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RESPONSE OF BASIL (*OCIMUM BASILICUM* L.) TO SUPERABSORBENT POLYMER UNDER VARIOUS IRRIGATION REGIMES

Mohammadreza Ghasemi Nezhad-raeini[1], Mahdiyeh Zare-Kohan[2*], Safar Marofi [3]

1. Department of Water Engineering, Islamic Azad University, Kerman Branch, Iran.
2. Department of Plant Breeding and Biotechnology, Sari Agricultural Sciences and Natural Resources University (SANRU), sari, Iran.
3. Department of Water Engineering, Bu-Ali Sina University, Hamedan, Iran.

ABSTRACT: The aim of this study was to assess the effect of various rates of superabsorbent polymer and drought stress on water use efficiency, antioxidant activity and some physiological traits in basil. The considered factors were four levels of irrigation including irrigation at 100, 80, 60 and 40 percentage of field capacity and three levels of superabsorbent polymer including 0, 3 and 5 grams polymer per kilogram of soil. Analysis of variance showed the significant effect of irrigation treatment on all the studied traits, and the interaction effects of two factors were not significant. The results revealed that water stress considerably reduced the plant height, number of leaves and branches per plant, fresh and dry weight and water use efficiency in basil, whereas the application of superabsorbent polymer compensated the negative effect of drought stress, particularly in high values of polymer application. Highest values for all traits and water use efficiency were observed in irrigation treatment via 80 percentage of field capacity. Furthermore, the water stress enhanced antioxidant activity at basil leaf tissues, and maximum amounts of antioxidant's activity were observed under irrigated with 40 percentage of field capacity. It could be concluded that larger values of superabsorbent decreased the negative effect of the drought, as well as, it reduced the adverse influence of reactive oxygen species scavenging, therefore, it could be helpful for developing and improvement of plants under water deficient conditions.

Keywords: Superabsorbent polymer, Drought stress, Basil, Reactive oxygen species.

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Corresponding Author: Dr. Mahdiyeh Zare-Kohan* Ph.D.
Department of Plant Breeding and Biotechnology,
Sari Agricultural Sciences and Natural Resources University (SANRU), sari, Iran.
Email address: mahdiyehzare65@gmail.com

1. INTRODUCTION

Water stress is one of the major abiotic stress, specifically in arid and semi-arid areas that has always reduced crop production. It is imperative to achieve the maximum yield in agriculture via available water to obtain high profit form per unit area, since the available agricultural land and irrigation water diminishing owing to the urban development and swift industrialization. Considering to water scarcity, optimizing and developing irrigation systems as well as cultivation of suitable crops are highly demanded. In other hand, the costly irrigation equipment and unfavorable irrigation layout capacity, also, water sources limitation are the significant causes that force many countries to reconsider irrigation scheme and water applications. Due to insufficient rainfall in the recent years in Iran, noticeably crop production reduced. Annual rainfall does not satisfy farming requirements. Thus, a suitable management is needed to receive an acceptable performance in plant cultivation. The appropriate irrigation treatments should be used usefully to enhance the sustainability of agriculture production systems [8]. Potential of the water deficiency tolerance and the economical merit of aromatic and medicinal plants, make them appropriate alternative for cultivation in dry lands [10]. One of the substantial aromatic plants that applied as flavor foods also usage in traditional medicines is basil (*Ocimum basilicum* L.) [34]. It is worth noting that active pharmaceutical ingredients production and growth in aromatic plants are affected via different environmental factors like water stress [7, 31]. Some endeavor are being accomplished to cope water stress problem mostly using the tolerance plants and chemical, physical and hormones treatments, as well as biological approaches. Dry and fresh matter, essential oil yield and nutrient content significantly reduced under water deficit [24]. Dry and fresh weights of basil (*Ocimum basilicum* L.) were diminished once the water deficiency increased [30]. In order to save soil moisture using some materials consist of plant residuals, straw, mulch plants, stubble, also some synthetic materials such as colophony hydroplus superabsorbent polymers (SAP) could be helpful. Owing to low cross-links in the SAP structure, it is highly hydrophilic [13]. It seems, the SAP have a good ability in reclamation and restoration of soil and saving water for plant growth and production [35]. Therefore, application of water-saving SAP for field-crop production has become a popular water saving technology in drought-affected areas. . In arid and semi-arid regions of the world, intensive research on water management is being carried out, and use of SAP may effectively increase water and fertilizer use efficiency in crops. The incorporation of SAP for stabilizing and improving physical properties of the soil may lead to enhance infiltration, water and fertilizer use efficiency, seed germination and emergence, crop growth and yield, also, decrease soil erosion and irrigation requirement of plants [2, 22, 33]. Once polymers are applied in the soil, it is supposed that they maintain immense quantities of nutrients and water, hence, improvement of plant growth will be happened by limited water supply [9, 21]. Johnson [15] demonstrated that enhancing of 171 to 402 percentage in water maintenance capacity once polymers were added in coarse sand. Furthermore, application of hydrophilic polymer materials to peat reduced water deficiency stress and enhanced the time to wilt [9], also, it was useful in decreeing undesired losses of fertilizer [23]. Thus, the application of SAP in the agricultural field has become a popular water-saving technology for many farmers in arid and semi-arid regions. In addition, reactive oxygen species (ROS) plays a crucial role in causing cellular damage under drought stress, which can result in increased production of antioxidants to overcome this problem. Antioxidants help the organisms in dealing with oxidative stress, caused by free-radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are very unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The ROS formed, such as superoxide anion, hydroxyl radical and hydrogen peroxide are greatly reactive and potentially damaging transient chemical species. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. One of the methods that are currently popular is based upon the use of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The aim of present study was to evaluate both the antioxidant activity using DPPH scavenging and the effectiveness of that applied polymer on basil plant under different watering regimes.

2. MATERIALS AND METHODS

2.1. Plant materials, cultural practice, experimental design and treatments

This study was conducted as a factorial experiment based in a randomized complete block design (RCBD) with two factors and three replications. The experiment was carried out at the agricultural research greenhouse of Hamedan University, Iran (48°31′E and 34°48′N and 1800 m of sea level). Four irrigation treatments, including irrigation at 100, 80, 60 and 40 % of field capacity (FC) were considered as first factor. Three levels of superabsorbent polymer including 0, 3 and 5 grams polymer per one kg of soil were considered as second factor. The treatments were as follows: watering 100 % of FC and without polymer (I_1 S_1), watering 100 % of FC and 3 grams polymer (I_1 S_2), watering 100 % of FC and 5 grams polymer (I_1 S_3), watering 80 % of FC and without polymer (I_2 S_1), watering 80 % of FC and 3 grams polymer (I_2 S_2), watering 80 % of FC and 5 grams polymer (I_2 S_3), watering 60 % of FC and without polymer (I_3 S_1), watering 60 % of FC and 3 grams polymer

(I_3 S_2), watering 60 % of FC and 5 grams polymer (I_3 S_3), watering 40 % of FC and without polymer (I_4 S_1), watering 40 % of FC and 3 grams polymer (I_4 S_2), watering 40 % of FC and 5 grams polymer (I_4 S_3). Each pot was filled with 17 kg of air dried soil, some properties of the soil used are presented in Table 1.

Table 1. Some properties of soil used during the experiment

Properties	Values	Unit
pH	7.69	-
EC	710.96	$\mu s.cm^{-1}$
Na	127.8	$mg.kg^{-1}$
P	3.02	$mg.kg^{-1}$
K	490.4	$mg.kg^{-1}$
C	1.73	%
Ca	266.66	$mg.kg^{-1}$
Fe	22.34	$mg.kg^{-1}$
Mn	0.56	$mg.kg^{-1}$
Cl	473.33	$mg.kg^{-1}$
Soil Texture	Sandy - Loam	

The polymer was added to pots before sowing stage. The pots were put at appropriate distances from each other to avoid of light absorption competition. At 4 leave stage seedlings were thinned and six plants in each pot were maintained, and drought stress was started in eight leave stage. Then appropriate amount of water corresponding to each treatment was added to pots and recorded. The growth parameters which recorded were number of branches per plant, plant height (cm), fresh and dry weights (g), number of leaves per plant of herb yield and water use efficiency (WUE). The number of leaves and branches per plant and plant height were measured after reproduction stage. After cutting, leaves were sealed in plastic bags and quickly transferred to the laboratory. Fresh weight was determined after excision, and dry weights were obtained after oven drying for 72 (hr) at 70°C. WUE determined through the following formula:

$$WUE\ (g.L^{-1}) = (\text{The general performance/ the used water}) \quad (1)$$

2.2. Determination of Radical Scavenging Activity

2.2.1. Sample preparation

The cut leaves of each treatment were milled to fine powder. The methanolic extracts were prepared by 0.5 g of leaf powder extracting with 3 ml of %85 methanol. The methanolic extracts were centrifuged at 5000 rpm for 10 minutes and filtered through whatman (No. 1) filter paper. Then 500 µl of each filtered extract was mixed with 500 µl of distilled water and were centrifuged at 10000 rpm for five minutes. In the last, extracts were filtered with filter paper (Whatman No. 1).

2.2.2. DPPH Radical Scavenging Activity

A DPPH free radical scavenging assay was done using Bozin et al. [3] approach by some modification. Different concentrations of each extract were added to 1 mL of 90 μM DPPH solution and made up with methanol (95% v/v) to a final volume of 3 ml. The mixture was shaken immediately after adding DPPH solution and was allowed to stand for 30 minutes at room temperature in the dark; then the absorbance was read at 517 nm against the blank (the same solution with no added extracts). Three replications were recorded for each sample.

The radical scavenging capacity (RSC) was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100 \quad (2)$$

Data analysis was done by using Excel and SAS softwares. Mean comparison carried out with least significant difference (LSD) at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Plant height

Drought stress had a significant effect on plant height, intensity increase of the drought stress caused a reduction in plant height (Table 2, 3). The highest value for plant height (55.56 cm) was observed in 80 % of FC (Table 3). The lowest value for plant height (30.9 cm) was observed in 40 % of FC (Table 3). Reduction in cell elongation was observed under low water supply [32]. Drought stress via reduction in cell turgidity, cell growth, cell volume and number of stem cells reduced plant height [5, 32]. Consequently, shortening in internode's distance caused a reduction in plant height [32]. With the increase in SAP content, plant height increased from 46.04 to 47.72 cm (Table 4). Furthermore, the positive effect of superabsorbent on stem elongation was reported by Brar et al. [6], Keshavars et al. [16], Jahan et al. [14] and Mohebi [25]. This happened due to high potential of superabsorbent to absorb water and store water in the soil [4]. Physical structural properties of soil were amended using superabsorbent polymer [4, 27].

Table 1. Analysis of variance of studied traits under drought stress and application of superabsorbent polymer in basil.

Source of variation	Df	PH (cm)	NL	NB	FW (g)	DW (g)	WUE ($g.L^{-1}$)	DPPH SA (%)
Replication	2	145.27	2280.08	6.15	1618.02	71.4	1.94	284.33
Irrigation (a)	3	1211.34**	7627.59**	19.63**	18814.52**	708.2**	7.27**	554.55**
Superabsorbent (b)	2	12.89	836.6	2.3	1556.2**	17.54	1.74**	592.64**
a × b	6	27.87	1224.13	2.08	540.53*	11.26	0.42	23.98
Error	22	49.42	490.93	3.8	194.3	13.31	0.24	45.24

* and **: significant at 5% and 1% probability levels, respectively. PH: Plant height, NL: Number of leaves per plant, NB: Number of branches per plant, FW: Fresh weight, DW: Dry weight, WUE: Water use efficiency, DPPH SA: DPPH scavenging activity.

Table 3. Mean comparison of studied traits in different levels of drought stress in basil.

Irrigation treatments	PH (cm)	NL	NB	FW (g)	DW (g)	WUE ($g.L^{-1}$)	DPPH SA (%)
I_1:100 (%) of FC	55.11^{a}	144.53^{ab}	15.35^{a}	129.5^{b}	26.35^{a}	3.7^{c}	28.33^{d}
I_2:80 (%) of FC	55.56^{a}	149.43^{a}	16.08^{a}	151.7^{a}	28.55^{a}	5.42^{a}	29.95^{c}
I_3:60 (%) of FC	44.55^{b}	125.31^{b}	15.78^{a}	91.77^{c}	17.08^{b}	4.37^{b}	38.38^{b}
I_4: 40 (%) of FC	30.9^{c}	85.38^{c}	12.84^{b}	47.41^{d}	9.3^{c}	3.4^{c}	45.22^{a}

The numbers in each columns have same alphabet aren't different significantly with together

Table 4. Mean comparison of studied traits in different levels of superabsorbent polymer in basil

Superabsorbent	PH (cm)	NL	NB	FW (g)	DW (g)	WUE ($g.L^{-1}$)	DPPH SA (%)
S_1: 0 ($g.kg^{-1}$) of soil (control)	46.04^{a}	120.24^{a}	14.71^{a}	91.96^{b}	18.93^{a}	3.8^{b}	42.28^{a}
S_2: 3 ($g.kg^{-1}$) of soil	45.82^{a}	135.71^{a}	14.74^{a}	111.07^{a}	20.87^{a}	4.41^{a}	35.87^{b}
S_3: 5 ($g.kg^{-1}$) of soil	47.72^{a}	122.53^{a}	15.6^{a}	112.24^{a}	21.15^{a}	4.47^{a}	28.25^{c}

The numbers in each column have same alphabet aren't different significantly with together

3.2. Number of leaves per plant

Analysis of variance illustrated that drought stress has a significant effect on number of leaves per plant (Table 2). Mean comparison revealed that enhancing drought tension caused a significant reduction in number of leaves per plant (Table 3). Number of leaves was decreased by an increase in water deficit (Table 3). The highest number of leaves (149.43) was observed in 80 % of FC (Table 3). Combination of low contents of polymer by intense drought stress caused leaf death, as well as, dryness of shrubs. On the other hand, long duration of water deficit caused leaf-drying with subsequent decrease in plant performance. However, high contents of polymer and irrigation treatment of 80 % of FC provided adequate conditions for plant growth and enhanced the number of leaves (Table 3, 4). The same result was reported by Keshavars et al. [16].

3.3. Number of branches per plant

Drought stress had a significant effect on number of branches per plant (Table 2). Number of branches per plant was diminished with the increase in water deficit (Table 3). This result was similar to the reports of Abbasian and Shirani-Rad [1]. The highest number of branches (16.08) was observed in 80 % of FC (Table 3). The lowest number of branches (12.84) was observed in 40 % of FC (Table 3). With an increase in SAP content, number of branches increased from 14.71 to 15.6 (Table 4). Thus high values of SAP with suitable treatments of irrigation increased branches of basil.

3.4. Fresh weight

Irrigation, superabsorbent and irrigation × superabsorbent had a significant effect on fresh weight (Table 2). Irrigation treatments reduced fresh weight, significantly (Table 3). The highest (112.24 g) and the least (9.96 g) values of fresh weight observed in 5 and 0 grams polymer per kilogram of soil, respectively. Therefore, High contents of superabsorbent polymer increased fresh weight of basil (Table 4). The same result was reported by Khalili Darini et al. [18].

3.5. Dry weight

Irrigation treatment had a significant effect on dry weight (Table 2). Water stress treatments reduced dry weight, significantly (Table 3). This result was similar to the reports of Munir et al. [26] and Goksoy et al. [11]. Application of polymer increased dry weight of basil in comparison with control treatment (Table 4). High quantity of polymer with water supply, caused long time opening of stomata [17]. Eventually, good fixation of CO_2 resulted in enhancement of dry matter in crop plants [17]. Similar to our results, polymer application into soil has enhanced dry weigh [12, 16, 18].

3.6. Water Use Efficiency (WUE)

Analysis of variance showed that irrigation treatments and superabsorbent had the significant effect on water use efficiency (Table 2). The maximum values for WUE (5.42 and 4.37 $g.L^{-1}$) were observed in 80 and 60 % of FC, respectively. The lowest value for WUE (3.4 $g.L^{-1}$) was observed in 40 % of FC (Table 3). Effect of water stress on WUE depends on plant species, phenological stage of plant, as well as, duration and intensity of drought stress [20]. High levels of polymer application increased WUE (Table 4). This result was similar to the reports of Keshavars et al. [16], Rahul et al. [28] and Jahan et al. [14]. The highest (4.47 $g.L^{-1}$) and the least (3.8 $g.L^{-1}$) values of WUE observed in 5 and 0 grams polymer per kilogram of soil, respectively. Sharifan et al. [29] showed that superabsorbent polymer is one of the appropriate solutions to enhance the water use efficiency in agriculture, leading to the enhanced quality of crop yield as well.

3.7. DPPH scavenging activity

The DPPH radical scavenging activity of basil was shown in figure 1. Irrigation and superabsorbent had a significant effect on DPPH scavenging activity (Table 2). Increase in drought stress caused the increase in DPPH scavenging activity (Table 3). The similar result reported by Khan et al. [19]. The highest value for DPPH scavenging activity (45.22%) was observed in 40 % of FC (Table 3). The lowest value (28.33%) was observed in 100 % of FC (Table 3). With the increase in SAP content, DPPH scavenging activity reduced from 42.28 to 28.25 % (Table 4). Our results indicated that superabsorbent polymer decreased the adverse effects of reactive oxygen species on plant resistance improvement to water stress.

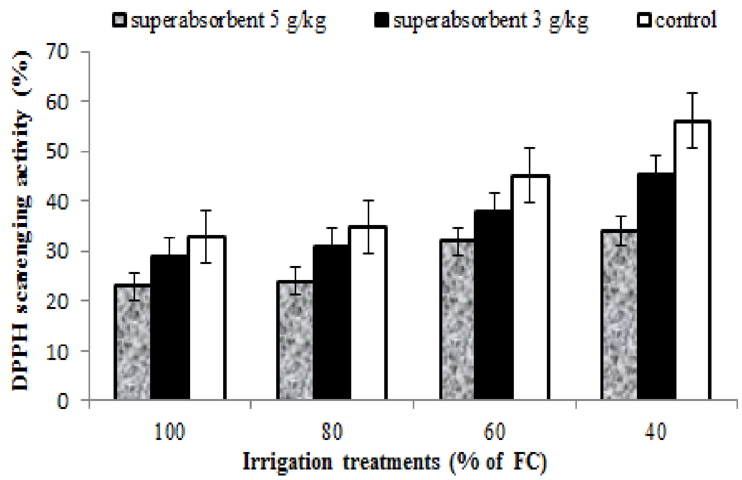

Figure 1. DPPH scavenging activity (%) under varied irrigation treatments.

4. CONCLUSION

Abiotic stresses such water deficiency adversely effect on agronomy, physiology and production of plant. Plants altering in various biological and physiological mechanism under abiotic stresses such as accumulation of reactive oxygen species in the cell caused by water deficiency. To cope ROS damaging the antioxidants defense system will be activated. Considering to the obtained results antioxidants play important role as a free radical scavenger, also antioxidants activity may be remarkably overcoming the ROS under stress conditions. High amount of antioxidants and as biochemical activity was observed in basil once irrigated with 40% water intensity. In the present study, the highest values for all studied traits and WUE were observed in irrigation treatment of 80 % of FC. Besides, with the increasing in SAP content the traits performance will be increased. It could be concluded that SAP reduced the negative effects of ROS may leading to improved plant resistance in arid and semi-arid regions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors have no conflict of interest

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