Physiological and biochemical responses of onion plants to deficit irrigation and humic acid application

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Abstract: Onion is an important crop with significant roles in human diets. The growth, yield, and quality of vegetable crops, including onions, are more vulnerable to water stress than other crops. In this study, different levels of deficit irrigation (DI) as factor A (a1: 80%, a2: 70%, and a3: 60% of soil field capacity [FC]) and humic acid (HA) as factor B (b1: without and b2: with HA application) were evaluated on onion growth characteristics in a factorial design with four replications. The results showed that the interaction of DI and HA was significant on leaf protein, peroxidase (POD), superoxide dismutase (SOD), and on bulb protein and potassium (K) concentrations. The highest record of these traits was observed in a3b2 (highest DI with HA application), and their lowest was in those at first level of DI (a1). Leaf protein and, to a lesser extent, bulb protein were increased by DI and HA applications. DI at 60% but not at 70% FC significantly reduced bulb fresh weight. There was a gradual increase in leaf proline, soluble sugars, protein, catalase (CAT), POD, SOD activity, and bulb K by application of DI; however, most of bulb traits including protein, iron (Fe), zinc (Zn), and CAT and POD activity were increased only under highest DI level (a3: 60% FC). However, application of HA further increased the soluble sugars and protein concentration as well as the POD and SOD activities of leaves, and protein, Fe, K concentrations, and CAT activity of bulbs under DI. The results indicated that HA benefitted onion growth particularly under DI conditions.

Keywords: antioxidant enzymes, biostimulant, deficit irrigation, drought, leaf protein, minerals

1 Introduction

Agriculture is facing the world’s most complicated challenges including climate change, water crises, drought, soil degradation, and food insecurity [1]. For adequate food production, adopting some techniques and strategies are necessary to avoid these challenges or to enhance plant production under the changing environment [2]. In many parts of the world, agricultural food production is restricted due to limited water supply or its low quality [3,4]. Effective practical approaches are required to avoid or diminish the water stress dimensions on crop production. These including application of modern irrigation systems, deficit irrigation (DI), changing the cultivation programs including cultivation date and season, crop types, cropping system, and application of some organic or inorganic effectors toward better water use efficiency or less crop water consumption [5,6]. Nevertheless, using all these techniques and strategies, water stress is not fully avoided particularly in arid and semi-arid regions [7], but instead, they can induce partial mitigation on their adverse effects [8]. DI or irrigation of crops below full crop water requirements as well as application of organic compounds such as humic acids (HAs), salicylic acid, amino acids, plant extracts, and inorganic ions and compounds such as silica, selenium, calcium (Ca), potassium (K), zinc (Zn), and boron have been extensively reported to reduce adverse effects of water stress on plants and to improve plant tolerance [9–13].

An enhanced knowledge on plant–water relation under DI and its effects on plant productivity can help to better manage the limited available water in agriculture.
DI or partial root zone drying are the two most studied techniques to assay plant growth responses to controlled water supply [14]. DI (by reduction in irrigation water volume or irrigation frequencies) either during a particular period or throughout the whole plant growing period can have several benefits in cropping systems [3,5,15]. Moreover, the combination of DI practice and foliar spray of antioxidant compounds has been attractive to scientists in this regard [6,8].

Onions represent one of the most important horticultural crops that their cultivation and production is constantly increasing [16]. Iran produces about 2.5–3 million tons onions annually in a nearly 60,000 hectare area, which ranks sixth after India, China, USA, Pakistan, and Turkey. Onions as vegetable crop are more sensitive to water stress than many other agronomic crops, so their production is threatened by recent climate change and water limitation [17]. It has been well established that DI represents a useful practical and simple tool to better manage the limited irrigation water toward more productivity and higher farmer’s economic return via saving in resources or increase in irrigated cultivation area. However, in many cases, a slight reduction in yields is not avoidable [5,8]. Therefore, in this study, the growth characteristics of onion plants were evaluated under DI and HA application.

2 Materials and methods

This study was done during spring and summer of 2019 under a protected greenhouse located in the suburb of Bojnord, Iran. The onion plants were exposed to different levels of DI throughout the growing season with or without HA application.

2.1 Soil preparation

In this study, a field soil with vegetable production background was used. The soil was mixed and passed through a 2 mm sieve and then was analyzed for physiochemical characteristics. The soil was calcareous lithosol with a loamy texture and a field capacity (FC) of 17.5%, pH of 7.8, electric conductivity of 1.2 dS/m, 0.9% organic carbon content, 3,200 mg/kg total nitrogen (N), 14.2 mg/kg phosphorus (P), 271 mg/kg K, and 453 mg/kg Ca. Thereafter, an amount of 100 mg/kg soil of an NPK (20-15-20) fertilizer was thoroughly mixed to the soils before pot filling and plantation.

2.2 Experimental setup

Onion seeds (Allium cepa var. Zargan) were sown in a mixture of peat moss and perlite under greenhouse conditions in the middle of January. Thereafter, the 8-week-old seedlings were transplanted to experimental pots at the end of March 2019. Black plastic pots (40 cm height and 32 cm diameter) were filled with 9 kg of dry soil, and then four onion seedlings were transplanted into the pots that later one of them was removed.

2.3 Treatments application

This study was arranged in factorial based on completely randomized design with two factors and four replications. Treatments were the interactions of DI as factor A (with levels of a1: 80%, a2: 70%, and a3: 60% of soil FC) and application of HA as factor B (with levels of b1: without and b2: with HA application). Each replication was a pot containing three onion seedlings. HA powder (INDOGULF Co., India) was purchased from the market and then was prepared and diluted in distilled water for usage. Its composition was 70% K humic, 15% fulvic, 12% K2O with a pH of 6.5–7.5, and 100% water solubility and 3.5% moisture. HA was supplied to pot soil three times and in a final concentration of 1 g/pot (nearly 0.1 g/kg soil). The DI levels were performed based on the soil FC and by weighing the pots every day at the evening to water and compensate the amount of lost water (weight loss of pots) and based on the treatments.

2.4 Morphophysiological and biochemical measurements

Onion plants were harvested in the middle of September 2019 and when plant leaves were turning to pale yellow color. Different growth traits were measured accordingly. Leaf sample for antioxidant enzymes determination was taken 4 weeks prior to harvest when the leaves were fully green and plant growth was active (at harvest time, yellow leaves have no metabolic activity except destructive enzymes). At harvest, plant shoot (leaves) was cut at 1–2 cm above soil surface and growth parameters including bulb fresh weight (FW) and dry weight (DW) percentage as well as their enzymes activity was determined. The bulbs FW was immediately measured using a digital scale after their cleaning. Thereafter, bulb DW
percentage was calculated after slicing and drying the bulbs in an oven at 70°C for 48 h.

For determination of bulb minerals, 0.5 g of bulb dry powder was burned to ash in a furnace at 550°C for 5 h. Then the ash was dissolved in HCl and HNO3 in a heating bath until full digestion and carbon removal. The rest was dissolved in distilled water and its K, iron (Fe), and Zn concentrations were determined by following Aslani and Souri [18] using flame photometry or atomic absorption spectroscopy.

The concentration of soluble sugars in onion leaves was determined using anthrone reagent and following the method used by Hatamian et al. [1]. Briefly, 0.6 g of leaf fresh tissues (from middle part of plant) was extracted twice in 3 mL ethanol 80% for 60 min. The extracts were then mixed, filtered, and the alcohol was removed by evaporation. The anthrone reagent was added to samples, and after thoroughly shaking, their absorption was measured at 625 nm. A standard curve of glucose concentrations of 0, 10, 20, 30, 40, and 50 mg/L was also used for calculation of carbohydrate concentration of leaves.

For determination of leaf proline, fresh leaf samples were grounded in liquid N and then extracted using methanol. The leaf proline concentration was then measured using 2 mL leaf extract, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid. The sample was mixed thoroughly, and its absorbance was measured using different concentrations of standard proline including 0, 4, 8, and 16 mg/L and with the help of a spectrophotometer at 520 nm.

The protein concentration of leaves and bulb was determined following the method used by Bradford [19]. Determination of leaf and bulb antioxidant enzymes was performed after grinding of fresh leaf and bulb samples in liquid N. An aliquot of tissue powder was extracted on ice using phosphate buffer. The tissue catalase (CAT) activity was determined following Pereira et al. [20], as the assay solution contained 50 mM phosphate buffer (pH 7.5) and 25 mM hydrogen peroxide. Then 0.1 mL of tissue enzyme extraction was added to the assay solution. CAT activity was assessed by recording the decrease in absorbance of hydrogen peroxide for 1 min at 240 nm and at a temperature of 25°C, and the CAT activity was expressed as U/mg protein/min. Peroxidase (POD) activity was determined using 4 mM acetate buffer (pH 5), 4 mM hydrogen peroxide (3%), and 0.2 mL benzidine 2% in methanol, while the mixture in a tube was placed in ice. Thereafter, 0.2 mL of protein enzymatic extract was added to each tube. The absorbance change was read at 530 nm, and POD activity was expressed as U/mg protein/min [20].

Leaf superoxide dismutase (SOD) was determined following Giannopolitis and Ries [21]. Briefly, 0.1 mM ethylene diaminetetra acetic acid, 50 mM phosphate buffer, 13 mM methionine, 75 µM nitroblue tetrazolium (NBT), and 0.21 mM riboflavin were added to enzymes supernatant. Then after 15 min slow vortex, they were subjected to fluorescence light, and thereafter the absorbance of the samples was read at 560 nm using spectrophotometer. The activity unit of SOD was the amount of enzyme that was needed for quenching the photochemical reduction of NBT.

2.5 Statistical analysis

The collected data were subjected to factorial analysis of variance (ANOVA) to assess the effect of different factors and their interaction using SAS software. The differences among the treatments were analyzed with least significant difference (LSD) test at $P < 0.05$.

3 Results

3.1 Bulb FW and DW percentage

DI (Table 1) had significant effect on bulb FW ($P < 0.05$) and on bulb DW percentage ($P < 0.01$). Bulb FW was significantly reduced only at highest DI level (a3; 60% FC) compared to a1 and a2 levels. However, bulb DW percentage was significantly higher under a2 and a3 (70 and 60% FC) than a1 (80% FC; Table 2), whereas application of HA significantly reduced this trait compared to those plants without HA application (Table 3).

3.2 Leaf protein and antioxidant enzymes

The results of ANOVA (Table 1) showed that the interaction effect of irrigation and HA was significant on leaf protein concentration and leaf POD and SOD enzymes activity at $P < 0.01$. The simple effect of both irrigation and HA was also significant on leaf protein, CAT, POD, and SOD activity at $P < 0.01$. Leaf protein was significantly highest in a3b2 (irrigation at 60% FC with HA application), whereas it was the lowest amount in a1b1 treatment (Figure 1). Leaf protein was significantly increased by decrease in irrigation water, as the highest and lowest amount was in a3 and a1, respectively (Table 2).
Application of HA also resulted in higher leaf protein compared to untreated plants (Table 3). Leaf POD enzyme activity (Figure 2) was significantly increased by application of DI (at 70 and 60% FC) and HA compared to those at first level of DI (80% FC). The significant highest leaf POD was in a3b2, and the significant lowest activity was in a1b2.
and a1b1 treatments, whereas there was no significant difference in leaf POD among a3b1, a2b2, and a2b1 combination treatments. Similarly, leaf SOD enzyme activity (Figure 3) was significantly highest under a3b2 treatment, followed by a3b1 and a2b2 treatments. The lowest leaf SOD activity was in a1b1 treatment that showed no significant difference with a1b2 and a2b1 treatments.

Moreover, the activity of leaf CAT, POD, and SOD enzymes was significantly increased by decrease in irrigation water from 80 to 60% of FC (Table 2). The highest activity of these antioxidant enzymes observed in plants treated with highest DI (a3), and their lowest activity was in plants treated with lowest DI (a1). Application of HA also significantly increased leaf activity of all these three antioxidant enzymes compared to those plants without HA treatment (Table 3).

### 3.3 Leaf proline and soluble sugars

Leaf soluble sugars were significantly influenced by simple effects of irrigation and HA at $P < 0.01$, whereas leaf proline was influenced only by irrigation water ($P < 0.01$). None of them was influenced by DI and HA interactions. Leaf
soluble sugars and proline concentrations were significantly increased with decrease in irrigation water from 80 to 60% FC, as the highest leaf soluble sugars and proline were in a3, and the lowest amounts were in a1 treatment (Table 2). However, leaf proline was not affected by HA application, but leaf soluble sugars were significantly increased by application of HA compared to untreated plants (Table 3).

### 3.4 Bulb protein and antioxidant enzymes

The results of ANOVA (Table 1) showed that the interaction effect of DI and HA was significant on bulb protein concentration at $P < 0.01$. The simple effect of irrigation was significant on bulb protein and CAT and POD enzymes activity at $P < 0.01$. The simple effect of HA was also significant on bulb CAT activity at $P < 0.01$ and on bulb protein at $P < 0.05$. Similar to leaf status, interaction of DI and HA showed that bulb protein (Figure 4) was highest in a3b2 treatment followed by a3b1 that showed significant difference compared to other combination treatments. There was no significant difference in bulb protein among a1b1, a1b2, a2b1, and a2b2 treatments (Figure 4). The results also showed that application of highest DI (a3) resulted in higher bulb protein concentration than other irrigation levels (a1 and a2; Table 2). Similarly, application of HA significantly increased bulb protein than untreated plants (Table 3).

![Figure 3](image1.png)

**Figure 3**: Leaf SOD activity of onions under combination treatments of irrigation (a) and HA (b). The irrigation levels include a1: 80%, a2: 70%, and a3: 60% FC, and HA levels include b1: without and b2: with application. Means with at least one common letter are not statistically significant at 5% level of LSD test.

![Figure 4](image2.png)

**Figure 4**: The protein concentration of onion bulbs under combination treatments of irrigation (a) and HA (b). The irrigation levels include a1: 80%, a2: 70%, and a3: 60% FC, and HA levels include b1: without and b2: with application. Means with at least one common letter are not statistically significant at 5% level of LSD test.
The results showed that bulb CAT and POD activity were significantly higher under highest DI (a3) than other two irrigation levels (a1 and a2; Table 2). However, bulb POD activity was not affected by HA application, while CAT activity was significantly increased by application of HA compared to untreated plants (Table 3).

3.5 Bulb K, Zn, and Fe concentrations

The results of ANOVA showed that the interaction effect of DI and HA was significant on bulb K concentration at $P < 0.05$ (Table 1). The simple effect of DI was significant on bulb Zn, K concentration at $P < 0.01$, and on bulb Fe concentration at $P < 0.05$. The simple effect of HA was significant on bulb Fe, K at $P < 0.01$. The interaction of DI and HA showed that the bulb K increased by application of HA under higher DI levels of a2 and a3 (70 and 60% of FC). There was no significant difference in bulb K among a2b1, a1b2, and a1b1 treatments (Figure 5). Application of higher DI at second (a2) and third (a3) levels gradually and significantly increased bulb K than first level of DI (Table 2). However, the application of HA significantly increased bulb K than untreated plants (Table 3). The results also showed that bulb Fe was significantly higher under a3 (highest DI) only compared to those at a2 (irrigated with 70% FC) level (Table 2). Application of HA also resulted in higher bulb Fe than untreated plants (Table 3). Bulb concentration of Zn was not affected by HA, but it was significantly higher under a3 and a2 (irrigation at 60 and 70% FC) than a1 (irrigation at 80% FC) level (Table 2).

4 Discussion

The growth characteristics of onion were affected by interaction of DI and HA application or by their simple effects. Bulb FW was significantly decreased only by highest DI level, and this was a coincidence with higher bulb DW percentage. The reduction in onion yield by drought or water deficit has been also reported [5,22,23]. Onions have a shallow root system and this makes them more sensitive to water stress and to application of strong or extended DI, particularly during bulb formation and enlargement [3,16]. The onion responses to water shortage depend mainly on the strength of DI and the growth stage that DI is applied [8,15]. In onions, the bulb yield generally increases with increasing in levels of irrigation water; however, actual evapotranspiration (ETc) also increases with more irrigation water [24]. It was reported that the application of 20 and 40% DI can save 19.2 and 41.7% water consumption; however, the yield is reduced by 20 and 32%, respectively [25]. Significant reduction in onion yield and size was observed by application of 40% DI but not at 20% DI level [15]. Moreover, the size of onion bulbs is in direct relationship to quantity of water applied; so, DI can be used to produce market-demanding onion sizes [15]. A relatively similar result was observed in our study in which 20%.

![Figure 5: The K concentration of onion bulbs under combination treatments of irrigation (a) and HA (b). The irrigation levels include a1: 80%, a2: 70%, and a3: 60% FC, and HA levels include b1: without and b2: with application. Means with at least one common letter are not statistically significant at 5% level of LSD test.](image)
reduction in irrigation water only reduced onion FW at a rate of 10% that is significant in arid regions regarding water saving. Nevertheless, it has been reported that severe water deficit can reduce the onion marketable quality such as bulb weight and size, whereas it increases the bulb protein content, total soluble sugar, total phenolics content, and pyruvic acid [8].

Application of DI particularly at its highest level (a3; 60% FC) significantly affected many physiological and biochemical characteristics of leaf and bulb of onion. These include higher amounts of leaf soluble sugars, proline, protein, antioxidant enzymes, and mineral elements. Most of these biochemical compounds are those associated with better tolerance of plants to stressful conditions [2,26]. Abiotic stresses including drought can change general plant metabolism [14]. Leaf proline and soluble sugars were increased particularly by highest DI and further by HA application. Proline and soluble sugars are among the most important osmoregulators that accumulate in cells under water stress [2,27]. The potential effect of exogenously applied proline (1 and 2 mM) under different irrigation regimes (120, 100, 80, and 60% of ETc) on onion growth in a saline calcareous soil showed that proline application significantly enhanced growth as well as plant–water status, photosynthetic efficiency, and osmoprotectants of drought-stressed onion, which consequently reflected in increased bulb yield [2]. However, we applied a mild DI, and it caused no strong morphological, yield, or physiological effects on onion plants compared to other studies that applied stronger DI [23,28]. Plants exposed to abiotic stresses such as salinity and drought accumulate some organic and inorganic osmoregulators that can better adjust plant cell osmotic, resulting in higher plant tolerance to stress conditions [6,29,30]. Moreover, these compounds are also actively involved in alleviation of oxidative stresses induced by environmental constraints [31,32]. Under soil salinity of 4.8 dS/m, foliar application of glycine betaine (GB) at 25 and 50 mM on onion growth showed that GB at higher level was more effective and significantly increased growth indices, bulb yield, and water use efficiency, endogenous osmoprotectants, and nonenzymatic antioxidants and enzymatic antioxidant, whereas glutathione reducing activity was reduced, and free proline and soluble sugars were not affected [29].

In this study, the application of HA showed significant positive effects on many physiological and biochemical traits of onion leaves and bulbs. The growth-enhancement effects of HA were more evident under higher DI levels (a2 and a3 than a1). The beneficial effects of HA on plant growth under different stresses have been well documented [4,10,33]. HA is a well-known beneficial or biostimulant compound that nowadays is extensively used in cropping systems [13,33,34]. Many inorganic and organic compounds can have biostimulation effects on plant growth under restricted growth conditions [31,35]. It has been shown that under saline conditions, foliar application of diluted bee-honey extract on onion growth showed that plant growth and biomass, bulb yield, and water use efficiency were significantly increased [35].

HA can benefit plant growth with several mechanisms; however, its efficiency depends on different internal and external factors [12,36]. HA can induce protective antioxidant responses in plants to avoid oxidative damages induced by stresses including drought [9,27]. It was shown that the pretreatment immersion of onion seedlings in HA solution improved early growth and bulb yield as well as the sugars and protein contents of bulbs; however, the increase in onion bulb yield was not related to nutrients uptake stimuli [34]. It was reported that the biostimulants can improve water use efficiency in onion plants and improve the average bulb yields by 10.1–25% [8]. Foliar application of salicylic acid enhanced drought stress tolerance in onion plants by improving photosynthetic efficiency and plant water status that resulted in yield recovery under drought stress [23].

The efficient use of available water resources is vital in agricultural systems. In many countries, agricultural production and food security is threatened by water availability. Limited irrigation water and soil salinity are the two major limiting factors in crops production and onions are highly sensitive to both of these stresses [4]. Water shortage or drought can build up salts in top soil and root zone that can significantly reduce onion growth and production. DI can maximize the irrigation water use efficiency per unit of applied water [3,15]. DI at the 50% potential plant ETc reduced onion yield, while the yield from DI at 75% was not much different from 100% ETc and produced a similar bulb size. So, application of DI at 75% ETc rate in onions can conserve water and produce high-price bulb sizes without reducing quality [37].

Our results showed that the highest DI levels reduced bulb yield at a rate of 10%, whereas it saved irrigation water almost 24% compared to those plants irrigated at 80% FC. It was shown that a 20% reduction in irrigation water has no significant effect on onion yield [5,23,38]. Similarly, application of 20% DI on onion plants saved about 11% in irrigation water, whereas no significant reduction in yield occurred [15]. DI at a moderate level can be beneficial for many crops including onions; however, gradual increase in DI can significantly reduce the bulb yield [8,23].
In conclusion, the results of this study showed that onion growth and yield were significantly reduced (about 10%) only when DI was used at 60% FC, whereas DI (at 70 and 60% FC) significantly influenced many physiological and biochemical traits of onion plants. Nowadays, with climate change and global warming, water shortage and water stress is very common in agricultural activities particularly in arid regions. So, a better management of the limited available waters is quite important. Our findings indicate that a 20% reduction in irrigation water can significantly save irrigation water and improve biochemical characteristics of onion bulbs, whereas the yield reduction is small or negligible.

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