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ORIGINAL PAPER

Salicylic acid affects mycorrhizal features, antioxidant enzyme activities and seed yield of linseed under water-deficit stress in open-field conditions

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Abstract

The research aims were to study salicylic acid (SA) effects on mycorrhiza [hyphal width (HW), vesicle diameter (VD) and mycorrhizal colonization (MC)] and interaction between them on greenness index (GI), drought tolerance index (DTI), antioxidant enzymes activities, and seed yield of linseed under drought. A factorial experiment was conducted in an open-field place with mycorrhiza [non-inoculation, *Funneliformis mosseae* (FM), and *Rhizoglomus intraradices* (RI)], SA (250 µM and non-SA), and irrigation levels [100%, 70%, and 40% field capacity (FC)] as treatments. Severe drought increased VD, MC, superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase activities while decreased GI, DTI, and yield. The RI-linseed had higher MC, GI, SOD, and glutathione reductase (GR) activities, but FM-linseed had greater VD and yield under drought. Inoculated linseed with both mycorrhiza showed a reduction in DTI and yield under SA than non-SA. In RI-linseed, SA increased GI, MC, HW, VD, catalase and GR, but decreased in FM-plants. Mycorrhiza (particularly RI) alleviated drought (40% FC)-caused negative effects on linseed via the improvement of SOD, APX, and GI. Regardless of other treatments, SA had negative effects on HW and VD, but SA effects varied depending on mycorrhizal species so that SA increased HW, VD, and MC in RI. Due to the positive correlation between MC and HW, SA reduces FM colonization by reducing the HW of FM. Totally, SA along with RI species can mitigate the harmful effects of drought and improve tolerance via increasing MC, HW, VD, catalase, peroxidase, and GR activities.

Keywords Catalase · *Funneliformis mosseae* · Greenness index · Hyphal width · Peroxidase

Abbreviations

AM	Arbuscular mycorrhiza
APX	Ascorbate peroxidase
CAT	Catalase
DTI	Drought tolerance index
FC	Field capacity
FM	*Funneliformis mosseae*
GI	Greenness index
GR	Glutathione reductase
HW	Hyphal width
MC	Mycorrhizal colonization
POX	Peroxidase
RI	Rhizoglomus intraradices
SA	Salicylic acid
SOD	Superoxide dismutase
VD	Vesicle diameter

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Introduction

Linseed or flaxseed (*Linum usitatissimum* L.) is one of the most important dual-purpose crops for the extraction of oil and fibers (Fila et al. 2018; Shivaraj et al. 2019).

Linseed is a susceptible plant to water stress at seedling, flowering, and early seed development stages due at least partially to its shallow root system (Aghdam et al. 2016; Ansari et al. 2016). Therefore, the alleviation of drought tolerance is vital in linseed production. Many researches are being carried out, to develop strategies to cope with drought (Aghdam et al. 2016; Ansari et al. 2016; He et al. 2017; Kamarudin et al. 2018). In drought-affected soils, plants are highly dependent on arbuscular mycorrhizal (AM) fungi, one of the beneficial microorganisms, belonging to the Glomeromycota phylum because of their ability to increase the metabolic activity of plants for stress tolerance (Marulanda et al. 2007; Hashem et al. 2018). These fungi form symbioses with the roots of over 80% of land plant species (Berruti et al. 2016). Mycorrhizal fungi are beneficial for agricultural, endangered, and medicinal plant species and have key roles in increasing water uptake, photosynthetic performance, plant growth, yield improvement, disease control, soil fertility, and nutrient management, as well as enhancing plant tolerance to environmental stresses like drought, heavy metals, and high temperature (Armada et al. 2015; Berruti et al. 2016; Hashem et al. 2018).

Salicylic acid (SA) is a universal metabolite which has various physiological functions including regulation of plant growth and development (Hara et al. 2012; Dempsey and Klessig 2017), increasing chlorophyll content (La et al. 2019), the increment of antioxidant activities and induction of plant resistance to biotic and abiotic stresses such as drought, salt, and heavy metals (Shi et al. 2009; Hara et al. 2012; La et al. 2019). Quiroga et al. (2018) represented that under drought stress, non-AM plants enhanced the percentage of apoplastic water flow by exogenous application of SA, while AM plants reduced it due to the differential effect of SA on root hydraulic conductivity in AM plants under drought. Rui-hong et al. (2009) and Li et al. (2010) concluded that AM and SA application could synergically induce salt-tolerance of strawberry and NO_2-tolerance of *Avena nuda*, respectively. Zhang et al. (2019) reported higher endogenous SA concentrations at the root of AM plant and also indicated that mycorrhizal networks transfer the SA signal from disease-infected to neighboring healthy seedlings, to activate defense system and supplying protection to neighboring seedlings against citrus disease. Medina et al. (2003) and Stacey et al. (2006) observed that SA concentrations are inversely correlated with the degree of root colonization and rhizobial infection, respectively. Despite many studies about the effects of AM and SA on different crops separately, there are a few studies about the SA effect on AM organs, and interaction between AM and SA on seed yield and antioxidant enzyme activities (Rui-hong et al. 2009; Li et al. 2010; Ansari et al. 2016; Garg and Bharti 2018). In our previous study, interaction between SA and AM fungi on photosynthetic pigments, relative water content, proline content, leaf area, phosphorus content, mycorrhizal colonization percentage, and mycorrhizal dependency was studied in a growth chamber (Ansari et al. 2016) and in the present study, we thought that exogenous SA increases the diameter of the mycorrhizal organs and thereby affects the AM colonization in open-field conditions, and also hypothesized that SA with AM synergistically enhances drought tolerance of linseed by improving antioxidant defense system. Therefore, the aims of the present research were to study the SA effect on the vesicle diameter and hyphal width, and the interaction between SA and AM on antioxidant enzyme activities and seed yield of linseed plant under different water levels in open-field conditions.

Materials and methods

Experimental design and treatments

A factorial arrangement on a randomized complete block design with six replications (three replications for seed yield and three replications for other traits) was conducted in an open-field place of College of Agriculture, Isfahan University of Technology, Iran. Treatments consisted of three irrigation regimes [100%, 70%, and 40% Field capacity (FC) moisture], mycorrhiza [non-inoculated and inoculated with two AM species: *Funneliformis mosseae* [FM] and *Rhizoglomus intraradices* [RI] syn. *Rhizophagus intraradices* (Baltruschat et al. 2019)] and salicylic acid (SA) treatment (seeds treated with 250 µM SA for 8 h and non-treated seeds).

The linseed plants were grown in an open-field place, having natural conditions: average sunshine duration 12 h, the average maximum temperature of 30.9 °C, the average minimum temperature of 16.1 °C, the average maximum relative humidity of 38.5%, the average minimum relative humidity of 10.5% with a light intensity of 2000 *µmol* photons m^{-2} s^{-1} at 1 p.m. (1600–2000 µmol photons m^{-2} s^{-1} at 11 a.m. to 1 p.m.) in the 51°31´41″ E, 32°43´00″ N and 1550.4 m altitude. Soil physical and chemical properties were determined (Page et al. 1982). The soil was silty clay loam with FC of 37%, pH of 7.9, available P of 4.6 mg/kg, total N of 0.09%, available K of 359 mg/kg, diethylenetriaminepentaacetic acid-Fe of 3.3 mg/kg, diethylenetriaminepentaacetic acid-Zn of 0.85 mg/kg, and an organic matter = 1.23%. The soil was sterilized for 1 h by steaming at 121 °C at 1.1 atm vapor pressure in an autoclave.

The AM inoculants consisted of FM and RI (formerly *Glomus mosseae* and *G. intraradices*, respectively, http://schuessler.userweb.mwn. de/amphylo) that were indigenous and dominant in the non-contaminated area of Anguran Mine, Zanjan, Iran. These two AM fungi were isolated

and identified by Zarei et al. (2008a) and Zarei et al. (2010) and prepared through the trap culture [culture medium of autoclaved soil/quartz sand (< 1 mm) (1:4, v/v)] of forage sorghum (*Sorghum bicolor* L.) with spores of FM and RI as single isolate. In general, in order to propagate the fungal species, there were three pots for the FM species, three pots for the RI species, and three pots for the control treatments (without fungal spores). After 4.5 months, the shoots of each pot were picked up at the onset of the reproductive stage, and the contents of pots were retained in the polyethylene bags at 4 °C and finally used to test on linseed plants. The potential of these inoculants (spore numbers of 10–12 spores/g substrate and root colonization of 80–85%) was measured based on the method described by Zarei et al. (2008b) for spore extraction and counting, and assessment of root colonization.

Seeds of linseed (*Linum usitatissimum* L., genotype SE-13) were disinfected with 5% sodium hypochlorite solution for 30 s and rinsed thrice with distilled water (Sauer and Burroughs 1986; Ditommaso and nurse RE 2004). Then in order to treat the seeds with SA, the seeds were soaked in 250 µM SA for 8 h, and in order not to treat, the seeds were used after disinfection. Twenty-five seeds were planted in each pot with 30 cm diameter and 25 cm height. Most portion of each pot was filled with soil, and 50 g of each AM inoculants was spread on top and covered with the 2 cm soil at sowing time. Control pots consisted of adding 50 g of media from control sorghum trap culture pots as described earlier. Then seeds were placed on the soil and finally covered with 2 cm soil. Due to the volume of the pot and in order to avoid the effect of competition, the plants were thinned to eight plants per pot at the three leaf stage. Plants were watered regularly for 40 days, and then, water stress treatments were imposed. Irrigation regimes were determined based on soil water holding capacity by weighting the pots initially and weighing again after 2 days, and the amount of water lost from the soil was replaced by watering the pots to bring the soil water status back to 100, 70 and 40% FC (Chemikosova et al. 2006; Ansari et al. 2016). In total, in this experiment, we had 18 treatments as follows: 1 = NM + 100% FC + non-SA, 2 = NM + 100% FC + with SA, 3 = NM + 70% FC + non-SA, 4 = NM + 70% FC + with SA, 5 = NM + 40% FC + non-SA, 6 = NM + 40% FC + with SA, 7 = FM + 100% FC + non-SA, 8 = FM + 100% FC + with SA, 9 = FM + 70% FC + non-SA, 10 = FM + 70% FC + with SA, 11 = FM + 40% FC + non-SA, 12 = FM + 40% FC + with SA, 13 = RI + 100% FC + non-SA, 14 = RI + 100% FC + with SA, 15 = RI + 70% FC + non-SA, 16 = RI + 70% FC + with SA, 17 = RI + 40% FC + non-SA, 18 = RI + 40% FC + with SA. Each of these treatments had six replications (three replications for measuring seed yield trait and three replications for measuring other traits). Generally, we had 54 pots used to measure seed yield and 54 pots for measuring other traits.

Measurements

At the flowering stage (80 days after planting), greenness index (GI) was measured using a Minolta SPAD-502 chlorophyll meter (Minolta Inc., Tokyo, Japan). Measurement of electrolyte leakage was taken according to the method of Lutts et al. (1996). In this method, fresh leaf samples were cut in small pieces and incubated at 25 °C for 24 h in test tubes containing 10 ml of double distilled water to measure initial electrical conductivity (EC1) using the conductivity meter. The samples were then placed in a water bath at 100 °C for 30 min to measure second electrical conductivity (EC2) after reaching room temperature. Then electrolytic leakage was calculated as the EC1 divided by the EC2 and expressed as a percentage. Leaf sampling was then performed to measure antioxidant enzyme activities. Enzyme extraction was carried out in 0–4 °C. Samples (100 mg fresh leaves) were frozen in liquid nitrogen and then homogenized in 1 ml of ice-cold derivation solution containing 50 mM sodium phosphate buffer (pH 7.8), 2 mM Dithiothreitol (DTT), 2 mM Ethylene diamine tetra acetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone-25 (PVP-25), 0.2% (w/v) Triton-× 100 and 50 mM Tris-hydrochloride (Tris–HCl) (Nakano and Asada 1981). The homogenate was centrifuged at 14,000 × *g* for 30 min at 4 °C (Centrifuge, Eppendorf 5810R), and the supernatant was collected for soluble protein, catalase (CAT), peroxidase (POX), glutathione reductase (GR), and superoxide dismutase (SOD) assays. For the ascorbate peroxidase (APX) assay, the extraction buffer contained 2 mM of Ascorbate in addition to the above substances. Soluble protein was determined at 595 nm by a spectrophotometer (HITACHI U-1800) according to the method described by Bradford (1976).

Activity of CAT was assayed as a decrease in absorbance at 240 nm for 2 min by a spectrophotometer (HITACHI U-1800) following the decomposition of H_2O_2 (Bergmeyer 1970). The reaction mixture contained 3 ml sodium phosphate buffer (50 mM, pH 7.0) and 4.51 µl H_2O_2 (30%) and 50 µl enzyme extract. The activity of POX was determined using guaiacol oxidation (Herzog and Fahimi 1973) in a reaction mixture containing 3 ml sodium phosphate buffer (50 mM, pH 7.0), 4.51 µl of H_2O_2 (30%), 3.35 µl guaiacol and 50 µl enzyme extract. The increase in absorbance was recorded with the addition of H_2O_2 at 470 nm for 2 min. Activities of APX and GR were assayed following the methods of Nakano and Asada (1981) and Herzog and Fahimi (1973) at 290 and 340 nm, respectively (Spectrophotometer, HITACHI U-1800). The activity of SOD was measured at 560 nm according to the method described by Beauchamp and Fridovich (1971). The reaction mixture contained sodium phosphate buffer (50 mM, pH 7.0), 2 mM EDTA, 13 mM methionine, 1 mM riboflavin, 1 mM Nitro blue tetrazolium (NBT), and 50 µl enzyme extract which illuminated

for 15 min. Control was the reaction mixture without enzyme extract that was placed in the light. Blank contained the reaction mixture without enzyme extract that was in the dark. One unit of SOD activity was calculated based on 50% inhibition of NBT light reduction and expressed as Unit/mg FW.

Drought tolerance index (DTI) of AM and non-AM linseed plants was assessed similar to Rabie and Almadini (2005) as the shoot dry weight in drought soils divided by the corresponding shoot dry weight on non-drought soil and expressed as a percentage. Roots were stained following the method of Zarei et al. (2008b). The roots were cleared with 10% KOH, acidified with 2% HCl, and stained with 1% (vol/vol) Pelikan blue ink in lactic acid. The mycorrhizal colonization (MC) in roots was determined with the grid line intersection method (McGonigle et al. 1990; Zarei et al. 2008b). An eye lens equipped with a micrometer ruler in a microscope (Nikon eclipss E600) with the 400 magnification was used to measure the hyphal width (HW) and vesicle diameter (VD) (Müller et al. 2017). In order to measure seed yield, the seeds were harvested at physiological maturity from the pots related to the seed yield and weighted after reaching a moisture content of 14%. Then the seed yield was determined as g plant^{-1}.

Statistical analysis

A three-way factorial ($3 \times 3 \times 2$) arranged in a randomized complete block design with three replications was used for data analysis. The first factor was three AM fungi, and the second and third factors were three water levels and two SA treatments, respectively. The GLM procedure of SAS statistical software (SAS Institute Inc. 2009) was used for Analysis of Variance (ANOVA). Data of the CAT, POX, GR, and APX activities were transformed and normalized before statistical analysis. When the effects were significant ($p \leq 0.05$), differences between means were evaluated for significance by using Duncan multiple range test ($p \leq 0.05$). The standard error of treatment means was used as a measure of the data spread. To study the relationship between investigated traits, Pearson's correlation coefficients (r) were calculated using SAS statistical software. The principal component analysis was performed to display the relationship of various parameters with the different treatments using SPSS.

Results

Analysis of variance for all the studied traits is specified in Table 1. In the open-field conditions, inoculation with FM increased seed yield, DTI, APX, and SOD activities, while inoculation with RI enhanced seed yield, GR activity, and GI in comparison with non-AM plants and had the highest MC (Table 1). Inoculation with both AM fungi, especially RI, decreased electrolyte leakage compared with the non-AM plants (Table 1). Vesicle diameter and HW had the greatest amount in FM species (Table 1). Reduction of FC from 100 to 40% decreased seed yield, GI, and DTI up to 61.5%, 22.8%, and 43.2%, respectively. Drought stress (40% FC) increased MC, VD, SOD, POX, and APX activities compared with 100% FC up to 10.4%, 35.3%, 3%, 90.8%, and 38.1%, respectively. Drought had no significant effect on HW, and CAT and GR activities (Table 1). Reduction of FC from 100 to 40% enhanced electrolyte leakage (Table 1). Treatment with SA decreased HW, DTI, POX, and APX activities up to 8.8%, 29%, 50.2%, and 34.4% compared with the non-SA, respectively. The decline in DTI may be due to decreased POX and APX activities in SA treatment (Table 1). Salicylic acid had no significant effects on MC, VD, GI, electrolyte leakage, and CAT and SOD activities (Table 1). The activity of GR increased significantly under the influence of SA by 73.5% compared with non-SA (Table 1). Interaction between AM fungi and drought stress showed that in non-AM, APX activity (Table 2), and SOD activity (Fig. 1a) were higher at 40% FC compared with 100% FC, while in FM and RI plants, APX and SOD activities were higher at 70% FC. At 40% FC, GI was higher in FM plants and at 100% FC it was greater in RI plants (Table 2). In the non-AM plants and AM-plants, seed yield decreased in drought conditions, especially at 40% FC (Fig. 1b). In non-AM plants, FM plants, and RI plants, 40% FC decreased DTI up to 89.8%, 18.9%, and 31.7% in comparison with 100% FC, respectively (Fig. 1c). At 40% and 100% FC, RI-plants had lower electrolyte leakage in comparison with non-AM plants, while at 70% FC, FM plants had lower electrolyte leakage (Table 2). At 70% and 100% FC, RI species had the highest VD, while FM species had the highest VD at 40% FC (Table 2). At 100% and 40% FC, RI species had the highest MC, while at 70% FC, FM species had the highest MC which had not significant differences with RI species (Table 2).

Interaction between AM fungi and SA indicated that SA increased SOD activity in comparison with non-SA in non-AM plants while decreased it in FM plants and RI plants up to 1.01% and 0.87% compared with non-SA, respectively, which the lowest decline was observed in RI plants (Table 3). Salicylic acid reduced GI in FM plants while increased it in non-AM plants and RI plants (Table 3). Salicylic acid increased electrolyte leakage in FM plants up to 5.3% compared with non-SA, while SA decreased it in RI plants and non-AM plants up to 6.7% and 6.4%, respectively (Table 3). In non-AM plants, SA increased seed yield, while in FM plants and RI plants, SA decreased it up to 18.3% and 12.95%, respectively (Table 3). Salicylic acid increased DTI compared with

Table 1 Analysis of variance and mean comparison of the main effects of AM, drought stress and SA on investigated traits of linseed plants in the open-field conditions

S.O.V	df	Electrolyte leakage (%)			CAT (ΔA240min-1 mg-1protein)			POX (ΔA470min-1 mg-1protein)			APX (ΔA290min-1 mg-1protein)		
		Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig
R	2	32.24	6.21^{**}	0.0050	0.0118	0.61^{ns}	0.5479	2.585	0.97^{ns}	0.3888	0.0012	0.42^{ns}	0.6627
M	2	1021.17	196.72^{***}	<.0001	0.0819	4.25^{*}	0.0226	32.764	12.31^{***}	<.0001	0.0111	3.9^{*}	0.0299
D	2	6459.39	1244.36^{***}	<.0001	0.006	0.31^{ns}	0.7365	24.76	9.3^{***}	0.0006	0.0116	4.05^{*}	0.0265
M×D	4	278.84	53.72^{***}	<.0001	0.022	1.14^{ns}	0.3541	7.37	2.77^{*}	0.0428	0.0085	2.97^{*}	0.0332
SA	1	12.04	2.32^{ns}	0.1371	0.0531	2.75^{ns}	0.1063	88.907	33.41^{***}	<.0001	0.0286	10^{**}	0.0033
M×SA	2	19.802	3.81^{*}	0.0320	0.0577	2.99^{ns}	0.0635	4.759	1.79^{ns}	0.1826	0.0059	2.08^{ns}	0.1404
D×SA	2	7.716	1.49^{ns}	0.2405	0.027	1.4^{ns}	0.2610	8.343	3.14^{ns}	0.0563	0.0041	1.44^{ns}	0.2500
M×D×SA	4	13.15	2.53^{ns}	0.0581	0.0694	3.6^{*}	0.0150	25.231	9.48^{***}	<.0001	0.0009	0.3^{ns}	0.8749
Error	34	5.191			0.0193			2.661			0.0029		
			Mean comparison of the main effects			Mean comparison of the main effects			Mean comparison of the main effects			Mean comparison of the main effects	
(M)													
	NM		39.4 ± 5.31^{a}			22.2 ± 4.09^{a}			71.7 ± 10.53^{a}			3.4 ± 0.52^{b}	
	FM		27 ± 3.06^{b}			19.3 ± 1.85^{a}			37.9 ± 8.05^{b}			5.1 ± 0.75^{a}	
	RI		25.8 ± 3.08^{b}			12.02 ± 1.47^{b}			36.1 ± 5.96^{b}			3.1 ± 0.34^{b}	
(D)													
	100% FC		13.7 ± 0.74^{c}			19.8 ± 4.27^{a}			32.8 ± 7.8^{b}			2.9 ± 0.51^{b}	
	70% FC		27.4 ± 1.08^{b}			17.6 ± 1.92^{a}			50.3 ± 8.27^{a}			4.7 ± 0.68^{a}	
	40% FC		51.1 ± 3.13^{a}			16.2 ± 1.82^{a}			62.6 ± 10.15^{a}			4 ± 0.52^{ab}	
(SA)													
	non-SA		31.2 ± 3.49^{a}			20.1 ± 2.83^{a}			64.8 ± 7.13^{a}			4.6 ± 0.53^{a}	
	with SA		30.3 ± 3.35^{a}			15.6 ± 1.66^{a}			32.3 ± 6.44^{b}			3.1 ± 0.38^{b}	

S.O.V	df	GR (ΔA340min-1 mg-1protein)			SOD (unit mg-1 FW)			GI			Seed yield (mg plant-1)		
		Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig
R	2	0.00014	1.06^{ns}	0.3588	0.00014	2.28^{ns}	0.1175	43.37	5.66^{**}	0.0076	50.892	0.6^{ns}	0.5561
M	2	0.00095	7.03^{**}	0.0028	0.0006	9.35^{***}	0.0006	285.4	37.22^{***}	<.0001	2935.33	34.44^{***}	<.0001
D	2	0.00012	0.91^{ns}	0.4130	0.00118	18.51^{***}	<.0001	255.9	33.38^{***}	<.0001	4596.26	53.92^{***}	<.0001
M×D	4	0.00027	2.03^{ns}	0.1127	0.000207	3.25^{*}	0.0232	113.7	14.83^{***}	<.0001	408.989	4.8^{**}	0.0035
SA	1	0.0007	5.17^{*}	0.0294	0.000004	0.07^{ns}	0.7967	21.13	2.76^{ns}	0.1061	95.425	1.12^{ns}	0.2975
M×SA	2	0.00013	0.93^{ns}	0.4028	0.00023	3.61^{*}	0.0379	96.8	12.63^{***}	<.0001	329.411	3.86^{*}	0.0307
D×SA	2	0.0002	1.5^{ns}	0.2381	0.000004	0.06^{ns}	0.9456	15.15	1.98^{ns}	0.1543	75.362	0.88^{ns}	0.4224
M×D×SA	4	0.00037	2.77^{*}	0.0429	0.00012	1.8^{ns}	0.1507	19.29	2.52^{ns}	0.0594	145.15	1.7^{ns}	0.1721

Table 1 (continued)

S.O.V	df	GR (ΔA340min-1 mg-1protein)			SOD (unit mg-1 FW)			GI			Seed yield (mg plant-1)		
		Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig
Error	34	0.00014			0.000064			7.6674			85.238		
		Mean comparison of the main effects			Mean comparison of the main effects			Mean comparison of the main effects			Mean comparison of the main effects		
(M)													
	NM		0.095 ± 0.0111^{b}			0.372 ± 0.0024^{b}			24.6 ± 1.8^{b}			21.9 ± 4.32^{b}	
	FM		0.098 ± 0.0112^{b}			0.383 ± 0.0035^{a}			30.5 ± 1.18^{a}			45.6 ± 3.94^{a}	
	RI		0.225 ± 0.0601^{a}			0.379 ± 0.0018^{a}			32.2 ± 0.84^{a}			41.9 ± 3.98^{a}	
(D)													
	100% FC		0.12 ± 0.016^{a}			0.369 ± 0.0017^{b}			32.3 ± 0.82^{a}			52.1 ± 3.45^{a}	
	70% FC		0.12 ± 0.018^{a}			0.384 ± 0.0036^{a}			30 ± 1.42^{b}			37.2 ± 3.44^{b}	
	40% FC		0.18 ± 0.061^{a}			0.38 ± 0.0014^{a}			24.9 ± 1.69^{c}			20.1 ± 3.93^{c}	
(SA)													
	non-SA		0.102 ± 0.0119^{b}			0.3776 ± 0.00278^{a}			29.7 ± 1.43^{a}			37.8 ± 4.19^{a}	
	with SA		0.177 ± 0.0414^{a}			0.3782 ± 0.00182^{a}			28.5 ± 1.03^{a}			35.1 ± 3.53^{a}	

		DTI (%)			HW (µm)			VD (µm)			MC (%)		
S.O.V	df	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig	Mean of squares	F Value	Sig
R	2	20,076.41	22.76^{***}	< .0001	0.0579	0.52^{ns}	0.5982	18.227	1.34^{ns}	0.2756	11.734	0.31^{ns}	0.7377
M	2	38,866.52	44.06^{***}	< .0001	110.69	997.51^{***}	< .0001	7217.357	530.16^{***}	< .0001	14,903.9	389.92^{***}	< .0001
D	2	14,580.9	16.53^{***}	< .0001	0.0491	0.44^{ns}	0.6461	244.639	17.97^{***}	< .0001	219.96	5.75^{**}	0.0070
M×D	4	4048.67	4.59^{**}	0.0045	0.0397	0.36^{ns}	0.8367	176.349	12.95^{***}	< .0001	191.175	5^{**}	0.0028
SA	1	16,706.19	18.94^{***}	0.0001	0.9316	8.4^{**}	0.0065	28.131	2.07^{ns}	0.1597	22.893	0.60^{ns}	0.4443
M×SA	2	6897.93	7.82^{**}	0.0016	1.022	9.21^{**}	0.0006	248.156	18.23^{***}	< .0001	178.61	4.67^{*}	0.0161
D×SA	2	259.79	0.29^{ns}	0.7468	0.2848	2.57^{ns}	0.0916	10.846	0.8^{ns}	0.4591	359.86	9.41^{***}	0.0006
M×D×SA	4	161.81	0.18^{ns}	0.9454	0.2084	1.88^{ns}	0.1369	9.997	0.73^{ns}	0.5750	271.989	7.12^{***}	0.0003
Error	34	882.135			0.11097			13.614			38.223		
		Mean comparison of the main effects			Mean comparison of the main effects			Mean comparison of the main effects			Mean comparison of the main effects		
(M)													
	NM		50.2 ± 11.96^{b}			0 ± 0^{c}			0 ± 0^{c}			0 ± 0^{c}	
	FM		136.3 ± 13.26^{a}			4.4 ± 0.15^{a}			36.38 ± 2.44^{a}			47.4 ± 2.92^{b}	
	RI		123.6 ± 12.73^{a}			4.12 ± 0.08^{b}			32.68 ± 1.22^{b}			52 ± 2.62^{a}	

Table 1 (continued)

	DTI (%)	HW (µm)	VD (µm)	MC (%)
(D)				
100% FC	125.4 ± 9.71^{a}	2.8 ± 0.49^{a}	20.08 ± 3.55^{b}	29.77 ± 5.76^{b}
70% FC	113 ± 19.15^{a}	2.83 ± 0.5^{a}	21.84 ± 4.02^{b}	36.75 ± 6.42^{a}
40% FC	71.2 ± 13.37^{c}	2.9 ± 0.51^{a}	27.16 ± 4.92^{a}	32.85 ± 6.08^{ab}
(SA)				
non-SA	120.9 ± 13.48^{a}	3 ± 0.42^{a}	23.74 ± 3.56^{a}	32.47 ± 4.63^{a}
with SA	85.8 ± 10.72^{b}	2.7 ± 0.38^{b}	22.3 ± 3.32^{a}	33.77 ± 5.26^{a}

R Block, *M* Mycorrhiza, *D* Drought, *SA* Salicylic acid, *NM* Non-mycorrhizal plants, *FM Funneliformis mosseae*, *RI Rhizoglomus intraradices*, *FC* Field capacity, *CAT* Catalase,*POX* Peroxidase,*APX* Ascorbate peroxidase. * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$); ns (not significant). The results are represented as the mean ($n=18$ for M, $n=18$ for D, and $n=27$ for SA) ± standard error. For each parameter, means with the same letter are not significantly different (Duncan's test $p<0.05$)

R Block, *M* Mycorrhiza, *D* Drought, *SA* Salicylic acid, *NM* Non-mycorrhizal plants, *FM Funneliformis mosseae*, *RI Rhizoglomus intraradices*, *FC* Field capacity, *DTI* Drought tolerance index, *MC* Mycorrhizal colonization, *HW* Hyphal width, *VD* Vesicle diameter. * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$); ns (not significant). The results are represented as the mean ($n=18$ for M, $n=18$ for D, and $n=27$ for SA) ± standard error. For each parameter, means with the same letter are not significantly different (Duncan's test $p<0.05$)

R Block, *M* Mycorrhiza, *D* Drought, *SA* Salicylic acid, *NM* Non-mycorrhizal plants, *FM Funneliformis mosseae*, *RI Rhizoglomus intraradices*, *FC* Field capacity, *DTI* Drought tolerance index, *MC* Mycorrhizal colonization, *HW* Hyphal width, *VD* Vesicle diameter. * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$); ns (not significant). The results are represented as the mean ($n=18$ for M, $n=18$ for D, and $n=27$ for SA) ± standard error. For each parameter, means with the same letter are not significantly different (Duncan's test $p<0.05$)

non-SA in non-AM plants while decreased it in FM plants and RI plants up to 43.3% and 23.4% compared with non-SA, respectively, in which the lowest decline was observed in RI plants (Fig. 2a). In the open-field conditions, SA decreased VD (Fig. 2b), HW (Fig. 2c), and MC (Table 3) in FM compared with non-SA up to 16.8%, 23.1%, and 8.6%, respectively [when the FM + with SA is compared with its own control (FM + non-SA)], but increased these in RI species up to 0.61%, 17.1%, and 17%, respectively [when the RI + with SA is compared with its own control (RI + non-SA)]. These results showed that the FM fungus had a negative response to SA, while RI fungus had a positive response to SA in comparison with FM due to the increase in the HW and VD in the application of SA. Lower reduction of DTI in RI plants with SA treatment could be due to the positive response of RI fungus to SA, the lower reduction of SOD activity, and increasing GI.

There was an interaction between AM fungi, drought and SA on MC, CAT, POX, and GR activities. In both RI and FM plants, SA increased MC at 40% FC, while at 100% FC, SA decreased MC in FM plants but increased it in RI plants (Fig. 3). Non-AM plants at 100% FC without SA had the highest CAT activity and RI plants at 100% FC with SA had the lowest CAT activity. In non-AM plants and RI plants, SA increased CAT activity in comparison with non-SA at 40% FC, while in FM plants, SA decreased CAT activity compared with non-SA at 40% FC (Table 4). Non-AM plants at 40% FC without SA had the highest POX activity and RI plants at 100% FC with SA had the lowest POX activity. In non-AM plants and FM plants, SA decreased POX activity compared with non-SA at 40% FC, while in RI plants, SA increased POX activity in comparison with non-SA at 40% FC (Table 4). In non-AM and AM plants, SA increased GR activity compared with non-SA at 40% FC so that the greatest increment was observed in RI plants (Table 4). The interaction showed that the FM fungus had a negative response to SA, especially in drought conditions, while RI fungus had a positive response to SA in comparison with FM due to the increase in the CAT, POX, and GR activities in the application of SA. Hence lower reduction of the DTI in RI plants with SA treatment could be due to the positive response of RI fungus to SA and the increase in the CAT, POX, and GR activities.

The results of the principal component analysis showed that the 12 studied traits were reduced to four components and the four components together account for 78.74% of the total data changes. The first component, the second component, the third component, and the fourth component explained 40.09%, 17.24%, 11.93%, and 9.48% of the total data changes, respectively (Table 5). In the first component, the traits of MC, HW, VD, GI, seed yield, and DTI had a positive factor coefficient, and POX activity and electrolyte leakage had a negative factor coefficient; therefore, the first

Table 2 Mean comparison for the interaction of AM and drought stress on investigated parameters of linseed plants in the open-field conditions

M	FC%	Electrolyte leakage (%)	APX ($\Delta A_{290} min^{-1} mg^{-1}{}_{protein}$)	GI	VD (µm)	MC (%)
NM	100	16.9 ± 0.51^{e}	2 ± 0.45^{c}	33.3 ± 0.91^{a}	0 ± 0^{c}	0 ± 0^{e}
NM	70	32.6 ± 1.12^{c}	2.9 ± 0.66^{bc}	23.6 ± 0.93^{c}	0 ± 0^{c}	0 ± 0^{e}
NM	40	68.7 ± 1.75^{a}	5.2 ± 1.02^{ab}	16.9 ± 2.12^{d}	0 ± 0^{c}	0 ± 0^{e}
FM	100	13.4 ± 1.02^{f}	4.7 ± 1.14^{ab}	29.4 ± 1.23^{b}	29.93 ± 1.74^{b}	39.43 ± 5.83^{d}
FM	70	24.4 ± 1.07^{d}	6.4 ± 1.67^{a}	33 ± 2.45^{a}	31.6 ± 3.58^{b}	58.34 ± 2.78^{a}
FM	40	43.2 ± 1.57^{b}	4.1 ± 0.98^{abc}	29 ± 2.17^{b}	47.61 ± 2.6^{a}	44.44 ± 2.61^{cd}
RI	100	10.7 ± 0.63^{g}	1.98 ± 0.39^{c}	34.2 ± 1.42^{a}	30.3 ± 2.17^{b}	49.88 ± 5.22^{bc}
RI	70	25.2 ± 1.15^{d}	4.7 ± 0.5^{ab}	33.5 ± 1.13^{a}	33.9 ± 2.92^{b}	51.9 ± 1.88^{abc}
RI	40	41.4 ± 1.03^{b}	2.7 ± 0.19^{bc}	28.9 ± 0.77^{b}	33.86 ± 0.62^{b}	54.1 ± 6.14^{ab}

M Mycorrhiza, *NM* Non-mycorrhizal plants, *FM Funneliformis mosseae, RI Rhizoglomus intraradices, FC* Field capacity, *APX* Ascorbate peroxidase, *GI* Greenness index, *DTI* Drought tolerance index, *MC* Mycorrhizal colonization, *VD* Vesicle diameter

The results are represented as the mean ($n = 6$) ± standard error. For each parameter, means with the same letter are not significantly different (Duncan's test $p < 0.05$)

component can be named as the component of drought tolerance and yield potential. Given that high values of MC, HW, VD, GI, seed yield, and DTI and low values of electrolyte leakage and POX activity are desirable, if the amount of the first component is high, treatments are selected that have less electrolyte leakage and POX activity and induce higher MC, HW, VD, GI, seed yield, and DTI (Table 5). The traits of APX activity, SOD activity, and electrolyte leakage had a positive effect on the second component; therefore, this component can be named as the drought stress sensitivity component. If the amount of the second component is high, treatment is selected that has high electrolyte leakage, APX activity, and SOD activity, in other words, induces drought sensitivity. Therefore, the lower value of this component will lead to the selection of treatments that induce drought tolerance and high yield under stress conditions (Table 5). In the third component, CAT, POX, and APX activities had a positive factor coefficient. The trait of GR activity had a positive effect on the fourth component (Table 5). The biplot diagram (Fig. 4) showed that the treatments 7, 9, 10, 13, 14, 15, and 16 are located in the area with the high value of the first component and the medium to the low value of the second component. The treatments 11, 12, 17, and 18 are located in the area with the medium first component and medium to high second component. The treatments 1, 2, and 8 are placed in an area with the medium first component and the low second component. The treatments 3 and 4 are located in an area with the medium to the low first component and the medium second component (Fig. 4). The treatments 5 and 6 are placed in an area with the low first component and the high second component. Due to the fact that the high value of the first component and the low value of the second component are important in choosing the effective treatment in this experiment, so treatment 7 (inoculation with FM without SA treatment at 100% FC) is the best choice and after that, treatment 14 (inoculation with RI and SA treatment at 100% FC) and then treatment 13 (inoculation with RI without SA treatment at 100% FC) is better (Fig. 4).

Discussion

In our study, drought enhanced oxidative stress by increasing electrolyte leakage and increased antioxidant enzymes in leaves of linseed considerably, which was confirmed by the increments in SOD, APX, and POX activities in linseed. Increased electrolyte leakage and activity of SOD, APX, and POX in stress conditions has been reported in other studies in maize, soybean, barley, and rice (Porcel and Ruiz-Lozano 2004; Abdel Latef and Chaoxing 2011; Yaghoubian et al. 2014; Harb et al. 2015; Quiroga et al. 2017; Kamarudin et al. 2018; Kim et al. 2018). Water deficit can increase the superoxide radical and SOD, as the first defense enzyme, can convert this radical to H_2O_2 to activate other defense mechanisms such as POX, APX, and CAT enzymes which can convert H_2O_2 to H_2O and O_2 (Kim et al. 2018). Catalase is less efficient compared to POX in H_2O_2 scavenging because of its low-substrate affinity, so as long as the stress is not too strong for the plant defense capacity (Shi et al. 2009), which was in agreement with our results about the CAT and POX under FC levels. Glutathione reductase and ascorbate are able to regenerate antioxidants. These enzymes are components of the ascorbate–glutathione pathway responsible for the removal of H_2O_2 in cellular compartments (Marulanda et al. 2007; Sofo et al. 2015). The decrease in seed yield under drought in this study was consistent with the results of Aghdam et al. (2016) who reported that drought stress (50% FC) resulted in a 48.1% reduction on seed yield of *Linum usitatissimum* (cv. Olajonzon). Water deficit just prior and during the early flowering stage can decrease the

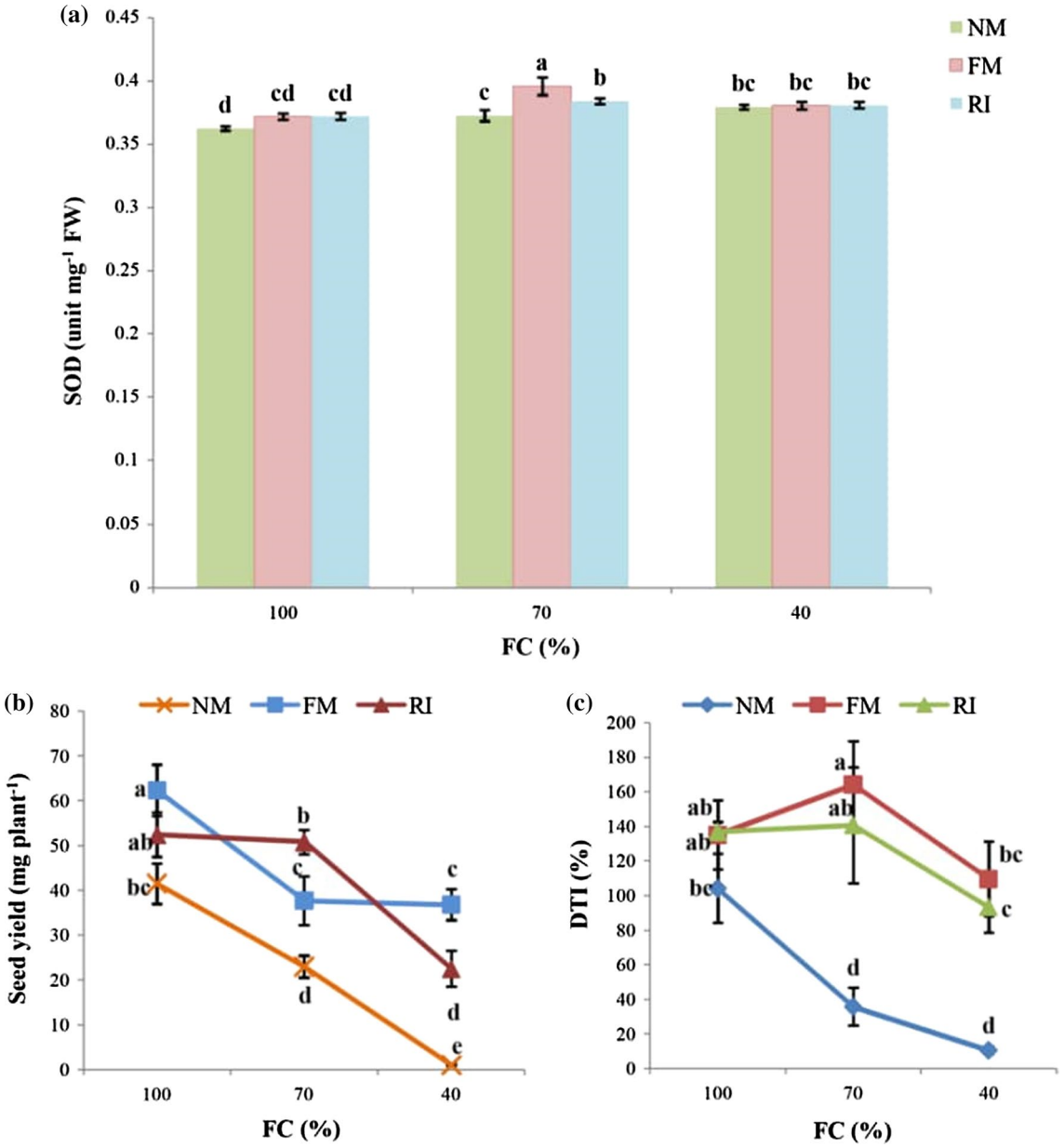

Fig. 1 Mean comparison for the superoxide dismutase (SOD) activity **a**, seed yield **b** and drought tolerance index (DTI) **c** in *Linum usitatissimum* L. when affected by drought (FC, field capacity) levels and mycorrhizal fungi in the open-field conditions. Non-mycorrhizal plants (NM), *Funneliformis mosseae* (FM), and *Rhizoglomus intraradices* (RI). *Vertical T bars* indicate standard error. For each parameter, data with the same letter are not significantly different (Duncan multiple range test $p < 0.05$)

number of fertile flowers, leading to reduced final seed yield production. The decline could also be associated with the reduction of leaf area, chlorophyll density, photosynthetic rate, and less nutrient absorption (Kamarudin et al. 2018). In this regard, there was a positive and higher correlation of seed yield with GI ($r = 0.68$, $p = 0.001$) which confirms the decrease in seed yield due to the reduction of chlorophyll density. Under drought conditions (especially 40% FC), VD increased in both mycorrhizal fungi (especially in FM species). The increase in the VD and HW under drought stress was consistent with the increase in the VD under lead stress studied by Alvarado-López et al. (2019) which reported that vesicle area increases under lead stress in *Glomus intraradices* when inoculated with *Daucus carota* L. plants.

The ameliorative effects of mycorrhiza on drought stress observed in this study are consistent with the results of Porcel and Ruiz-Lozano (2004), Ceccarelli et al. (2010), and He et al. (2017). The dissimilar behavior of AM fungi in relation to several plant enzymatic activities has been often reported (Porcel and Ruiz-Lozano 2004; He et al. 2017). It seems that

Table 3 Mean comparison for the interaction of AM and SA on investigated parameters of linseed plants in the open-field conditions

M	SA	Electrolyte leakage (%)	SOD (unit mg^{-1} FW)	GI	Seed yield (mg $plant^{-1}$)	MC (%)
NM	non-SA	40.7 ± 7.99^{a}	0.3673 ± 0.00285^{c}	23.4 ± 2.83^{c}	18.4 ± 5.17^{c}	0 ± 0^{c}
NM	with SA	38.1 ± 7.45^{b}	0.3761 ± 0.00327^{b}	25.8 ± 2.34^{bc}	25.4 ± 7.04^{c}	0 ± 0^{c}
FM	non-SA	26.3 ± 4.29^{cd}	0.385 ± 0.0059^{a}	33.7 ± 1.58^{a}	50.2 ± 6.75^{a}	49.52 ± 2.27^{b}
FM	with SA	27.7 ± 4.61^{c}	0.381 ± 0.0042^{ab}	27.2 ± 0.87^{b}	41 ± 3.89^{ab}	45.28 ± 5.47^{b}
RI	non-SA	26.7 ± 4.42^{cd}	0.3808 ± 0.00342^{ab}	32.1 ± 1.33^{a}	44.8 ± 5.13^{ab}	47.89 ± 2.51^{b}
RI	with SA	24.9 ± 4.56^{d}	0.3775 ± 0.00148^{ab}	32.4 ± 1.12^{a}	39 ± 6.24^{b}	56.04 ± 4.34^{a}

M Mycorrhiza, *SA* Salicylic acid, *NM* Non-mycorrhizal plants, *FM Funneliformis mosseae*, *RI Rhizoglomus intraradices*, *SOD* Superoxide dismutase,*GI* Greenness index, *MC* Mycorrhizal colonization

The results are represented as the mean ($n = 9$) ± standard error. For each parameter, means with the same letter are not significantly different (Duncan's test $p < 0.05$)

specific antioxidant mechanisms (CAT, POX, APX, SOD, and GR activities) according to the fungus involved in the symbiotic association are indicative of drought tolerance in inoculated linseed plants. In our previous study, we showed that the roots of linseed were well colonized by AM species in growth chamber conditions (Ansari et al. 2016), and RI species (with 85.5%) showed higher colonization in comparison with FM species (with 72.4%). In this study, the application of both RI and FM increased MC in the open-field conditions, but RI with 52% was more efficacious which was in agreement with results of Ceccarelli et al. (2010) and Ansari et al. (2016). The difference in the amount of colonization in these two studies could be due to differences in environmental conditions between the two locations (growth chamber and open-field). Increased APX activity in FM plants has been reported by Abdel Latef and Chaoxing (2011) and Yaghoubian et al. (2014). The obtained results of SOD and GR activities in AM plants were concordant with the results of the Wu et al. (2006) and He et al. (2017). Both AM fungi, especially FM species under 40% FC increased seed yield and DTI in linseed plants through the increase in the GI and the decrease of the membrane electrolyte leakage, which these were concordant with the results of Rabie and Almadini (2005), Quiroga et al. (2017), and Hashem et al. (2018). Positive and higher correlations of MC with GI ($r = 0.5$, $p = 0.001$), SOD ($r = 0.43$, $p = 0.01$), GR ($r = 0.25$, $p = 0.05$), DTI ($r = 0.58$, $p = 0.001$), and seed yield ($r = 0.42$, $p = 0.01$) were recorded in this study which indicating that MC has direct and positive effects on these traits. Majidi et al. (2015) reported that increasing chlorophyll content can increase DTI. Positive and higher correlations of DTI with GI ($r = 0.6$, $p = 0.001$), HW ($r = 0.63$, $p = 0.001$), and VD ($r = 0.56$, $p = 0.001$) observed in this study, which confirms that any factor (such as AM fungi) that increases GI can increase DTI. Thompson (1996) and Rahimzadeh and Pirzad (2017) demonstrated that AM fungi are responsible for increasing growth and seed yield of linseed, which was concordant with our results about seed yield in AM plants. Also in our previous study, it was demonstrated that AM fungi, especially FM fungi improved dry weight accumulation of linseed (Ansari et al. 2016). Improved dry weight accumulation by AM fungi was reported by Ma et al. (2019) and Ozgonen et al. (2001) in cowpea and tomato, respectively. Improvement of dry matter leads to an increase in seed yield of plants under stressful and non-stressful conditions. An increment in the uptake and transfer of water and nutrients, especially P, which leads to better osmoregulation, improved antioxidant system, protection of photosynthesis, and increased hydraulic conductivity are among the reasons for the increase in seed yield by AM fungi. Improvements in these traits may be due to enhanced water uptake in AM plants which is due to the presence of extra-radical hyphae (Hashem et al. 2018).

Porcel and Ruiz-Lozano (2004) and He et al. (2017) expressed that drought stress increased SOD, POX, and APX activities in both AM and non-AM plants compared with well-watered treatments. Also, Porcel and Ruiz-Lozano (2004) represented that APX activity was higher in the non-AM plant compared with AM plant which was in agreement with our results at 40% FC but was not similar to the other water regime (100% and 70% FC). On the other hand, FM caused an increment in APX activity in comparison with non-AM plants when the plants were exposed to well-watered (100% FC) and drought-stressed (70% FC) conditions, which was in agreement with results of Wu et al. (2006). In this study, AM effect on GI, especially by RI, was in agreement with the results of Baslam and Goicoechea (2012) and Nasaruddin and Ridwan (2018) who expressed that GI increased in AM plants. Both AM fungi, especially RI, assisted to stabilize the membranes of the cells and decrease electrolyte leakage in both drought and well-watered conditions, which was in agreement with the results of Quiroga et al. (2017) who reported that the higher stability of cell membrane is mostly related to lower malondialdehyde contents in AM plants. Cekic et al. (2012) reported that the response of antioxidant enzymes can be

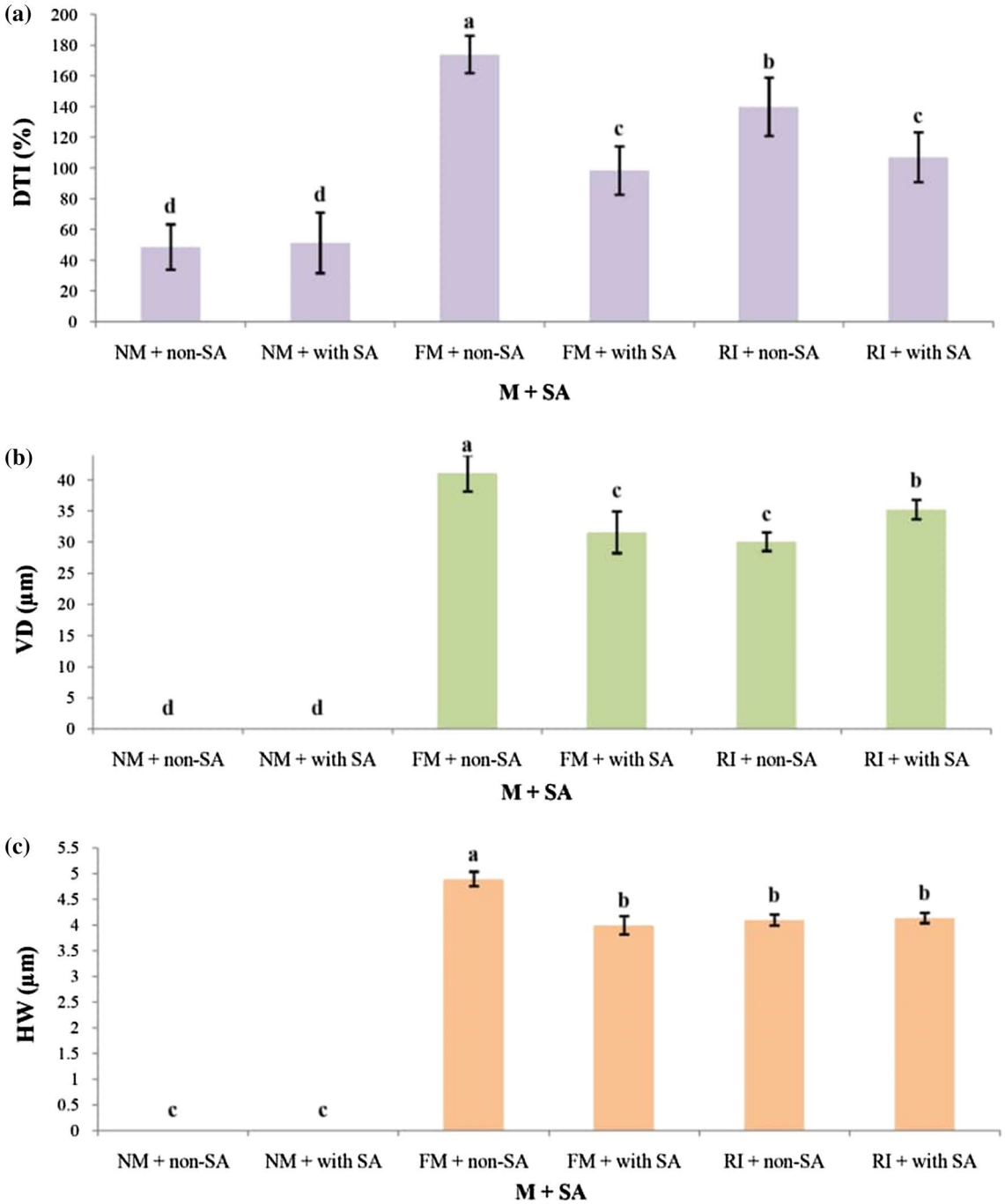

Fig. 2 Mean comparison for the drought tolerance index (DTI) of *Linum usitatissimum* L. **a** vesicle diameter (VD) **b** and hyphal width (HW) **c** of mycorrhizal fungi (M) when affected by salicylic acid (SA) in the open-field conditions. Non-mycorrhizal plants (NM), *Funneliformis mosseae* (FM), and *Rhizoglomus intraradices* (RI). *Vertical T bars* indicate standard error. For each parameter, data with the same letter are not significantly different (Duncan multiple range test $p < 0.05$)

altered according to the plants, AM fungi and drought conditions. It seems that the increase in antioxidant enzyme activities and improved seed yield of AM plants in drought may be attributed to the contribution of fungal hyphae in diffusing nutrient ions which serve as a co-factor for these enzymes like Zn and Cu (Subramanian et al. 2011). Herein, AM fungi might increase nutrients and water uptake by the improvement of root conductivity to water flow and/or via extra-radical mycelium being able to transport nutrients and water to the AM roots (Marulanda et al. 2007; Rahimzadeh and Pirzad 2017). Our results showed that the RI species were more effective compared with the FM on seed yield and

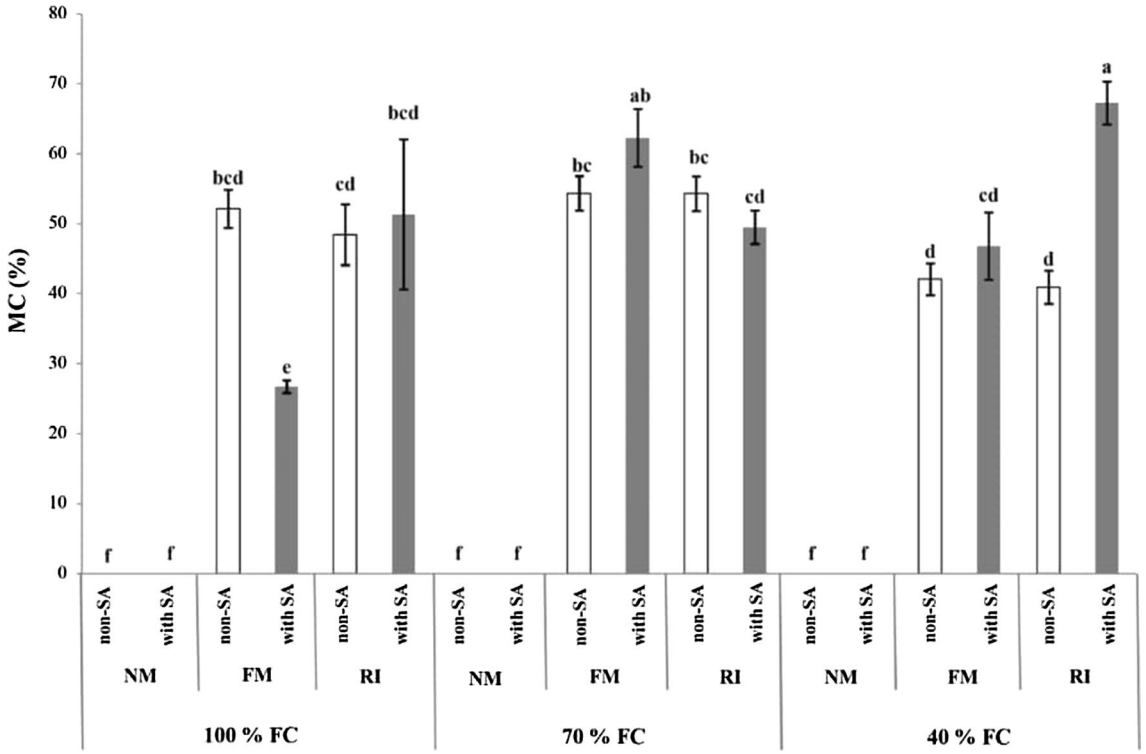

Fig. 3 Mean comparison for the interaction of drought (FC, field capacity) and salicylic acid (SA) on mycorrhizal colonization (MC) of linseed plants in the open-field conditions. Non-mycorrhizal plants (NM), *Funneliformis mosseae* (FM), and *Rhizoglomus intraradices* (RI). *Vertical bars* indicate standard error. Bars with the same letter are not significantly different (Duncan's test $p < 0.05$)

Table 4 Mean comparison for the interaction of AM and drought stress and SA on investigated parameters of linseed plants in the open-field conditions

M	FC%	SA	CAT ($\Delta A_{240} min^{-1} mg^{-1}{}_{protein}$)	POX ($\Delta A_{470} min^{-1} mg^{-1}{}_{protein}$)	GR ($\Delta A_{340} min^{-1} mg^{-1}{}_{protein}$)
NM	100	non-SA	49.6 ± 15.69^{a}	93.1 ± 14.43^{ab}	0.087 ± 0.0072^{b}
NM	100	with SA	12.8 ± 6.84^{b}	14.8 ± 7.79^{ef}	0.071 ± 0.0089^{b}
NM	70	non-SA	22.6 ± 2.5^{b}	85.2 ± 30.76^{ab}	0.068 ± 0.0036^{b}
NM	70	with SA	15.1 ± 8.43^{b}	38 ± 17.37^{cde}	0.18 ± 0.0394^{b}
NM	40	non-SA	15.2 ± 1.67^{b}	101.1 ± 1.05^{a}	0.078 ± 0.0089^{b}
NM	40	with SA	18.1 ± 1.67^{b}	97.8 ± 29.29^{ab}	0.087 ± 0.0036^{b}
FM	100	non-SA	13.5 ± 1.12^{b}	13.2 ± 0.52^{ef}	0.081 ± 0^{b}
FM	100	with SA	24.4 ± 0.68^{b}	24.9 ± 8.92^{def}	0.18 ± 0.0251^{b}
FM	70	non-SA	24.1 ± 5.36^{b}	64.2 ± 11.86^{abc}	0.087 ± 0.0072^{b}
FM	70	with SA	17.8 ± 4.39^{b}	10.9 ± 0^{ef}	0.043 ± 0.0072^{b}
FM	40	non-SA	23.2 ± 2.51^{b}	97.1 ± 0.42^{ab}	0.081 ± 0^{b}
FM	40	with SA	12.7 ± 6.89^{b}	17.2 ± 4.27^{def}	0.115 ± 0.0197^{b}
RI	100	non-SA	10.8 ± 2.33^{b}	47 ± 5.82^{bcd}	0.102 ± 0.0197^{b}
RI	100	with SA	7.5 ± 3.61^{b}	4.1 ± 0.99^{f}	0.198 ± 0.0716^{b}
RI	70	non-SA	13.6 ± 0.5^{b}	67.1 ± 7.7^{abc}	0.189 ± 0.0734^{b}
RI	70	with SA	12.3 ± 2.09^{b}	36.2 ± 15.48^{cde}	0.127 ± 0.0018^{b}
RI	40	non-SA	8.1 ± 4.51^{b}	15.2 ± 7.14^{ef}	0.146 ± 0.0662^{b}
RI	40	with SA	19.8 ± 4.05^{b}	47.1 ± 9.64^{bcd}	0.589 ± 0.2935^{a}

M Mycorrhiza, SA Salicylic acid, *NM* Non-mycorrhizal plants, *FM Funneliformis mosseae*, *RI Rhizoglomus intraradices*, *FC* Field capacity, *CAT* Catalase, *POX* Peroxidase, *GR* Glutathione reductase. The results are represented as the mean ($n = 3$) ± standard error. For each parameter, means with the same letter are not significantly different (Duncan's test $p < 0.05$)

Table 5 Results of principal components analysis for studied traits in mycorrhizal inoculated linseed and treated with salicylic acid under drought stress

Traits	Component			
	1	2	3	4
Mycorrhizal colonization	0.863	0.339	−0.101	0.131
Hyphal width	0.901	0.308	−0.067	0.086
Vesicle diameter	0.831	0.353	−0.034	0.111
Greenness index	0.749	−0.313	0.209	0.240
Catalase activity	−0.231	−0.251	0.667	0.427
Peroxidase activity	−0.528	0.180	0.502	0.399
Ascorbate peroxidase activity	0.113	0.504	0.559	−0.168
Glutathione reductase activity	0.086	0.170	−0.449	0.790
Superoxide dismutase activity	0.309	0.731	0.156	−0.155
Seed yield	0.750	−0.491	0.085	−0.133
Drought tolerance index	0.769	−0.111	0.358	−0.083
Electrolyte leakage	−0.634	0.686	−0.042	−0.015
Relative variance	40.090	17.240	11.930	9.480
Cumulative variance	40.090	57.340	69.260	78.740

antioxidant mechanisms and reduced electrolyte leakage in drought. Also, RI species had more colonization compared with FM that's about it, we represented high colonization of RI species with the roots of the linseed under drought stress and non-stress conditions (Ansari et al. 2016).

Irrespective of AM status, SA can mitigate oxidative stress in severe drought as evidenced by an increase in GR activity at 40% FC (Table 4) that was according to the study of Ahmad et al. (2018). In non-AM plants and RI plants, SA increased CAT activity at 40% FC while decreased it at 100% FC which was in agreement with the results of Kim et al. (2018) especially at 100% and 70% FC. Uzunova and Popova (2000) indicated that exogenous application of SA to plant exerts diverse physiological effects, such as the internal destruction of the plastids, inhibition of dry weight accumulation, and alterations in leaf expansion and anatomy. Shi et al. (2009) expressed that SA do not alter photosynthetic pigments in hemp plants which was in agreement with our results about the GI.

We hypothesized that reducing the VD and HW under SA treatment could have a negative effect on AM fungi and their function in plants. Salicylic acid caused a significant reduction on MC, HW and VD in FM species, but increased them in RI species which indicating the negative effect of SA on FM. Salicylic acid decreased colonization percentage in FM plants while increased it in RI plants (Ansari et al. 2016). Therefore, a mycorrhizal fungus and its colonization percentage were affected by SA treatment. Positive and higher correlations of MC with HW ($r=0.92$, $p=0.001$) and VD ($r=0.84$, $p=0.001$) were recorded in this study. Hence our investigation demonstrated that SA can affect MC via altering HW and VD that the type of change (decrease or increase) depends on the species of mycorrhizal fungus and its ability and genome. In this regard, Garg and Bharti (2018) reported that SA enhances the root colonization by significantly increasing the number of arbuscules and vesicles under salt stress which was in agreement with our results about the RI fungus. Also, Medina et al. (2003) expressed that SA may delay root colonization by AM fungi. Ozgonen et al. (2001) represented that SA and related compounds have a negative effect on pathogens and also on AM fungi. Also, Bhat et al. (2017) reported that SA functions as negative regulators in AM symbiosis. Temporary increases in CAT and POX activities in AM tobacco roots occur at the same time as the temporary increase of free SA (Blilou et al. 2000). In this study, CAT and GR activities were increased in RI plants with SA. Interaction between AM and drought and SA showed that RI plants with SA had higher CAT and POX activities in severe drought indicating the positive and synergistic effect between SA and RI species, while FM plants with SA had less CAT activity in severe drought indicating a negative effect between SA and FM species. Li et al. (2010) and Soltani Kazemi et al. (2018) showed that AM with SA increased CAT and POX activities in response to NO_2 exposure and salinity, respectively, which this was in agreement with our results about the CAT and POX activities in RI plants with SA. Garg and Bharti (2018) demonstrated that the combination of SA and AM fungus (*Rhizoglomus intraradices*) strengthens the plasma membrane and decreases electrolyte leakage under salt stress which was in agreement with our results about the RI fungus. Increasing the concentration of calcium ions in plants treated with both SA and AM fungus can reduce electrolyte leakage and favor AM symbiosis (Garg and Bharti 2018) because higher concentrations of calcium ion increase the sporulation and colonization of AM fungi (Jarstfer et al. 1998). Ozgonen et al. (2001) indicated that the deterrent effect of SA on the progress of *Fusarium oxysporum f.sp. lycopersici* enhances linearly with increasing SA concentration.

It is clear that SA altered beneficial effects of AM fungi in growth and yield of AM plants and AM symbiosis. Salicylic acid can have negative or positive effects on AM fungi which may be related to the SA effect on fungal hyphae and vesicle, and on root colonization of linseed by AM fungi. Li et al. (2010) represented that colonization by FM with SA can increase *Avena nuda* plant tolerance to increased NO_2 exposure. In our study, RI fungus had a positive response to SA through the lower reduction of seed yield, DTI and SOD, increasing GI and POX, CAT, and GR activities, and decreasing electrolyte leakage. Salicylic acid decreased the ability of linseed to AM symbiosis via decreasing HW and VD; hence the beneficial effects of AM reduced on the growth and yield of AM linseed plants. In general

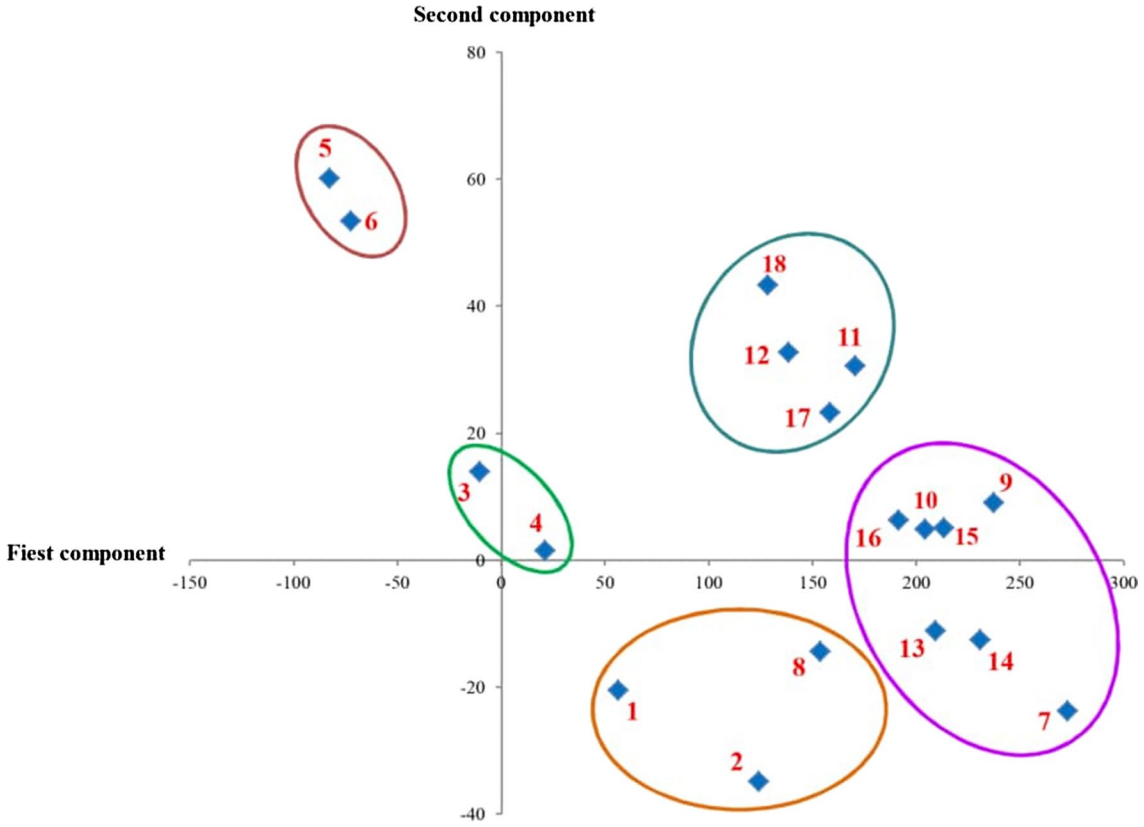

Fig. 4 Biplot of principal component analysis for the studied traits of linseed inoculated with mycorrhizal fungi and treated with salicylic acid under drought stress based on the first and second components.1: NM + 100% FC + non-SA, 2: NM + 100% FC + with SA, 3: NM + 70% FC + non-SA, 4: NM + 70% FC + with SA, 5: NM + 40% FC + non-SA, 6: NM + 40% FC + with SA, 7: FM + 100% FC + non-SA, 8: FM + 100% FC + with SA, 9: FM + 70% FC + non-SA, 10: FM + 70% FC + with SA, 11: FM + 40% FC + non-SA, 12: FM + 40% FC + with SA, 13: RI + 100% FC + non-SA, 14: RI + 100% FC + with SA, 15: RI + 70% FC + non-SA, 16: RI + 70% FC + with SA, 17: RI + 40% FC + non-SA, 18: RI + 40% FC + with SA. Salicylic acid (SA), Non-mycorrhizal plants (NM), *Funneliformis mosseae* (FM), *Rhizoglomus intraradices* (RI), and Field capacity (FC)

SA reduced fungal HW (Table 1). However, SA increased VD and HW in RI, but decreased them in FM which demonstrated that FM is the most sensitive species to SA and RI had a synergistic response to SA. Medina et al. (2003) reported that changed SA in plants have an effect on FM during the establishment of the fungus, but do not affect the rate of final root colonization; they also represented that the status was not observed in RI and suggested that this might be due to the different root colonization pattern with this fungus in their experiments. Based on the results of principal component analysis, FM-linseed without SA at 100% FC, and RI-linseed treated with SA at 100% FC (with the highest amount of the first component and the low second component) had the lowest electrolyte leakage and POX activity and the highest DTI, GI, seed yield, and MC. This also showed that FM species did not have a synergistic effect with SA. On the other hand, RI-linseed without SA at 40% FC (with the medium first component and the medium second component) is the best choice under severe drought conditions.

Conclusion for future biology

Linseed plants subjected to drought stress showed a significant reduction in growth, seed yield and DTI and a significant increment in oxidative damage. However, seed treatment with SA singly can ameliorate the antioxidant system, GI, seed yield, and DTI, while decrease electrolyte leakage that can be applied as a beneficial approach for elevating the drought tolerance of linseed in the future. The combination of SA and RI strengthens plant growth and performance compared with the non-AM and non-SA by decreasing electrolyte leakage, the stimulation of antioxidant system, the higher MC, VD, and HW followed by the better uptake of ions and water, and the higher chlorophyll density followed by more photosynthesis rate, which are responsible for drought tolerance. This can be significant to increase linseed production under drought conditions in the future, however, the use of RI and FM species singly can be more effective in the future linseed production under drought conditions compared to the combination of RI and SA. Also, these results

would be the main step in other dry agro-ecological regions wherein cost-effective AM products and SA treatment could be applied for sustainable production of plants growing in that specific region. In addition, these findings encourage researchers to evaluate plant growth and yield as well as AM features (e.g., HW and VD) in terms of genes involved in the interaction pathway of SA with AM fungi.

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Author contributions AA contributed to conceptualization, investigation, experimental work, collected samples, methodology, data curation, statistical analysis, and writing-original draft preparation. JR was involved in supervision, resources, and methodology. MZ contributed to conceptualization, methodology, validation, and writing-reviewing. HK was involved in conceptualization. All authors read and approved the final manuscript.

Data accessibility The data generated during and/or analyzed during the current study are available from the authors on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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