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ORIGINAL PAPER

EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGUS (AMF) AND WHEY APPLICATIONS ON THE GRAPEVINE (*VITIS VINIFERA* L.) CUTTINGS EXPOSED TO SALT STRESS

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Abstract

This study was carried out to determine some morphological and physiological reactions to the application of organic bio-stimulants, arbuscular mycorrhizal fungus (AMF) and whey (W), which were used against the negative results of salt stress in the cuttings of the Ercis grapevine cultivar. The cuttings were rooted in pots filled with perlite with no drainage. Once the cuttings were rooted, the buds were formed and the nodes extended, the salt application was initiated. The budded cuttings were irrigated with 1% Hoagland Nutrient Solution added with three different NaCl concentrations (0, 50 and 100 mmol). Moreover, AMF, W, and AMF+W were applied and the response of the budded cuttings against salt stress was monitored. As well as making analyses of macro- and microelements (P, K, Ca, Na, Mg, Fe, Mn, and Zn) in the shoots and roots, some parameters, such as shoot diameter, shoot height, root width, root length, number of leaves, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and leaf water content (LWC) were investigated, and the salt amount in the growth media was determined. At the end of the study, it was shown that AMF, W, and their combination (AMF + W), which had been applied against the physiological and morphological changes induced by salt stress and its adverse effects, had a positive effect on the majority of the parameters compared to the control group. Moreover, it was demonstrated that the use of AMF and W in the context of sustainable viticulture would be beneficial in terms of promoting the rooting and development of cuttings and protecting the plants against soil salinity that creates abiotic stress.

Keywords: arbuscular mycorrhizal fungus, grapevine cuttings, salt stress, sustainable viticulture, whey.

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INTRODUCTION

The loss caused by abiotic stress in the arable areas also significantly affects the yield and quality of the crops (Golldack et al. 2011, Kamiloglu et al. 2014). Salinity stress is one of the most important and common abiotic stress factors in arid and semiarid region that causes significant physiological and metabolic changes in various plants, negatively affecting the growth and development of crops, and decreasing the quality and quantity of yields (Esfandiari et al. 2015, Kurtar et al. 2016, Alp, Kabay 2017).

The physiological changes promoted by salt-induced osmotic stress and the roles of these changes in salt resistance have been investigated in grapevines, and it was determined that salt applications generally decreased the transpiration rate and stoma conductance while increasing leaf temperature (Sivritepe 1995). In a study investigating salt problems in grapevines, it was found that salinity levels of 0.6-2 milimhos, EC (1 milimhos cm^{-1} EC = 640 g kg^{-1}) were harmless; salinity at 3 milimhos could be tolerated with good drainage; but 3 to 6 milimhos could cause loss of fruit size, quality, and quantity with loss of blight and vitality on the leaves; above 6 milimhos could result in death of grapevines (Halsey et al. 1963).

Mycorrhiza (arbuscular mycorrhizal fungi – AMF is the largest group) is a mutualistic symbiotic life between plant roots, and some AMF enhance the root development of a plant as well as extend the area of the host plant roots through external hyphae. This increase contributes significantly to the transport of minerals and water to the plant. AMF can affect the uptake of several mineral nutrients, especially phosphorus. AMF have been also found to increase plant resistance against environmental and cultural stress factors, such as drought, salinity, pH, soil structure, heavy metals or toxicity (Hayman, Mosse 1972, Bola 1991). In agricultural ecosystems, investigation of AMF interactions and revealing suitable combinations are considered to be very important in terms of improving plant development and survival (Sensoy et al. 2007, Demir et al. 2015, Kabay et al. 2017). Whey is one of the organic materials that could be important in plant development although there might be some disadvantages of its use, such as causing crop damage due to the rapid consumption of soil oxygen and the cost of transporting the material (Prazeres et al. 2012). Whey, which is an important dairy by-product, can be used in soil fertilization. Whey does not only provide nutrients to the soil but also encourages aggregation in the growing media (Demir, Ozrenk 2009, Sensoy et al. 2013, Demir et al. 2015).

Both AMF and whey can be useful in agricultural systems (Sensoy et al. 2013, Demir et al. 2015). The present study aimed to determine the tolerance level for salinity and salinity induced morphological changes of the Ercis grapevine cultivar, which is an important cultivar in Lake Van Basin. Moreover, the effects of the AMF and whey applications were examined to reveal the removal of salt stress damage or reduction of the damage threshold.

MATERIALS AND METHODS

The cuttings of the Ercis grapevine cultivar were planted in a greenhouse, in drainage-free pots with 3 liters of perlite, where they were budded and rooted. The experiment was established according to the randomized factorial experimental design with twelve applications (3 salt doses in the control, AMF, whey, and AMF + whey treatments) with three replications, each having 10 cuttings. The isolate of *Glomus intraradices* was used as AMF. Unrooted cuttings were planted by treating them with AMF. The intensity of *Glomus intraradices* was set to be 25 spores g^{-1}. A low dose of whey (50 mL kg^{-1}) was applied twice: immediately after planting and 1 month later. The composition of the whey used was N (%) – 0.140; P (g kg^{-1}) – 36.38; Fe (g kg^{-1}) – 0.80; Zn (g kg^{-1}) – 4.33; Mn (g kg^{-1}) – 0.82; Mg (g kg^{-1}) – 42.15; K (g kg^{-1}) – 956.57; Ca (g kg^{-1}) – 268.75; Na (g kg^{-1}) – 228.77. The cuttings with three buds from one-year-old branches were irrigated with 1% Hoagland Nutrient Solution added with three different NaCl concentrations (0, 50 and 100 mmol) divided into three parts and applied at four-day-interval (Sivritepe 1995, Sivritepe, Eris 1998*a*, 1998*b*). The salt applications were carried out 2 months after AMF, and whey applications were performed when the cuttings were rooted and one of buds sprouted about 10 cm with at least a full leaf. The response to the salt stress was monitored 3 weeks after the salt application was completed.

The analysis of macro- and microelements (K, Ca, Na, Mg, Fe, Mn, and Zn) was made with an atomic absorption spectrophotometer, and phosphorous was determined with vanadium-molybdate phosphoric yellow method in a spectrophotometer (Kacar, Inal 2008). Some parameters, such as shoot diameter, shoot height, root width, root length, number of leaves, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and leaf water content (LWC), were investigated and the salt amount in the growth media was determined (Kusvuran 2010, Kabay et al. 2017):

$$LWC = (FW\text{-}DW) / (TW\text{-}DW) \times 100\ FW,$$

where: FW – fresh weight, DW – dry weight, TW – turgid weight.

A modified 0-5 scale (0 – no effect at all, 1 – growth retardation, 2 – onset of wilting in the lower leaves, 3 – curling and wilting in the upper leaves, 4 – severe wilting and chlorosis in the leaves, the onset of drying of leaf margins 5 – wilting of the plants and drying in the lower leaves) was employed in the study (Kusvuran 2010).

Statistical analysis: Data were analyzed using the SPSS Statistics 20 program, with the multivariate analysis of variance conducted for all data, and the differences between the averages were determined according to the Duncan's multiple comparison test (Eckstein 2012).

RESULTS AND DISCUSSION

The present study aimed to determine the effect of arbuscular mycorrhizal fungus (AMF) and whey (W) applications on the grapevine (*Vitis vinifera* L. cv. Ercis) cuttings exposed to salt stress. Another aim was to examine the physiological changes induced by the salt-induced osmotic stress according to either single (AMF and W) or combined application (AMF+W).

Salt stress caused some detrimental effects on some studied parameters (Tables 1, 2). The EC values of the growth media, i.e. perlite, were significantly increased by the salt application as expected (Table 2). Grapevine development was negatively affected by the salt applications based on the 0-5 scale results. Moreover, the root diameter was also significantly decreased by the salt application (Tables 1, 2).

In the AMF, W, and AMF+W applications, there were improvements in the shoot and root growths in the salt compared to the control treatment (Tables 1, 2). The highest average shoot height (12.54 cm) was obtained from the combined AMF+W application followed by the W application (10.77 cm). The height of plants in the sole AMF application (9.14 cm) was also higher than in the control application (7.15 cm). Regarding the shoot width, the highest average values were obtained from the AMF and the AMF+W applications, 4.03 cm and 3.78 cm, respectively, while the lowest value was observed in the control application (2.92 cm). As for the root length, the AMF application at 100 mmol salt dose and the W application at 50 mmol salt dose stood out in their groups. The highest average leaf number (6.72) was obtained from the combined AMF+W application followed by the sole W application (6.50). The value of this parameter after the sole AMF application (5.70) was also higher than in the control application (4.12). With respect to the leaf fresh and dry weights, the highest average values were obtained from the W and the AMF+W applications, at 4.12 g - 0.75 g and 3.94 g - 0.71 g, respectively, while the lowest value was obtained from the control application (1.63 g - 0.35 g) – Table 1. As for the shoot fresh and dry weight, besides the root fresh weight, the average values in the AMF, W, and AMF+W treatment were significantly higher than the value in the control (Table 2).

Arbuscular mycorrhizal fungi (AMF) could enhance the grapevine's growth deteriorated by salt stress. Nogales et al. (2021) stated that grapevines are highly dependent on AMF for normal growth and development. Bybordi (2012) studied the tolerance of two grape cultivars against various NaCl salinity levels (0, 50, 100, 150, 200 and 250 mmol), and reported that there were significant effects of high salinity levels on several traits, including dry weight of the stem and root; concentrations of elements such as nitrogen, phosphorus, potassium, chloride; plant height; leaf area; and relative leaf water content due to increasing the negative osmotic pressure in the root growth zone as well as the toxic effect of a high salt concentra-

Table 1

Effects of arbuscular mycorrhizal fungus (AMF) and whey (W) applications on shoot height, shoot width, root length, root diameter, leaf number, leaf area, leaf fresh weight, and leaf dry weight of the cuttings of the Ercis cultivar exposed to different salinity levels

Applications	Salt application (mmol)	Shoot height (cm)	Shoot width (mm)	Root length (cm)	Root diameter (cm)	Leaf number	Leaf area (cm^2)	Leaf fresh weight (g)	Leaf dry weight (g)
Control	0	8.67±0.46 ns	3.14±0.34 ns	22.67±3.71 ns	13.33±1.20 a*	4.29±0.36 ns	52.95±11.02 ns	1.97±0.44 ns	0.40±0.10 ns
	50	7.75±0.55	3.53±0.28	22.00 ±2.00	12.33±1.20 ab	5.15±0.15	42.04±3.98	1.92±0.11	0.40±0.03
	100	5.02±2.52	2.10±1.06	13.67±4.26	6.67±2.33 b	2.93±1.05	36.43±8.32	0.99±0.77	0.25±0.14
	mean	7.15±0.93 C**	2.92±0.40 B*	19.44±2.26 NS	10.78±1.33 NS	4.12±0.46 C**	43.81±4.80 NS	1.63±0.30 B*	0.35±0.06 B*
AMF	0	9.25±1.46 ns	4.07±0.19 ab**	19.67±3.7 ab*	10.33±1.86 ns	6.86±0.56 ns	43.85±5.98 ns	3.90±1.28 ns	0.73±0.24 ns
	50	9.58±1.42	3.44±0.22 b	14.67±0.33 b	9.00±1.00	5.50±0.76	43.86±3.09	2.08±0.57	0.37±0.11
	100	8.60±0.98	4.57±0.15 a	25.00±2.65 a	10.67±0.67	4.75±0.19	46.55±7.94	3.14±0.55	0.60±0.12
	mean	9.14±0.67 BC	4.03±0.19 A	19.78±1.99	10.00±0.69	5.70±0.42 B	44.75±3.04	3.04±0.51 AB	0.57±0.10 AB
W	0	11.91±0.33 ns	3.45±0.10 ns	19.33±2.67 ab*	11.67±0.88 ns	7.46±0.54 a*	49.17±10.98 ns	5.21±2.06 ns	0.99±0.41 ns
	50	10.98±0.65	3.61±0.10	21.00±1.00 a	14.00±2.31	6.52±0.29 ab	54.93±12.68	4.14±1.35	0.71±0.23
	100	9.41±1.05	3.62±0.18	13.67±1.45 b	11.00±2.08	5.51±0.33 b	58.42±6.88	3.01±1.12	0.55±0.20
	mean	10.77±0.52 AB	3.56±0.07 AB	18.00±1.44	12.22±1.04	6.50±0.35 AB	54.17±5.40	4.12±0.85 A	0.75±0.16 A
AMF+W	0	13.13.33±2.02 ns	3.93±0.45 ns	15.00±2.52 ns	12.67±0.67 ns	6.58±0.61 ns	39.69±1.74 ns	3.83±0.97 ns	0.69±0.20 ns
	50	10.10.65±0.85	3.49±0.23	15.33±0.33	11.33±1.45	7.42±0.56	66.26±18.49	3.66±0.24	0.68±0.06
	100	13.13.64±1.85	3.93±0.45	16.00±2.89	11.33±1.76	6.17±0.35	44.32±8.58	4.31±0.59	0.76±0.11
	mean	12 12.54±0.95 A	3.78±0.21 A	15.44±1.12	11.78±0.72	6.72±0.32 A	50.09±7.19	3.94±0.35 A	0.71±0.07 A
Mean (salt applications)	0	10.9.79 NS	3.65 NS	19.17 NS	12.00 NS	6.30 A**	46.41 NS	3.73 NS	0.70 NS
	50	9.79.74	3.52	18.25	11.67	6.15 A	51.77	2.95	0.54
	100	9.19.17	3.56	17.08	9.92	4.84B	46.43	2.86	0.54

Different capital letters indicate significant differences among the means based on the Duncan's multiple comparison test at ** ($p \leq 0.01$) or * ($p \leq 0.05$), and small letters are for individual applications.

Table 2

Effects of arbuscular mycorrhizal fungus (AMF) and whey (W) applications on shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, leaf water content (LWC), perlite EC and 0-5 scale of the cuttings of the Ercis cultivar exposed to different salinity levels

Applications	Salt application (mmol)	Shoot Fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	LWC (%)	Perlite EC (µS)	0-5 scale
Control	0	2.06±0.20 ns	0.34±0.02 ns	2.87±1.25 ns	0.46±0.12 ns	86.14±11.77 ns	2.90±0.08 c**	0.00±0.00 c**
	50	2.31±0.33	0.40±0.07	2.26±0.25	0.37±0.03	78.80±2.32	6.28±0.16 b	3.00±0.58 b
	100	1.26±0.52	0.30±0.03	1.58±1.09	0.33±0.16	71.56±7.52	11.16±1.64 a	4.33±0.33 a
	mean	1.88±0.25 B**	0.34±0.03 B**	2.24±0.52 B**	0.39±0.06 NS	78.83±4.60 NS	6.78±1.29 NS	2.44±0.67 A**
AMF	0	3.30±0.61 ns	0.55±0.09 ns	7.30±2.19 ns	0.66±0.25 ns	87.10±3.31 ns	2.74±0.24 c**	0.00±0.00 c**
	50	2.49±0.41	0.39±0.05	5.27±1.68	0.49±0.07	81.40±3.53	5.18±0.47 b	2.67±0.67 b
	100	3.07±0.42	0.51±0.05	6.77±0.78	0.68±0.25	84.27±5.82	8.71±0.88 a	4.33±0.33 a
	mean	2.95±0.27 A	0.48±0.04 A	6.45±0.88 A	0.61±0.11	84.26±2.33	5.54±0.92	2.33±0.67 A
W	0	4.07±0.96 ns	0.57±0.13 ns	9.13±1.61 ns	0.77±0.17 ns	85.95±3.47 ns	2.58±0.16 b**	0.00±0.00 b**
	50	3.56±0.68	0.50±0.09	8.31±2.63	0.82±0.24	80.54±1.26	8.58±1.52 a	2.33±0.33 a
	100	2.99±0.40	0.50±0.07	2.75±0.58	0.55±0.11	74.74±3.64	10.18±1.56 a	2.67±0.33 a
	mean	3.54±0.39 A	0.53±0.05 A	6.73±1.35 A	0.71±0.10	80.41±2.20	7.11±1.32	1.67±0.44 B
AMF+W	0	3.79±0.45 ns	0.54±0.08 ns	4.81±0.51 ns	0.34±0.06 ns	85.13±3.78 ns	4.81±1.02 ns	0.00±0.00 b**
	50	3.71±0.22	0.54±0.04	7.38±1.27	0.48±0.11	84.57±2.49	7.23±2.79	2.33±0.33 a
	100	3.68±0.49	0.56±0.11	4.84±1.54	0.64±0.08	91.68±7.02	10.57±0.67	2.00±0.58 a
	mean	3.72±0.20 A	0.55±0.04 A	5.68±0.73 A	0.49±0.06	87.13±2.67	7.54±1.21	1.44±0.41 B
Mean (salt applications)	0	3.31 NS	0.50 NS	6.03 NS	0.56 NS	86.08NS	3.26 C**	0.00 C**
	50	3.02	0.46	5.81	0.54	81.33	6.82 B	2.58 B
	100	2.75	0.47	3.98	0.55	80.56	10.15 A	3.33 A

Different capital letters indicate significant differences among the means based on the Duncan's multiple comparison test at ** ($p \leq 0.01$) or * ($p \leq 0.05$), and small letters are for individual applications.

tion, which creates unfavorable conditions for the root growth and stem development. Cramer et al. (2007) reviewed that salinity, causing ion toxicity and lowering the water potential of plants, affected a higher percentage of transcripts involved in transcription, protein synthesis, and protein fate in grapevines. Cetin et al. (2019) studied injury degree, shoot length, shoot weight, average leaf number per shoot, leaf area, rooting ratio, root length, number of roots and membrane injury, leaf proportional water content, chlorophyll, proline, lipid peroxidation, hydrogen peroxide, total phenolic compound, soluble protein content and antioxidant enzyme activities of grapevine rootstocks in drought stress conditions, and demonstrated that AMF had a positive effect on plant development, biochemical traits, and antioxidant enzyme activities. Korkutal et al. (2020) applied two different mycorrhiza cocktails by different methods (control, planting mixture, root, root + planting mixture) on grafted rooted vines (*Vitis vinifera* L.), testing the sapling performance and growth traits, and concluded that the most beneficial application to young grapevine in both grafting combinations and with either of the different mycorrhizal preparations was the application to the soil mixture. Schreiner (2007) studied the effects of various arbuscular mycorrhizal fungi on the growth and nutrient uptake of Pinot noir (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus, and reported that Pinot noir grapevines were heavily dependent on AMF to achieve normal growth in the low P, JY soil due to enhanced P uptake and uptake of most other nutrients by mycorrhizal vines.

In our experiment, in the AMF, W, and AMF+W applications, there was some improvement in the uptake of some nutrients by the shoots and roots of the grapevine in the salt treatments compared to the control (Tables 3, 4). The highest average Fe content in the shoots (90.90 g kg^{-1}) was obtained from the combined AMF+W application followed by the sole W and AMF applications (75.93 g kg^{-1} and 72.02 g kg^{-1}, respectively). The control application had an approximately 35% lower Fe content compared to the AMF+W application. The highest average Fe content in the roots (580.22 g kg^{-1}) was obtained from the AMF application, followed by the W application (419.19 g kg^{-1}). On the other hand, the value obtained in the combined AMF + W application (328.91 g kg^{-1}) was insignificantly higher than the value in the control (297.49 g kg^{-1}). As regards the Mn content in the shoots, the W application at 50 mmol salt dose resulted in a significantly higher value (71.53 g kg^{-1}) than the value from the control application (34.71 g kg^{-1}). The highest average Mn content in the roots (51.01 g kg^{-1}) was obtained from the combined AMF+W application, while the lowest value (35.10 g kg^{-1}) was obtained from the control application. As for the Zn content in the shoots, the W application at 50 mmol salt dose caused a significantly higher value (34.15 g kg^{-1}) than the value of the control (26.41 g kg^{-1}). The highest average Zn content in the roots (34.76 g kg^{-1}) was obtained in response to the combined AMF+W application. It was also noticed that the salt applications decreased the root Zn content in the W and AMF+W applications. Concern-

Table 3

Effects of AMF and W applications on the shoot and root iron (Fe), manganese (Mn), zinc (Zn), magnesium (Mg) and phosphorous (P) content of the cuttings of the Ercis cultivar exposed to different salinity levels

Applications	Salt applications (mmol)	Shoot					Root				
		macro nutrients		micro nutrients			macro nutrients		micro nutrients		
		P (%)	Mg (%)	Fe (g kg^{-1})	Mn (g kg^{-1})	Zn (g kg^{-1})	P (%)	Mg (%)	Fe (g kg^{-1})	Mn (g kg^{-1})	Zn (g kg^{-1})
Control	0	0.49±0.06 ns	0.24±0.02 ns	64.06±7.87 ns	56.00±10.67 ns	24.72±0.12 ns	0.64±0.05 a*	0.44±0.14 ns	308.49±21.13 ns	44.41±3.24 a*	37.40±0.28 ns
	50	1,18±0.23	0.30±0.06	66.36±10.45	47.92±7.61	34.13±0.23	0.43±0.04 b	0.33±0.07	308.28±62.67	33.80±3.80 ab	33.41±1.68
	100	1,14±0.83	0.29±0.08	47.01±6.11	45.62±14.57	36.59±0.91	0.37±0.07 b	0.28±0.05	275.70±77.50	27.09±4.04 b	27.01±4.90
	mean	0.94±0.15 NS	0.28±0.03 NS	59.15±5.17 B**	49.85±5.87 NS	31.81±0.91 NS	0.48±0.05 C**	0.35±0.05 NS	297.49±29.91 B**	35.10±3.13 C**	32.60±2.13 AB*
AMF	0	1.06±0.10 ns	0.35±0.12 ns	85.29±18.97 ns	76.59±31.61 ns	27.65±0.26 ns	1.43±0.16 ns	0.29±0.01 ns	537.99±171.19 ns	49.88±8.04 ns	25.34±2.48 ns
	50	1.21±0.29	0.31±0.03	60.52±1.47	74.51±29.21	39.45±0.30	1.28±0.10	0.28±0.01	562.85±152.81	44.60±2.41	30.97±1.27
	100	0.64±0.18	0.32±0.04	70.26±8.43	49.37±15.08	31.73±11.18	1.16±0.37	0.33±0.17	639.82±118.00	47.20±2.95	25.75±2.04
	mean	0.97±0.13	0.33±0.04	72.02±7.01 AB	66.82±13.87	32.94±0.90	1.29±0.13 A	0.30±0.05	580.22±76.05 A	47.23±2.68 AB	27.35±1.35 B
W	0	0.60±0.12 ns	0.27±0.05 ns	70.09±11.61 ns	34.71±6.04 b*	26.41±0.29 b**	0.82±0.28 ns	0.41±0.17 ns	363.46±48.19 ns	42.24±1.77 ns	33.54±2.53 a**
	50	0.75±0.11	0.37±0.06	82.89±8.48	71.53±25.64 a	34.15±0.13 a	0.80±0.29	0.22±0.07	470.48±130.66	37.91±5.17	28.39±0.40 b
	100	0.79±0.16	0.28±0.01	74.81±8.25	41.63±9.33 ab	28.93±0.34 ab	0.54±0.33	0.18±0.03	423.63±35.10	34.77±2.38	24.42±1.96 c
	mean	0.72±0.07	0.31±0.03	75.93±5.14 AB	49.29±9.85	29.83±0.92	0.72±0.16 BC	0.27±0.06	419.19±44.26 AB	38.31±2.03 BC	28.78±1.62 B
AMF+W	0	0,93±0,15ns	0.33±0.03 ns	85.29±18.54 ns	70.38±15.92 ns	30.60±0.23 ns	1.04±0.34	0.35±0.03	300.97±8.16 ns	53.51±8.54 ns	42.32±6.57 a*
	50	0,92±0,10	0.32±0.05	103.14±3.55	67.87±6.20	34.99±0.17	1.07±0.33	0.27±0.05	330.63±62.83	50.80±9.63	31.91±3.91 b
	100	0,95±0,01	0.45±0.05	84.27±13.44	68.99±5.39	34.07±0.57	0.95±0.37	0.28±0.09	355.12±101.80	48.74±13.90	30.05±5.38 b
	mean	0,93±0,05	0.37±0.03	90.90±7.36 A	69.08±5.18	33.22±0.64	1.02±0.17 AB	0.30±0.03	328.91±35.49 B	51.01±5.51 A	34.76±3.30 A
Mean (salt applications)	0	0.77 NS	0.30 NS	76.18 NS	59.42 NS	27.35 B*	0.98 NS	0.37 NS	377.73 NS	47.51 NS	34.65 A**
	50	1.01	0.33	78.23	65.46	35.68 A	0.89	0.28	418.06	41.78	31.17 AB
	100	0.88	0.33	69.09	51.40	32.83 AB	0.75	0.26	423.57	39.45	26.81 B

Different capital letters indicate significant differences among the means based on the Duncan's multiple comparison test at ** ($p \leq 0.01$) or * ($p \leq 0.05$), and small letters are for individual applications.

Table 4

Effects of AMF and W applications on the shoot and root potassium (K), calcium (Ca), sodium (Na) contents and K/Na and Ca/Na ratios of the cuttings of the Ercis cultivar exposed to different salinity levels

Applications	Salt application (mmol)	Shoot					Root				
		K (%)	Ca (%)	Na (%)	K/Na	Ca/Na	K (%)	Ca (%)	Na (%)	K/Na	Ca/Na
Control	0	1.30±0.16 ns	0.37±0.07 ns	0.25±0.09 ns	6.44±1.94 ns	1.90±0.66 ns	2.24±0.22 a**	0.73±0.07 a**	3.78±1.11 ns	0.67±0.14 a*	0.22±0.04 a*
	50	1.59±0.63	0.40±0.11	0.50±0.09	3.45±1.63	0.88±0.31	1.38±0.14 b	0.43±0.07 b	4.80±1.47	0.34±0.09 ab	0.10±0.02 b
	100	1.33±0.37	0.37±0.09	0.52±0.11	2.66±0.60	0.74±0.12	1.16±0.14 b	0.36±0.01 b	6.04±1.54	0.22±0.06 b	0.07±0.02 b
	mean	1.41±0.22 AB*	0.38±0.05 C**	0.42±0.06 NS	4.18±0.95 B*	1.18±0.28 B*	1.59±0.19 A**	0.51±0.06 NS	4.87±0.77 NS	0.41±0.08 NS	0.13±0.03 B**
AMF	0	1.75±0.12 ns	0.59±0.09 ns	0.18±0.03 ns	10.43±2.25 ns	3.49±0.76 ns	1.73±0.14 ns	0.50±0.06 ns	1.91±0.39 ns	0.98±0.19 ns	0.27±0.04 ns
	50	1.78±0.15	0.47±0.04	0.30±0.14	8.731±2.93	2.22±0.72	1.22±0.23	0.50±0.04	2.41±0.33	0.56±0.19	0.21±0.03
	100	1.43±0.33	0.46±0.03	0.35±0.10	4.25±0.27	1.49±0.31	1.15±0.12	0.58±0.12	3.17±0.82	0.41±0.08	0.19±0.01
	mean	1.65±0.12 AB	0.50±0.04 BC	0.28±0.06	7.80±1.41 A	2.40±0.43 A	1.37±0.12 AB	0.53±0.04	2.50±0.33	0.65±0.12	0.23±0.02 A
W	0	1.54±0.29 ns	0.52±0.01 ns	0.40±0.17 ns	5,75±2.52 ns	1.58±0.50 ns	1.34±.0.15 a*	0.51±0.13 ns	2.07±0.42 ns	0.71±0.12 a**	0.26±0.03
	50	1.12±0.09	0.61±0.06	0.41±0.09	3,20±1.03	1.69±0.52	1.07±0.07 ab	0.46±0.09	2.32±0.37	0.53±0.14 ab	0.23±0.07
	100	0.94±0.11	0.49±0.02	0.46±0.21	2,68±0.78	1.69±0.57	0.86±0.08 b	0.57±0.02	2.36±0.04	0.37±0.06 b	0.24±0.01
	mean	1.20±0.13 B	0.54±0.03 AB	0.42±0.08	3,88± 0.95 B	1.65±0.27 AB	1.09±0.08 B	0.51±0.05	2.25±0.18	0.54±0.08	0.24±0.02 A
AMF+W	0	1.58±0.14	0.76±0.13ns	0.30±0.13 ns	6,56±0.02 ns	3.02±0.62 ns	2.12±0.42 a*	0.75±0.06	2.44±0.13 ns	0.89±0.19ns	0.31±0.02
	50	1.69±0.43	0.59±0.10	0.33±0.08	5.76±0.03	1.97±0.48	1.46±0.08 b	0.55±0.12	3.20±0.04	0.46±0.02	0.17±0.04
	100	2.33±0.05	0.64±0.09	0.36±0.08	7.48±0.03	2.03±0.55	1.45±0.19 b	0.69±0.38	3.43±0.60	0.48±0.15	0.17±0.05
	mean	1.87±0.18 A	0.66±0.06 A	0.33±0.05	6.60±0.01 AB	2.34±0.33 A	1.68±0.18 A	0.66±0.12	3.02±0.22	0.61±0.10	0.22±0.03 A
Mean (salt applications)	0	1.54 NS	0.56 NS	0.28 NS	7.29 A*	2.50 A*	1.86 A**	0.62 NS	2.55 NS	0.81 A**	0.27 A**
	50	1.55	0.52	0.38	5.29 AB	1.69 B	1.28 B	0.49	3.18	0.47 B	0.18 B
	100	1.51	0.49	0.43	4.27 B	1.49 B	1.15 B	0.55	3.75	0.37 B	0.17 B

Different capital letters indicate significant differences among the means based on the Duncan's multiple comparison test at ** ($p \leq 0.01$) or * ($p \leq 0.05$), and small letters are for individual applications.

ing the Mg content in the shoots, the W application at 50 mmol salt dose and AMF+W application at 100 mmol salt dose led to significantly higher values (0.37% and 0.45%) than the other applications. In terms of the Mg content in the roots, the W application at 0 mmol salt dose induced the highest value (0.41%), while W application at 100 mmol salt application caused the lowest value of this element (0.18%).

There were no significant effects of the applications on the P content in the shoots, but there were significant effects of AMF and W applications on the root P content. The highest average P content in the roots (1.29%) was obtained from the sole AMF application, followed by the combined AMF+W and W applications (1.02% and 0.72%, respectively); the control application resulted in the lowest P content (0.48%). There were significant effects of AMF and W applications on the shoot K content; the highest average K content in the shoots (1.87%) was obtained from the combined AMF+W application followed by the sole AMF and control applications (1.65% and 1.41%, respectively). Salt applications had significantly reduced the shoots' K contents. Moreover, the highest average K contents in the roots (1.68% and 1. 59%) were obtained from AMF+W and control applications, respectively; the lowest one was obtained from W application (1.09%). The highest average Ca content in the shoots (0.66%) was obtained from the combined AMF+W application followed by the W and AMF applications (0.54% and 0.50%, respectively); the control application had the lowest Ca content (0.38%). Moreover, there were significant effects of the applications on the shoot K/Na ratio. The highest salt dose (100 mmol) caused the lowest K/Na ratio in the shoot (4.27), compared to that of the control application (7.29). The highest average K/Na ratio in the shoots (7.80) was obtained from the sole AMF application, followed by the combined AMF+W application (6.60); the control and W applications led to the lowest K/Na ratios (4.18 and 3.88, respectively). Similarly, the highest average Ca/Na ratios in the shoots were obtained from the sole AMF and the combined AMF+W applications (2.40 and 2.34), followed by the ratio due to the W application (1.65). In the roots, the salt doses caused significant decreases in the K/Na and Ca/Na ratios. Moreover, all applications (AMF, W, and AMF+W) increased the Ca/Na ratio in the roots.

Cangi and Kilic (2020) studied the effects of AMF applications on nutrient content of saplings in grafted and potted grapevine sapling production with five grapevine rootstocks (140 Ru, 110 R, 41 B, 1103 P, and 5 BB) and Narince grape cultivar cuttings. These researchers reported that the effect of AMF on the P, K, Zn, Ca, Fe, and Mg content in grapevine leaves varied depending on the rootstocks and AMF types; and AMF generally had a positive impact on nutrient intake. Trouvelot et al. (2015) indicated that AMF applications improved the regular uptake of N, P and other nutrients from the soil, and also reduced the amount of P given to the soil. Atceken et al. (2011) reported that AMF increased the uptake of N and P from soil. Cabral et al. (2015) also stated that AMF helped plants in the uptake of micro-

elements from the soil. Moreover, Goddard et al. (2021) revealed that symbiosis between AMF and grapevines triggers major changes in primary metabolism together with modification of defence responses and signaling in both roots and leaves, and there are enhanced levels of several unsaturated fatty acids in grapevine roots and leaves, together with higher levels of SA and JA in leaves and PR protein accumulation in roots, which have the potential to confer better resistance to various pathogens in AMF treated plants.

Whey is a by-product of dairy industry and could be an environmental problem if not effectively disposed of; therefore, studies have been carried out to evaluate the potential use of whey in various crops (Bettiol et al. 2008). Demir et al. (2015) reported that whey at low application dose has been shown to cause no adverse effect on plant growth. Ocak and Demir (2012) reviewed that whey and AMF could be important sources of soil fertility and increase the nutrient uptake by plants because whey contains N, P, K, S, Ca, Na, Mg, lactose and proteins for manuring and for increasing useful microbiological growth, while AMF is one of the most widespread mycorrhizal associations between soil microorganisms and plants, which provides the host plant with an increased capacity to absorb water and nutrients from the soil while the host plant provides the fungus with soluble carbon sources. Considering the composition of whey, it has been revealed by various researchers that the protein nitrogen in this product is converted to inorganic nitrogen by 30-60% by microorganisms in the soil and that lactose is an energy source for microorganisms (Iwabuchi, Yamauchi 1987). It has been also reported that whey has stimulating effects on microbial growth, especially on the symbiont microorganism population in the soil (Reddy et al. 1987). As a result of the present study, it has been demonstrated that arbuscular mycorrhizal fungus (AMF) and whey (W) applications are more effective in terms of plant growth and alleviation of the negative effects of salt stress in plants, especially where both applications are used simultaneously.

In conclusion, the present study has demonstrated that arbuscular mycorrhizal fungi (AMF) and whey (W) and their combination could fight against salt stress. It is seen that either sole applications of AMF and W, and their combined application (AMF + W), which has been used against the physiological and morphological changes induced by salt stress and its adverse effects, have a positive effect on the majority of parameters compared to the control group. AMF and W are important bio-stimulants owing to their positive effects on plant growth parameters and the environment, especially in terms of sustainable agricultural practices. Within the scope of agricultural practices, the use of beneficial biological agents and especially organic materials considered as waste material comes to the fore with their positive effects on plant growth, helping to grow healthy crops and ensure food safety. The effects of AMF and W on plant growth and saline soil conditions have been studied and the positive effects of these organic applications have been determined; therefore, it could be essential to investigate them in detail in future abiotic stress research.

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