Journal of Plant Growth Regulation
https://doi.org/10.1007/s00344-024-11508-3

Strigolactone (GR24) and Abscisic Acid Induced Drought Tolerance in Wheat by Ameliorating Nutrient Uptake

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Received: 10 September 2023 / Accepted: 20 September 2024

Abstract

Cross-talk between strigolactones (SLs) and abscisic acid (ABA) in the presence of arbuscular mycorrhizal fungi (AMF) plays an important role in plants resistance to drought stress. SLs and ABA can be involved in plant-AMF interaction through nutrient uptake and physio-biochemical characteristics. Therefore, the present study was conducted to investigate the effects of wheat seed priming with GR24 (0, 2.5, 5 and 10 µM), AMF (without and with AMF) and ABA foliar application (0 and 5 mg L^{-1}) under drought stress conditions during the vegetative growth stage. The results showed that the application of GR24 at a concentration of 5 µM in the presence of AMF increased the tolerance of the wheat plant to drought stress by improving the root area, nutrient uptake and physiological and biochemical characteristics. These treatments increased the root area, and uptake of P, K and Ca by 66, 13, 56 and 32%, respectively. The third level of GR24 (5 µM) with AMF also increased the physio-biochemical parameters, including relative water content, soluble sugar, proline and total phenol by 20, 45, 63 and 65%, respectively, and reduced electrolyte leakage by 50%. Moreover, ABA increased the effectiveness of GR24 in the presence of the fungus in some parameters such as P and K uptake, electrolyte leakage and proline. Therefore, the findings of this study indicated that the application of GR24, ABA and AMF can be considered as a strategy to increase nutrient uptake and wheat growth under drought stress conditions.

Handling Editor: Parvaiz Ahmad.

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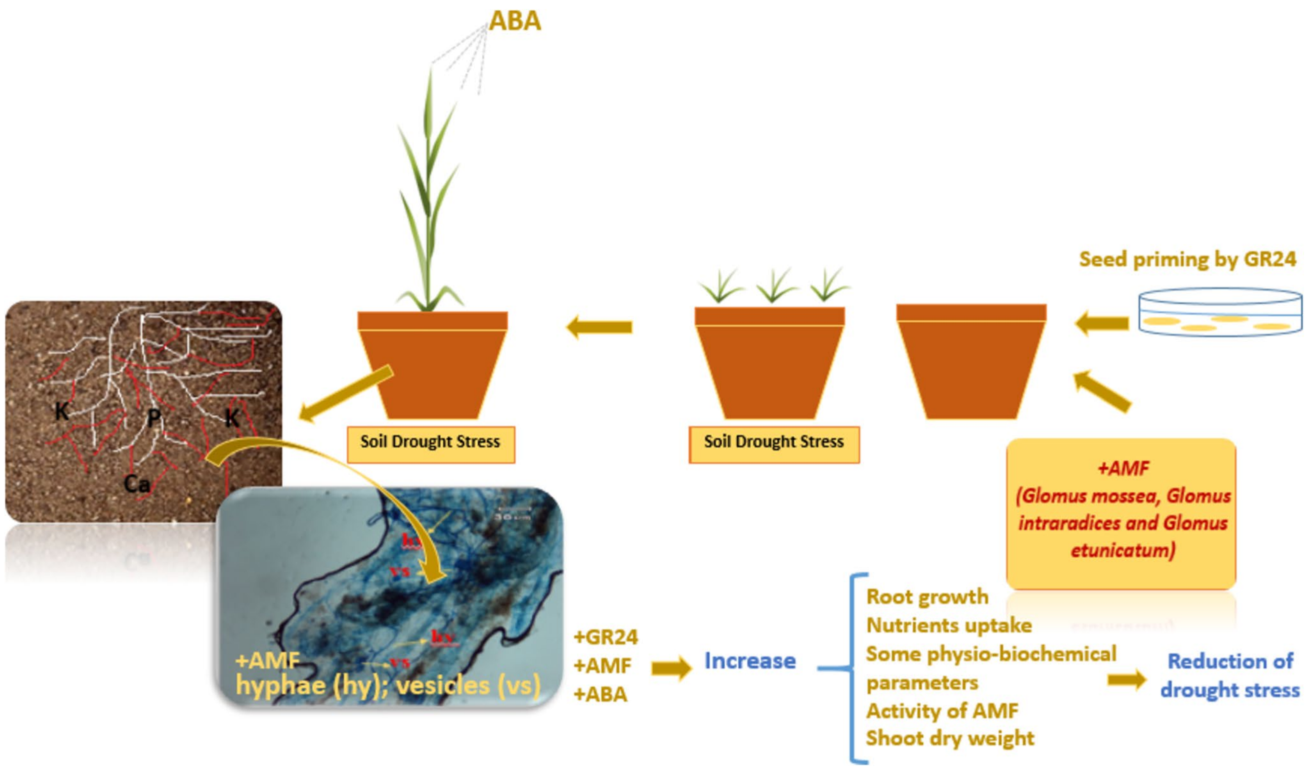
Published online: 15 October 2024

Graphical Abstract



Schematic model of the effect of seed priming with GR24, abscisic acid (ABA) foliar application and arbuscular mycorrhizal fungi (AMF) on improving the growth of wheat plant under drought stress conditions

Keywords AMF symbiosis · Foliar application · Moisture stress · Plant nutrition · Seed priming

Introduction

Drought stress, as one of the most widespread abiotic stresses in the soils of dry regions, negatively affects the growth of plants such as wheat by reducing the uptake of water and nutrients (Karimzadeh et al. 2021; Kaya et al. 2020; Kosar et al. 2021). Therefore, considering the increase in the world population and the supply of food resources, the use of suitable nutritional strategies can improve the yield of wheat cultivars under drought stress conditions (Salim and Raza 2020). Studies have shown that the use of drought resistant and nutrient-efficient cultivars can be a sustainable way to deal with drought stress (de Souza Campos et al. 2019; Neji et al. 2019). Drought resistant plants adapt to stress by activating different metabolic pathways, including the biosynthesis of secondary metabolites, phytohormones, sugars and other responses (Campos et al. 2018; de Souza Campos et al. 2019; Jan et al. 2019; Ahammed et al. 2021; Duvnjak et al. 2023). Phytohormones can effectively alleviate various abiotic stresses such as drought and salt stresses. Strigolactones (SLs), a class of carotenoid-derived phytohormones, play an important role in improving plant growth under drought stress. SLs have been observed in different amounts in the root secretions of monocotyledonous and dicotyledonous plants (Yoneyama et al. 2012; Zwanenburg et al. 2016; Singh et al. 2023). SLs increase root size and symbiosis between plants and arbuscular mycorrhizal fungi under nutrient deficiency conditions (Yoneyama et al. 2012; de Souza Campos et al. 2019). Due to the production and low stability of SLs in plants, synthetic analogs are used. Synthetic SL analogs include GR5, GR24, and GR7 and the first is mostly used in scientific research (Koltai and Prandi 2014; Zwanenburg et al. 2016). Studies have shown that GR24 increases the resistance of plants against abiotic stresses by increasing root growth, shoot dry weight, seed germination and physio-biochemical characteristics of plants such as the relative content of leaves water, total phenolic, sugar, cell membrane stability and antioxidant activity (Yoneyama et al. 2012; Ha et al. 2014; Trabelsi et al. 2017; Min et al. 2019; Sedaghat et al. 2020; Wani et al. 2022; Al-Amri et al. 2023; Singh et al. 2023). Due to the difference in GR24 structure with other synthetic SL analogs, it is also widely used in biological activities (Akiyama et al. 2005; Zwanenburg et al. 2016).

Plants use different strategies to deal with stress, including symbiosis with soil microorganisms. Arbuscular mycorrhizal fungi (AMF) in the rhizosphere increases plant growth under various environmental stresses. Furthermore, SL plays an essential role in plant symbiosis relations, especially with AMF (Wani et al. 2020; Dowarah et al. 2022). Various studies showed that AMF symbiosis enhances plant growth by improving water uptake, osmotic regulation, photosynthetic efficiency, soil structure and reducing oxidative stress under drought stress conditions (Campos et al. 2018; Zhang et al. 2018a, b; Begum et al. 2019a, b; Hamidian et al. 2023; Ould Amer et al. 2023). The beneficial effects of AMF have been reported in different crops such as wheat, maize and soybean under drought stress conditions (Grümberg et al. 2015; Begum et al. 2019a, b; Garcia de Leon et al. 2020; Ould Amer et al. 2023). AMF is very effective in essential nutrient uptake, especially phosphorus, by increasing root growth under drought stress. Therefore, mycorrhizal symbiosis improves plant yield (Smith and Smith 2011; Zhang et al. 2018a, b; Dowarah et al. 2022). Furthermore, studies have found that symbiosis of roots with AMF in the presence of SL reduces the effects of drought stress on the plant. SLs can assist symbiotic relationships between plants and AMF by activating processes related to colonization, including spore germination and hyphal branching (López-Ráez 2016; Ruiz-Lozano et al. 2016; Lanfranco et al. 2018). Various reports have demonstrated that ABA is also necessary for AMF symbiosis establishment (Pozo et al. 2015; López-Ráez 2016). Therefore, the SL-ABA interaction in the presence of AMF can be a strategy to cope with drought stress. Furthermore, AMF symbiosis establishment under drought stress conditions depends on the fungal species, environmental conditions and stress level (Begum et al. 2019a, b; Dowarah et al. 2022).

ABA is one of the other signals in plants that affect the growth of plants under drought conditions (Ton et al. 2009; Hong et al. 2013; Wei et al. 2015a, b; Mega et al. 2019; Duvnjak et al. 2023). Several studies stated that the use of ABA increases plant resistance in abiotic stress conditions by improving some plant physiological processes such as osmotic regulation, antioxidant protection and stomatal regulation (Chen et al. 2018; Jan et al. 2019; Takahashi et al. 2020; Ilyas et al. 2021; Mao et al. 2022). ABA increases the amount of soluble sugars and proline in the plant under drought stress conditions (Pattanagul 2011; Huai et al. 2019; Park et al. 2021). The optimal concentration of ABA also stimulates root growth (Li et al. 2017; Muhammad Aslam et al. 2022). Studies have shown that the effectiveness of SL under drought stress conditions can be influenced by ABA (Ren et al. 2018; Wani et al. 2020). Aroca et al. (2013) reported that a positive interaction between ABA and SLs was observed in mycorrhizal plants under stress conditions.

There are different methods for using phytohormones to improve plant growth, but the most effective method is not clearly defined. One of the economic methods for using GR24 in plants is seed priming. Seed priming is the treatment of seeds with various materials of natural and synthetic (Aswathi et al. 2022). Many studies have been conducted on the priming of different seeds with various phytohormones (Jisha et al. 2013; Wei et al. 2015a, b; Kausar and Shahbaz 2017; Jaiswal et al. 2022; Mujahid et al. 2023). However, few studies have been done with GR24 to improve root growth, uptake of nutrients and physiological and biochemical characteristics in plants. In addition, GR24, ABA and AMF reduce the effects of drought stress in plants by improving root growth and physio-biochemical characteristics. GR24 and ABA can also increase plant-AMF interaction. Hence, it is important to explore the role of GR24 and ABA in regulating the morphological, physiological and biochemical characteristics involved in AMF symbiosis and plant growth under drought stress conditions. Therefore, the present study was carried out to investigate the effect of GR24 and ABA along with AMF on drought-sensitive wheat variety under drought stress conditions in the vegetative growth stage.

Materials and Methods

Plant and Chemical Materials

Seeds of spring wheat (*Triticum aestivum* L.), cv. Parsi, (as a drought sensitive cultivar) were obtained from Khorasan Razavi Agricultural Research Center, Mashhad, Iran. The synthetic strigolactone analogue GR24 (CX23880; (3aR*,8bS*,E)-3-(((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one, M. wt. 298.2) and abscisic acid (ABA) (M. wt. 264.32) in the form of 2-cis, 4-trans-Abscisic acid were purchased from Chiralix, the Netherlands and Sigma, the USA Company, respectively. Arbuscular mycorrhizal fungi (AMF) (a mixture of *Glomus* spp. including *G. mossea, G. intraradices and G. etunicatum*) was procured from the soil microbiology and biotechnology department of Iran Soil and Water Research Institute. AMF inoculation was obtained from the host plant of sorghum and corn in a sandy soil bed.

Experimental Design and Treatments

In 2019, a pot experiment was performed in a greenhouse located at the Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran (36°18′19"N, 59°31′41"E) under controlled conditions (Day/night 32/22 °C, 31/60% RH, and 14/10 h light/dark). The experiment was factorial based on a completely randomized design with three replications and three factors during the vegetative growth period under drought stress of 65% field capacity (FC). Factors included SL (GR24), ABA (with and without ABA) and AMF (with and without AMF). According to our previous study, a level of 65% FC provided the necessary condition for drought stress. Therefore, this level was considered for drought stress.

In this study, wheat seeds were primed with GR24. To prepare GR24, pure GR24 was dissolved in a few drops of acetone and then diluted with deionized water to obtain concentrations of 0, 2.5, 5 and 10 µM. After that, the sterilized seeds were primed with levels of GR24 in Petri dishes for 12 h at 15 °C under dark conditions. The treatments of AMF were applied at two levels of 0 (without AMF) and 75 g (mixed *Glomus* spp.) in the soil before seed sowing. ABA was also sprayed at two levels of 0 and 5 mg L^{-1}. Pure ABA was dissolved in a few drops of ethanol and then diluted with deionized water to the desired concentration (Fig. 1).

Growth Conditions

In this experiment, soil samples were collected from a depth of 0–30 cm. The texture and some physical and chemical properties of the soil were then tested (Table 1). The soil field capacity (FC) was determined by the pot weighing method according to Shirani Bidabadi and Sharifi (2021). Before planting, the soil was sterilized for three consecutive days (121 °C, 20 min). Nutrients, including phosphorus from mono-calcium phosphate monohydrate source ($Ca(H_2PO_4)_2 \cdot H_2O$) in the amount of 15 mg kg^{-1}, nitrogen from ammonium nitrate source in the amount of 80 mg kg^{-1}, and potassium from the source of potassium sulfate in the amount of 70 mg kg^{-1} were added to the soil of each pot before planting. Phosphorus, nitrogen and potassium elements were used in all the pots based on the soil test results of the study area. After examining the percentage and speed of seed germination in the germinator, healthy seeds of the same size were selected. The seeds were disinfected with a 5% (v/v) sodium hypochlorite solution and then washed with distilled water. After applying GR24 and AMF levels, wheat seeds were sown at a depth of 2 cm in plastic pots containing 5 kg of sterilized soil. Four days after sowing, the number of seedlings per pot was reduced to five to maintain the desired plant population density. Then the drought stress treatment was performed by daily weighing of pots after fourteen days of seed sowing. The first day after drought stress was recorded as the first day of the experiment. Furthermore, seedlings in two growth stages of wheat GS31 (Stem elongation) and GS41 (Booting) (Zadoks et al. 1974) were sprayed with ABA at sunset. To increase the absorption of ABA by leaves, Tween-20 was used. The control pots (without GR24 and ABA) were treated with an equal amount of distilled water plus ethanol and acetone, respectively.

Determination of Dry weight, Root Area and Length, Concentration of Nutrients and Nutrient Uptake

Two months after sowing, the plants were harvested from pots at the end of the vegetative growth stage to study dry and fresh weight, morphological traits, concentration and uptake of nutrients. The dry weight of the shoot was determined after oven drying at 70 °C for 48 h. The root

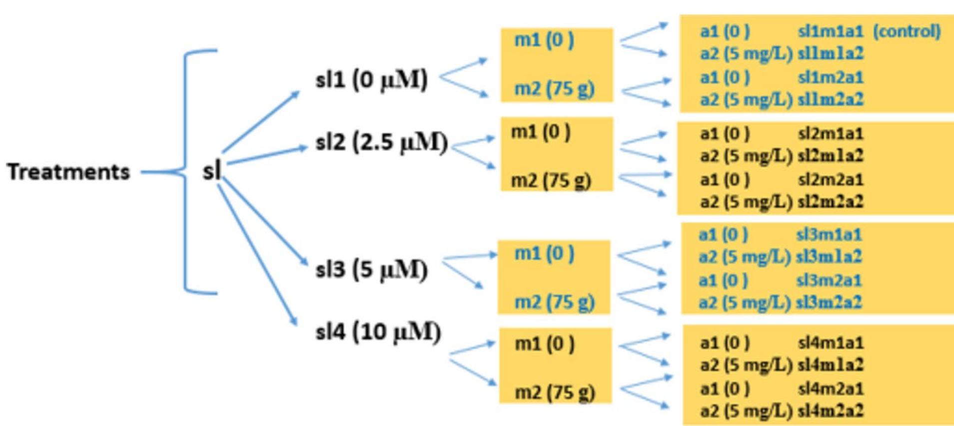


Fig. 1 Pattern of treatments used on the growth of wheat plants under drought stress conditions. The amount of GR24 (Sl), abscisic acid (a) and arbuscular mycorrhizal fungi (m) respectively 0, 2.5, 5 and 10 µM, 0 and 5 mg L^{-1}, 0 and 75 g were used

Table 1 Some physico-chemical properties of the soil sampled in the experiment

EC (ds m^{-1})	Calcium carbonate equivalent (%)	Available potassium (mg kg^{-1})	Available Phosphorus (mg kg^{-1})	Total Nitrogen (mg kg^{-1})	Organic carbon (%)	pH	FC (%)	Texture
1.46	13.75	147.51	8.57	707	0.35	7.8	19	Clay loam

samples were also scanned using a Delta-T root scanner and WinRHIZO software (V5.0, Regent Instruments, Quebec, Canada) for root size system characteristics (root area and length) (Deng et al. 2018; Neji et al. 2019). Furthermore, the plant samples were extracted by the dry digestion method to measure the concentration of nutrients. The phosphorus concentration of shoots and roots was then measured by colorimetry (by Ammonium Vanadate-Ammonium Molybdate yellow color method) using a spectrophotometer at 420-nm wavelength (Dynamica, UV–VIS) (Chapman and Pratt 1961). Moreover, the concentration of K and Ca was determined by inductively coupled plasma optical emission spectroscopy (Spectro Arcos ICP-OES, Spectro Analytical Instruments, Kleve, Germany). The uptake of nutrients in the shoot was also determined by the following equation (Deng et al. 2018; Karimzadeh et al. 2021):

$$\text{Nutrient uptake}\left(\text{mg pot}^{-1}\right) = \text{C}\left(\text{mg kg}^{-1}\right) \times \text{SDW}\left(\text{kg pot}^{-1}\right) \tag{1}$$

C: concentration, SDW: shoot dry weight.

Measurement of Relative Water Content (RWC), Electrolyte Leakage (EL), Total Phenol Content (TPC), Proline and Total Soluble Sugar (TSS)

The leaves relative water content (RWC) assay was based on the method suggested by Teulat et al. (2003). Flag leaves were used for measuring RWC. RWC was determined by the following equation:

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100 \tag{2}$$

FW: fresh weight, DW: dry weight, TW: turgid weigh.

Electrolyte leakage (EL) was determined according to the method of Jambunathan (2010). For this purpose, flag leaves discs were transferred to the test tube containing 5 mL of de-ionized water. The tubes were placed in a shaker for 4 h at room temperature and electrical conductivity (R1) in the solution was measured. Samples were then autoclaved at 121 °C for 20 min to determine EC (R2). The EL was determined by the following equation:

$$\%\ \text{EL} = \frac{\text{R1}}{\text{R2}} \times 100 \tag{3}$$

R1: EC (before autoclaving), R2: EC (after autoclaving).

Total phenol content (TPC) was measured using the method described by Singleton and Rossi (1965). Extract of fresh leaves (0.2 mL) was added to 1.5 ml of freshly diluted tenfold Foline-Ciocalteu reagent. The mixture was allowed to equilibrate for 5 min and then 1.5 ml of sodium carbonate solution (7.5% w/v, in deionized water) was added to it. After incubation at room temperature (90 min), the absorbance of the samples was measured using a spectrophotometer at a wavelength of 765 nm. Gallic acid was used as a standard for the calibration curve. The results were then expressed based on mg of gallic acid g^{-1} of leaves fresh weight.

Proline content was determined according to the method of Bates et al. (1973). For estimation of proline, 0.1 g of fresh leaves was homogenized in 5 mL of 3% sulfo-salicylic acid. After centrifugation at 1200 g for 5 min, the extract was filtered. Then 2 ml of the extract was treated with 2 ml of glacial acetic acid and 2 ml of acid-ninhydrin for 1 h at 100 °C. After cooling, 4 mL of toluene was added to the mixture. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance was recorded at 520 nm.

The amount of total soluble sugar (TSS) was determined by the phenol–sulfuric acid method and using a spectrophotometer at A480 nm wavelength (Dynamica, UV–VIS) (Dubois et al. 1956). The total soluble sugar quantity of the samples was calculated with a standard curve of glucose and expressed as mg g^{-1} dry wt.

Microscopic Observations and Estimation of Mycorrhizal Colonization

To observe arbuscular mycorrhizal fungi, roots were cut into 1-cm-long segments. Roots were then cleared by soaking in 10% KOH at 90 °C for 1 h. After placing the roots in 1% HCl for 4 min, roots were stained with trypan blue (0.05% w/v) in lactoglycerol (lactic acid–glycerol–water, 14:1:1 v/v) (Phillips and Hayman 1970). Root colonization was then assessed under a stereo microscope (Luxeo 4Z, USA). The percentage of root colonization was also determined using the grid-line intersect method (Giovannetti and Mosse 1980). Furthermore, photographs of roots along with AM fungi were taken with a digital camera attached to the microscope (Olympus, U-TV0.5xc-2, Japan).

Statistical Analysis

All data presented are the mean of three replicates. The data were statistically analyzed by SAS software and a comparison of means was carried out with the Duncan's multiple range test at $P < 0.05$. The corresponding graphs were also drawn using Microsoft EXCEL software.

Table 2 The results of variance analysis of the effect of strigolactone (GR24), abscisic acid (ABA) and arbuscular mycorrhizal fungi (AMF) on various morphological, physiological and biochemical attributes of Parsi wheat cultivar under drought stress conditions

S.O.V	df	Shoot dry weight	Root area	Mycorrhizal colonization	Phosphorus uptake	Potassium uptake	Calcium uptake	Relative water content (RWC)	Electrolyte leakage (EL)	Total Phenol Content (TPC)	Total Soluble Sugar (TSS)
GR24	3	13.75**	0.007**	156.35**	31.88**	6232.04**	148.07**	120.88**	54.44**	176.93**	363.26**
ABA	1	0.91^{ns}	0.0003^{ns}	3.52^{ns}	41.21**	5683.89**	8.31^{ns}	50.24**	62**	3.92^{ns}	34.57**
AMF	1	16.57**	0.0258**	23,310.82**	13.78^{ns}	26,872.00**	790.63**	436.51**	152**	367.13**	1069.77**
GR24 × ABA	3	1.09^{ns}	0.0013*	8.22^{ns}	7.50^{ns}	1429.61*	41.85^{ns}	14.96^{ns}	3.86*	54.09**	3.82^{ns}
GR24 × AMF	3	3.26**	0.0013*	156.35**	5.84^{ns}	1671.75*	112.73**	17.31*	3.31^{ns}	43.63*	8.86*
ABA × AMF	1	1.15^{ns}	0.0007^{ns}	3.52^{ns}	100.73**	395.87^{ns}	0.15^{ns}	7.40^{ns}	7.19*	13.15^{ns}	0.71^{ns}
GR24 × ABA × AMF	3	0.83^{ns}	0.0010^{ns}	8.22^{ns}	10.62^{ns}	1622.39*	31.60^{ns}	4.07^{ns}	19.74**	13.75^{ns}	5.07^{ns}
Error	30	0.44	0.0004	6.16	3.83	435.84	14.77	5.52	1.30	11.52	2.38
CV (%)		3.66	8.67	11.26	6.80	4.76	5.38	3.13	6.15	9.91	3.61

SOV source of variance, *CV* coefficient of variation

^{ns} non-significant; *, **Significant at the 5% and 1% probability levels, respectively

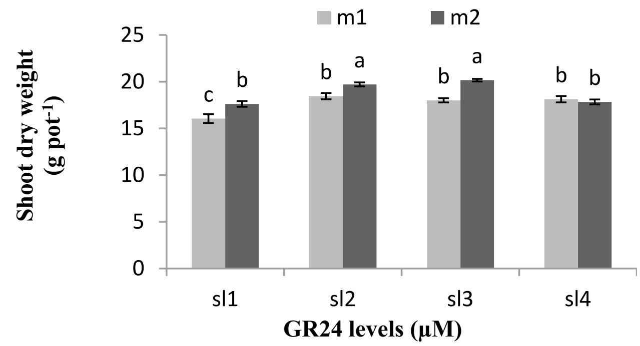


Fig. 2 Effect of different doses of strigolactone (GR24) and arbuscular mycorrhizal fungi (AMF) on shoot dry weight of Parsi wheat cultivar Sl (GR24), Sl1: 0, Sl2: 2.5 μM, Sl3: 5 μM, Sl4: 10 μM; m (AMF), m1: 0, m2: 75 g; Error bars show SE; Data represent mean of three replicates for each treatment

Results

Dry Weight

The shoot dry weight was affected by GR24 and AMF levels and their interactions (Table 2). The results showed that shoot dry weight decreased in the control treatment (sl1m1) under drought stress conditions, while the application of GR24 and AMF increased shoot dry weight. However, the highest value for dry weight was observed in the sl2m2 and sl3m2 treatments. The second and third levels of GR24 along with AMF (sl2m2 and sl3m2) increased shoot dry weight by 25% compared to the control (Fig. 2). As shown in Fig. 2, sl4m2 treatment compared to sl2m2 and sl3m2 treatments decreased dry weight. Based on the results, increasing the concentration of GR24 is not necessary to improve the dry weight of the plant (Fig. 2). This study also showed that the application of ABA had no significant effect on the shoot dry weight (Table 2).

Cumulative Root Length and Root Area

Based on the results, the root area was affected by GR24 and AMF levels and the interaction of GR24 with AMF and GR24 with ABA (Table 2). Drought stress decreased root area and cumulative root length, while GR24 and AMF application increased these parameters (Figs. 3 and 4). In addition, the interaction between GR24 and ABA increased root area compared to the control treatment. However, there was no significant effect between GR24 and ABA levels (Fig. 3b). According to the results, the highest value for root area was observed in the sl3m2 treatment by 66% (Fig. 3a). The increased root area can be due to the growth of the cumulative root length under drought stress conditions (Fig. 4).

Root Colonization

The percentage of root colonization with fungi was affected by GR24 and AMF levels and their interactions (Table 2). The results showed that the effect of sl1, sl2, sl3 and sl4 levels along with AMF on the percentage of root colonization under drought stress was 34.5, 49.80, 49.49 and 42.50%, respectively. As the results and microscopic

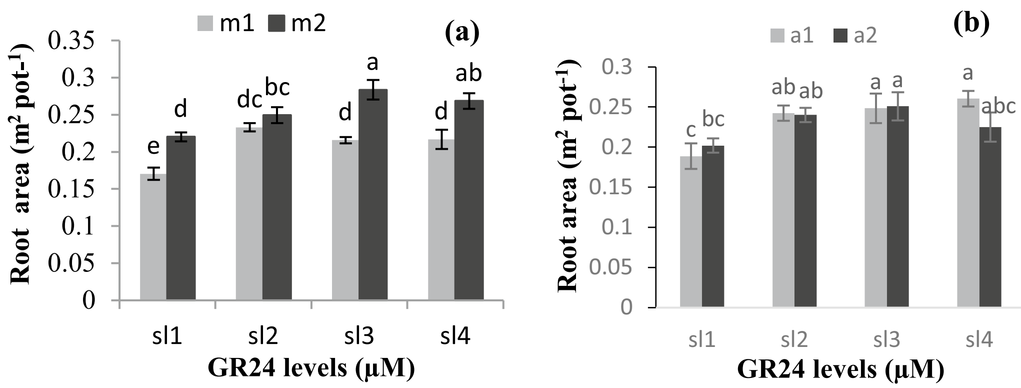


Fig. 3 Effect of different doses of strigolactone (GR24), arbuscular mycorrhizal fungi (AMF) and abscisic acid (ABA) on root area of Parsi wheat cultivar Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g; a (ABA), a1: 0, a2: 5 mgL^{-1}, Error bars show SE; Data represent mean of three replicates for each treatment

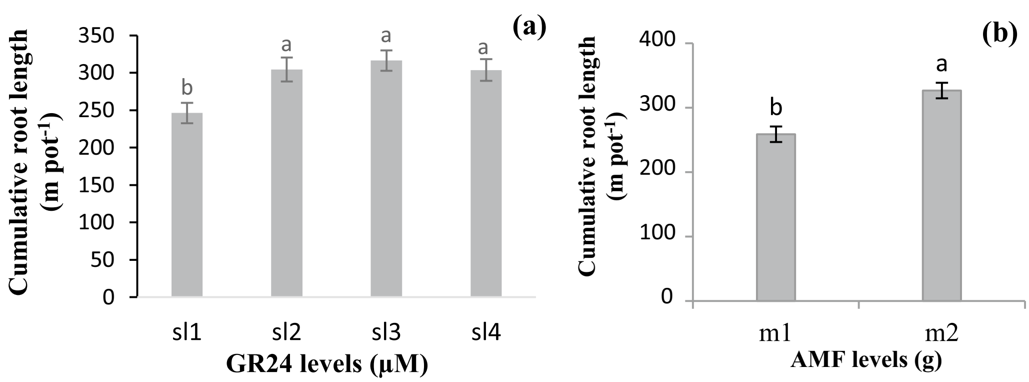


Fig. 4 Effect of different doses of strigolactone (GR24) and arbuscular mycorrhizal fungi (AMF) on cumulative root length of Parsi wheat cultivar Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g; Error bars show SE; Data represent mean of three replicates for each treatment

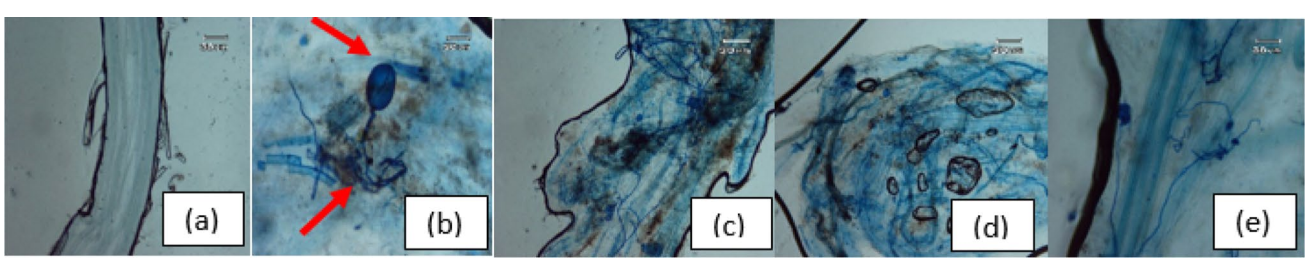


Fig. 5 Effect of different doses of GR24 in the establishment of arbuscular mycorrhizal fungi (AMF) on the root of Parsi wheat cultivar (hyphae and vesicle organs of AMF inside the root); control (**a**); sl1m2 (**b**); sl2m2 (**c**); sl3m2 (**d**); sl4m2 (**e**) Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g

observations indicated (Fig. 5), the second and third levels of GR24 (sl2, sl3) had a considerable impact on root colonization compared to the fourth level of GR24 (sl4). These treatments (sl2 and sl3) increased root colonization by 45% compared to the control. In this study, the increased AMF symbiosis could be due to the application of GR24 which has provided suitable conditions by improving the physio-biochemical characteristics.

Nutrient Uptake

The difference in the uptake of nutrients in plants can be associated with different soil conditions, types and varieties of plants and their growth stages. The uptake of P in the shoot was affected by GR24 and ABA levels and the interaction of ABA with AMF (Table 2). Drought stress decreased P uptake, while the use of GR24 (sl3 and sl4) and ABA increased it (Fig. 6). As shown in Fig. 6, the highest value for P uptake was obtained (13.26%) after the application of the GR24 third level. The fourth level of GR24 also increased P uptake. Nevertheless, it did not have much effect on increasing dry weight (Fig. 2). These results show that the optimal concentration of GR24 and nutrient use efficiency play an important role in increasing wheat growth. According to the results, the interaction of ABA and AMF also increased P uptake by 10% in wheat plant (Fig. 6).

The uptake of K was affected by the levels of GR24, AMF, ABA and their interactions (Table 2). As shown in Fig. 7, the third level of GR24 (sl3) and the second level of ABA (a2) in the presence of AMF (m2) had a considerable impact on K uptake. These treatments increased the uptake of K by 56.12% compared to the sl1m1 treatment.

The uptake of Ca was affected by the levels of GR24, AMF and their interactions (Table 2). The third level of GR24 (sl3) along with the second level of AMF (m2) increased Ca uptake by 32% compared to the control (Fig. 7).

Relative Water Content (RWC) and Electrolyte Leakage

Relative water content was affected by ABA, GR24 and AMF levels and the interaction between GR24 and AMF (Table 2). Non-application of GR24 and AMF (sl1m1) decreased RWC under drought stress conditions. Nevertheless, the application of GR24 along with AMF had a positive impact on RWC (Fig. 8a). As shown in Fig. 8a, the third and fourth levels of GR24 (5 and 10 µM) along

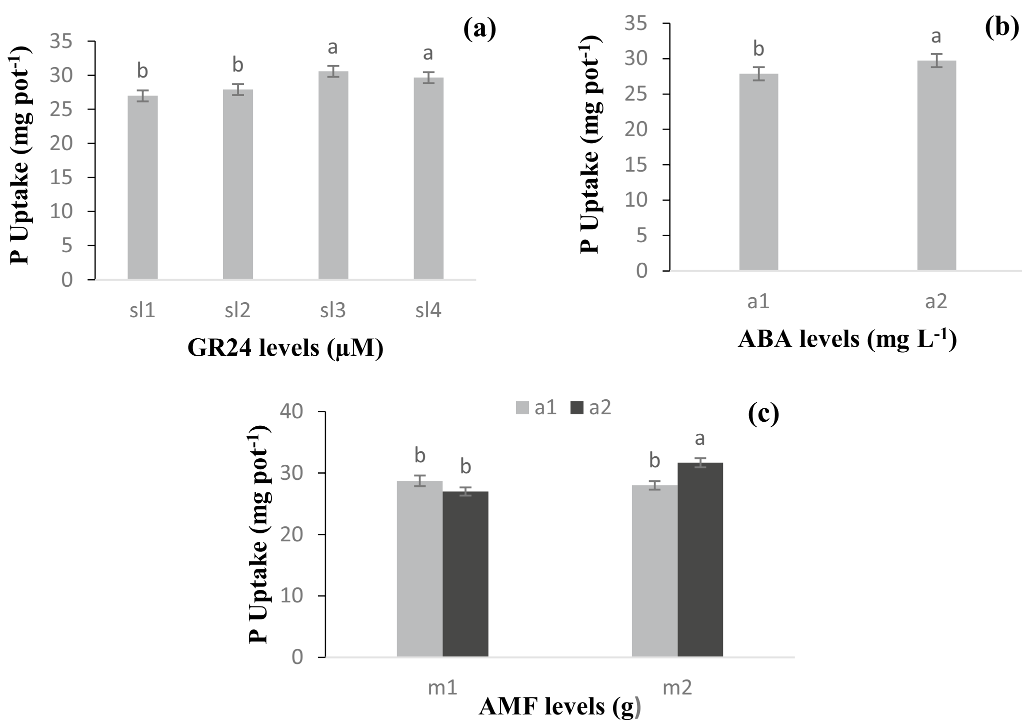


Fig. 6 Effect of different doses of strigolactone (GR24), arbuscular mycorrhizal fungi (AMF) and abscisic acid (ABA) on phosphorus (P) uptake of Parsi wheat cultivar Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g; a (ABA), a1: 0, a2: 5 mgL^{-1}; Error bars show SE; Data represent mean of three replicates for each treatment

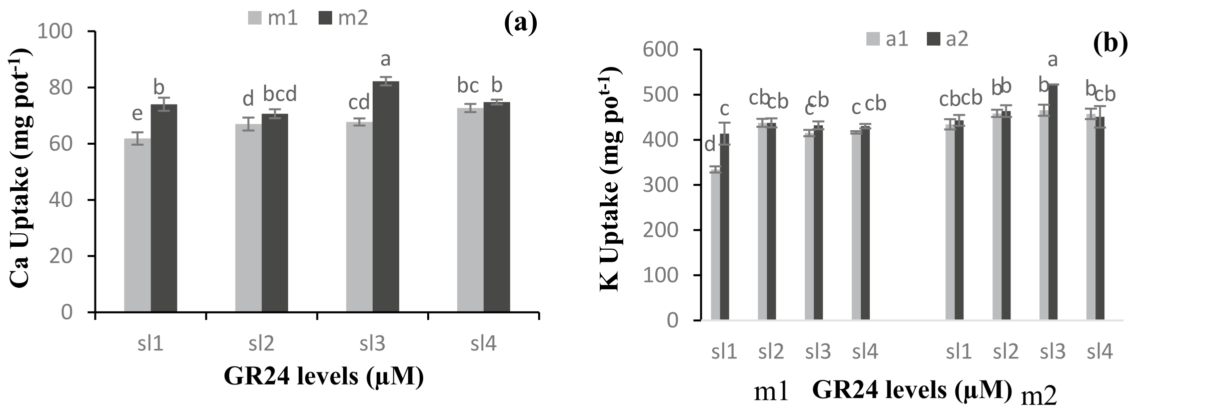


Fig. 7 Effect of different doses of strigolactone (GR24), and abscisic acid (ABA) and arbuscular mycorrhizal fungi (AMF) on calcium (Ca) uptake (**a**) and potassium (K) uptake of Parsi wheat cultivar (**b**) Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; a1: 0, a2: 5 mgL^{-1}; m (AMF), m1: 0, m2: 75 g; Error bars show SE; Data represent mean of three replicates for each treatment

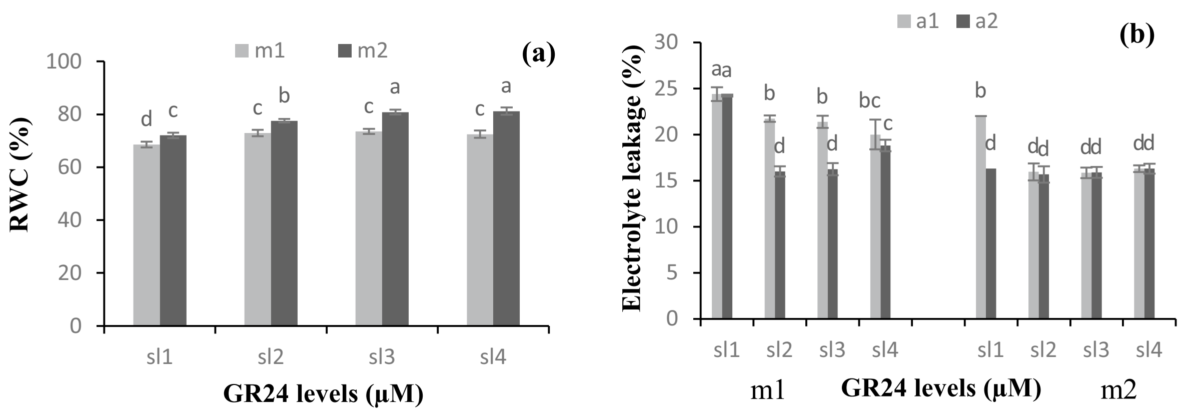


Fig. 8 Effect of different doses of strigolactone (GR24) and arbuscular mycorrhizal fungi (AMF) on and relative water content (RWC) (**a**) and effect of different doses of GR24, AMF and abscisic acid (ABA) on electrolyte leakage of Parsi wheat cultivar (EL) (**b**) Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g; a (ABA), a1: 0, a2: 5 mg L^{-1}; Error bars show SE; Data represent mean of three replicates for each treatment

with the second level of AMF (m2) increased RWC by 20% compared to the control.

Electrolyte leakage was affected by GR24, AMF and ABA levels and their interactions (Table 2). As Fig. 8b shows, the application of GR24, ABA and AMF significantly reduced leaves electrolyte leakage under drought stress conditions. The second and third levels of GR24 (2.5 and 5 µM) and the second level of ABA in the presence of AMF reduced electrolyte leakage by 50% compared to the control (Fig. 8b).

Total Phenol Content (TPC)

Total phenol content (TPC) was affected by GR24 and AMF levels and the interaction of GR24 with AMF and GR24 with ABA (Table 2). Application of GR24 along with AMF increased TPC under drought stress conditions (Fig. 9).

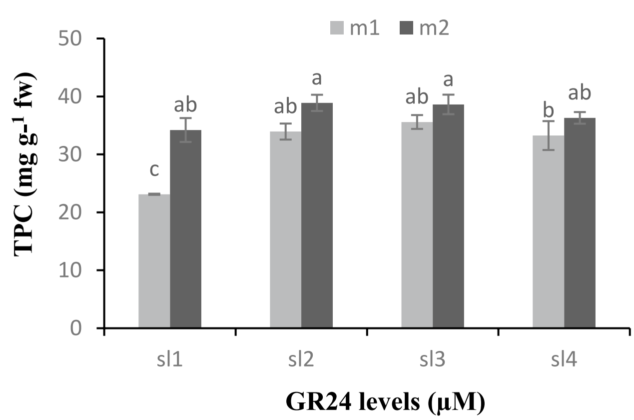


Fig. 9 Effect of different doses of strigolactone (GR24) and arbuscular mycorrhizal fungi (AMF) on total phenol content of Parsi wheat cultivar (TPC) Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g; Error bars show SE; Data represent mean of three replicates for each treatment

Nonetheless, the highest value for TPC was observed in the sl2m2 and sl3m2 treatments. As shown in Fig. 9, the second and third levels of GR24 (2.5 and 5 µM) along with the second level of AMF (75 g) increased TPC by 65% compared to sl1m1 treatment.

Total Soluble Sugar (TSS) and Proline Content

The amount of total soluble sugar (TSS) was affected by GR24, AMF and ABA levels and the interaction between GR24 and AMF (Table 2). According to the results, the combined application of GR24 and AMF was significantly more effective than using them separately (Fig. 10a). As shown in Fig. 10a, the second, third and fourth levels of GR24 (2.5, 5 and 10 µM) along with the second level of AMF (75 g) increased TSS by 45% compared to the control.

Proline content was affected by GR24, AMF and ABA levels and their interactions (Table 2). The addition of GR24, ABA and AMF significantly increased proline under drought stress conditions. As shown in Fig. 10b, the third level of GR24 (5 µM) along with ABA in the presence of AMF increased proline by 63% compared to the control.

Discussion

Drought stress disrupts physiological functions and the activity of soil microorganisms, thereby reducing plant growth. In our investigation, the measurement of morphological and physio-biochemical parameters and nutrient uptake showed that the relationship between GR24 and ABA in the presence of AMF can reduce the effects of drought stress in wheat.

According to the results, non-application of GR24 and AMF decreased shoot dry weight under drought stress conditions. The use of GR24 alone or together with AMF increased shoot dry weight (Fig. 2). The reduction in shoot dry weight under drought stress conditions can be related to the decrease in cell development and division, physiological and photosynthesis disorders, which is consistent with the results of Ahammed et al. (2021) and Sohag et al. (2020). In addition, our results in this research showed that the application of GR24 alone or together with AMF increased root growth (root area) (Fig. 3), uptake of nutrients from the soil (Figs. 6 and 7) and physio-biochemical characteristics (Figs. 8, 9 and 10). Increasing these parameters can be effective in improving the dry weight of the plant, which is consistent with the results of Koltai and Kapulnik (2011), Yoneyama et al. (2012), Wani et al. (2022) and Al-Amri et al. (2023). Sedaghat et al. (2020) reported that GR24 plays an important role in the improvement of wheat biomass by increasing physiological characteristics. Studies have shown that AMF improves the growth of plants such as wheat and maize by increasing the uptake of nutrients, root growth and physio-biochemical characteristics under drought stress conditions (Zhang et al. 2018a, b; Begum et al. 2019a, b; Abdi et al. 2021; Ould Amer et al. 2023), which is in accordance with our findings in this study. In addition, the appropriate concentration of SL in seed priming may be one of the reasons for increasing the symbiosis of the fungus and plant growth. Our results in this study showed that the absorption of GR24 in 2.5 and 5 µM concentrations by the seed has probably led to the activation of metabolic processes in the seeds. Therefore, the increase in plant growth may be due to the effect of GR24 in improving seed physiology. Al-Amri et al. (2023) reported that GR24-pretreated seeds increased root and shoot growth by improving seed

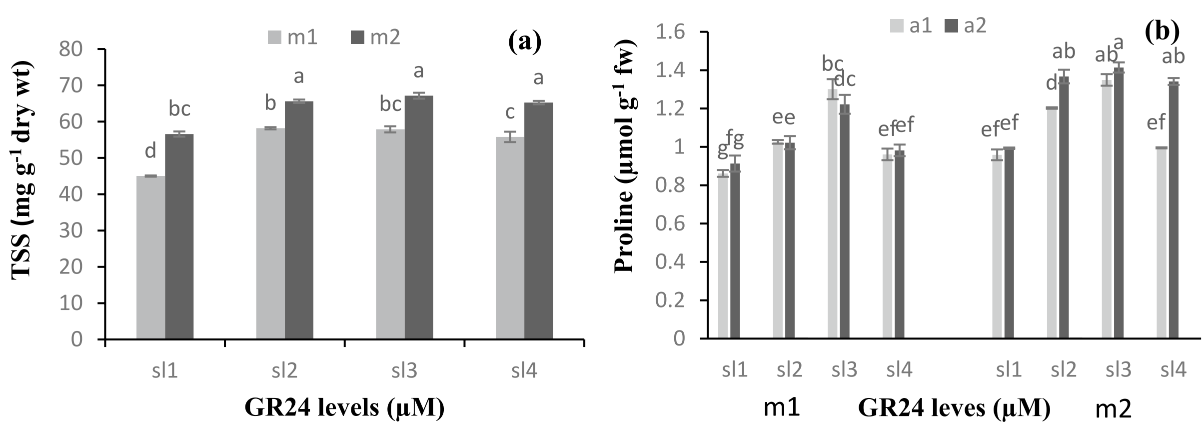


Fig. 10 Effect of different doses of strigolactone (GR24) and arbuscular mycorrhizal fungi (AMF) on total soluble sugar (TSS) and effect of different doses of GR24, AMF and abscisic acid (ABA) on proline of Parsi wheat cultivar Sl (GR24), Sl1: 0, Sl2: 2.5 µM, Sl3: 5 µM, Sl4: 10 µM; m (AMF), m1: 0, m2: 75 g; a (ABA), a1: 0, a2: 5 mg L^{-1}; Error bars show SE; Data represent mean of three replicates for each treatment

physiology. Furthermore, the results showed that ABA and GR24 increased phosphorus uptake and root area (Figs. 3 and 6). Since P and ABA increase root and shoot growth in drought stress conditions (Tariq et al. 2018; de Souza Campos et al. 2019; Neji et al. 2019; De Smet et al. 2006; Li et al. 2017; Muhammad Aslam et al. 2022). Therefore, phosphorus uptake could be one of the effects of GR24 and ABA in improving plant growth.

Based on the results, the application of GR24 increased root colonization. The highest values of root colonization were observed in the second (2.5 μM) and third (5 μM) levels of GR24. The increased root colonization in Sl2 and Sl3 may be due to providing suitable conditions for activity and symbiosis establishment of the AMF in the rhizosphere. Sl2m2 and Sl3m2 treatments enhanced plant growth, nutrient uptake, and morphological and physio-biochemical parameters, while the fourth level of GR24 (Sl4m2) had a less significant impact on these parameters. Min et al. (2019), Sadaqat et al. (2020) and Wani et al. (2022) reported that the appropriate concentration of GR24 is essential for increasing plant growth. Therefore, the concentrations of GR24 used in research can be one of the reasons for its different effects on improving plant growth. SLs also increase mycorrhizal colonization in plants by hyphal growth in AMF, thereby enhancing nutrient uptake. Therefore, the effect of nutrients such as phosphorus and potassium on the improvement of photosynthesis provides organic carbon in the form of sugars for the fungus. Besides this, there is a positive correlation between AMF and the accumulation of soluble sugar, which can increase the symbiosis of the fungus with the plant roots (Wang et al. 2017; Alvi et al. 2022), which is in accordance with our findings in this study.

In the present study, GR24, AMF and ABA enhanced nutrient uptake in wheat (Figs. 6 and 7). The root system architecture is an important factor in nutrient uptake and reduced consumption of fertilizers (Zhou et al. 2016; Campos et al. 2018; Sidhu et al. 2018). GR24, AMF and ABA increase plant root growth which allows plants to absorb more nutrients and water from the soil. Studies have demonstrated that GR24 increases the growth of wheat roots under drought stress conditions (Song et al. 2023). Chen et al. (2017) and Begum et al. (2019a, b) reported that AMF inoculation can effectively increase the nutrient uptake of host plants by improving root growth, which is consistent with our findings. There is also a positive correlation between root cation exchange capacity and nutrient uptake. Among nutrients, P increases root cation exchange capacity (Sharma et al. 1990). Therefore, the increased root growth could be one of the mechanisms of GR24, ABA and AMF to improve nutrient uptake, which may be even more important under drought stress conditions. In addition, nutrients may be involved in mitigating drought stress by improving dry matter production and its distribution between root and shoot. Studies showed that the increase in plant growth under drought stress can be due to the improvement of some physio-biochemical characteristics such as plant water status, cell membrane stability, chlorophyll content, photosynthesis and sugar and proline accumulation (Ayyaz et al. 2021; Bano et al. 2021).

The relative water content (RWC) is a useful variable to evaluate the water status of plants, which can show the ability of plants to tolerate drought stress (Kadioglu et al. 2011). The present study showed that the non-application of GR24 and AMF under drought stress conditions reduced RWC (Fig. 8a). The reduction in RWC under drought stress can be due to the decrease in water content of the plant tissue, the loss of turgor in the leaves and the closing of the stomata. Therefore, drought stress probably caused a decrease in plant performance through disruption of the photosynthesis process, which is consistent with the results of Dąbrowski et al. (2019) and Sedaghat et al. (2021). According to the results, the application of GR24 along with AMF increased RWC (Fig. 8a). An increase in RWC in response to GR24 foliar application in drought stress conditions has been reported in wheat plants (Sedaghat et al. 2017). GR24 also improves water uptake by increasing the expression of genes involved in cell wall biogenesis and root architecture (Song et al. 2023). Moreover, Zhang et al. (2018a, b) and Begum et al. (2019a, b) reported that the symbiosis of wheat and maize roots with AMF improves RWC by increasing root growth, nutrient uptake, proline and sugars. Studies have shown that P and K also play an important role in increasing RWC under drought stress conditions (Zahoor et al. 2017; Tariq et al. 2018), which confirms our findings. Therefore, the enhancement of RWC in this study may be due to the effect of GR24 and AMF on root growth, the accumulation of sugar and proline (Figs. 3 and 10) and the uptake of P and K (Figs. 6 and 7).

In the present study, the electrolyte leakage increased under drought stress conditions. Nevertheless, the application of GR24, ABA and AMF decreased this parameter (Fig. 8b). Furthermore, the use of GR24, ABA and AMF had a positive impact on root growth (Fig. 3) and the uptake of P, K and Ca (Figs. 6 and 7). The increased electrolyte leakage in plants is related to disruption and destruction of the cell membrane. Studies have shown that cell membrane stability is affected by several items such as ABA (Parveen et al. 2021), GR24 (Sedaghat et al. 2017), AMF (Abdi et al. 2021), P, K and Ca (Demidchik et al. 2018; Tariq et al. 2018; Thor. 2019; Anokye et al. 2021; Fang et al. 2022). Sedaghat et al. (2020), Park et al. (2021) and Mujahid et al. (2023) reported that GR24 and ABA increase the stability of the cell membrane through the synthesis of phenolic compounds and osmotic regulation substances such as proline and soluble sugars, which is consistent with our results. Therefore, the decreased electrolyte leakage in wheat could be due to the effects of GR24, AMF and ABA on increasing cell membrane stability.

To adapt to abiotic stresses, plants use complex mechanisms, including the biosynthesis of phenolic compounds under drought stress conditions (Shirani Bidabadi and Sharifi 2020). Increasing antioxidants such as phenol compounds can be one of the efficient strategies to deal with drought-induced oxidative stress. Based on the results, the application of GR24 (2.5 and 5 μM) along with AMF increased the amount of phenol by 65% in wheat (Fig. 9). Our results showed that the wheat plant strengthened its antioxidant defense system by increasing phenolic compounds to deal with drought stress. Mujahid et al. (2023) reported that the application of GR24 increased phenol in the ajwain plant. Furthermore, various studies have demonstrated that AMF (Begum et al. 2019a, b), K (Ahanger and Agarwal 2017), and P (Loudari et al. 2023) enhance the amount of phenol under abiotic stress conditions. Abdi et al. (2021) and Nahuelcura et al. (2022) showed that the effect of AMF on phenol amounts in wheat cultivars under drought stress depended on the rate of AMF symbiosis with plant roots, plant resistance, and cultivar type. In the present study, GR24 has probably increased the amount of phenol in wheat under drought stress by improving the function of AMF in the rhizosphere and nutrient uptake.

Proline, a metabolite and signaling molecule, increases water uptake and root growth under drought stress conditions (Biancucci et al. 2015; Hosseinifard et al. 2022; Patanè et al. 2022). Proline also protects the structure of proteins and the cell membrane under drought stress conditions. Proline plays a key role in the activity of antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Ali et al. 2023). Studies have shown that SL (GR24) and ABA enhance proline by pyrroline-5-carboxylate synthetase (P5CS) enzyme activity (Kaur and Asthir 2020; Al-Amri et al. 2023). According to the results, the application of GR24 and ABA in the presence of AMF increased proline in wheat. Consistent with our results, it was shown that ABA and GR24 increase proline in wheat under drought stress conditions (Kaur and Asthir. 2020; Sedaghat et al. 2020). Abdi et al. (2021) and Begum et al. (2019a, b) also reported that AMF increased the amount of proline in wheat and maize. Therefore, the increased root growth, water uptake and plant resistance to drought in this study may be due to proline accumulation.

Total soluble sugar (TSS) plays an important role in increasing the resistance of plants, root growth and water uptake under drought stress conditions (Du et al. 2020). Our results demonstrated that GR24 and AMF increased the amount of TSS, and the uptake of P and K (Figs. 6, 7 and 10). Studies have shown that phosphorus and potassium increase the amount of TSS in plants by improving physiological processes such as photosynthesis (Ahanger and Agarwal 2017; Loudari et al. 2023). Therefore, the role of GR24 and AMF in increasing the amount of TSS under drought stress may be due to the uptake of P and K. Similar to our results, Sedaghat et al. (2020) and Yang et al. (2023) observed that GR24 increased the amount of TSS in wheat and alfalfa under drought stress conditions. Yooyongwech et al. (2016), Begum et al. (2019a, b), and Abdi et al. (2021) demonstrated that AMF also increases the amount of TSS in plants, which accords with our findings in this study. Furthermore, in the present study, the improvement of AMF symbiosis could be due to the ability of GR24 to accumulate sugar under drought stress conditions.

Conclusion

In this study, the application of GR24 alone and together with AMF reduced the effects of drought stress by increasing some morphological characteristics, uptake of nutrients and physio-biochemical parameters. Moreover, this study highlighted the positive role of GR24 and AMF in the growth of wheat through nutrient uptake under drought stress conditions. According to the results, the seeds primed with GR24 at the third level (5 μM) along with AMF under drought stress of 65% FC increased the root area, uptake of P, K and Ca, RWC, total soluble sugar, proline and total phenol and reduced electrolyte leakage. Therefore, the appropriate concentration of GR24 can significantly influence the growth of wheat by increasing the symbiosis between AMF and root. It was also found that the second level of GR24 (2.5 μM) had almost the same performance as the third level of GR24 (5 μM), and economically, the second level of GR24 can also be useful. Moreover, the results showed that ABA had a positive impact on wheat growth under drought stress conditions by increasing the amount of proline, root expansion, and P and K uptake. In addition, this research showed that the application of GR24 and ABA increased the interaction between AMF and plants by improving root growth, nutrient uptake, and physio-biochemical characteristics such as accumulation of soluble sugar. Therefore, the application of GR24 and ABA in the vegetative growth stage increased the growth of wheat plants by improving the physio-biochemical characteristics, root growth, nutrient uptake and interaction between plants and fungi under drought stress conditions.

Acknowledgements Authors acknowledge the Vice president for Research and Technology of Ferdowsi University of Mashhad, Iran for financial support of this project through the grant number of 3/48696.

Author Contributions MM: Conducted the experiment and performed lab analyses. MM and RK wrote, reviewed, and edited the manuscript. RK was the project supervisor, designed the research and provided all the technical support during the laboratory work. RK: Main idea. RTA helped in the use of seed priming. All authors read and approved final version of manuscript.

Funding The present study was financially supported by the Vice president for Research and Technology of Ferdowsi University of Mashhad, Iran (grant number of 3/48696).

Data Availability This work is part of Maryam Moosavi PhD thesis in the Department of Soil Sciences, Ferdowsi University of Mashhad, Iran. The data that support this study cannot be publicly shared due to ethical or privacy reasons and may be shared upon reasonable request to the corresponding author if appropriate.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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