The Effect of Nitroxin Application and Drought Stress on Growth and Yield of Two Persian and Iraqi Celery Populations

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Abstract

To investigate the effect of drought stress and nitroxin biological fertilizer on two Iranian and Iraqi celery populations, an experiment was designed and done in the research greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad in 2016-2017. This three-full randomized factorial experiment was performed with three replications. The first factor is drought stress in three levels include: control (100% of field capacity), moderate stress (70% of field capacity) and severe stress (40% of field capacity), the second factor is using nitroxin in seed treatment in three concentrations of zero (control), 15 mM and 30 mM and the third factor is the cultivar type at two levels (Iranian celery mass, Iraqi celery mass). According to the results of this study, it was found that different studied cultivar type showed different responses. Use of drought stress in the plant in both type compared to the control treatment reduced the growth traits. In Iraqi celery population, use of nitroxin at 15 mM concentration and in Persian celery population, use of 30 mM nitroxin concentration had the greatest effect on growth traits such as: leaf area, height, plant dry weight, leaf chlorophyll content and plants Stomatal conductance. The fresh weight of the plant was strongly affected by drought stress and use of nitroxin at different levels of drought stress prevented from the significant reduction in fresh weight of the plant in both Iranian and Iraqi celery population.

Keyword: Morphological traits; Physiological traits; Bio fertilizer.

1. Introduction

Chemical fertilizers are one of the main factors in maintaining soil fertility; however, excessive use of them, especially when combined with improper management operations such as burning crop residues, greatly reduces the amount of soil organic matter. This issue affected properties such as physical, chemical and biological of soil and increases the possibility of erosion in these soils [1]. Today, due to the increasing importance of environmental issues, more attention has been paid to biological fertilizers to replace the chemical fertilizers [2]. Biological fertilizers contain a dense mass of one or more types of beneficial soil organisms that are produced to provide the nutrients needed by the plant [3]. Today, the use of biological fertilizers, especially in nutrients poor soils, is an inevitable necessity to maintain soil quality [4]. Nitroxin is one of the biological fertilizers that have many microorganisms [5]. Nitroxin biological fertilizer contains a set of nitrogen-fixing bacteria such as Azotobacter and Azospirillium, which causes the growth and development of roots and aerial parts of plants [6]. In addition to, Nitroxin can stabilizing atmospheric nitrogen in the plant’s root section, and has the ability to produce and secrete some biologically active substances such as B vitamins, nicotinic acid, pantothenic acid, auxins and gibberellins, which improve root growth and thus increase the absorption of water and nutrients and finally, plant performance increases ([2] and [7]).

Drought stress is one of the most important factors limiting plant growth worldwide and is one of the most common environmental stresses. It has been found that the effect of dehydration stress on plant growth and performance depends on the plant genotype [8]. Environmental conditions and plant management also determine quantitative and qualitative performance in plants [8]. By exposing the plant to drought stress, root access to nutrients is affected. Proper plant nutrition in these conditions is one of the important factors affecting plant performance [9]. Celery is a biennial plant belonging to the Apiaceae family. This plant produces leaves in the first year and in the second year the flowering aerial stem appears and gives seeds [10]. The importance of celery plant is due to its various purposes consumption. All different parts of this plant have aromatic substances, which makes it used as a condiment in raw, frozen and dried form. Also, the active substances in celery have antifungal [11] and antibacterial properties [12,13]. In addition, its antioxidant properties have been confirmed ([14] and [15]). Celery fruits and leaves contain 2 to 3% and 0.09 to 0.43% of active ingredient, respectively [16]. The effect of nitroxin fertilizer uses on performance and performance components of safflower with using top dress and seed treated method showed that many performance components increased compared to the control treatment [17]. Also increased growth and performance in thyme [18], Milk thistle [18,19] are confirmed under nitroxin biofertilizer and Drought stress conditions. Proper nutrition and management of plant nutrition in drought stress conditions is one of the determinant elements of growth.
Therefore, in this study, we try to investigate the effect of using seed nitroxin biological fertilizer on two Iranian and Iraqi celery populations under different drought stress conditions and its effects on morphological and physiological factors that should be investigated and reported, to be able to use the results of this study and similar studies to provide a suitable strategy for planting in water shortage conditions without much reduction in performance and other factors.

2. Material and Methods

Test location and treatments: to investigate the effect of drought stress and nitroxin biological fertilizer on two Iranian and Iraqi celery populations, an experiment was conducted in the research greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad in 2017-2018. The experiment designed in three-factor factorial randomized with three replications. The first factor is drought stress in three levels include: control (100% of field capacity), moderate stress (70% of field capacity) and severe stress (40% of field capacity), the second factor is using nitroxin in seed treatment in three concentrations of zero (control), 15 mM and 30 mM and the third factor is the cultivar type at two levels (Iranian celery mass, Iraqi celery mass).

Procedure the test: This experiment was designed and performed in the pot. Nitroxin fertilizer was applied in seeds. Nitroxin fertilizer used in the experiment was also purchased ready-made from Mehr Asia Biotechnology Company. And its active ingredient is a collection of nitrogen-fixing bacteria of the genus Pseudomonas and Entrobacter cloacea. After treatment the seeds with nitroxin, the seeds were planted in pots. Seeds were planted in January 2016; Stress started two months after sowing seeds and the plants were harvested after stress and in June. Drought stress was applied by weight method based on pot weight at 100% of field capacity. In this way, the weight of the pot was measured at 100% of the field capacity, then its dry weight was measured after placed in the oven. By putting the obtained numbers in the formula, the amount of moisture was calculated at 100% of the field capacity. Based on the obtained number and the weight of each pot (all of the pots had the same weight), the amount of moisture at 70 and 40% of the field capacity was calculated. The pots were weighed daily and according to the amount of moisture for each treatment, the weight of the pots was kept constant during the experiment.

Morphological traits: At the end of the experiment, morphological traits including leaf area, petiole length, plant height, plant fresh weight and plant dry weight were measured. A ruler was used to measure the petiole length and plant height. Leaf area was measured after sampling of the plant using Model Le-COR_1300 SA.

TSS rate: Tss rate in the plant was read by a refractometer at the end of the experiment.

Proline: Bates (1973) method was used to measure free leaf proline. 0.5 g of fresh leaves was eroded with 10 ml of 3% sulfosalicylic acid. After centrifugation the samples, 2 ml of upper clear solution was added to the reagent of Nine Hydrin and Acetic Acid and placed in a hot water bath at 90 °C for one hour. After cooling the solution, toluene was added. Then the optical absorption of the supernatant at 520 nm was read using a blank solution of toluene and the concentration of free proline amino acid in the sample was determined using a standard curve of pure proline and its amount was calculated based on the following equation.

\[
\text{µmole proline/g f. w. material} = \frac{\text{µg proline/ml} \times \text{ml toluene}}{115.5 \text{µg/µmole}} / \text{g sample}
\]

Leaf chlorophyll content: To measure the amount of chlorophyll, 200 mg of fresh leaves were separated from fully developed young leaves and pigments were extracted by using 99% methanol. The absorbance was recorded at 470, 653 and 666 nm using a spectrophotometer. Finally, the amount of chlorophyll a, b and total were calculated based on mathematical relations.

Electrolyte leakage: Electrolyte leakage index was used to determine the membrane stability of leaf cells. In this method, at the first, leaf pieces with a size of 2 cm were prepared. After washing, these pieces were placed in tubes with 10 ml of distilled water. Then, the tubes were shaken vigorously (160 rpm) for 17 to 18 hours. At this stage, the electrical conductivity of the test (E1) was recorded. The tubes were then transferred to autoclave at 121 °C for 15 minutes to kill leaf cells. Thus, the electrical conductivity (E2) was measured at this stage and after the contents of the tubes was cooled. Finally, the electrolyte leakage values were calculated using the following equation.

\[\text{EL} = \left(\frac{E_1}{E_2}\right) \times 100\]

Relative leaf water content: Barrs and Weatherley (1962) method was used to measure and calculate the relative leaf water content, which included measuring fresh weight (FW), Turgor weight (TW) and then dry weight (DW) and placed them in the following equation.

\[\% \text{ RWC} = \left(\frac{\text{FW-DW}}{\text{TW-DW}}\right) \times 100\]

Stomatal conductance: Leaf Stomatal conductance was measured by Leaf porometer model SC-1.

Greenness index: Leaf greenness index was measured by chlorophyll-meter (SPAD 502 (Konica-Minolta-Tokyo)).
Statistical analysis was performed using JMP-8 software and means comparisons were performed using LSD test.

3. Results and Discussion

According to the results of variance analysis of the traits in the experiment of the study, it was found that growth traits were affected by the simple and interaction effects of the experimental treatments (Table 1). Petiole length in different celery types was affected by different concentrations of nitroxin. It is important that the increase in petiole length was more evident during the use of nitroxin in the Iraqi celery population and the highest petiole length was observed in this type at 15 mM concentration of nitroxin (Table 2). About plant height, the results showed the same as petiole length and the best response to the use of nitroxin was observed in Iraqi celery population at 15 mM concentration. In rapeseed, the use of Pseudomonas bacteria improved plant height under salinity stress [21]. Under the same environmental conditions, the provision of nutrients to the plant by different fertilizers can increase plant growth and then increase plant height. The plant's access to sufficient water and nutrients, especially nitrogen, is very effective in increasing plant height by affecting cell division and growth. The reason for the increase in height due to nitroxin consumption can be attributed to increased nutrient absorption and improved Photosynthesis [22]. In basil (Ocimum basilicum L.) and fennel (Foeniculum vulgare L.) the use of biological and organic fertilizers improved the growth traits of the plant [23]. In another study, inoculation of sunflower seeds with growth-promoting bacteria increased plant height [24]. The leaf area of different celery type was affected by different levels of nitroxin. The use of nitroxin in Iraqi celery population increased leaf area compared to the control treatment (without use of nitroxin). In Persian celery population, the use of nitroxin increased the leaf area but without statistically significant difference with the control treatment (without use of nitroxin) (Table 2). Also, the use of 15 mM nitroxin concentration in the Iraqi celery type and 30 mM concentration in the Iranian celery type produce the highest dry weight in the plant. Also, the use of low concentration of nitroxin in Iraqi celery population and high concentration of nitroxin in Persian celery population significantly increased the chlorophyll content of the plant compared to the control treatment (without use of nitroxin). Similar results were observed in relation to plant greenness index (Table 2). Also, the highest amount of Stomatal conductance in the plant was observed in 15 mM concentration of nitroxin and in the type of Iraqi celery. However, with using nitroxin, the amount of Stomatal conductance in Persian celery population was not significantly different from the non-use of nitroxin treatment (Table 2). The relative leaf water content in both Iranian and Iraqi celery population increased when nitroxin was used (Table 2). In confirmation of the present study’s results, the use of Pseudomonas bacteria in Common sage, increased the dry weight of roots and aerial organs of the plant. Also, the use of biofertilizer containing Pseudomonas fluorescens and Azotobacter chroococcum with mycorrhizal fungus in Pot marigold improved plant performance under drought stress [25]. Nitrogen is one of the most important nutrients for plant growth. This element is the basis for the formation of proteins and nucleic acids. Due to the importance of this element, it is very essential to provide the required amount for the plant [26]. Nitroxin contains nitrogen-fixing bacteria and also induces the growth of Azotobacter and Azospirillum. These plant-symbiosis microorganisms provide more and better plant growth either through nitrogen fixation or through the production of growth hormones [27,28]. Therefore, it can be concluded that the beneficial effect of bacteria may be due to their participation in increasing plant growth through nitrogen fixation and production of plant hormones, which can stimulate more nutrient absorption by the plant and improve plant growth quality [29]. Leaf area in Persian celery population decreased due to drought stress. It should also be noted that the decrease in leaf area in the Persian celery population was more evident compared to the Iraqi celery population. Also, the decrease in leaf area in Iraqi celery population was not statistically significant compared to the control treatment (Table 3). According to Table 4, it was found that drought stress in the plant, strongly affected the growth traits. Petiole length, plant height, plant dry weight, relative leaf water content, plant greenness index, leaf chlorophyll content and the amount of Stomatal conductance in the plant, decreased with decreasing soil moisture compared to the control treatment. In contrast, amount of leaf TSS, leaf proline and electrolyte leakage in the plant increased with drought stress (Table 5).Reduction of growth traits have also been observed in basil [30], Fennel flower (Nigella sativa L.) [31]. Sunflower [32] and chamomile which is aligned with the results of this study. The balance of nutrients in plants depends on the presence of soil moisture, lack of soil moisture causes nutritional imbalance [33]. [34] Investigating the effect of low irrigation on shoots dry weight of three safflower genotypes and they reported that with increasing irrigation time, shoot dry weight of all three genotypes decreased. The reduction of plant growth traits in conditions of dehydration stress compared to optimal irrigation can be attributed to the reduction of Photosynthesis and materialization in the plant under stress conditions; Because the reduction of pure Photosynthesis and the reduction of nutrients are the consequences of water shortage stress, which reduces the growth traits of the plant [33]. The use of nitroxin under drought stress conditions in different Iranian and Iraqi celery population was effective on the fresh weight of the plant. Drought stress severely reduced the fresh weight of the plant, in contrast, the use of nitroxin in drought stress conditions prevented from losing the leaf weight in both Iranian and Iraqi types. The use of 15 mM nitroxin in Iraqi celery population under drought stress condition increased compared to the control treatment (without use of nitroxin) (Table 6). In a study, the use of Enterobacter aerogenes effectively moderated the negative effects of salinity stress on the length and dry weight of alfalfa [35]. Also in rapeseed, the use of different strains of Pseudomonas under salinity stress improved the growth traits of the plant [21]. The use of biofertilizer in the form of seed treatments causes to easily absorb the required nutrients in the
plant, including nitrogen [36]. Also, growth-promoting bacteria improves plant growth conditions, directly by fixing nitrogen and producing growth hormones, reducing the potential of root membranes, producing some enzymes effective in nutrient absorption and phosphorus dissolution, and indirectly by reducing or preventing the harmful effects of pathogenic microorganisms by producing a variety of antibiotics, antifungals, and siderophores [37]. Indoleacetic acid with cytokinin absorption and phosphorus dissolution, and indirectly by reducing the potential of root membranes, producing some enzymes effective in nutrient plant, including nitrogen [36]. Also, growth-promoting bacteria improves plant growth conditions, directly by fixing nitrogen and producing growth hormones, reducing the potential of root membranes, producing some enzymes effective in nutrient absorption and phosphorus dissolution, and indirectly by reducing or preventing the harmful effects of pathogenic microorganisms by producing a variety of antibiotics, antifungals, and siderophores [37]. Indoleacetic acid with cytokinin absorption and phosphorus dissolution, and indirectly by reducing the potential of root membranes, producing some enzymes effective in nutrient absorption and phosphorus dissolution, and indirectly by reducing or preventing the harmful effects of pathogenic microorganisms by producing a variety of antibiotics, antifungals, and siderophores [37]. Indoleacetic acid with cytokinin absorption and phosphorus dissolution, and indirectly by reducing or preventing the harmful effects of pathogenic microorganisms by producing a variety of antibiotics, antifungals, and siderophores [37].

Table 2. Results of analysis of variance (mean squares), the effect of experimental treatments on morphological and physiological properties of celery

| S.O.V       | df | Leaf area | Petiole length | Height | Dry weight | Fresh weight | EL | Stomatal conductance |
|-------------|----|-----------|----------------|--------|------------|--------------|----|----------------------|
| Cultivar (C)| 1  | 479326641 | 272.3**        | 3240.6 | 8.86**     | 392.4**      | 547.7** | 322.6**              |
| Nitroxin (N)| 2  | 542340158 | 672.9**        | 1836.8 | 21.17**    | 2076.2**     | 481.48** | 42.70**              |
| Stress (S)  | 2  | 580390460 | 752.8**        | 926.5**| 35.92**    | 1059.3**     | 2004.0** | 105.94**             |
| C×N         | 2  | 430944933 | 594.7**        | 1394.3 | 13.70**    | 1740.9**     | 339.4**  | 46.90**              |
| N×S         | 4  | 165102288 | 35.52**        | 30.49**| 3.41**     | 416.6**      | 147.2**  | 5.91**               |
| C×S         | 5  | 227157763 | 82.04**        | 70.10**| 0.06**     | 204.8**      | 249.7**  | 30.52**              |
| C×N×S       | 4  | 684618189 | 39.19**        | 17.59**| 1.95**     | 427.2**      | 227.6**  | 4.91**               |
| Error       | 36 | 43341268  | 85.84          | 104.5  | 2.59       | 51.88        | 276.5    | 10.48                |

Table 2. Morphological and physiological traits studied in two Persian and Iraqi Celery Populations under the influence of different concentrations of nitroxin

| Cultivar | Nitroxin | Leaf area (cm²) | Petiole length (cm) | Height (cm) | Dry weight (g) | RWC (%) | SPA D | Cla (mg/g FW) | Clb (mg/g FW) | CI (mg/g FW) | Stomatal conductance (mol/m/s) |
|----------|----------|-----------------|---------------------|-------------|----------------|---------|-------|--------------|---------------|--------------|-------------------------------|
|          |          | 2388.4          | 18.08               | 21.11       | 0.99           | 85.33   | 45.92  | 4.60         | 3.05b         | 7.65         | 10.82                         |
|          |          | d               | c                   | c           | c              | b       | b     | c            | d             | b            |
|          | 15       | 21862.3         | 39.44               | 58.00       | 4.08           | 95.33   | 51.09  | 6.09         | 3.87a         | 9.96         | 15.12                         |
|          |          | a               | a                   | a           | a              | a       | a     | b            | b             | a            |
|          |          | 30              | 21056.5             | 23.72       | 36.88          | 2.07    | 91.22  | 45.60        | 3.24b         | 8.55         | 9.34                          |

ns: Non-significant, ** and * significant at 5% and 1% level of probability, respectively.
### Table 3. The effect of drought stress on leaf area of Persian and Iraqi Celery Populations.

| Cultivar              | Drought stress (FC%) | Leaf area (cm²) |
|-----------------------|----------------------|-----------------|
|                       | 100                  | 13535.63b       |
| Iraqi Celery Population | 70                   | 11526.41b       |
|                       | 40                   | 9033.25b        |
| Persian Celery Population | 100                | 27604.78a       |
|                       | 70                   | 14499.66b       |
|                       | 40                   | 9033.25b        |

In each column, numbers with similar letters do not differ significantly at the 5% level based on the LSD test.

### Table 4. The effect of drought stress on the morphological and physiological traits that studied in celery.

| Drought stress (FC%) | Petiole length (cm) | Height (cm) | Dry weight (g) | TSS (%) | RWC (%) | Prolin (μmol/e FW) | SPA D (%) | EL (%) | Cla (mg/g FW) | Clb (mg/g FW) | CI (mg/g FW) | Stomatal conductance (mol/m²s) |
|----------------------|---------------------|-------------|----------------|---------|---------|-------------------|-----------|--------|----------------|----------------|-------------|-----------------------------|
| 100                  | 32.07               | 38.69       | 4.36           | 2.5     | 6.0     | 0.003             | 52.6      | 37.2   | 6.82           | 3.78           | 10.60       | 12.02                      |
| 70                   | 22.83               | 29.51       | 2.38           | 2.8     | 7.2ab   | 0.019             | 46.7      | 45.1   | 5.73           | 3.10           | 8.84        | 8.60                       |
| 40                   | 19.61               | 24.54       | 1.62           | 3.2     | 1.6a    | 0.065             | 41.8      | 58.1   | 4.89           | 2.64           | 7.53        | 7.33                       |

In each column, numbers with similar letters do not differ significantly at the 5% level based on the LSD test.

### Table 5. Effect of using nitroxin on drought stress conditions Persian and Iraqi Celery Populations on fresh weight of plant.

| Cultivar              | Nitroxin | Drought stress (FC%) | fresh weight (g) |
|-----------------------|----------|----------------------|------------------|
|                       | 100      | 7.24ij               |                  |
| Iraqi Celery Population | 0       | 70                   | 6.38j            |
|                       | 40       |                      | 4.62j            |
|                | 100 | 77.06a |
|----------------|-----|--------|
| 15             | 70  | 31.18bc|
| 40             | 19.38cdefgh |
| 100            | 13.00fghij |
| 30             | 70  | 11.47ghij |
| 40             | 9.16hij |
| 100            | 23.99cdef |
| 0              | 70  | 18.89defghi |
| 40             | 15.17efghij |
| 100            | 30.23bcd |
| Persian Celery Population | 15  | 24.67bcdef |
| 40             | 21.74cdefg |
| 100            | 36.03b |
| 30             | 70  | 30.98bc |
| 40             | 26.31bcde |

In each column, numbers with similar letters do not differ significantly at the 5% level based on the LSD test.

**Conclusion**

The use of nitroxin at a concentration of 15 mM in Iraqi celery population and at a concentration of 30 mM in Persian celery population could affect the studied morphological and physiological traits and improve plant growth. Drought stress caused a decrease in fresh weight of the plant in both celery types. However, reducing the fresh weight of the plant when using nitroxin, did not show a significant difference with the non-nitroxin treatment in these conditions; this issue indicates the positive effect of this substance in improving plant growth and increasing its resistance. According to the results, it was found that the use of nitroxin as a seed treatment, by increasing growth rate and improving nutrient absorption, accelerates plant growth and improves plant resistance to various environmental stresses, including drought stress.

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