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Evaluation of physiological response and yield function to bio-fertilizer and potassium consumption in melon (*Cucumis melo* L.) under water deficit conditions

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ABSTRACT

Tarbat-e Jam is one of the major areas of melon production. Recent droughts and shortage of water have reduced melon yield. In this research, the role of nitroxin bio-fertilizer and potassium on mitigating the adverse effects of drought stress on melon yield was evaluated during the years of 2019 and 2020. This experiment was designed as a split-plot factorial with three replicates based on a randomized complete block design. The factors in the split-factorial design including: irrigation (I), bio-fertilizer (B) and Potassium (K). Irrigation levels (I_{100}, I_{80}, and I_{60}) were kept at 60%, 80%, and 100% of crop evapotranspiration. The Nitroxin was used as seed coating (B_1) and none seed coating (B_0). The highest proline content and electrolyte leakage were obtained in I_{60} whit combination of B_0 and K_0. The highest and the lowest value of CAT activity were obtained in I_{60} + B_0 and K_2+B_1 treatments respectively. The highest peroxidase activity was achieved in the I_{60}. The seeds inoculated with nitroxin and Potassium application significantly decreased POD activity. Relative water content and total chlorophyll were decreased under drought stress but they increased significantly by using bio-fertilizer and potassium. The highest TSS was obtained in the K_2 and in the I_{80}+B_1 treatment. Potassium application increased the yield significantly. The highest and lowest yields were recorded in the I_{100}+B_1 and I_{60}+B_0 respectively. In this research, the use of bio-fertilizer and potassium moderated the effect of drought stress and reduced its negative effects, and the yield improved under drought stress.

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1. Introduction

Water stress is one of the most critical environmental stresses that limits crop production, especially in arid and semi-arid regions (Dar *et al*., 2021). Torbat-e Jam is the largest melon production area in Iran, which has an arid climate and 18,000 ha on average cultivated yearly. Melon production has significant economic importance and employment for the people of this region. Still, in recent years water deficiency and decreased rainfall have periled the cultivation of this valuable crop(Nastari Nasrabadi and Saberali, 2020).

The Cucurbitaceae family, due to having large leaves, fast growth, and a superficial root system, requires a lot of water for development and crop production (Korkmaz *et al*., 2007). Optimal use of water is crucial to crop production and increased yield. Several studies have shown that melon yield decreased under drought stress(Cabello *et al*., 2009; Hossein *et al*., 2015; Yavuz *et al*., 2021). Some strategies reported tolerance to drought stress, such as applying antiperspirant materials(Burme *et al*., 2011; del Amor *et al*., 2010; Moftah and Al-Humaid, 2005), different mulches(Aa *et al*., 2011; Behzad Nejad *et al*., 2018; Shokri *et al*., 2015) (Samaila *et al*, 2011; Shokri *et al*., 2016; Behzad Nejad *et al*., 2018), and proper plant nutrition under water deficit conditions(Al-Fraihat, 2011; Merghany *et al*., 2015; Tuna *et al*., 2010; Zahedyan *et al*., 2022). Under water stress, plant growth and yield are reduced due to reduced water and nutrient uptake. In these conditions, active adsorption, transportation, and membrane permeability are disrupted. In addition, nutrient diffusion, and surface absorption decreases(Hu and Schmidhalter, 2005).

Potassium is one of the most essential macronutrients in plant nutrition, which has various roles in plant growth and development. Potassium also plays a role in cell expansion, maintaining the turgor pressure, as well as the osmoregulation of cells. It also helps in the opening and closing of stomata, and activates more than 60 enzymes (Hawkesford *et al*., 2012). (Wang *et al*., 2013) reported that plants need more potassium under water stress conditions. Various studies showed that plant growth and yield could increase by providing potassium in drought stress conditions (Damon and Rengel, 2007; Sangakkara *et al*., 2001; Zhao *et al*., 2001).

Bio-fertilizers can increase nutrient uptake by developing plant root systems, improve soil structure and water holding capacity, reduce sodium uptake, and increase resistance to drought and salinity stress(Bacilio *et al*., 2004; Karlidag *et al*., 2007; Patten and Glick, 2002; Timmusk and Wagner, 1999). Nitroxin bio-fertilizer contains nitrogen-fixing bacteria such as *Azotobacter* and *Azospirillum*. These are usually found near and even inside the roots of plants*(Nazari Nasi et al*., 2018). Nitroxin has been reported to increase grain yield in medicinal pumpkins under drought stress(Najafi *et al*., 2021). Hasanpour *et al*., (2011) reported that supernitroplus fertilizer increased growth and yield in three sesame cultivars. The application of *Azotobacter* and *Azospirillum* increased the growth parameters of melon seedlings under salinity stress(Nastari Nasrabadi and Saberali, 2020).

Kord Zanganeh and Marashi (2018) in study the effects of combined application of chemical and biological fertilizers of potassium on yield and yield components of wheat under soil moisture shortage reported that the interaction effects between irrigation regimes and combined use of chemical and biological fertilizers on grain yield and biological yield were significant. Amujoyegbe *et al*., (2007) in their experiment showed the combined use of chemical and biological fertilizers increased the corn yield. The application of biofertilizer and chemical potassium fertilizer together increased flower yield by increasing the number of flowers, fresh weight of flowers and dry weight of stigma in saffron (Mohammad Ghasemi *et al*., 2022).

In this study, the impact of nitroxin and potassium on melon growth and yield was studied to explore if they can reduce water consumption and increase water use efficiency in arid and semi-arid regions.

2. Materials and Methods

2.1. Study area

This study was conducted at Torbat-e Jam, Khorasan Razavi Province, Iran (35˚ 23′ 17″ N, 60˚ 64′ 01″ E) during the spring and summer of 2019 and 2020 to determine the effect of potassium and bio-fertilizer on growth and yield of melon (*Cucumis melo* L.) under limited irrigation conditions. The soil was sandy loam, and the soil characteristics are provided in table 1. This area has an arid climate. Some meteorological variables during the experimental years and long-term averages were acquired from Torbat-e-Jam synoptic station (Table 2).

Table1. Soil characteristics of experimental plots.

Property	2019	2020
pH	7.98	7.91
EC (mS cm^{-1})	0.81	0.82
Organic carbon (%)	0.21	0.18
N (%)	0.02	0.03
P (ppm)	5.15	4.98
K (ppm)	85.00	76.00
Sand (%)	66.00	63.00
Silt (%)	23.00	24.00
Clay	11.00	13.00

Table 2. Some climate parameters of the study area.

Year	Month	T (˚C)	RH (%)	P (mm)	R_s (MJ m^{-2} day^{-1})	U_2 (m s^{-1})
2019	May	21.90	48.60	25.40	24.20	7.70
	June	25.80	35.40	13.00	28.50	8.80
	July	31.00	24.50	0.00	28.20	10.60
	August	27.00	26.00	0.00	26.50	10.40
	September	23.40	31.00	0.00	21.60	7.90
020	May	21.80	46.40	7.50	24.60	7.90
	June	27.40	24.00	0.00	28.50	7.30
	July	28.90	25.90	0.00	28.10	8.80
	August	26.70	31.00	0.00	25.60	8.30
	September	20.90	29.90	0.00	22.20	7.90
Long-term average (1993-2020)	May	22.50	38.00	13.07	24.10	4.70
	June	27.30	27.60	1.58	27.70	5.80
	July	29.20	25.70	0.12	28.10	6.90
	August	27.20	24.70	0.17	25.70	6.50
	September	22.50	28.30	0.67	21.80	4.90

T: Daily mean temperature, RH: Relative Humidity, P: Precipitation, R_s: Solar Radiation, U_2: Wind speed at 2 m Height.

2.2. Experimental design

This experiment was designed as a split-plot factorial in two years with three replicates based on a randomized complete block design. The factors in the split-factorial design including: irrigation (I), bio-fertilizer (B) and potassium (K).

1- Main plot: The main plot included irrigation treatment. The irrigation treatment was determined at three levels based on the water requirement of melon plant. 100% water requirement (I_{100}) was used as a control and 80% (I_{80}) and 60% (I_{60}) water requirement were determined as medium and severe water stress respectively.

2- Sub-plot: Bio-fertilizer at two levels (B_0: none application, and B_1: seed treatment) and potassium treatments at three levels: 0, 100, and 150 kg ha-1 (K0, K1, and K2) were applied factorial in the sub-plot (B_0 K_0, B_0 K_1, B_0 K_2, B_1 K_0, B_1 K_1 and B_1 K_2).

The nitroxin was used as a bio-fertilizer. It was used at a rate of 2 L/h for seed coating. The seeds were coated in the shade and sown immediately after the seeds dried. Potassium sulfate was used as a source of potash.

Each subplot included three blocks with six rows in each block, and there were six plants in each row. Before planting, one-third of nitrogen fertilizer from a urea source (50 kg N ha^{-1}) and total dose of phosphorus (triple superphosphate, 100 kg P ha^{-1}) along with potassium based on determined treatments combined with soil. The remaining of nitrogen fertilizer was top-dressed at (50 kg N ha^{-1}) before flowering and before the color change. The melon seeds (Khatooni cultivar) were sown in the field on 5 May; 2019, and 9 May; 2020. According to the custom of farmers, seeds were planted 70 cm in a row and 2.8 m between rows. The growing season of Khatooni melon in this study area was divided into four stages (Table 3) (Nastari Nasrabadi *et al*., 2012).

Table 3. Phenological stages growth of Khatooni melon.

Stage	Description	Days after sowing
1	From seed germination to the beginning of flowering	22
2	From flowering to production small fruits	17
3	From small fruits to the beginning of fruit color change	32
4	From the beginning of color change to the end of second harvest	39

3.2. Irrigation methodology and system

The amount of evapotranspiration of the reference plant was calculated using the FAO Penman-Mantith method(Richard *et al.*, 1998). The crop coefficient (Kc) of melon obtained from (Richard *et al*., 1998), and evapotranspiration of melon (ETc) was calculated according to equation 1:

$$ET_c = ET_p \times K_c \tag{1}$$

Water requirement was calculated by adequate rainfall for each irrigation period. Irrigation efficiency was estimated at 85% according to the regional conditions. The daily water requirement of melon was estimated from 5 May; to 17 August; 2019 and 9 May; to 21 August; 2020. The weekly water requirement for each treatment (100, 80, and 60% ET_C) was calculated (Table 4). The irrigation system consisted of a drip line in each crop row and volumetric meters.

Table 4. Reference evapotranspiration (ETo), crop coefficients, crop evapotranspiration (ETc), weekly irrigation water amount actually applied over the scheduling irrigation period in the different treatments in 2019 (A) and 2020 (B).

Dates of Irrigation in 2019 (A)	ET_o	K_C	ET_C	I_{60} (cm)	I_{80} (cm)	I_{100} (cm)
5 May to 11 May	70.14	0.50	35.07	24.76	33.01	41.26
12 May to 18 May	67.34	0.50	33.67	23.77	31.69	39.61
19 May to 25 May	50.38	0.50	25.19	17.78	23.71	29.63
26 May to 1 June	65.99	0.60	39.03	27.74	36.99	46.23
2 June to 8 June	71.67	0.82	59.45	41.57	55.43	69.29
9 June to 15 June	78.16	1.02	79.80	56.34	75.12	93.90
16 June to 22 June	85.87	1.05	90.16	63.64	84.86	106.07
23 June to 29 June	99.56	1.05	104.54	73.79	98.39	122.98
30 June to 6 July	97.88	1.05	102.78	72.55	96.73	120.92
7 July to 13 July	108.70	1.05	114.13	80.56	107.42	134.27
14 July to 20 July	127.68	1.03	130.97	92.51	123.35	154.19
21 July to 27 July	125.46	0.97	121.83	86.01	114.68	143.35
28 July to 3 August	103.13	0.92	94.46	66.68	88.91	111.14
4 August to 10 August	108.41	0.86	93.36	65.87	87.82	109.78
11 August to 17 August	88.96	0.81	71.79	50.58	67.44	84.30
Dates of Irrigation in 2020 (B)	ET_o	K_C	ET_C	I_{60} (cm)	I_{80} (cm)	I_{100} (cm)
9 May to 15 May	53.14	0.50	26.57	18.76	25.01	31.26
16 May to 22 May	56.30	0.50	28.15	19.87	26.49	33.12
23 May to 29 May	89.65	0.50	44.82	31.64	42.19	52.73
30 May to 5 June	94.15	0.60	56.21	39.58	52.77	65.96
6 June to 12 June	84.01	0.82	68.65	48.73	64.97	81.21
13 June to 19 June	93.47	1.02	95.73	67.38	89.84	112.30
20 June to 26 June	92.07	1.05	96.67	68.24	90.98	113.73
27 June to 3 July	94.35	1.05	99.07	69.93	93.24	116.55
4 July to 10 July	91.16	1.05	95.72	67.57	90.09	112.61
11 July to 17 July	98.13	1.05	103.04	72.73	96.98	121.22
18 July to 24 July	106.04	1.03	108.70	76.83	102.44	128.06
25 July to 31 July	97.15	0.97	94.28	66.61	88.81	111.01
1 August to 7 August	83.38	1.60	76.34	94.35	125.80	157.25
8 August to 14 August	88.27	0.86	75.93	53.63	71.50	89.38
15 August to 21 August	84.65	0.81	68.26	48.13	64.17	80.22

In this experiment, leaf relative water content (RWC) was determined following Yamasaki & Dillenburg, (1999), chlorophyll concentrations were calculated following Strain & Svec, (1966), proline was determined following the protocol of Bates *et al*., (1973), electrolyte leakage (E.L) measured based on the method of (Zhao *et al*., 1992), catalase (CAT) and peroxidase (POD) activity were measured by following (Havir and McHale, 1987) and(Tuna *et al*., 2008) respectively, total soluble solids (TSS) were measured by hand-refractometer (RHB0-80). Fruit weight was measured to determine the total yield.

To obtain production functions under seed coating (B_1) and non-seed coating (B_0)

conditions, yield and TSS were considered as output, and different amounts of water in various stages of growth, and the amount of potassium fertilizer were as input parameters. In this research, simple linear (2), logarithmic (3), quadratic (4), and transcendental (5) production functions were used to determine the production functions based on changes in the amount of potassium fertilizer (K) (kg ha^{-1}) and the amount of irrigation water (I) (cm).

$$Y = a_0 + a_1 I + a_2 K \quad (2)$$

$$Y = a_0 I^{a_1} K^{a_2} \quad (3)$$

$$Y = a_0 + a_1 I + a_2 I^2 + a_3 K + a_4 K^2 + a_5 IK \quad (4)$$

$$Y = a_0 I^{a_1} K^{a_2} \, exp(a_3 I + a_4 K) \quad (5)$$

In order to evaluate the best production function, used widely accepted statistical indices such as correlation coefficient (CC), root mean square error (RMSE), model efficiency (EF), and maximum error (ME) (Equations 6-9).

$$\text{CC} = \frac{\frac{1}{N}\sum_{n=1}^{N}(P_n - \bar{P})(O_n - \bar{O})}{\sqrt{\frac{1}{N}\sum_{n=1}^{N}(P_n - \bar{P})^2}\sqrt{\frac{1}{N}\sum_{n=1}^{N}(O_n - \bar{O})^2}} \quad (6)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{n=1}^{N}(P_i - O_i)^2} \quad (7)$$

$$EF = (\sum_{i=1}^{n}(O_i - \sigma)^2 - \sum_{i=1}^{n}(P_i - O_i)^2) / \sum_{i=1}^{n}(O_i - \sigma)^2) \quad (8)$$

$$ME = MAX_{i=1}^{n}|O_i - P_i| \quad (9)$$

In these equations, O and P was the observed and predicted performance values, respectively, and n was the number of observations.

The data was first tested for normality of residual distribution using the Shapiro-Wilk test (PROC UNIVARIATE; SAS) and a Q–Q plot. Bartlett's test was then conducted to confirm the homogeneity of error variances among treatments and across years for combined analysis. Analysis of variance was performed using the SAS general linear model procedures (SAS Institute, 2007). The means were compared by Duncan's multiple range test at a 5% probability level. Excel software was used to analyze production functions.

3. Results

3.1. Proline Content

Based on the results of analysis of variance (Table 5), it was determined, that the interaction effects of irrigation, biofertilizer and potassium had significant effects at 1% probability level. Results showed (Table 6) that proline content significantly increased with the decreasing amount of irrigation. The highest amount of proline (1.89 mg/g fw) was obtained in the 60% irrigation treatment (I_{60}), without bio-fertilizer (B_0) and potassium (K_0), which showed a 52.14% increase compared to the control (I_{100} B_0 K_0). The seed coating with nitroxin (B_1) and different levels of potassium (K_1 and K_2) reduced proline production compared to the B_0+K_0 under different irrigation levels. The lowest amount of proline was recorded in the combination of 150 kg ha^{-1} of potash with B_1, by 13.70%, 25%, and 25.92% reduced compared to the B_0+K_0 in each irrigation regime, respectively.

3.2. Electrolyte leakage

According to the results of analysis of variance (Table 5) it was found, that the interaction effects of irrigation, bio-fertilizer and potassium had significant effects at 5% probability level.

Data in table (6) showed that electrolyte leakage significantly increased by increasing drought stress. The highest electrolyte leakage (28.85%) was recorded in the most severe drought stress (I_{60}) whit combination of B_0 and K_0. Results showed (Table 6) that the application of nitroxin (B_1) and different levels of potassium reduced electrolyte leakage under all irrigation treatments. The combination of B_1 and K_2 (150 kg ha^{-1}) significantly reduced the electrolyte leakage value in each irrigation treatment, which achieved relative decrement of 48.53%, 57.59%, and 39.96% as compared to the control (B_0 + K_0) in 100, 80, and 60% irrigation, respectively.

Table 5. Variance analysis (mean square) of studied traits of melon plant by using bio-fertilizer (B), and potassium (K) at different irrigation (I) levels in two consecutive years (Y).

Sov	df	Proline	Electrolyte leakage	Catalase	Peroxidase	Relative water content	Total chlorophyll	Total soluble solids	Yield
Y	1	0.0075 ns	109.55 ns	0.01 ns	0.081 ns	10.53 ns	3.27 ns	0.37 ns	4.00 ns
R	2	0.1615 **	2.06 ns	0.02 ns	0.010 ns	4.26 ns	2.17 *	1.10 ns	16.16 **
E(Y)	2	0.2639	6.32	0.02	0.005	12.02	3.40	0.48	0.22
W	2	1.1257 **	1192.46 **	53.98 **	0.216 **	1616.27 **	15.81 **	6.52 **	1264.30 **
Y.W	2	0.0015 ns	14.03 ns	0.03 ns	0.001 ns	7.77 ns	0.69 ns	0.47 ns	0.15 ns
E(a)	8	0.0119	5.73	0.02	0.002	6.46	0.49	0.35	2.81
B	1	0.3780 **	93.54 **	5.21 **	0.047 **	157.91 **	4.89 **	17.28 **	46.68 **
K	2	0.3470 **	754.68 **	1.90 **	0.024 **	215.34 **	5.98 **	11.56 **	16.66 **
Y×B	1	0.0004 ns	6.01 ns	0.01 ns	0.001 ns	0.0001 ns	0.01 ns	0.16 ns	0.03 ns
Y×K	2	0.0006 ns	10.09 ns	0.02 ns	0.001 ns	23.97 ns	0.15 ns	0.33 ns	0.03 ns
B×K	2	0.0451 **	2.74 ns	0.32 **	0.003 ns	4.21 ns	0.33 ns	0.55 ns	0.18 ns
W×B	2	0.0230 ns	9.42 ns	0.31 **	0.001 ns	11.76 ns	0.55 ns	1.69 *	10.18 **
W×K	4	0.0524 **	47.81 **	0.24 **	0.001 ns	20.92 ns	0.76 ns	0.46 ns	1.05 ns
W×B×K	4	0.0444 **	23.40 *	0.07 ns	0.001 ns	9.08 ns	0.06 ns	0.60 ns	1.52 ns
Y×B×K	2	0.0001 ns	1.27 ns	0.05 ns	0.001 ns	10.67 ns	0.21 ns	0.04 ns	0.13 ns
Y×W×K	4	0.0006 ns	6.48 ns	0.01 ns	0.001 ns	4.36 ns	0.21 ns	0.17 ns	0.07 ns
Y×W×B	2	0.0004 ns	2.86 ns	0.02 ns	0.001 ns	3.82 ns	0.36 ns	0.80 ns	0.09 ns
Y×W×B×K	4	0.0001 ns	3.35 ns	0.03 ns	0.001 ns	9.62 ns	0.19 ns	0.07 ns	0.06 ns
E	60	0.0075	8.91	0.04	0.002	10.02	0.46	0.49	1.24
%C.V		6.38	17.45	3.96	9.13	4.25	8.33	5.82	4.50

ns none significant difference, * and ** are significant at %5 and %1probability levels respectively.

3.3. Catalase (CAT) and peroxidase (POD) activity

Based on the results (Table 5), it was determined that the double interaction effects of treatments on CAT activity were significant at 5% probability level. Mean comparison of data showed (Table 7) the highest value of CAT activity (6.77 µmol $H_2O_2.g^{-1}$ $FW.min^{-1}$) was obtained in the combination of I_{60} and B_0 treatments, with a 60.88% increase compared with the control (I_{100}+B_0). Based on the mean comparison of treatments (Table 8-A), it found that the lowest value of CAT activity (4.69 µmol H_2O_2.g^{-1} FW.min^{-1}) was obtained in the combination of K_2 (150 K/ha) treatment with seeds inoculated with nitroxin (B_1), by 15.49% decreased comparing with the control (B_0+K_0). Potassium application significantly decreased CAT activity in all irrigation treatments (Table 8-B). The lowest CAT activity was observed in the K_2 treatment (100 kg/ha) in each irrigation treatment.

The results showed that the simple effects of treatments on POD activity were significant at 1% probability level (Table 5). According to the results (Table 9), the POD activity significantly increased by increasing drought stress, the highest POD activity (0.54 units.g^{-1} FW.min^{-1}) was achieved in the I_{60} treatment. The seeds inoculated with nitroxin (B_1) had significantly lower POD activity. Potassium application significantly decreased POD activity, and the lowest value (0.43 units.g^{-1} FW.min^{-1}) was obtained in the K_2 (150 kg/ha) treatment.

3.4. Relative water content (RWC)

The simple effects of irrigation, bio-fertilizer, and potassium treatments had significant effects on RWC (Table 5). The results showed (Table 9) that the RWC decreased significantly with increasing drought stress. The lowest value (69.16%) was obtained in the most severe stress (I_{60}), with a 15.68% decrease compared with the control (I_{100}). Nitroxin (B_1) significantly increased the RWC. Also, the results showed (Table 9) that by increasing the amount of potassium fertilizer, the RWC increased significantly.

3.5. Total chlorophyll

The simple effects of the treatments on the total chlorophyll content were significant (Table 5). The results showed (Table 10) that the total chlorophyll content decreased with increasing drought stress. The lowest value (7.35 mg/g fw) was obtained in the I_{60} treatment, by a 14.53% decrease compared with the control (I_{100}). There was no significant difference between I_{100} and I_{80} treatments. Nitroxin (B_1) significantly increased chlorophyll content compared to the control (B_0) (Table 6). The results showed that total chlorophyll content improved significantly by increasing potassium levels. Total chlorophyll content under K_1 and K_2 treatments increased by 6.25% and 10.55% compared with the control (K_0), respectively.

3.6. Total soluble solids (TSS)

Simple effect of potassium (at 1% probability level) and interaction between irrigation regimes and bio-fertilizer (at 5% probability level) significantly affected TSS content (Table 5). The TSS increased by increasing potassium levels (Table 10), and the highest value of TSS (12.55%) was obtained in the K_2 treatment. The results showed (Table 7) that the highest value (12.83%) of TSS was obtained in the I_{80}+B_1 treatment with a 17.06% increase compared with the control (I_{100}+B_0). The TSS content in all irrigation treatments increased by seed coating with nitroxin (B_1), but this increase was not significant in the I_{60} treatment (Table 7).

3.7. Yield

According to the results of analysis of variance (Table 5), it was determined, that the Simple effect of potassium and interaction effects of irrigation and bio-fertilizer had significant effects at 1% probability level. The results showed (Table 10) that potassium application increased the yield significantly, and the highest yield (25.42 t/ha) was obtained in the K_2 (150 kg ha^{-1}) treatment. The interaction effects of irrigation and bio-fertilizer (Table 7) were showed the yield increased with B_1, and this increase was significant in the I_{100} and I_{80} treatments. The highest (30.14 t/ha) and lowest (17.90 t/ha) yield were recorded in the I_{100}+B_1 and I_{60}+B_0 treatments respectively

Table 6. Interaction effects of irrigation, bio-fertilizer, and potassium on proline content and Electrolyte leakage over two growing seasons.

Irrigation (I)	Biofertilizer (B)	Potassium (K)	Proline (mg/g fw)	Electrolyte leakage (%)
I_{100}	B_0	K_0	1.24 ghi	16.09 ef
		K_1	1.19 hi	12.54 fgh
		K_2	1.26 gh	10.72 hi
	B_1	K_0	1.19 hi	12.03 hi
		K_1	1.18 hi	10.31 hi
		K_2	1.07 j	8.28 i
I_{80}	B_0	K_0	1.52 bc	23.18 bc
		K_1	1.35 efg	15.74 ef
		K_2	1.27 gh	12.00 ghi
	B_1	K_0	1.40 def	21.95 c
		K_1	1.31 fg	16.55 de
		K_2	1.14 ij	9.83 hi
I_{60}	B_0	K_0	1.89 a	28.85 a
		K_1	1.54 b	20.10 cd
		K_2	1.45 bcde	17.73 de
	B_1	K_0	1.50 bcd	28.68 a
		K_1	1.45 bcde	26.10 ab
		K_2	1.41 cdef	17.32 de

Means in the same column with different letters differ significantly at 0.05 probability level according to Duncan's multiple range test.

3.8. Production functions of yield and TSS

Coefficients of production functions for yield and TSS under with and without bio-fertilizer showed in table 11. The production functions ranked based on the calculated values of RMSE, EF, ME, and CC statistical indicators (Table 12). The linear regression function was the best function for yield and TSS content. The higher correlation coefficient (CC) and model efficiency (EF) and the lower ME value in the linear regression function indicated the high efficiency of the model in estimating the desired values. The results showed that the yield decreased with a decrease in potassium level and the irrigation depth (Figure 1). The yield in water shortage conditions responded with a steeper slope, and by increasing the amount of irrigation water, the trend of yield increasing slowed down, and yield reduction was less by using bio-fertilizer (Figure 1). The yield contour lines (Figure 2) showed the geometric location of different combinations of irrigation water and potassium fertilizer, which produced the exact yield in melon plants. The yield increased by increasing the available water in the same amount of potassium fertilizer. The results showed that TSS would be higher by using bio-fertilizer (figure 3-A). In a certain amount of irrigation, the TSS had an increasing trend by increasing potassium fertilizer up to about 100 kg ha^{-1} (Figure 3). Then it continued with a low slope, especially without bio-fertilizer. In a constant potassium fertilizer, by increasing the depth of irrigation (about 120 cm), TSS had an increasing trend, which decreased by increasing irrigation.

Table 7. Interaction effects of irrigation and bio-fertilizer on catalase (CAT), total soluble solids (TSS) and yield over two growing seasons.

Treatment		CAT (μmol $H_2O_2.g^{-1}$ FW.min^{-1})	Total soluble solids (%)	Yield (t/ha)
I_{100}	B_0	4.21 e	10.96 c	29.23 b
	B_1	3.98 f	12.06 b	30.14 a
I_{80}	B_0	5.26 c	11.83 b	25.03 d
	B_1	4.67 d	12.83 a	27.55 c
I_{60}	B_0	6.77 a	11.94 b	17.90 e
	B_1	6.26 b	12.25 b	18.41 e

Means in the same column with different letters differ significantly at 0.05 probability level according to Duncan's multiple range test.

Table 8. A- Interaction effects of bio-fertilizer and potassium and B- Interaction effects of irrigation and potassium on catalase over two growing seasons.

Treatment		CAT (μmol $H_2O_2.g^{-1}$ FW.min^{-1})
	A	
B_0	K_0	5.55 a
	K_1	5.43 ab
	K_2	5.26 c
B_1	K_0	5.32 bc
	K_1	4.91 d
	K_2	4.69 e
	B	
I_{100}	K_0	4.20 f
	K_1	4.07 fg
	K_2	4.02 g
I_{80}	K_0	5.25 d
	K_1	4.89 e
	K_2	4.76 e
I_{60}	K_0	6.86 a
	K_1	6.54 b
	K_2	6.15 c

Means in the same column with different letters differ significantly at 0.05 probability level according to Duncan's multiple range test.

Table 9. Simple effects of irrigation, bio-fertilizer, and potassium on peroxidase, relative water content and total chlorophyll over two growing seasons.

Treatment	POD (units.g^{-1} FW.min^{-1})	Relative water content (%)	Total chlorophyll (mg/g fw)
I_{100}	0.39 c	82.02 a	8.60 a
I_{80}	0.45 b	72.33 b	8.36 a
I_{60}	0.54 a	69.16 c	7.35 b
B_0	0.48 a	73.29 b	7.89 b
B_1	0.44 b	75.71 a	8.32 a
K_0	0.48 a	72.16 c	7.68 c
K_1	0.46 a	74.31 b	8.16 b
K_2	0.43 c	77.04 a	8.49 a

Means in the same column with different letters differ significantly at 0.05 probability level according to Duncan's multiple range test.

Table 10. Simple effects of potassium on total soluble solids and yield over two growing seasons.

Treatment	Total soluble solids (%)	Yield (t/ha)
K_0	11.42 c	24.07 c
K_1	11.97 b	24.63 b
K_2	12.55 a	25.42 a

Means in the same column with different letters differ significantly at 0.05 probability level according to Duncan's multiple range test.

Table 11. Coefficients of production functions for yield and TSS under with and without bio-fertilizer.

Fertilizer	treatment	Production functions	Coefficients						F
			a_0	a_1	a_2	a_3	a_4	a_5	
B0	Yield	Liner	0.615	0.194	0.009	-	-	-	358.06 **
		Logarithmic	0.225	0.898	0.085	-	-	-	36.600 **
		Quadratic	-22.65	0.603	0.002	0.008	0	0	4709.5 ns
		transcendental	0.469	-0.757	0	0.054	0.002	-	35.810 **
	TSS	Liner	13.075	-0.017	0.006	-	-	-	16.218 **
		Logarithmic	17.142	0.167	0.087	-	-	-	37.870 **
		Quadratic	7.113	0.088	0	0.004	0	0	2546.4 ns
		transcendental	2.319	0.621	-0.156	-0.007	0.002	-	4786.5 **
B1	Yield	Liner	1.277	0.200	0.008	-	-	-	144.05 **
		Logarithmic	0.223	0.925	0.072	-	-	-	35.910 **
		Quadratic	-47.25	1.083	-0.004	-0.002	0	0	3332.0 ns
		transcendental	1.011	-0.764	0	0.049	0.002	-	40.690 **
	TSS	Liner	12.032	-0.003	0.009	-	-	-	15.217 **
		Logarithmic	6.667	-0.009	0.143	-	-	-	39.710 **
		Quadratic	2.197	0.180	-0.001	-0.007	0	0	3231.0 ns
		transcendental	0.025	1.944	-0.297	-0.017	0.004	-	3523.0 **

Table 12. Calculated statistical parameters to evaluate the validity of production functions for yield and TSS under with and without bio-fertilizer.

Fertilizer	treatment	Production functions	Parameters				Rank
			RMSE	EF	ME	CC	
B0	Yield	Linear	5.17	0.16	3.35	0.97	1
		Logarithmic	34.52	-2.36	17.19	0.51	4
		Quadratic	18.13	-0.18	9593.73	0.97	2
		transcendental	57.35	-10.17	17.63	0.85	3
	TSS	Linear	5.85	0.59	2.16	0.62	1
		Logarithmic	34.17	-22.41	9.01	0.39	2
		Quadratic	57.81	-50.30	10.12	-0.41	3
		transcendental	223.6	-909	48.8	-0.29	4
B1	Yield	Linear	7.98	0.21	4.31	0.92	1
		Logarithmic	32.60	-1.89	17.87	0.53	3
		Quadratic	21.44	-0.52	10.57	0.87	2
		transcendental	55.43	-8.9	20.79	0.78	4
	TSS	Linear	5.81	0.58	1.87	0.61	1
		Logarithmic	43.88	-42.30	10.12	0.55	2
		Quadratic	34.84	-20.46	8.15	0	3
		transcendental	1194	-28014	267.56	-0.51	4

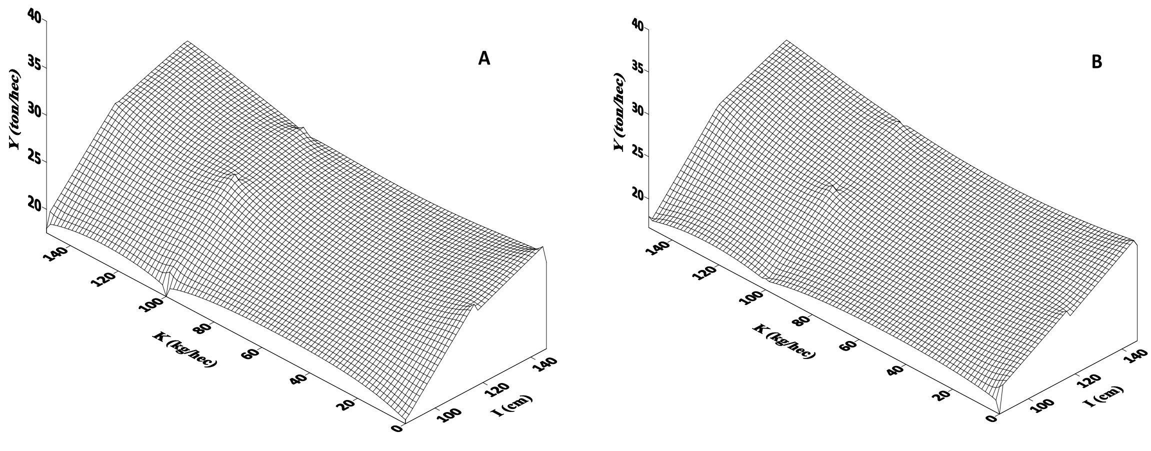

Fig. 1. Yield changes to the amount of irrigation water and potassium (K) under seed treatment with bio-fertilizer (A) and without bio-fertilizer (B).

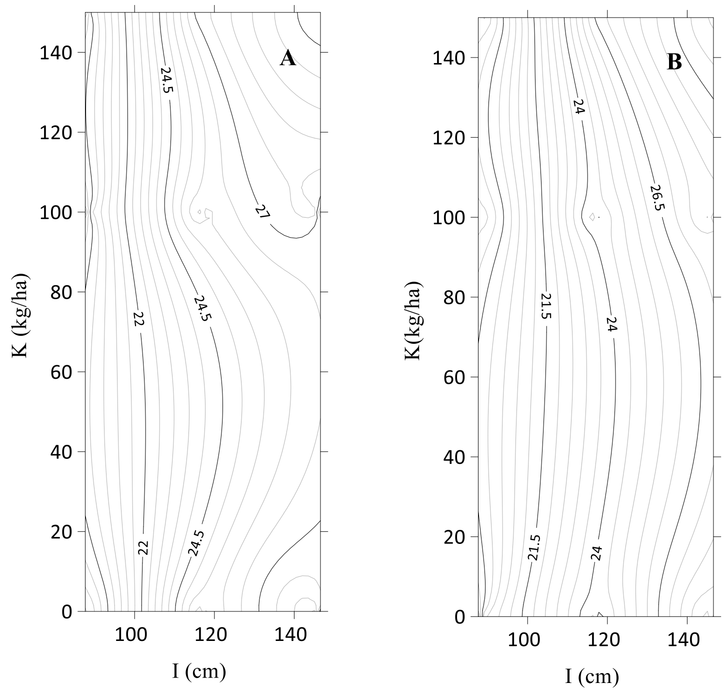

Fig. 2. Yield contour lines under seed treatment with bio-fertilizer (A) and without bio-fertilizer (B).

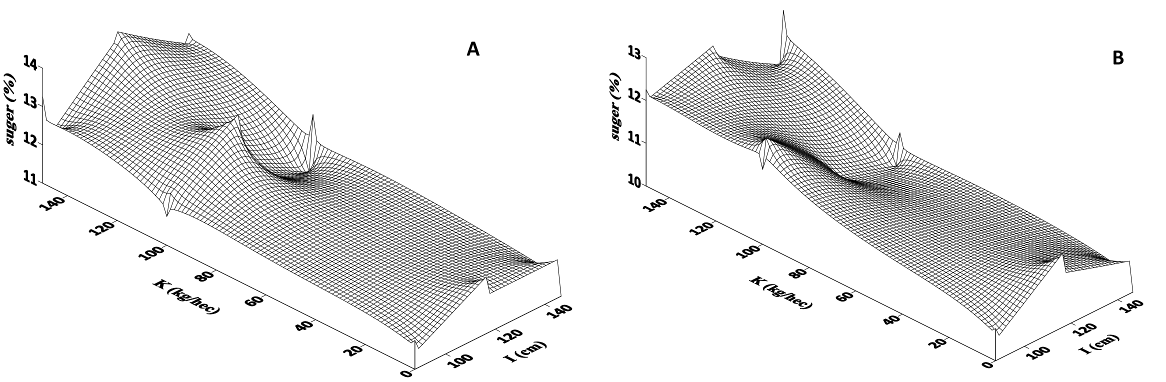

Fig. 3. TSS changes to the amount of irrigation water (I) and potassium (K) under seed treatment with bio-fertilizer (A) and without bio-fertilizer (B).

4. Discussion

The proline content is an indicator of drought tolerance in plant tissue under drought stress. The proline accumulation helps to increase the osmotic level of plant cells under water deficiency. It enables the plant to survive for a short period after drought stress, and to be able to recover its growth after removing the stress (Tang *et al*., 2009). Proline accumulation in response to stress is widely reported, also in this experiment, proline content increased with increasing drought stress, similar to the results of other researchers (Barzegar *et al*., 2017; Tuna *et al*., 2010; Zahedyan *et al*., 2022). In this research, the effects of drought stress were reduced by using bio-fertilizers and potassium, therefore proline production also decreased (Levent Tuna *et al*., 2010; Zahedyan *et al*., 2022).

The cytoplasmic membrane of plant cells under stress has little stability, therefore the electrolyte leakage of intracellular substances increases in them. Drought stress by stimulating oxidative stress and producing free radicals induces peroxidation of fatty acids in the cell membrane and increases membrane permeability and ion leakage (Nastari Nasrabadi *et al*., 2023). Zahdian *et al*., (2022) reported that electrolyte leakage decreased by using bio-fertilizers in melon under drought stress. Aksu and Altay (2020) in study the effects of potassium applications on drought stress in Sugar Beet reported that potassium reduced electrolyte leakage and cell membrane damage was reduced under drought condition.

The importance of water in the plant to maintain turgor pressure has been thoroughly proven for growth and development(Barnabás *et al*., 2008; Zhang *et al*., 2012). The RWC is measured as a vital factor in expressing the water status of the plant and indicates the deficit of water in the plant. A critical defense mechanism during drought stress is maintaining plant water status to maintain enough water by minimizing water loss and maximizing water absorption*(Barnabás et al.*, 2008). The RWC decreased significantly by increasing drought stress, consistent with similar results(Bahrami-Rad and Hajiboland, 2017; Barzegar *et al*., 2017; Zahedyan *et al*., 2022).

Under drought stress conditions, root growth and nutrient absorption are disturbed, partly because the decrease in soil moisture reduces the transfer of nutrients to the root surface *(Dar et al*., 2021; Tuna *et al*., 2010). Potassium element plays an essential role in plant water relations. This nutritional element is actively and passively absorbed by plant roots, and its concentration in the cytosol is higher than other cations surface (Dar *et al*., 2021; Tuna *et al*., 2010). The potassium application increases the resistance to drought stress. It increases water absorption by reducing the osmotic potential of root cells and also plays a role in regulating stomata activity. If there is enough potassium in the stomatal guard cells, the water content of these cells increases, and the leaf stomata open, therefor CO_2 quickly penetrates, and photosynthesis performs better. Under dry conditions, potassium is released from the stomata guard cells, and the stomata close to prevent water loss. If plants have a potassium deficiency, water loss will increase, because stomata activity slows down*(Cui and Tcherkez, 2021; de Bang et al., 2021; Sardans and Peñuelas, 2021).*

Growth-promoting bacteria can increase plant tolerance to environmental stresses by developing root system, increasing the absorption of nutrients, improving soil structure, and reducing sodium absorption (Patten and Glick, 2002). Bio-fertilizers produce phytohormones (auxin, cytokinin, and gibberellin) and l-aminocyclopropane-l-carboxylate (ACC) deaminase (German *et al*., 2000; Glick *et al*., 2007; Shaharoona *et al*., 2006). The auxin group plays a very influential role in root system production, the most important of which is indole-3-acetic acid (IAA) (Patten and Glick, 2002). A large number of plant symbiotic bacteria, including *Azospirillum*, produce IAA. These effects have been demonstrated by *Azospirillum* inoculation in wheat, corn, and sorghum (German *et al*., 2000). The ethylene level reduction is another mechanism of rhizosphere bacteria to stimulate plant growth. The ACC is a substrate for ethylene production and is converted to ethylene by ACC oxidase(Glick *et al*., 2007). The bio-fertilizers with ACC deaminase enzyme, such as *Azospirillum, Rhizobium, Agrobacterium, Achromobacter, Burkholderia, Ralstonia, Pseudomonas,* and *Enterobacter,* use ACC as the only source of nitrogen, and they can control root growth by reducing the negative effects of ethylene (Blaha *et al*., 2006). These bacteria protect plants from the adverse impacts of ethylene accumulation in environmental stress conditions such as drought, salinity, heat, cold, diseases, and toxic metals by reducing ethylene(Ghosh *et al*., 2003; Glick *et al*., 2007; Hontzeas *et al*., 2004). The seed coating with nitroxin and using potassium fertilizer, especially K_2 (150 kg/ha), created that better conditions for root growth and water absorption. Therefore, the RWC increased in all irrigation treatments. Under drought stress, the plants treated with B_2+K_2 had lower proline content than the control plants in each irrigation treatment. It can say that the photosynthetic materials, instead of converting to proline for osmotic regulation, use for plant growth and transfer to the fruits.

Biological membranes are the first target of many environmental stresses. It is generally accepted that maintaining the integrity and stability of membranes under water stress is the central part of drought resistance in plants(Bajji *et al*., 2002). The drought stress, by instigating oxidative stress and free radicals, causes peroxidation of cell membrane fatty acids and increases the membrane permeability and ion leakage(Hasanuzzaman *et al*., 2020).

In this research, the activity of CAT and POD increased under drought stress. Several studies showed that antioxidant activity increased under water deficit (Aksu and Altay, 2020; Barzegar *et al*., 2017; Eliaspour *et al*., 2020; Munsif *et al*., 2022). The antioxidant enzymes such as CAT and POX play an essential role in drought tolerance. (Eliaspour *et al*., 2020) and (Omar *et al*., 2009) reported, antioxidant enzymes activity in maize and barley decreased by seed inoculation.(Aksu and Altay, 2020) also reported a decline in non-enzymatic antioxidants in sugar beet with potassium application under drought conditions. In contrast to this study (Munsif *et al*., 2022) reported that potassium increased CAT and POD activity in wheat. Also,(Zhu *et al*., 2011) showed the activity of these enzymes increased in maize under bio-fertilizer application. It can be said, using potassium and bio-fertilizer under drought stress can increase water and nutrient absorption. Therefore, antioxidant activity reduces for defense purposes when faced with plant stress.

According to the results, the chlorophyll content decreased by increasing water stress. Chlorophyll reduction under drought stress is an index of oxidative stress in plants. The chloroplast destruction increases by closing the stomata under drought conditions for a long time. Nitrogen and glutamate (substrate for chlorophyll and proline production) consumption are the reasons for chlorophyll reduction under drought stress, which causes more proline production and accumulation(Bybordi, 2012; Rostami *et al*., 2015). The results showed, that the plants inoculated by nitroxin (B_1) and also with potassium application (especially K_2) had a significantly higher chlorophyll content, which is in agreement with the results of *Rezaienia et al*., (2017) and Zahedyan *et al*., (2022). Chlorophyll biosynthesis increases with an increasing amount of nitrogen and water availability in plants. Nitrogen is the main component of chlorophyll and many chlorophyll synthesizing enzymes (Fageria *et al*., 2010). In this experiment, nitroxin increased the chlorophyll content at different levels of drought stress. This can be due to the role of nitrogen-fixing bacteria, which increased the chlorophyll content of melon. The chlorophyll content of melon increased by potassium application, especially the application of a high concentration of K (150 kg ha^{-1}). Lotfi *et al*., (2022) reported that K application (150 kg ha^{-1}) had a beneficial effect on the Chlorophyll fluorescence efficiency of wheat under dryland conditions. Alagarsamy & Kumar, (2008) reported that the relative content of chlorophyll in bananas increased by increasing potassium sulfate consumption. They stated that the increase in photosynthetic activity could be due to the role of potassium in the synthesis of the precursor of chlorophyll pigments, and the transfer of radiation energy into primary chemical energy in the form of ATP and NADPH in the chloroplasts improved by increased chlorophyll content.

In our study, the highest value of TSS was obtained in medium water stress (I_{80}). *Zahedyan et al*., (2022) reported that drought stress increased TSS in melons, but Cui-hua *et al*., (2016) stated that TSS decreased in melons by increased irrigation intervals. (Cabello *et al*., 2009) reported that Water stress had no significant effect on the TSS content of melon. The TSS increasing with drought stress has also been reported by other researchers(Alam *et al*., 2021; Hossein *et al*., 2015). This increase can be due to the decrease in water absorption by the fruit and the increase in the sugar ratio. Moreover, reducing invertase enzyme activity during drought stress increases the accumulation of sucrose and TSS in fruits (Alam *et al*., 2021).

The yield significantly decreased under drought stress. Our results are consistent with many other studies on melons*(Barzegar et al., 2017; Hossein et al., 2015; Tuna et al., 2010; Yavuz et al*., 2021; Yildirim *et al*., 2009). Adequate available soil moisture within the root zone increases the various physiological processes, such as better uptake of nutrients, plant growth, and photosynthesis rates. Therefore, the highest yield was obtained. In this study, the yield increased under drought stress by using potassium and nitroxin bio-fertilizer, which can be due to the reduction of the effects of drought on physiological processes (German *et al.,* 2000*;* Merghany *et al*., 2015; Najafi *et al*., 2021; Tuna *et al*., 2010; Zahedyan *et al*., 2022).

In this study, drought stress increased proline, electrolyte leakage, catalase and peroxidase, decreased relative water content and decreased total chlorophyll in melon. Under drought stress, assimilate accumulation were used to increase drought resistance, so yield decreased, which is the same with similar studies (Aksu and Altay, 2020; Barzegar *et al*., 2017; Eliaspour *et al*., 2020; Munsif *et al*., 2022). In this research, the use of bio-fertilizer and potassium moderated the effect of drought stress and reduced its negative effects, and the yield improved under water stress condition.

After yield, TSS is the most important essential characteristic of melon. The linear regression function was the best function for yield and TSS content. Rashki *et al*., (2020) stated that the decrease in yield due to the decline in irrigation depth could be compensated by increasing the amount of potassium fertilizer. The positive effect of potassium on TSS increase has been reported by other researchers (Bouzo *et al*., 2018; Lin *et al*., 2004). Also results showed that bio-fertilizer increased TSS. These results were consistent with (Zahedyan *et al*., 2022) and (Al-Fraihat, 2011) studies.

5. Conclusion

A large land area in the Torbat-e Jam region is dedicated to melon cultivation yearly (more than 18000 ha). On the other hand, this region has been suffering from drought and reduced rainfall for years. Therefore, to reduce the drought risks, it is necessary to use new agricultural methods to reduce water consumption and keep the living of this region. According to these results, using nitroxin and potassium fertilizer can play a significant role in increasing yield, especially under water deficit conditions, by reducing the destructive physiological and biochemical effects of water stress. Based on the local statistics, the average melon yield in this region is reported to be about 17-19 t ha-1. It can suggest that by calculating the depths of irrigation index and applying water deficit in the limited water sources conditions and by using biological fertilizer and potassium fertilizer, not only yield increase but also can save water.

Author Contributions

All authors contributed equally to the conceptualization of the article and writing of the original and subsequent drafts.

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Ethical Statement

As this manuscript does not involve research on humans or animals, nor does it include vulnerable populations, an ethical statement is not applicable.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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