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Research article

The morpho-physiological responses of a tolerant and sensitive wheat (*Triticum aestivum* L.) cultivar to drought stress and exogenous methyl jasmonate

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Abstract

One of the most significant abiotic factors that has a negative impact on wheat productivity globally is drought. To comprehend the impacts of drought on wheat and propose remedies, numerous studies are carried out on various wheat varieties. In this study, 50 µM of methyl jasmonate (MeJA) was applied to tolerant Gün 91 and sensitive Bezostaja wheat cultivars and they were exposed to drought stress for 15 days. The responses of MeJA application on wheat development and physiology, as indicators of drought tolerance, were investigated comparatively. Wheat's morphology was negatively impacted by drought stress, which also decreased the crop's relative water content (RWC) and protein content while raising its soluble sugar level. Furthermore, Gün 91, a tolerant cultivar, came to the fore as the cultivar with higher shoot-root length, RWC, total soluble sugar and protein contents compared to Bezostaja cultivar as a result of drought application. Exogenous MeJA application, cause to increase in content of osmolytes (total soluble sugar, protein) compared to the drought group and had an improving effect in maintaining the water status of wheat seedlings. Hence, the RWC increased from 48.90% to 66.87% in the tolerant Gün 91 cultivar, but no change was observed in Bezostaja cultivar. Applying 50 µM of MeJA increased the protein by 4.42%, total soluble sugar by 19.92%, and RWC by 36.74% in Gün 91 cultivar while increasing protein by 3.11% and total soluble sugar by 11.02% in Bezostaja cultivar. Moreover, there is not any significant effect of MeJA observed on the shoot-root length of both cultivars and the RWC of Bezostaja cultivar. When all results are evaluated together, exogenous MeJA application may positively affect the response of wheat seedlings, and minimize the damaging effects so we can suggest using MeJA and cultivars that are resistant to drought stress for wheat yield.

Keywords: *Drought; methyl jasmonate; protein; sugar; wheat*

1. Introduction

One of the most extensively cultivated grains is wheat (*Triticum aestivum*) ranging from temperate to subtropical regions (Ahmed et al., 2019). Generally, the basic food of the world's population (approximately one-third of) is wheat and as the first cereal product in most developing countries. It has the potential to be used in numerous fields such as flour, bread, biscuits, cakes, cookies, pasta, noodles, beverages, and biofuel production (Knott et al., 2009). It is very important to increase wheat production for its use in many fields and providing the sufficient food for the rapidly increasing world population. Food and Agriculture Organization (FAO) reported "A further increase in population will lead to inadequate levels of food consumption and put pressure on food resources to increase even more". Based on these predictions, efforts have been made to develop product varieties that can cope with different types of stress and provide higher yields for a long time (FAO, 2023).

Drought has gained relevance because of the effects of recent global climate change, and it is one of the main factors limiting world wheat yield. It is predicted that global warming and climatic fluctuations will lead to significant losses in wheat yield by increasing the frequency of droughts in the coming years (Rijal et al., 2021). Drought stress delays plant growth and

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crop yield by affecting the morphological, biochemical, physiological, and molecular properties of plants. Additionally, it results in a significant decrease in the efficiency of photosynthetic activity, water use, stomatal conductance and leaf area (Farooq et al., 2019). Plants undergo morphological changes that are critical to respond to water deficiency, such as a decrease in growth rate, a deep root system and altered root-to-shoot ratio are used to prevent drying out. Furthermore, plant cells exposed to drought accumulate some organic compounds (proline, glycine-betaine, soluble amino acids, sugars, etc.), in order to maintain their turgor status, reduce their osmotic potential, and facilitate water uptake from the external medium (Vinod, 2012). Methyl jasmonate (MeJA), a naturally occurring phytohormone that is a methyl ester of jasmonic acid, controls plant development and growth by preserving the biochemical, morphological, and physiological process of plants. It functions in some signal transduction systems as a signaling chemical, activating specific enzymes that catalyze biosynthetic events to produce protective substances such polyphenols, alkaloids, and antioxidants. Additionally, it has been shown that these signaling molecules function systemically, causing the activation of several defense genes that have been activated by a stressful environment. As a result, when exposure exogenously, these signaling molecules increase abiotic stress tolerance (Walia et al., 2007; Fahad et al., 2014). Some plant growth regulators synthesis is reduced by abiotic stressors. Therefore, for reducing the detrimental effects of abiotic stress, the exogenous usage of growth regulators can make up for the plant's endogenous shortage and enhance its tolerance (Ashraf and Foolad, 2007). While the exogenous use of methyl jasmonate regulates various plant physiological responses that lead to the development of tolerance to stress, these effects of MeJA vary depending on the tissue, plant species, severity, the exposure time of drought stress, and the application amount (Karami et al., 2013).

The growing demand for wheat and the fact that consumption in sub-Saharan Africa has recently reached around 650 million tons per year have put further pressure on the demand for wheat (Mason et al., 2012). In this manner, creating drought-tolerant wheat cultivars with higher water utilize productivity is of best need, particularly within the setting of food supportability. The using of tolerant cultivars is known as an effective method for increasing yield and operating in arid and semi-arid regions. Finding strategies to boost output under various environmental situations, such as the selection of tolerable cultivars to drought stress, may benefit from additional research on physiological and biochemical features and the exogenous application of suitable growth regulators. The aim of the study is; to investigate the morphological and physiological properties of two different wheat cultivars in various irrigation regimes with the application of methyl jasmonate exogenously.

2. Materials and methods

2.1. Growth conditions and MeJA application

Two wheat cultivars, including drought stress-sensitive Bezostaja and tolerant Gün 91 cultivars, were used in the study. Sodium hypochlorite (5%) was used for surface sterilization. The soil that was composed of peat and perlite (1:1) added to the pots in which the seeds would be planted, and each of them was watered until reaching the field capacity. Fifty of the sterilized seeds were planted in each pot and grown in the plant growth chamber under 4000 lux light intensity, 25°C temperature, and 16/8 hours light/dark photoperiod conditions for 43 days. All pot experiments were irrigated with ¼ Hoagland nutrient solution based on the field water capacity for 28 days, and drought stress applications were initiated after this period. At this stage, while the control group continued to be irrigated, the application groups were left to develop without irrigation for 15 days. On the 14th day after the start of the application, 50 µM MeJA was applied exogenously to the plants under drought stress by spraying it. All plants were harvested 24 hours after the MeJA application, and their root-shoot lengths and RWC were determined. Some fresh material samples from the control and application groups were left to dry in the shade and at room temperature to be used in the analysis of total soluble sugar content.

2.2. Root-shoot length

During the plant harvesting of the cultivars in the control and drought stress-applied groups, the root and shoot lengths were measured by a millimeter ruler in 10 repetitions and expressed as cm plant^{-1}.

2.3. Relative water content (RWC%)

Immediately after the plants were harvested, fresh weights of randomly selected plant samples in the control and application groups were measured and recorded. The plant materials, whose fresh weights were measured, were kept in distilled water for 6 hours so that they would reach osmotic balance, and at the end of this period, their turgor weights were recorded. At the next stage, the seedlings were dried at 50°C, dry weights were recorded by weighing them again. The -RWC of the materials in the control and application groups were calculated according to the formula below, based on these three measured values (Hu et al., 2010).

RWC (%) = [(Fresh Weight-Dry Weight) / (Turgor Weight-Dry Weight)] x 100

2.4. Total soluble sugar content

By using the phenol-sulfuric acid method, the amount of total soluble sugar was measured (Dubois et al., 1956). 50 mg sample was weighed at and ethanol (5 ml) was added before being placed in a bath at 80°C for 60 minutes. Test tubes were centrifuged at 5000 rpm for 20 minutes. Then, 1 ml of supernatant, 1 ml of 5% phenol, and 5 ml of concentrated sulfuric acid (H_2SO_4) were added and mixed by vortex. The total soluble sugar content was then reported as mg g^{-1} dry matter by using a UV-Vis spectrophotometer readings at a wavelength of 490 nm.

2.5. Protein content

0.5 g sample of the fresh plant material was homogenized in 100 mM phosphate buffer (pH 7.0) and centrifuged at 14000 rpm for 20 minutes at +4°C. After adding 480 ml of distilled water and 5000 ml of Bradford solution to 20 µl of the supernatant, UV-Vis spectrophotometer was used to detect the absorbance at a 595 nm wavelength. A standard curve made with bovine serum albumin (BSA) and protein contents were expressed as µg g^{-1} fresh weight (Bradford, 1976).

2.6. Statistical analysis

Three replications of the analysis were conducted using a random design. The data acquired from the analyses were analysed by one-way analysis of variance (ANOVA). Also, Duncan's test using the statistical analysis application SPSS 21.0. Each wheat cultivar was subjected to statistical analysis on its own. Statistics were considered as significant at a *p*-value of ≤0.05.

3. Results and discussion

In the present study, tolerant and sensitive wheat cultivars were selected, and their morphological and physiological responses and the effects of MeJA application were determined\ comparatively. Drought stress-sensitive Bezostaja and tolerant Gün 91 cultivars were chosen as the plant materials. The agricultural and climatic information on the two cultivars is available at www.wheatatlas.org (Wheatatlas, 2023). As a result of drought applications, the shoot length in both wheat cultivars increased less compared to the control, whereas the root length increased due to stress. In the control group, the plants continued to develop since irrigation was maintained. However, since there was a slowdown in development due to lack of water in drought-applied plants, the shoot length was shorter. The shoot length of Gün 91 was not statistically significant but showed greater improvement over Bezostaja (Table 1).

Table 1
Effect of MeJA on shoot and root length of wheat cultivars grown under drought stress conditions.

Treatment		Shoot length cm plant^{-1}	Root length cm plant^{-1}
	Control	50.95±0.89^{a}	12.70±0.56^{b}
Gün-91	**Drought**	44.75±1.35^{b}	16.30±0.21^{a}
	Drought + MeJA	42.10±1.14^{b}	16.10±0.23^{a}
	Control	47.30±1.42^{a}	11.20±0.65^{b}
Bezostaja	**Drought**	43.10±1.22^{b}	14.30±0.32^{a}
	Drought + MeJA	43.25±0.75^{b}	14.10±0.44^{a}

*All values are given as Mean ± Standard Deviation. The same wheat variety and different character in the same column indicate that the difference between the means is significant ($p \leq 0.05$)

Wheat undergoes a number of morphological changes in response to drought, which are clearly visible at different plant development stages. In general, the shoot part and the root part of the wheat morphological response can be separated. The reduction in leaf size, shape, area, pubescence, and shoot length is included in the shoot component (Denčić et al., 2000). The growth of plants that continue to develop under normal conditions occurs in the presence of cell division, cell expansion, and turgor pressure (Rijal et al., 2021). In this study, it was considered that the water losses resulting from the drought application might lead to insufficient turgor formation and a possible decrease in the amount of auxin/cytokinin hormones adversely affected the plant growth. Our results on the effect of drought stress on growth are parallel with other studies. The responses of drought stress on the wheat reported that water deficiency led to a reduction in shoot length (Abdalla and El-Khoshiban, 2007). Likewise, many other researchers have reported a reduction in shoot growth when wheat is exposed to drought conditions (Azooz and Youssef, 2010; Farooq et al., 2013).

Plant roots penetrate the depths of the soil to absorb water in water shortage. Numerous studies have suggested that plant drought tolerance is correlated with root volume, weight, length, and density. To combat drought stress, wheat uses its roots' osmotic adjustment, greater root penetration and root density into the soil, and increased root-to-shoot ratio (Ali et al., 2020). If water potential decreases, it is ensured that the turgor level is maintained up to a certain level by making osmotic adjustments in the root, and potential gradient of water is rearranged for intaking of water. In our study, drought application promoted the increase in root length in both sensitive and tolerant cultivars. The root length, which increased due to drought stress, was more in tolerant wheat cultivar according to sensitive one (Table 1). As a result; it can be said that root length increased in the wheat cultivars so that plants could reach the water deep in the soil and use this water efficiently. In the comparative study of the standard Hartog and the Seri wheat genotype, which forms denser roots, the Seri genotype had a longer root length and thus contributed to the increase in yield by allowing more water uptake in deeper soil layers (Manschadi et al., 2006). While Ahmed et al. (2019) revelated that root length was longer in drought-tolerant wheat genotypes, Kato et al. (2007) and Kuru et al. (2021) indicated that, root length increased in case of water deficiency in different rice cultivars. Furthermore, it was observed that the MeJA application had no effect on root and shoot length in both wheat cultivars. The morphological changes due to drought occur as a result of a long duration of drought. When plants are exposed to short-term water deficiency, they close their stomata as the first response before the above-mentioned morphological responses and prevent water loss (Mahajan and Tuteja, 2005). Since the MeJA application was performed for a short time (24 hours) in our study, it was normal that there were no morphological changes such as root and shoot length.

The RWC is a useful measurement for estimating the water status of the plant and provides information about a genotype's capacity for absorption of water from the soil. In our study, drought stress reduced the RWC in both cultivars; however, the reduction rate was higher in the drought-sensitive Bezostaja cultivar. Moreover, while MeJA application improved RWC in the tolerant Gün 91 cultivar, it had no positive effect on the sensitive Bezostaja cultivar (Fig. 1). Based on protoplasmic permeability, RWC is a measure of a plant's sensitivity to drought stress, and higher RWC indicates a drought-tolerant plant (Raja et al., 2020). According to Pazirandeh et al. (2015)'s study on barley genotypes, the RWC decreases during drought stress, although the usage of methyl jasmonate improved the decline. Drought-tolerant Sirvan and sensitive Pishtaz wheat cultivars were exposed to drought, and MeJA was applied, and consequently, it was reported that drought-tolerant Sirvan cultivar had a higher RWC rate and low-concentration MeJA application had an improving effect in Sirvan cultivar (Javadipour et al., 2021). These literature data are parallel with the results of our study. Furthermore, according to Ahmad et al. (2017) and Bali et al. (2019), the administration of jasmonic acid enhanced the RWC of tomato and broad bean seedlings during heavy metal stress. By influencing plant stomatal cells, methyl jasmonate raises the RWC of the leaf and improves the water status, membrane stability, and water transport system of stressed plants. Closing the stoma likely causes an increase in RWC since less water is lost from the plant cells as a result (Javadipour et al., 2021).

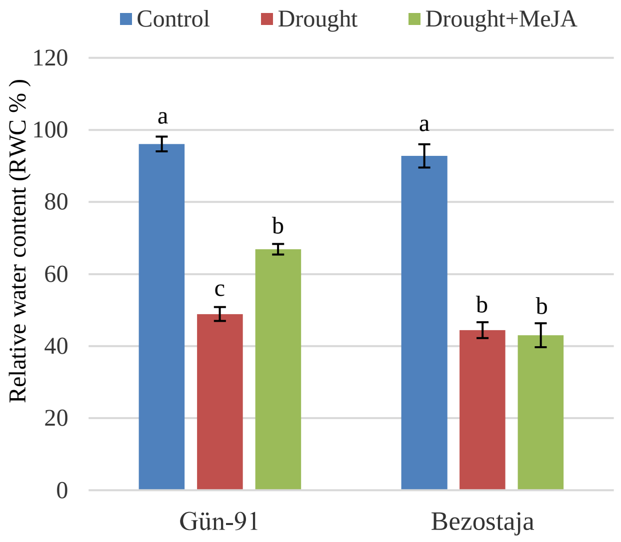

Fig. 1. Effect of MeJA on RWC of wheat cultivars grown drought stress conditions. Vertical bars indicate ±SE.

In general, sugars often function as osmoprotectants and membrane stabilizers in abiotic stress conditions. Furthermore, they also maintain the leaf water content and osmotic compatibility of plants exposed to drought stress conditions (Xu et al., 2007). Under drought stress, glucose causes stomatal closure and improves plant adaptability (Osakabe et al., 2013). In our study, drought stress increased the soluble sugar content in both cultivars, but the rate of increase in the drought-tolerant cultivar was higher. Furthermore, MeJA application increased the soluble sugar content in both cultivars compared to drought application, and the rate of increase was 19.92% in Gün 91 cultivar and 11.02% in Bezostaja cultivar (Fig. 2). In a study, sugar accumulation in the wheat cultivar increased due to drought, which was stated to have contributed to high wheat yield (Shi et al., 2016). Drought stress cause to increase the soluble sugar content of wheat genotypes, Sids 1 and Beni-Suef 5, however jasmonic acid use during drought considerably decreased the soluble carbohydrate content in both cultivars (Abeed et al., 2021). Contrary to these findings, other researches have shown that the exogenous administration of methyl jasmonate raises the soluble sugar concentration (Wu et al., 2012; Tayyab et al., 2020). This difference may be associated with the balance between starch accumulation in plants and sucrose, the transport form of carbohydrate in plants (Xu et al., 2015). The findings of our investigation are in agreement with those of Wu et al. (2012) and Tayyab et al. (2020). It can be said that the MeJA application attempted to preserve the osmotic balance against drought by increasing the accumulation of soluble sugar and, thus, contributed to the more efficient uptake of water from the soil by plants.

Drought, among other environmental stresses, harms proteins, membrane lipids, and other biological components by causing oxidative stress and the formation of free oxygen radicals (Dąbrowski et al., 2019). The results of our study revealed that protein content decreased in wheat cultivars exposed to drought stress. The decrease in protein content due to water deficiency was reported by many researchers (Yang et al., 2015; Faraji and Sepehri, 2020). MeJA applied with drought application cause to an increase in protein content in two cultivars compared to the drought application group (Fig. 3). The rate of increase was found to be 4.42% in Gün 91 cultivar and 3.11% in Bezostaja cultivar. Soaking corn seeds in MeJA may reduce the negative effects of drought stress by increasing antioxidant activities, proline and carbohydrate content as well as total protein. (Abdelgawad et al., 2014). Application of MeJA has been reported to play a protective role against water stress and increase protein levels in many plants (Tayyab et al., 2020; Abeed et al., 2021).

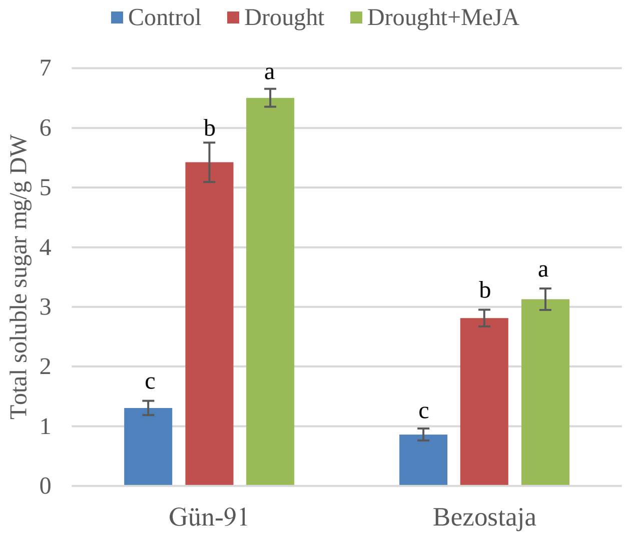

Fig. 2. Effect of MeJA on total soluble sugar of wheat cultivars grown drought stress conditions. Vertical bars indicate ±SE.

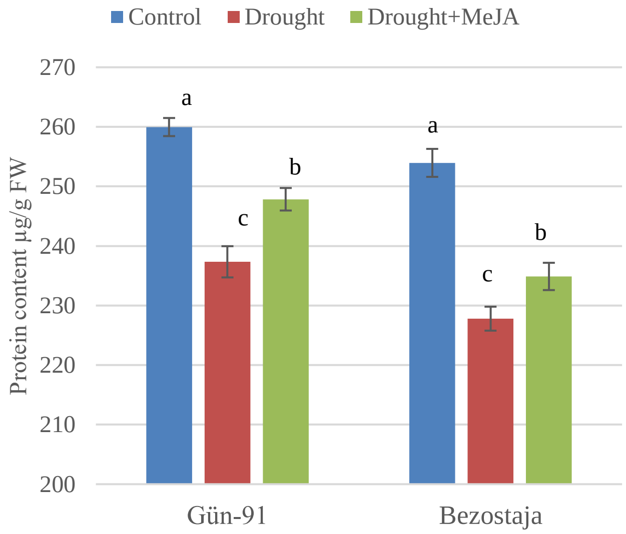

Fig. 3. Effect of MeJA on proteins of wheat cultivars grown drought stress conditions. Vertical bars indicate ±SE.

4. Conclusion

Both wheat cultivars were negatively affected by the lack of irrigation, which also changed the morphological and physiological characteristics. Nevertheless, the methyl jasmonate application had positive effects on the RWC, soluble sugar and protein contents. Exogenous applications of MeJA can mostly avoid the destructive impacts of drought-induced osmotic stress in wheat seedlings by balancing osmolytes such as sugar. Furthermore, they can also increase drought tolerance in wheat by preventing protein denaturation due to stress. The fact that Gün 91 cultivar had higher RWC, soluble sugar and protein contents after the MeJA application revealed that it was more tolerant compared to Bezostaja cultivar. In conclusion, using MeJA is an alternative to change adverse results of drought stress together with the selection of appropriate cultivars. The results of our study will support research programs that attempt to develop anti-drought stress applications in order to increase the yield of wheat cultivars.

Conflict of interest: The author declares that he has no conflict of interests.

Informed consent: The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

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