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Research Article

The effects of strigolactones on some biochemical traits in calcified media on grapevine

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Abstract

Plants are stressed in different ways when they are in environments unsuitable for them. Among these stresses, abiotic factors are common. Calcified soil is also a stress factor. In this type of soil, there are problems with the nutrient intake of the plant, nutrient deficiencies, or toxicity. In a stress environment, plants try to survive by reacting differently. One of these reactions is that plants increase the synthesis of phenolic compounds. These compounds perform various physiological functions in adapting to environmental problems. It is known that the reactions of plants to stress are associated with endogenous hormones. One of these hormones is strigolactone (SL), which is produced in plant roots and introduced as a new generation of hormones. In this study, the effects of SL applications in grapevine grown in calcified environments were examined with regard to the content of phenolic compounds and mineral element intake. 'Hasandede' grape variety grafted on 1103 P American rootstock were grown in environments containing control, 10% and 25% calcium oxide (CaO) and applied with SL (control, 1, 3 and 5 µM) in different doses. Total phenolic compounds were found statistically much higher especially in the majority of plants applied with 3 µM and 5 µM SL is considered to be the effect of SLs in stress prevention. It was concluded that mineral compounds intakes generally responded positively to SL applications. These results are considered to prove the effects of SLs on plant nutrition physiology and stress tolerance.

Keywords: calcium oxide; grapevine; mineral compounds; phenolic compounds; stress; strigolactone

Introduction

Plants are exposed to various stress environments called biotic and abiotic throughout their lives. This affects the performance and survival of plants (Hirayama and Shinoza, 2010). There may occur a resistance to mild damage in plants. However, stresses generally weaken the plant and it leads to a chronic disease or irreversible damage on the end of the plant capacity (Ozcan *et al*., 2004). Some environmental factors such as strong light, ultraviolet, high and low temperature, drought and salinity, which are abiotic stresses, cause low yields of up to 50% (Mahajan and Tuteja, 2005). High pH and calcification are important abiotic stress factors that cause imbalances in nutrient intake in plants. In these environments, there occur problems with plant nutrition, and signs of stress are observed as a result of limiting the intake of certain mineral elements or, on

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the contrary, the resolution of some elements becoming increasingly toxic. One of the most common symptoms is the change in plant phenolic compound content. That is because phenolic compounds perform various physiological functions in adapting plants to adverse environmental conditions.

Plant hormones are great importance in their response to various stresses (Perez Torres *et al*., 2008; Evelin *et al*., 2009). For instance, in the event of an injury, it is known that plants detect signals from these injured cells, activate the salicylic acid signalling pathway and interact with other plant hormones and react differently. Abscisic acid (ABA), another hormone, was found to play an important role as a defence mechanism against drought stress (Christmann *et al*., 2006). It is also known that in the stress environment, the plant closes its stoma to reduce the rate of photosynthesis and reduces CO_2 intake in parallel with the increase in ABA level (Wilkinson *et al*., 2001; Raghavendra *et al*., 2010). In cases where cytokinin levels were low, it was determined that there were more severe symptoms of nutrient deficiencies (Salama and Wareing, 1979), and that there were significant reactions to the roots to increase phosphate intake in the environments where cytokinin was added (Martin *et al*., 2000). It is also known that auxin hormone increases in case of stress caused by low phosphate content (Lopez Bucio *et al*., 2002; Perez Torres *et al*., 2008). Therefore, it is understood that hormones play a major role in classifying plants as "sensitive" or "tolerant" in responses to different stresses. A hormone contained in these substances and introduced as a new generation of hormones is strigolactone (SL), a substance derived from carotenoids and produced at plant roots. SLs consist of an ABC tricyclic lactone attached to unsaturated α, β-furanon part (D ring) through an enol ether bridge as a molecular structure. It was first detected in the root secretions of cotton plants as strigol and strigyl acetate in 1966 (Cook *et al*., 1966). Studies have revealed that SLs were found in relatively high amounts in plant roots, while other plant tissues such as hypocholine, trunk and leaf were found in very low concentrations or even absent in some (Yoneyama *et al*., 2007). Before its emergence as a plant hormone, SLs were thought to be a substance that promotes germination only in some root parasite plants, such as witch grass (Striga) and monster grass (*Orobanche, Phelipanche* species) (Cook *et al*., 1966), and no studies have been carried out on this hormone for many years. It was later understood that SLs also acted as a signal in the regulation of arbuscular mycorrhizal fungi-plant symbiosis, which plays an important role in the development of plants (Akiyama *et al*., 2005; Matusova *et al*., 2005; Foo and Reid, 2012; Yoshida *et al*., 2012). In optimum growth conditions, it was determined that lateral root formation was suppressed in the plant due to the effect of SL (Kapulnik *et al*., 2011), and SL levels increased in order to comply with these conditions in the face of adverse environmental factors (Umehara *et al*., 2008; Kohlen *et al*., 2011). Studies on SL increased after these studies, thus new functions were revealed, and it was also determined that this substance was associated with other hormones (Lopez Raez *et al*., 2010). SLs were found to act together with auxins in secondary development, rooting and tuber formation (Roumeliotis *et al*., 2012; Foo, 2013; Liu *et al*., 2013; Shinohara *et al*., 2013; Dierck *et al*., 2016).

There are a limited number of studies in the international literature on the relationship between exogenous SL applications and stress. In one of them, Einav *et al*. (2010) indicated that GR24 application, an SL form for mutant tomato plants lacking in SL, also positively affected the reaction of the roots to indole acetic acid. In an *in vitro* study on *Arabidopsis* mutants, the effects of SL on drought stress were examined physiologically and molecularly. Explants were developed in a temperature of 22 °C for 14 days and in bright/dark environment for 16/8 hours. 100 µM ABA was applied for drought stress. 5 µM SL was applied to the plants in the form of sprays. As a result of the research, it was determined that SL application to mutants inadequate in SL increases tolerance in those who are sensitive to the drought (Ha *et al*., 2014).

Torres Vera *et al*. (2014) examined the effects of SL on plant defense mechanism in their study and determined that Slccd8 mutant tomato line with SL deficiencies, was more susceptible to *Botrytis* mold (*Botrytis cinerea*) and *Alternaria* rot (*Alternaria alternata*) than its wild types. In a study on germination of wheat seeds under salty conditions, Kausar and Shahbaz (2017) examined the effect of pre-application of SL

(GR24). GR24 were applied to two wheat varieties in different concentrations (0.001; 0.01 and 0.1 mg/L) for 16 hours. It was reported that the application of 150 mM NaCl did not have a significant effect on fresh and dry weight of the seedlings and root length, and the exact CO_2 assimilation rate increased due to GR24 application. In a study on the exogenous applications to determine the effect of SLs on drought stress, the objective was to determine the effect of SL and salicylic acid on drought in two winter wheat genotypes that were sensitive and resistant to drought. In the study, SL (GR24) and salicylic acid were applied to leaves as foliar and it was determined that plants with SL and salicylic acid had a higher tolerance (Sedaghat *et al*., 2017). These studies confirm that SL plays a role as a positive regulator in the response to stress. As a matter of fact, based on these results, the researchers emphasized that SL can be used as an alternative to drought-tolerant transgenic plants in the future.

In a master's thesis study conducted on the effects of SLs in the tolerance of sea daffodil plant to salinity stress, the effects of SL applications on antioxidant enzyme activities were examined. GR24 (0, 10, 20 µM) was applied to sea daffodil seedlings and then salt application (0, 150, 300 mM NaCl) was applied. 10 days after stress application, seedling morphology and super oxide dismutase, catalase and ascorbate peroxidase activity and isozymes of these enzymes and changes in tiobarbituric acid and hydrogen peroxide contents were examined. As a result of the research, it was determined that GR24 pre-application has the potential to stimulate the antioxidant defense system of plants and to ensure resistance to salty conditions (Gök Ozel, 2018).

Onay (2019) applied salt stress (0; 200 and 300 mM NaCl) to the seeds in wheat varieties sensitive/tolerant to salt stress and planted them in different ways: dry planting, irrigated planting, GR24 pre-applied cultivation (20µM GR24). As a result of the research, it was determined that the pre-application of 20 µM GR24 to seeds has an encouraging role in tolerance to salt in wheat plant. Issah (2021), who examined the effect of SLs on the accumulation of phenolic substances in callus cultures of the caper (*Capparis spinosa* L.), indicated that caper calli taken into medium with containing 2 mg/L NAA, 1 mg/L BAP and 0.1 µM GR24 were the best composition in the accumulation of chlorogenic acid, rutin and quercetin from phenolic compounds, and emphasized that GR24 in lower concentrations was actually effective, but that much more work was required in this field.

Ensuring stress resistance is even more important, especially in plants with high economic value. One of these plants is the grapevine. In addition to its commercial importance, the vine and its product, grapes, are an extremely important fruit in terms of medicine and pharmacy with its important phytochemical content. Grapes contain more than 1600 important compounds, mainly resveratrol and anthocyanin (Pezzuto, 2008) and thus play an important role in many areas ranging from the reduction of low-density lipoprotein (LDL) to reducing cardiovascular diseases, regulating blood sugar and preventing cancer formation, and even preventing diseases such as Alzheimer (German and Walzem, 2000; Middleton *et al*., 2000). There is limited number of studies on SL applications in grapevine. In one of these few studies, Ferrero *et al*. (2018) examined the effect of SL and ABA application on the accumulation of anthocyanin in berries. As a result of the research, the co-application of GR24 and ABA delayed the accumulation of anthocyanin and also reduced gene expressions responsible for the biosynthesis of anthocyanin. GR24 increased expression of ABA hydrolysis genes, while affecting the expressions of ABA transport genes downward. In another reference reached in the comprehensive literature research, the effects of SL applications on drought stress on grapevine were examined (Min *et al*., 2019). GR24 (1 µM, 3 µM and 5 µM) and 7% polyethylene glycol (PEG-6000) were applied to two-year-old plants in 3 different concentrations. GR24 application was performed for 7 days with an interval of 24 hours. Leaf samples were taken in the 2nd, 12th, 24th, 72nd, 96th and 120th hours after PEG application. It was determined that electrolyte leakage was less in plants applied with GR24, also stoma expansion, ROT values, relative water content, chlorophyll content and photosynthesis rates changed to tolerate drought. GR24 application also reduced indole acetic acid levels and zeatin ribosite, while increasing abscisic acid levels in both roots and leaves. As a result of the research, it was concluded that GR24 application can improve the negative effects of drought.

A recent study of grapevine also examined the ability of SLs to prevent the branching of shoot (Ren *et al*., 2020). They studied the CRISPR/Cas9 system for regulating VvCCD7 and VvCCD8 gene expressions in 41B rootstock. Researchers transformed 41B embryogenic cells, and sequence analyses determined that transformation was successful in both VvCCD7 and VvCCD8 genes. After regeneration, six transgenic 41B plants carrying CCD8-sgRNA were identified. Four of them were identified as CCD8 mutant. It was reported that the branching of shoots in these mutants increased compared to wild types. No studies have been found in which determine the effect of SL applications on stress tolerance on calcareous soils in the grapevine. This study examined the effects of SL applications on phenolic compounds and macro and micro element quantities in the leaves of the 'Hasandede' variety grafted on 1103 P, which is grown in environments containing different levels of CaO, and tried to clarify some aspects of this mechanism.

Materials and Methods

Material

'Hasandede' variety graft to 1103 P American grapevine rootstock is used as plant material. 1103 P rootstock is a strong one. It can withstand up to 17% active lime. It has high level of drought resistance. It is widely used as a rootstock in viticulture. 'Hasandede' is a white, wine grape variety. It has thin skinned, round and medium-size berries. 'Hasandede' has been cultivated in Yozgat, Kırıkkale, Ankara, Çankırı and Çorum provinces in Turkey for many years.

Methods

The scion belonging to 'Hasandede' grape variety were grafted onto 1103 P rootstock with omega machines in the second week of March at Yozgat Bozok University Faculty of Agriculture, Grafted Vine Sapling Production Unit. Grafted cuttings were planted in 2 L pots containing 1:1 pearlite/turf and taken to the production greenhouse. In the second week of June, in grafted seedlings were given applications (Figure 1). In the whole process, the plants were irrigated with the Hoagland solution (Hoagland and Arnon, 1950).

Figure 1. Overview of plants in the greenhouse

CaO was applied to the root area of the plant at one time with a rate of 10% and 25%. The control application was made with the same amount of pure water. SL applications were made after CaO application. GR24, an SL analogue, was used in this study. GR24 (Chiralix, Netherlands) was dissolved in 3% acetone and applied to the root area of the plant on 5 times in 1, 3 and 5 µM concentrations on the 2nd, 4th, 6th, 8th and 10th days after CaO application. Since the 10th day of the end of SL application, the last application time was evaluated as the zeroth hour, and samples were taken from the plants in the 2nd, 12th, 24th, 48th and 96th hours.

Experimental procedures

Extraction of phenolic compounds

Phenolic compounds in grapevine were examined according to the following methods. Ethyl alcohol (10 mL 96%) was added on the leaf sample taken as 1 g for phenolic substance extraction and it was homogenized for 2 minutes. It was then soaked in a water bath at 45 °C, centrifuged at 4000 rpm for 5 minutes and evaporated in a rotary evaporator at 45 °C by removing the liquid part containing phenolic compounds. The remaining part was dissolved in 1 mL methanol and used in phenolic compounds analysis (Kiselev *et al*., 2007).

Determination of total phenolic compounds

The total amounts of phenolic compounds were made according to Singleton and Rossi (1965) using Folin Ciocalteu colorimetric method. Spectrophotometer were performed at a wavelength of 765 nm, using the curve prepared from the standard gallic acid solution and the amount of total phenolic compounds were determined as mg g^{-1} (gallic acid equivalent (GAE)) (Figure 2).

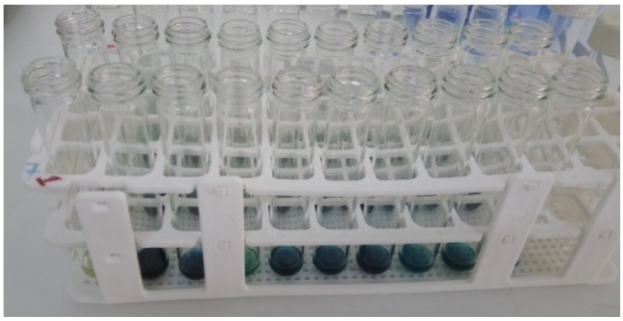

Figure 2. View of samples in total phenolic compound analyses

Determination of total flavonoids

The total flavonoids content was made according to Dai *et al*. (1995) using Neu solution. The total amount of flavonoids was determined as mg g^{-1} as rutin equivalent (RE) using the curve prepared from the rutin standard (Figure 3).

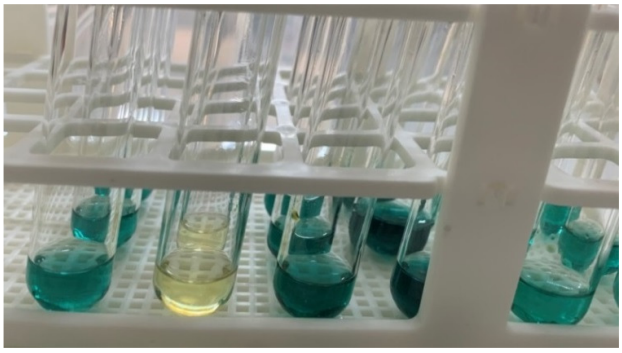

Figure 3. View of samples in total flavonoids analyses

Determination of total flavanols

Total flavanols were performed using the 4-dimethyl amino cinnamaldehyde (DMACA) method according to Arnous *et al*. (2001). Results were given in mg g^{-1} as catechin equivalent (CE) using the curve prepared from the catechin standard (Figure 4).

Figure 4. View of samples in total flavanol analyses

Determination of mineral compounds

The amount of nitrogen (N) was determined as total nitrogen using Kjeldahl device. N analyses were performed in three repetitions, and the results were given as percentage (%) (Figure 5).

Figure 5. Determination of the total amount of nitrogen in the Kjeldahl device

Phosphorus, potassium, calcium, magnesium, iron, zinc, boron and manganese contents were also determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Perkin Elmer Optima-8000). Leaf samples were washed using 0.1% detergent solution and distilled water, and after drying on blotting paper. Plant samples were burned in an ash oven at 600 °C (Figure 6).

Figure 6. Burning samples in ash oven for ICP-OES analyses

After the device cooled down, samples were acidification and readings were made in the ICP-OES device. The conditions of the device 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: PTFE 1 mm i.d.; Sample vials: Polypropylene; Source equilibrium delay: 15 sec; Plasma viewing: Axial; Processing mode: Peak area; Gases: Argon and Nitrogen; Shear Gas: Air. The wavelengths (nm) in mineral substances were as follows: phosphorus: 214.9; potassium: 766.4; calcium: 315.8; magnesium: 279.0; iron: 238.2; zinc: 213.8; boron: 249.6; manganese: 257.6.

Statistical analysis

The research was carried out with 3 repetitions and 10 plants (10 pots) each time. 3 (dose of CaO)*4 (dose of SL)* 5 (sapling time)*30= 1800 plants were used in total. The data obtained at the end of the experiment was evaluated in the SPSS 20 statistical program according to the randomized blocks trial pattern, and the differences between the averages were determined according to the Duncan multiple comparison test ($p \leq 0.05$).

Results and Discussion

One of the most important defense mechanisms in biotic and abiotic stress conditions in plants is the high synthesis of phenolic compounds. Plant phenolics perform a wide range of physiological functions for the survival of plants and adaptation to environmental problems (Andersen, 2003; Lattanzio *et al*., 2009). The accumulation of phenolic compounds in plant tissues is an indication that the plant is stressed or struggling to cope with stress. This accumulation is due to the increased activity of phenylalanine ammonia lyase, chalcone synthase and other enzymes. Here, phosphoenolpyruvate-carboxylase activity also increases, which activates the transition from primary metabolism to secondary metabolism, i.e., processes that support defense, and the repair mechanism. In this study, the contents of phenolic compounds in the leaves were determined as total phenolic compounds, total flavonoids and total flavanols, and the data obtained were presented in Table 1. Significant differences were found in terms of phenolic compounds examined between CaO doses, SL applications and times ($p \leq 0.05$).

In terms of total phenolic compound, total flavonoids and total flavanols, it is seen that these contents are statistically low levels in all plants that do not use CaO (Table 1). It is seen that all phenolic compounds are synthesized at higher levels in the highest dose (25% CaO). It was determined that the majority of plants applied 3 µM and 5 µM SL showed statistically high values with samples taken in only 12 hours of 1 µM SL application for total phenolic compounds in this CaO dose. In terms of total flavonoids, statistically high contents were detected in all plants in the group that did not apply SL and in the plants sampled in the 96th hour and 3 µM SL. In terms of total flavanols values, although the sample varies according to the reception times, it is noted that the values are statistically high only in the 3 µM and 5 µM groups of SL.

Although the varieties of the *Vitis vinifera* species are resistant to lime, they have to be grafted on vine rootstocks with lower sensitivity due to the necessity of using American rootstock in modern viticulture. Therefore, the effects of SL applications on mineral substance intake of grapevine in lime soils were also examined in this study. Thus, it was tried to determine the extent to which the mineral substance intake of plants is affected in these environments. In the literature reviews, no studies have been found on the effect of SL applications on mineral substance intake.

Table 1. Effects of SL application on phenolic compounds

CaO (%)	SL (µM)	Time (hour)	TPC (mg g^{-1} GAE)	Total flavonoids (mg g^{-1} RE)	Total flavanols (mg g^{-1} CE)
0	0	2	3.11 m-o*	1.19 h-s	0.72 f-j
		12	2.68 no	1.03 n-s	0.63 g-l
		24	3.86 k-n	1.24 f-s	0.84 d-g
		48	4.73 j-m	0.90 rs	0.72 f-j
		96	3.98 k-n	1.50 d-n	0.48 kl
	1	2	5.05 i-l	1.15 i-s	0.68 g-k
		12	3.09 m-o	1.35 e-r	0.65 g-l
		24	4.17 k-n	1.38 e-p	0.72 f-j
		48	2.60 no	1.39 e-p	0.68 g-k
		96	2.09 o	0.88 s	0.67 g-k
	3	2	3.08m-o	1.00 o-s	0.63 g-l
		12	3.47 l-o	1.19 h-s	0.57 i-l
		24	3.29 m-o	1.17 h-s	0.73 f-i
		48	2.97 no	1.24 f-s	0.57 i-l
		96	4.18 k-n	1.12 j-s	0.58 i-l
	5	2	3.85k-n	0.82 s	0.55 i-l
		12	3.43 l-o	1.08 l-s	0.60 h-l
		24	2.81 no	1.06 m-s	0.70 f-k
		48	3.98 k-n	1.11 k-s	0.53 i-l
		96	3.99 k-n	1.75 de	0.62 g-l
10	0	2	3.97 k-n	1.70 d-f	0.57 i-l
		12	3.56 l-o	0.94 p-s	0.55 i-l
		24	3.85 k-n	1.16 i-s	0.64 g-l
		48	4.13 k-n	1.39 e-p	0.63 g-l
		96	4.04 k-n	1.53 d-l	0.63 g-l
	1	2	2.97 no	1.56 d-k	0.58 i-l
		12	4.07 k-n	1.64 d-h	0.82 e-h
		24	3.77 k-o	1.39 e-p	0.70 f-j
		48	3.15 m-o	1.19 h-s	0.50 j-l
		96	3.87 k-n	1.39 e-p	0.72 f-j
	3	2	7.33 e-g	1.67 d-g	0.63 g-l
		12	7.63 d-g	1.61 d-I	0.83 d-g
		24	6.68 f-I	1.67 d-g	0.64 g-l
		48	7.65 d-g	2.40 ab	0.63 g-l
		96	6.78 f-h	1.67 d-g	0.63 g-l
	5	2	7.51 d-g	1.23 g-s	0.62 g-l
		12	6.17 g-j	2.25 bc	0.83 d-g
		24	6.26 g-j	1.54 d-l	0.65 g-l
		48	6.16 g-j	2.28 a-c	0.44 l
		96	7.50 d-g	1.46 d-o	0.60 h-l
25	0	2	6.44 g-I	2.57 ab	0.54 i-l
		12	6.47 g-I	2.39 ab	0.59 i-l
		24	6.43 g-I	2.44 ab	0.59 i-l
		48	5.43 h-k	2.71 a	0.73 f-i
		96	7.54 d-g	2.35 ab	0.75 e-i
	1	2	8.28 c-g	1.50 d-n	0.59 i-l
		12	10.03 ab	1.59 d-j	0.60 h-l
		24	8.89 b-e	2.18 bc	0.60 h-l
		48	8.68 b-e	1.23 g-s	0.62 g-l
		96	7.55 d-g	1.66 d-g	0.51 j-l
	3	2	9.16 a-d	1.56 d-k	0.94 de
		12	9.46 a-d	1.53 d-m	0.90 d-f
		24	9.10 b-e	1.41 e-o	1.46 a
		48	10.71 a	1.72 de	1.41 a
		96	8.50 b-f	2.35 ab	1.22 b
	5	2	9.54 a-c	1.91 cd	1.03 cd
		12	9.88 a-c	1.51 d-m	1.50 a
		24	9.77 a-c	1.70 d-f	1.54 a
		48	7.52 d-g	1.23 g-s	1.14 bc
		96	8.83 b-e	1.07 l-s	1.41 a

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

As is known, plants need at least 17 plant nutrients in order to continue their growth and development. Since hydrogen, carbon and oxygen are mostly taken from air and water, they are thought to be non-mineral plant nutrients (White, 2006; Gardiner and Miller, 2008; Fageria, 2009). The other 14 essential elements are taken directly from the soil. These elements, which are elemental for plants, are basically divided into macro and micro elements. Macro elements are those more needed by the plant than other elements. In this research, the effects of SL applications on the intake of macro elements (Table 2) and micro elements (Table 3) in calcareous environments were also examined. It was determined that there were significant differences between CaO doses, SL applications and times in all macro elements examined ($p \leq 0.05$). Nitrogen (N) is one of the main nutrients that control plant growth. The main source of N in the soil is organic matter. The organic content of the soil is broken down over time and as a result, the N contained in its composition is made available to plants. N deficiency is observed in most of the soils on earth. However, N plays a role in a wide range of physiological and biochemical cases; proteins, amino acids, nucleic acids, enzymes, chlorophyll (Gardiner and Miller, 2008; McCauley *et al*., 2009). It is of great importance that the plant is adequate and balanced in terms of N in its growth and development, as well as its tolerance to the stress environment (Fageria, 2009). The plant, which cannot be fed enough N, initially has a light green appearance, rather than dark and vibrant green, and in advanced cases browning and death occurs with chlorosis in the leaves.

It is noteworthy that when an examination was carried out in terms of the value determined as total N in the Kjeldahl device and expressed as percentage (%), low levels of N were found in all plants regardless of whether SL application was carried out at the highest dose of CaO and regardless of the times. Although there is no statistical difference, the highest amount of N (3.54%) in numbers was obtained from plants grown in an environment containing 10% CaO, applied 1 µM SL and taken in the 12th hour.

Phosphorus (P) is mainly formed by the breakdown of rocks and minerals, plays an active role in many events, from cell division to flowering, fruit formation and ripening. Another important function is that the plant increases its resistance to biotic and abiotic different stresses. It is also known that the plant is effective in establishing the water scheme (McCauley *et al*., 2009). It is noted that P content is high only in plants grown in a CaO-free environment. However, when the environment in question is examined, it is seen that only the P contents are high in the 48th and 96th hour samples, and statistically high P levels are detected in groups containing 1 µM SL (96th hour) and 3 µM SL (48th and 96th hours).

Potassium (K) is also playing a major role in the resistance of plants to diseases, allows the development of the root system of the plant, is important in establishing water balance in the plant and is also effective in the formation of chlorophyll (Brady, 1990; McCauley *et al*., 2009). In this research, it can be seen that the plants in the CaO-free group have much higher content statistically, as in K-level P. Here 0% CaO, 0 µM SL and 48th hour; 0% CaO, 3 µM SL, 24th, 48th and 96th hours and 0% CaO, 5 µM SL and 24th hour sampled plants were identified as leaves with the highest K value.

It is seen that the highest content in calcium (Ca) is obtained from 25% CaO application and these plants are plants that are applied 5 µM SL and taken in the 96th hour, the last sample intake period.

The reason why this value is high is naturally due to the fact that CaO is given in addition to the environment during application. Calcium is actually the third most used nutrient element of plants. The plant is an element in the position of the building block in the firmness of the cell wall (Plaster, 1992; McCauley *et al*., 2009). Ca, in the intake of nutrients, also plays a role in the precipitation of toxic substances found in plants and soil. It is known that they are more resistant to diseases if the plant is adequately fed in terms of this nutrient element. They also play an important role in protein formation and the transport of carbohydrates (Plaster, 1992). However, it is known to have antagonistic effect by converting P, K and Fe and some other elements into forms that plants cannot benefit from if they are found in large quantities in arid climatic conditions.

Table 2. Effects of SL application on macro elements

CaO (%)	SL (µM)	Time (hour)	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
0	0	2	2.95 a-e*	9432.21 c-f	8502.80 f	6185.76 m-o	3798.88 f-h
		12	2.80 a-i	9357.00 d-f	9695.00 b-e	6587.33 kl	4015.40 e-g
		24	2.84 a-h	9402.78 d-f	9527.78 c-e	6702.47 j-l	4657.41 cd
		48	2.80 a-i	10722.63 a	10059.31 a-d	6798.54 jk	4240.88 de
		96	2.73 b-l	10591.92 a	9888.48 b-d	6127.94 m-o	5111.28 b
	1	2	2.68 b-m	9692.12 b-e	7153.52 g-m	6968.96 i-k	4424.50 c-e
		12	2.60 b-o	7637.43 s-u	7166.92 g-l	6592.81 kl	3732.04 f-i
		24	2.86 a-h	8103.73 op	6316.54 l-t	6722.64 jk	4237.56 de
		48	2.80 a-i	8107.52 op	8654.10 f	6995.22 ij	4542.35 cd
		96	2.62 b-n	10504.59 a	6757.76 h-p	7569.88 h	5080.75 b
	3	2	3.12 a-c	9875.00 b	9232.01 d-f	8395.83 cd	4803.03 bc
		12	2.93 a-f	7452.09 s-y	7593.06 gh	8325.69 c-e	3413.89 h-m
		24	2.95 a-e	9108.56 f-h	9964.34 a-d	8165.44 d-f	5101.10 b
		48	2.63 b-m	10643.37 a	10761.49 a	9109.91 b	5545.05 a
		96	2.66 b-m	10556.67 a	10201.00 a-c	8520.00 cd	4424.00 c-e
	5	2	2.95 a-e	9060.81 f-i	9068.13 ef	7040.54 ij	4565.32 cd
		12	2.59 b-p	8830.13 g-k	7701.92 g	7059.29 ij	3794.87 f-h
		24	3.18 ab	10065.45 b	10395.94 ab	7777.49 gh	3675.39 f-j
		48	2.86 a-h	7383.34 s-y	6255.88 n-u	5333.33 s-u	3799.02 f-h
		96	2.69 b-m	7278.39 t-y	7523.41 gh	5703.69 p-s	3591.63 g-k
10	0	2	3.02 a-d	8295.78 m-o	7420.19 g-i	6673.12 j-l	2847.42 o-u
		12	2.96 a-e	7411.31 s-y	7013.79 g-o	5406.25 s-u	2124.08 vy
		24	2.86 a-h	9284.24 ef	6513.54 j-s	6916.40 i-k	3578.03 h-k
		48	2.91 a-f	8455.56 k-o	6420.83 k-s	6231.25 mn	3283.33 j-o
		96	3.12 a-c	8034.60 o-r	6925.78 g-p	5621.65 r-t	3679.69 f-j
	1	2	2.74 b-k	9391.67 d-f	5468.63 tu	6344.12 lm	3128.43 l-r
		12	3.54 a	8725.62 h-l	5702.13 r-u	5897.16 n-r	2711.88 r-u
		24	2.79 a-j	8458.34 k-o	6261.36 n-u	6037.34 m-p	3031.39 l-s
		48	2.87 a-g	7502.27 s-y	6740.94 h-p	4646.29 v	2424.82 uv
		96	2.63 b-m	8234.79 no	7361.11 g-j	6114.42 m-o	2878.31 n-t
	3	2	2.30 d-p	8579.38 j-n	6119.60 p-u	5568.09 r-u	2937.37 n-s
		12	2.52 b-p	7734.86 p-s	7230.20 g-k	5585.17 r-u	2194.10 vy
		24	2.46 b-p	7470.72 s-y	6108.92 r-u	5338.90 s-u	2803.32 p-u
		48	2.53 b-p	7726.19 p-s	6171.80 o-u	5219.52 u	3049.09 l-s
		96	2.40 b-p	7164.89 vy	6773.59 h-p	5359.91 s-u	2892.69 n-s
	5	2	2.41 b-p	8176.46 no	5847.90 r-u	5840.05 o-r	2949.03 n-s
		12	2.23 d-p	8060.98 op	7019.79 g-o	5921.35 n-r	2806.25 p-u
		24	2.35 c-p	8694.53 i-m	7159.76 g-m	6104.29 m-o	3463.02 h-l
		48	2.32 d-p	9698.94 b-e	7160.13 g-m	5286.44 tu	2770.10 r-u
		96	2.18 e-p	8324.08 l-o	5815.33 r-u	5243.11 tu	3002.26 m-s
25	0	2	2.03 i-p	7175.46 vy	5690.70 r-u	7229.27 i	1776.38 y
		12	2.09 g-p	7075.30 y	5632.53 s-u	8364.46 cd	2022.31 vy
		24	1.93 l-p	7397.68 s-y	6277.66 m-t	7723.27 gh	2041.89 vy
		48	1.83 n-p	7528.38 s-v	5669.68 s-u	8246.17 de	2010.02 vy
		96	2.01 i-p	7155.41 vy	5759.57 r-u	9169.93 b	2093.47 vy
	1	2	2.08 g-p	7364.33 s-y	5495.00 tu	6087.00 m-o	2238.33 v
		12	1.97 k-p	7544.36 s-v	6577.62 i-r	5271.17 tu	2448.59 t-v
		24	1.81 op	7229.96 u-y	9488.40 c-e	9358.65 b	2245.78 v
		48	1.81 op	8888.89 g-j	8978.17 ef	9001.98 b	2417.66 uv
		96	1.91 m-p	8180.02 no	7532.77 gh	7996.84 e-g	2669.90 s-u
	3	2	2.14 f-p	9345.00 ef	6881.91 g-p	6024.29 m-p	3101.43 l-s
		12	2.22 d-p	9766.19 b-d	5697.54 r-u	8645.73 c	2127.72 vy
		24	1.79 p	9210.81 f-g	5376.06 u	9108.05 b	2742.59 r-u
		48	1.99 j-p	9679.17 b-e	7506.94 gh	8674.31 c	3002.78 m-s
		96	2.32 d-p	7626.44 s-u	5641.96 s-u	7824.57 f-h	3308.91 i-n
	5	2	1.97 k-p	8673.37 i-m	6293.48 l-t	6837.50 jk	4251.09 de
		12	1.91 m-p	7324.45 s-y	6272.98 m-t	6990.81 ij	4834.56 bc
		24	2.07 h-p	9814.75 bc	6121.15 p-u	6723.08 jk	3275.64 j-o
		48	1.93 l-p	7665.33 r-t	5798.17 r-u	5609.63 r-u	4097.59 ef
		96	2.02 i-p	8104.51 op	7081.97 g-n	9706.97 a	3218.58 k-p

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

Table 3. Effects of SL application on micro elements

CaO (%)	SL (µM)	Time (hour)	Fe (ppm)	Zn (ppm)	B (ppm)	Mn (ppm)
0	0	2	511.32 cd	23.65 k	29.12 ij	42.74 ij
		12	524.93 bc	22.03 m-q	29.41 i	43.28 hi
		24	460.33 e	22.17 m-q	29.29 i	40.98 lm
		48	548.91 ab	27.40 j	29.24 i	44.13 gh
		96	418.82 f-h	23.05 k-m	28.46 k	42.24 jk
	1	2	413.34 f-j	21.12 q-x	26.64 m	40.12 m
		12	340.42 no	27.10 j	24.75 o	43.61 hi
		24	295.44 rs	23.41 kl	29.45 i	25.48 u
		48	498.93 d	21.03 q-y	24.30 op	33.48 q
		96	533.00 bc	20.47 s-α	24.88 o	28.79 s
	3	2	396.21 g-l	27.50 j	35.67 f	51.03 cd
		12	414.44 f-i	29.27 i	31.67 g	53.13 b
		24	391.55 h-l	31.17 h	30.10 h	51.27 c
		48	345.77 mn	21.32 q-w	28.89 i-k	43.01 ij
		96	560.80 a	20.53 r-z	28.50 jk	37.73 no
	5	2	311.33 p-s	23.53 kl	27.57 l	40.36 m
		12	323.93 n-r	21.65 n-r	25.76 n	43.43 hi
		24	381.24 kl	21.72 n-q	21.69 r	42.68 i-k
		48	388.75 i-l	20.47 s-α	18.19 t	37.93 no
		96	369.52 lm	20.46 t-β	17.98 t	32.62 r
10	0	2	439.43 ef	21.33 q-w	16.23 v	41.31 l
		12	305.92 p-s	21.69 n-q	15.05 w	38.26 n
		24	370.48 k-m	22.15 m-q	22.40 q	41.83 kl
		48	317.49 o-r	20.33 v-β	21.34 r	37.32 o
		96	437.06 ef	19.58 y-β	18.51 t	24.61 vw
	1	2	330.20 n-p	20.52 r-z	14.37 xy	35.37 p
		12	314.38 o-s	20.02 x-β	14.03 y	44.51 g
		24	372.08 k-m	21.55 o-t	16.28 v	41.82 kl
		48	390.48 h-l	19.30 αβ	16.24 v	27.56 t
		96	396.22 g-l	21.13 q-x	21.96 qr	35.34 p
	3	2	369.33 lm	47.37 d	37.13 e	51.47 c
		12	384.98 j-l	41.05 f	35.38 f	50.24 de
		24	422.54 fg	53.58 b	41.21 c	46.13 f
		48	399.17 g-k	50.60 c	43.15 b	45.36 f
		96	308.53 p-s	51.27 c	45.33 a	43.38 hi
	5	2	374.64 kl	57.42 a	40.52 d	45.71 f
		12	424.63 fg	45.43 e	43.37 b	51.11 c
		24	551.71 ab	40.56 f	45.37 a	50.17 e
		48	392.96 h-l	38.22 g	41.58 c	54.55 a
		96	545.96 ab	47.29 d	45.26 a	51.23 c
25	0	2	194.54 xy	19.35 αβ	13.06 z	10.73 ε
		12	221.15 wx	20.25 w-β	12.09 α	10.79 ε
		24	207.52 w-y	19.51 z-β	12.07 α	10.89 δ
		48	203.37 w-y	19.58 y-β	12.14 α	10.16 ε
		96	195.63 xy	19.98 y-β	12.45 zα	11.75 γδ
	1	2	197.80 xy	23.58 kl	17.32 u	16.40 αβ
		12	213.75 w-y	21.61 o-s	19.28 s	16.53 αβ
		24	228.17 vw	20.37 u-β	19.56 s	17.42 z
		48	184.80 y	19.28 β	12.45 zα	12.56 γ
		96	212.41 w-y	20.03 x-β	14.74 wx	13.28 β
	3	2	267.42 tu	21.42 p-v	19.34 s	18.71 z
		12	257.56 u	19.23 β	16.43 v	17.33 α
		24	346.35 mn	21.49 o-u	16.16 v	17.24 α
		48	304.17 p-s	22.77 k-n	19.42 s	17.12 α
		96	317.17 o-r	22.06 m-q	22.57 q	19.49 y
	5	2	248.63 uv	22.52 l-p	17.31 u	20.49 x
		12	258.99 u	23.28 kl	30.54 h	18.64 z
		24	286.58 st	22.15 m-q	23.78 p	23.52 w
		48	261.23 tu	23.14 k-m	24.87 o	25.17 v
		96	248.23 uv	22.59 k-o	27.56 l	22.75 x

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

Magnesium (Mg), the building block due to the fact that chlorophyll is the central atom, is also one of the main nutrients that ensures the continuation of vitality in the plant (Foth, 1984). It also acts in CO_2 assimilation, protein synthesis (McCauley *et al*., 2009) and intake of other nutrients. This mineral substance (Plaster, 1992; Gardiner and Miller, 2008), which is also responsible for the activity of a large number of enzymes, and its contents against SL application in calcareous environments on the grapevine were also examined in this research. Plants that did not contain CaO but were sampled in the 48th hour by applying 3 µM SL stood out as plants with the highest magnesium (Mg) content.

Other nutrients examined in the study are some elements in the micro element class (Table 3). It is possible to infer that the effect of SL in micro element has been observed more clearly. Fe levels were statistically highest in 96th hour samples (560.80 ppm) that do not contain CaO but have a 3 µM SL applied, and also in the 24th (551.71 ppm) and 96th hour (545.96 ppm) samples with 5 µM SL containing 10% CaO. Although iron is actually an element that is not included in the structure of chlorophyll, it is also known that there is a close relationship between the adequate or inadequate iron in plant nutrition and the scope of chlorophyll.

Although iron is a nutrient element that is found in the soils in total, chlorosis is very common as a result of its lack of plants. As a result of iron deficiency or inhibited intake, there is also a decrease in photosynthetic activity in the leaves in parallel with the lower chlorophyll content (Bavaresco and Poni, 2003). The absolute deficiency of iron in the soil is rarely seen as the cause of iron chlorosis in plants. This type of Fe chlorosis is especially common in sandy soils and peat soils. High pH of the soil, calcium carbonate, excessive Ca^{+2} and HCO_3-ion concentration in soil solution and interaction of iron with other elements inhibit the absorption of iron, transport and metabolism in the plant (Shalau, 2010).

Zn is effective in nitrogen metabolism, starch formation and especially in the elongation of plant sizes and chokes in relation to growth hormones (Gardiner and Miller, 2008; McCauley *et al*., 2009). As examined in this research, some limy and alkali are those where Zn deficiencies are seen in large quantities (Gardiner and Miller, 2008). However, in case of lack of Zn, problems arise in the functions mentioned above, as well as the contents of chlorophyll fall in parallel with the deficiencies and the formation of new leaves is negatively affected (Plaster, 1992).

Boron (B) is the only element among micro elements that is ametallic. B promotes the formation of cell walls in the plant. B is an effective element in activating certain dehydrogenase enzymes, carbohydrate biosynthesis and protein synthesis. It is also of great importance in sugar transport. Mn plays an important role in photosynthesis, nitrogen metabolism, Fe, Ca and Mg absorption. It is also an effective mineral in the formation of chlorophyll, fruit ripening (Plaster, 1992; Gardiner and Miller, 2008).

In Table 3, it is seen that all three micro elements (Zn, B and Mn) remain at low levels in terms of content in all plants with the highest dose of CaO. The 10% CaO medium was identified as the environment where higher contents were exhibited in terms of these elements. Statistically, the highest Zn content is 10% CaO and 5 µM SL is applied and sampled plants (57.42 ppm) in 2 hours. Plants with the highest B content contain 10% CaO; 3 µM SL (96^{th} hour) applied and 5 µM SL (24 and 96 th hours) applied in the same way as Fe contents with sampled plants in the same CaO dose and SL dose at 96th hour. In the analysis conducted with ICP-OES, the highest Mn content (54.55 ppm) was determined in the plants sampled in the 48th hour by applying 5 µM SL containing 10% CaO.

Conclusions

It is known that plants, which are an indispensable part of life, are subjected to different stresses in increasing diversity and intensity today. While this stress can sometimes be tolerated by the plant, it sometimes leads to loss of yield and quality, and in some cases it causes the end of plant vitality. Today, serious studies are also being carried out in the field of genetic engineering to develop varieties resistant to these stress factors in order to minimize product losses. However, it is impractical due to the fact that the methods used are expensive

and require a great knowledge. Therefore, it is extremely important to find natural, easy-to-use, practical and harmless alternatives to human health that will increase the stress tolerance of plants without changing their genetic structure. In fact, it is also known that some compounds in the bodies of plants are effective in gaining this tolerance. In recent years, studies have been carried out on strigolactones contained in these compounds and their new functions are coming to the fore every day. Therefore, studies examining the effect of this compound on different plants and different stress factors should be continued in a multifaceted way. This research, based on the effect of strigolactones on many other stress factors, examined the changes that SL applications show on plant nutrient intake and phenolic compounds in the calcareous environment in the grapevine. The results show that strigolactones have the potential to be used to prevent or mitigate damage from lime on the grapevine. However, in environments without stress, effects to support plant development have been detected.

Authors' Contributions

Emine Sema CETIN contributed to this work in the experimental design and setup, lab processing of samples, data analysis, manuscript writing and discussion. Birol KOÇ contributed to lab processing of samples, data interpretation, manuscript writing and discussion. Authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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