$

Lt: EC value of leaf before autoclave / EC value of leaf after the autoclave in the stress (treated) application; Lc: EC value of leaf before the autoclave / EC value of leaf after the autoclave in control plants.

Proline

The proline amount was determined in the samples according to Bates et al. (1973). Samples were homogenized with 3 % sulfosalicylic acid. The acid ninhydrin and glacial acetic acid were added to the filtered homogenate, and after keeping the samples in a water bath at 100 °C for 1 h, they were placed in an ice bath. After extracting this mixture with toluene, the toluene absorbance was read in a spectrophotometer at a wavelength of 520 nm.

Lipid peroxidation

The lipid peroxidation degree was determined by measuring the malondialdehyde (MDA) level, the final oxidation product (Madhava Rao and Sresty, 2000). A leaf sample of 0.5 g was homogenized with 10 % trichloroacetic acid (TCA) and then centrifuged. Then, the supernatant was mixed with 20 % TCA solution containing 0.5 % thiobarbituric acid. After, the mixture was kept in a 100 °C water bath for 20 min and read in a spectrophotometer at 532 and 600 nm wavelengths. The MDA concentration was calculated with the extinction coefficient (ε: 155 mM^{-1} cm^{-1}) and determined as nmol g^{-1} fresh weight.

Soluble protein

The homogenates were centrifuged at 1,047.198 rad s^{-1} for 10 min at 4 °C after homogenizing the leaf samples with 4 mL 50 mM potassium-phosphate solution (pH = 7.0) containing 2 mM Na-EDTA and 1 % polyvinylpyrrolidone. The supernatants obtained were used to determine soluble protein and antioxidant enzyme analyses (Ozden et al., 2009). The soluble protein content was determined by using Bovine Serum Albumin as a standard, according to the Bradford (1976) method.

Antioxidant enzyme activities

Extracts prepared for protein extraction were also used to determine the antioxidant enzyme activities. We also determined the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX).

Superoxide dismutase (SOD; EC 1.15.1.1): in the samples, the SOD enzyme activity was determined according to the method of Gong et al. (2005). The reaction mixture included (3 mL) 50 mM K-phosphate solution (pH = 7.3), 13 mM L-methionine, 75 µM Nitro Blue Tetrazolium (NBT), 0.1 mM EDTA, 4 µM riboflavin and 0.25 mL enzyme extract. The reaction mixture was

kept under 48 µmol photons $m^2 s^{-1}$ light intensity for 10 min. Then, the absorbance values were measured at 560 nm in the spectrophotometer, and the calculations were given in terms of U min^{-1} mg^{-1} protein. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50 % of the photoreduction of NBT in the presence of riboflavin and light (Giannopolitis and Ries, 1977).

Ascorbate peroxidase (APX; EC 1.11.1.11): the APX enzyme activity was determined according to the method of Nakano and Asada (1981), which is based on determining the decrease in absorbance at 290 nm of ascorbate oxidized by the enzyme in the sample by a spectrophotometer. The reaction mixture was composed of (2 mL) 50 mM of K-phosphate buffer solution (pH = 7.0), 0.5 mM of ascorbic acid, 1 mM of EDTA-Na_2, 0.1 mM of H_2O_2, and 50 µL of enzyme extract. The ascorbate oxidation was initiated by adding the enzyme extract to the reaction mixture, which was monitored for 3 min and read kinetically. The results were given in µmol min^{-1} mg^{-1} protein.

Phenolic compounds

The phenolic compound was extracted based on Kiselev et al. (2007). Accordingly, a 1 g leaf sample was taken, and 10 mL 96 % ethyl alcohol was added and then broken in a homogenizer for 2 min. Afterward, it was kept in a water bath at 45 °C for one night. At the end of this period, the samples were centrifuged at 418.879 rad s^{-1} for 5 min, and the liquid part containing the phenolic compounds was removed and evaporated at 45 °C in a rotary evaporator. Then, the extracts were dissolved in 1 mL of methanol and used to determine the total phenolic matter (spectrophotometrically) and the contents of phenolic compounds (HPLC). The total phenolic compound (TFC) contents were obtained using the Folin Ciocalteu colorimetric method, according to Singleton and Rossi (1965). The readings in the spectrophotometer were carried out at a wavelength of 765 nm, and the total phenolic matter was determined in mg g^{-1} in terms of gallic acid by using the curves prepared from the standard gallic acid solution. Identifing phenolic compounds was carried out at HPLC with modifications of the method by Caponio et al. (1999). The results were given as µg g^{-1}. The HPLC conditions were as follows: Shimadzu Prominence brand HPLC, CBM = 20ACBM; Detector = DAD (SPD-M20A); Column oven = CTO-10ASVp; Pump = LC20 AT; Autosampler = SIL 20ACHT; Computer program = LC Solution; Mobil phase: A = 3 % Formic acid; B = Methanol; Column = Zorbax Eclips XDB-C18 (250 × 4.6 mm, 5µ); Column temperature = 30 °C.

Plant hormones

Indole acetic acid (IAA) and Gibberellic acid (GA_3) were extracted according to Caponio et al. (1999) and HPLC determined the hormone contents (Cheikh and Jones, 1994).

Mineral elements

The amount of nitrogen (N) from mineral elements was determined as total N using the Kjeldahl device. The N analyses were performed in three repetitions and the results were given as percentage (%).

The contents of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), boron (B), zinc (Zn), and sodium (Na) were also determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Perkin Elmer Optima-8000). The ICP-OES conditions were as follows: Rf power (W) 1450; Injector = Alumina 2 mm i.d.; Sample tubing = Standard 0.76 mm i.d; Drain tubing = Standard 1.14 mm i.d.; Quartz torch = Single slot; Sample capillary = Polytetrafluoroethylene (PTFE) 1 mm i.d.; Sample vials = Polypropylene; Source equilibrium delay = 15 s; Plasma viewing = Axial; Processing mode = Peak area; Gases = Argon and Nitrogen; and Shear Gas = Air.

Statistical analysis

The analyzes were carried out in three repetitions. The data obtained as the test results were evaluated in SPSS (version 24) statistical program according to randomized block design, and differences between averages were determined according to the Duncan multiple comparison test.

Results

The effects of GR24 applications on drought stress in 1103 P American grapevine rootstock were analyzed, and the results are presented below.

The shoot length and weight data were not different (Table 1).

There is no difference in the number of leaves in plants under drought conditions. However, as in other physical traits, more new leaves were formed in drought conditions and in plants treated with 10 µM GR24 (Table 1).

The effects of GR24 treatment on some biochemical parameters were also studied. The chlorophyll content under both conditions was not statistically different (Table 2), but in plants treated with 10 µM GR24 and

Table 1 – Effects of GR24 applications on some physical parameters.

	GR24	Shoot length	Shoot weight	Average number of leaves per shoot (piece)
	µM	cm	g	
Irrigated	0	23.16^{ns}	5.70^{ns}	8.60 ab*
	5	24.80	6.23	7.80 b
	10	25.79	6.91	9.63 ab
Drought	0	25.48	5.20	8.63 ab
	5	26.90	7.00	8.97 ab
	10	28.27	7.01	10.23 a

*There is a difference between the means with different letters in the same column ($p < 0.05$).

grown under drought stress had a higher chlorophyll content. The chlorophyll content in leaves declines under most biotic and abiotic stress conditions. However, in this study, SL-treated plants had a higher chlorophyll content even under drought conditions. Likewise, the positive effect of the SL application is observed even if the environment is not under drought stress.

Membrane damage is a property determined based on measuring the electrolytes released by the cells and is a critical damage indicator, meaning that a high value indicates that the damage caused by stress is also high. Whether plants were under drought stress or not, the highest level of membrane damage was observed in the control plants that were not treated with GR24 (Table 2).

Proline is one of the most widely used parameters to measure the damage caused by different plants stress sources. Proline is synthesized in plants to tolerate stress as an osmoprotectant. In this study, the proline content was determined at low values in all irrigated plants. The highest proline content was recorded in plants under drought stress and treated with 5 µM GR24, and this value was 14.25 times higher than the lowest proline content of irrigated and 5 µM GR24 treated plants. Considering this parameter, it is possible to infer that the GR24 application is efficient to tolerate stress.

Lipid peroxidation is described as the oxidative damage of unsaturated fats in cell membranes. Depending on the stress severity, this oxidation in stressed cells can reach serious levels and may eventually cause plant death. The product of oxidation is malondialdehyde (MDA) and peroxidation severity in lipids can be estimated based on the principle of measuring the end product.

The treatment with GR24 reduced oxidation even in irrigated plants, although it varied according to the concentration. On the other hand, GR24 treatment was more effective than irrigated plants in terms of oxidation in stressed plants. The lowest amount of lipid peroxidation (5.122 nmol g^{-1}) was observed in irrigated and high-concentration GR24-treated plants, while the highest peroxidation (8.117 nmol g^{-1}) was observed in stressed plants without the GR24 treatment, indicating that SLs are effective to prevent stress (Table 2).

Protein synthesis is another negative effect on the plant under stress. This study achieved the highest protein content in irrigated plants treated with 10 µM GR24 (0.483 mg g^{-1}). However, the lowest protein content was also recorded in irrigated plants without or with a low GR24 concentration. In stressed plants, the 5 µM GR24 treatment contained a higher protein content than the GR24-treated plants at the same concentration but not exposed to stress. Therefore, it can be inferred that GR24 exerts its effect more clearly under stress conditions.

Whether under stress or not, plants tend to keep oxidant and antioxidant molecules in their cells in balance. Antioxidants are structures that delay or prevent oxidation and are grouped as enzymatic and non-enzymatic. While the superoxide dismutase enzyme converts O_2 to H_2O_2, APX keeps H_2O_2 in water-water and in ascorbate-glutathione cycles and uses ascorbic acid as an electron donor. It lowers H_2O_2 in water, and thus, it plays an important role in the antioxidant system of plants. In this study, changes in superoxide dismutase and ascorbate peroxidase enzymes, which are enzymatic antioxidants, were also studied.

Data on the SOD enzyme showed that the highest enzyme synthesis occurred in irrigated plants and plants treated with a high GR24 concentration (6.960 U min^{-1} mg^{-1} protein). The effect of GR24 application in drought conditions was observed with high SOD activity in both doses. The SOD synthesis was twice as high in the treated plants compared to the control plants in which GR24 was not applied under drought conditions, indicating the effect of the GR24 treatment to establish the balance under stress, as mentioned above.

Another antioxidant enzyme is ascorbate peroxidase (APX), and the positive effect of GR24 on APX activity is very significant. The highest APX synthesis was recorded under drought conditions and at 10 µM GR24 dose (0.938 µmol min^{-1} mg^{-1} protein), and this value was about three times higher in irrigated plants than in the control and in the 5 µM GR24 treatment.

The synthesis of phenolic compounds is one of the most important defense mechanisms in plants under biotic and abiotic stress conditions, and they are secondary metabolites in high amounts. Table 3 shows the effects of GR24 applications on TPC (mg g^{-1}) and individual phenolic compounds (µg g^{-1}).

The synthesis of TPC, p-coumaric acid, ferulic acid, and rutin increased more in plants cultivated under drought conditions and in plants treated with 5 µM GR24. The ferulic acid content was also synthesized at high levels in plants grown under irrigated and drought

Table 2 – Effects of GR24 applications on some biochemical parameters.

	GR24	Chl	MII	Proline	Lipid peroxidation	Soluble protein	SOD	APX
	µM	SPAD	%	µmol g^{-1}	nmol g^{-1}	mg g^{-1}	U min^{-1} mg^{-1} protein	µmol min^{-1} mg^{-1} protein
Irrigated	0	19.97^{ns}	11.41 a*	0.010 b	6.649 ab	0.245 c	2.305 b	0.301 b
	5	20.42	8.80 b	0.004 b	6.219 ab	0.236 c	2.904 b	0.354 b
	10	20.70	8.78 b	0.010 b	5.122 b	0.483 a	6.960 a	0.623 ab
Drought	0	20.48	12.04 a	0.018 ab	8.117 a	0.380 b	2.045 b	0.481 ab
	5	20.79	8.55 b	0.057 a	6.148 ab	0.311 bc	4.633 ab	0.824 a
	10	21.46	9.60 b	0.027 ab	6.524 ab	0.327 b	4.554 ab	0.938 a

*There is a difference between the means with different letters in the same column ($p < 0.05$). Chl = chlorophyll; MII = membrane injury index; SOD = superoxide dismutase; APX = ascorbate peroxidase.

conditions and in 10 µM GR24 treatment. Rutin synthesis was also at high levels in irrigated and 5 µM GR24-treated plants (Table 3).

The contents of gallic acid, 2,5 dihydroxy benzoic acid, 3,4 dihydroxy benzoic acid, and chlorogenic acid were also high in 10 µM GR24 treated and regularly irrigated plants. The synthesis of 2,5 dihydroxy benzoic acid was also significantly higher with 5 µM GR24 application under the same conditions. This treatment (irrigated regularly and treated with 5 µM GR24) also promoted the synthesis of caffeic acid. The application of 10 µM GR24 also increased the synthesis of 4-hydroxy benzoic acid, vanillic acid, and epicatechin in 1103 P American grapevine rootstock in arid conditions.

Data on TPC determined spectrophotometrically, and synthesis of phenolic compounds determined by HPLC can be summarized as follows: the synthesis of these compounds increased with the GR24 treatment with or without stress conditions.

Changes in indole acetic acid (IAA) and gibberellic acid (GA_3) levels were also examined to evaluate the relationship between strigolactones and other plant hormones (Table 4).

This study also examined the relationship between SL and auxin. Only in regularly irrigated plants, the GR24 treatment at a dose of 5 µM more than doubled the level of IAA compared to the control; however, auxin levels again significantly decreased with higher SL doses (Figure 2). Under drought conditions, the impact of SL on auxin was more serious and decreased gradually from the control to the GR24 treatments at 5 and 10 µM doses.

This study also examined th effect of the GR24 treatment on GA_3 (Table 4). Here, the change in GA_3 synthesis in response to SL application was similar to the auxin synthesis. The lowest GA_3 level was detected in plants grown under arid conditions and treated with high GR24 doses (Figure 3).

The effects of the GR24 treatment on the intake of macro and micro elements were also evaluated. Irrigated plants, but not GR24-treated plants, showed the lowest

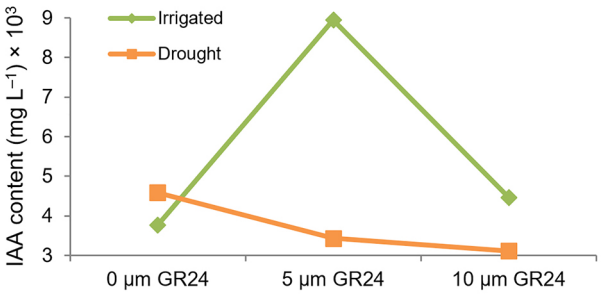

Figure 2 – Changes of the indole acetic acid (IAA) content in GR24 applications.

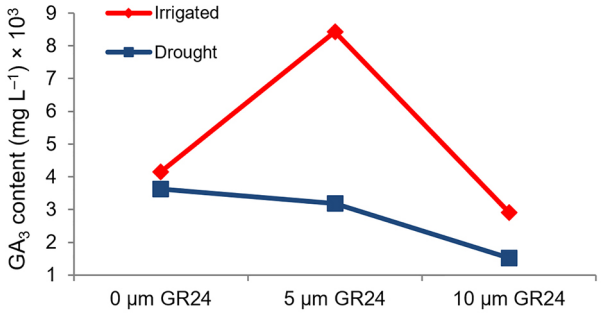

Figure 3 – Changes of the gibberellic acid (GA3) content in GR24 applications.

Table 3 – Effects of GR24 applications on phenolic compounds.

	GR24 (µM)	Total phenolic compound	Gallic acid	4-hydroxy benzoic acid	2,5 dihydroxy benzoic acid	3,4 dihydroxy benzoic acid	Klorogenic acid	Vanillic acid	Caffeic acid	Epicatechin	p-coumaric acid	Ferulic acid	Rutin
Irrigated	0	11.68 bc*	17.08 b	108.04 c	2772.74 d	33.82 d	126.46 cd	254.26 d	12624.93 c	656.69 d	133.13 d	1363.19 c	60.17 b
	5	13.11 b	18.17 b	98.77 d	3952.32 a	40.96 bc	136.04 c	237.06 e	16207.96 a	850.49 c	138.53 cd	1442.09 c	82.17 a
	10	12.63 b	21.87 a	116.15 b	3546.20 ab	47.93 a	188.85 a	272.92 c	14447.88 b	858.87 c	142.18 c	2090.85 a	64.80 b
Drought	0	9.53 d	17.60 b	119.14 b	3039.22 cd	39.35 c	116.84 d	286.08 c	1177.35 e	578.25 e	155.47 b	1684.08 b	50.08 c
	5	16.85 a	17.71 b	119.05 b	3218.74 bc	43.24 b	158.93 b	305.54 b	11212.10 d	1267.61 b	204.62 a	2167.61 a	83.94 a
	10	10.01 cd	17.62 b	127.96 a	3371.02 bc	43.81 b	169.15 b	424.24 a	1124.27 e	2562.00 a	157.55 b	2093.76 a	45.54 c

*There is a difference between the means with different letters in the same column ($p < 0.05$).

Table 4 – Effects of GR24 applications on hormones and mineral elements.

	GR24	IAA	GA_3	N	P	K	Ca	Mg	Cu	B	Zn	Na
	µM	------- mg L^{-1} -------		%	---------- mg kg^{-1} ----------							
Irrigated	0	3769.47 c*	4153.05 b	0.62 d	972.21 f	10651.20 a	8315.32 c	2336.51 c	1.66 ab	28.69 a	17.83 b	479.36 a
	5	8939.24 a	8434.49 a	0.67 b-d	999.06 e	10721.16 a	9812.73 b	3455.99 b	1.50 b	28.74 a	19.25 a	445.88 a
	10	4457.98 b	2906.59 d	0.84 a	1132.95 b	11055.06 a	10141.74 b	3362.72 b	1.88 ab	28.29 a	18.79 a	439.84 a
Drought	0	4582.07 b	3619.08 c	0.63 cd	1040.27 d	8618.85 c	8262.30 c	2778.69 c	1.91 ab	25.59 b	16.77 c	326.63 b
	5	3429.46 cd	3181.46 cd	0.70 b	1086.69 c	9764.23 b	9750.20 b	3627.03 b	2.21 a	28.33 a	19.61 a	354.98 b
	10	3110.66 d	1528.01 e	0.69 bc	1508.33 a	9389.71 b	13311.27 a	4718.14 a	2.25 a	28.66 a	17.60 bc	334.97 b

*There is a difference between the means with different letters in the same column ($p < 0.05$). IAA = indole acetic acid; GA_3 = gibberellic acid.

level of the N content determined as percent (%), while a slight increase was observed in 5 µM GR24 application (Table 4). Under irrigated conditions, the highest N level was reached with 10 µM GR24 application. Droughts negatively affected the N content. However, while the N content was 0.63 % in the control plants without GR24 application under drought conditions, the N content was higher in the 5 µM GR24 application in the same conditions (Table 4).

The P content increased 1.55 fold compared to the lowest value (irrigated but not SL-treated), Ca increased 1.6 fold compared to the lowest value (drought conditions but not SL-treated), and Mg increased two-fold compared to the lowest value (irrigated but not SL treated). Remarkably, the highest K values were reached in irrigated plants, regardless of whether SL was applied. A similar change was observed for Na, a microelement. A high Na content was detected in all irrigated plants. Therefore, the effect of irrigation is prominent in both elements. Except for the irrigated and 5 µM GR24-treated plants, all other plants had high Cu levels. The B levels were high in all plants except those not treated with GR24 and those treated under drought. Therefore, despite the negative effect of drought conditions, the GR24 application promoted B uptake even under drought conditions. The Zn uptake was high in all plants except for GR24 untreated and irrigated plants and GR24 untreated and high dose GR24 treated plants under drought conditions. The GR24 treatment with irrigation increased Zn uptake in comparison to the control. As for Na, like K, irrigated plants had the highest content.

Discussion

This study evaluated the effects of GR24 applications on 1103P rootstock in arid conditions, and the changes in physical and biochemical traits were analyzed. Although it varies according to the GR24 dose, most analyses proved that GR24 could be successfully used in arid conditions. Shoot length and shoot weight in plants were not different; however, numerically longer and heavier shoots were obtained in plants treated with GR24. The exogenous SL application increased grain weight in wheat (Wang et al., 2022). The highest value of membrane damage, an important stress indicator, was seen in the control plants. Similarly, it was reported that exogenous SL application decreased the MDA amount in wheat leaves (Wang et al., 2022).

In the present study, the positive effects of GR24 application on the antioxidant enzyme activities were seen. Similarly, it was also stated that the exogenous SL application changes the hormone balance in the leaves, increases the CO_2 fixation rate, and increases the SOD and peroxidase (POD) activity in wheat (Wang et al., 2022).

The changes in IAA and GA_3 contents in rootstocks treated with or without GR24 were evaluated. The effects of GR24 on IAA were more pronounced in arid conditions and decreased gradually from the control plants to the GR24 treatments. Plant development is a process characterized by events, such as the formation of initial models of organs in the apical shoot, branching of roots and shoots, emergence and growth of leaves, and the establishment of the connection of newly formed organs with the conduction system (Zhang et al., 2020). In this process, events such as cell division, cell growth, and cell and tissue differentiation are specifically associated to auxin behaviors in the plant (Adamowski and Friml, 2015). In a process called auxin canalization, narrow auxin transport pathways are formed from cells and tissues with higher auxin concentrations to parts where auxin is consumed intensively (Sauer et al., 2006; Bennett et al., 2016). A self-reinforcing system exists to direct the channel. In this system, auxin feeds back these auxin transporters by promoting the expression of *PIN* genes and recruiting *PIN*s to the plasma membrane facing the auxin pool (Balla et al., 2011; Bennett et al., 2016). While the mechanisms by which auxin controls the polarization of *PIN*s are still conceptually unclear (Zhang et al., 2020), several plant hormones, such as SLs, are involved in this process (Crawford et al., 2010). Most events affected by SLs require auxin canalization (Shinohara et al., 2013). Therefore, the relationship between auxin and SL is attempted to be elucidated. The accumulation of *PIN1* proteins responsible for auxin transport at the plasma membrane is inhibited by SL (Bennett et al., 2006; Crawford et al., 2010; Shinohara et al., 2013). Therefore, auxin transport is reduced, and shoot development is inhibited (Bennett et al., 2006; Ongaro and Leyser, 2008; Crawford et al., 2010). SLs inhibit shoots indirectly by reducing auxin canalization rather than directly inhibiting them (Waldie et al., 2014).

A study was conducted to determine the effect of SL treatments on IAA synthesis under drought stress (Cetin et al., 2022). It revealed that auxin synthesis was higher in grapevine plants treated with GR24 (10 µM) and highlighted that high SL doses positively affected the auxin content, regardless of the presence or absence of stress in the environment. This difference in auxin response to strigolactone between the studies may arise from the genotypical mechanisms. In addition, the presence of a stress condition in the environment and the type of stress factor may also alter the interaction between the two hormones. While auxin decreased continuously in the presence of GR24 under drought conditions, the increases and decreases in auxin against GR24 under irrigated conditions could explain that this situation is caused by the stress factor. In the period when the present study was first planned, the lack of many studies to be considered as a reference in dose determination in SL applications hindered the planning in terms of creating variations of doses and determining the upper dose. However, after the interpretation of the data obtained, examining the effects of higher dosages is also required.

Gibberellins are hormones with active roles in plants, primarily stimulating seed germination, providing stem elongation, accelerating fruit growth, and regulating flower formation. In this study, the effect of

GR24 application in GA_3 synthesis was similar to auxin synthesis. Some studies have investigated the interactions between SL and GA_3. GA_3 and SLs showed synergistic effects to regulate seed germination in *Arabidopsis thaliana* (L.) Heynh (Toh et al., 2012). However, the GR24 treatment did not increase gibberellin-3-oxidase 2 transcription, a key enzyme in gibberellin biosynthesis. This result showed that the effect of SL is mediated by the regulation of other steps in gibberellin biosynthesis (Zhang et al., 2013). Although various cross-talk has been reported between SLs and other hormones in physiological assays, the relationship between gibberellin and SLs must be fully understood. A study on peas also suggested that SL and gibberellin signaling are independent (Germain et al., 2013). However, studies on rice indicated that GA_3 suppresses SL biosynthesis by inhibiting the induction of SL biosynthesis genes; moreover, gibberellin was identified as a new SL regulatory molecule (Ito et al., 2017). Previous studies have demonstrated that gibberellin and SL signaling show remarkable similarities at the molecular and mechanistic levels (Santner and Estelle, 2009; Santner et al., 2009; Waters et al., 2017). The similarity of gibberellin and SL signaling pathways suggests a common evolutionary origin and a potential molecular interaction between signaling components (Lantzouni et al., 2017). Gibberelin is perceived by the *gid1* family of receptor proteins. The binding of GA leads to the activation of *gid1* and coupling of an SCF^{Skp1} (SKP1-CULLIN1-F-box protein) type E3 ubiquitin ligase complex with *SLY1* (SLEEPY1) or *SNE* (SNEEZY) in *Arabidopsis* and *gid2* F-box proteins in rice (Ariizumi et al., 2011; Davière and Achard, 2016). In *Arabidopsis*, the D14 receptor is known to function together with the F-box protein *MAX2* (MORE AXILLARY BRANCHES2) to promote the degradation of S*MXL7* (SUPPRESSOR of *max2 1-LIKE7*) and its paralogs SMXL6 and SMXL8 (Soundappan et al., 2015; Wang et al., 2015). Some studies suggest that DELLA proteins are ubiquitous through the SL pathway and that SL promotes gibberellin signaling (Nakamura et al., 2013). It was also reported that SL does not affect the degradation of DELLA proteins in *Arabidopsis* shoots (Bennett et al., 2016). Therefore, it is possible to say that the mechanism between SL and gibberellin is still not fully understood.

The structure and characteristics of strigolactones remain the subject of various new studies. To date, studies in which exogen SL and analogs (GR24 etc) are applied or which aim to determine amount of endogenous SL have been conducted on eucalyptus, willow, Japanese maple, apple and orange trees, sunflower and wheat (Agusti et al., 2011; Ward et al., 2013; Zheng et al., 2018, Özel and Sağlam 2022; Wang et al., 2022). A study conducted to determine the effects of SLs on plant defense mechanism reported that the mutant tomato line Slccd8 with SL deficiency was more sensitive to the fungal pathogens *Botrytis cinerea* Pers. and *Alternaria alternata* (Fr.) Keissl. compared to the types without SL deficiency (Torres-Vera et al., 2014). However, few studies have been conducted to determine the impact of SL on drought stress. One of these studies was carried out on wheat, which attempted to determine the effect of SL and salicylic acid on drought in two winter wheat genotypes sensitive and resistant to drought. In the study, SL and salicylic acid were applied from the leaf, and it was determined that plants that received the application were more tolerant to drought (Sedaghat et al., 2017). The authors also verified that SL had the role of a positive stimulator in its reaction against stress. These results show that SLs could be used as an alternative to transgenic plants against drought.

A study examined the effects of ABA and GR24 in *A. thaliana* under drought conditions (Van Ha et al., 2014). The authors kept the explants in a GM nutrient medium for 14 d under a temperature level of 22 °C for 8-16 h light and dark periods. For drought stress, 100 µM ABA, 5 mL 5 µM GR24 was applied to the plants from 7^{th} day until 13^{th} day two times a day at 10h00 and 16h00 in a spray form. The results showed that SL application to mutants was insufficient with respect to SL increased tolerance. SLs play an important role in responses against different plant stresses, such as saltiness, drought, and low temperatures (Van Ha et al., 2014; Xiong et al., 2002; Kapulnik and Koltai, 2014); nevertheless, more studies are needed for a fully understanding of these mechanisms.

The present study examined the effects of SL applications on drought stress in grapevine rootstock were examined. The data obtained show that SLs have high potential in this respect. This study provided important information for a better understanding of SL hormonal interaction at various regulation levels; however, investigations at both cellular and molecular levels are required to fill critical information gaps.

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