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The Effects of Zinc Oxide Nanoparticles on Enzymatic and Osmoprotectant Alternations in Different *Moringa peregrina* Populations Under Drought Stress

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Abstract

Introduction: *Moringa peregrina* (Forssk.) Fiori as a desert tree has economic, nutritional, and medicinal properties, and is constantly encountered with drought stress. In addition, the role of zinc oxide nanoparticles (ZnO-NPs) in regulating the drought stress which induces biochemical processes is uncertain in this plant. Therefore, this study aimed to investigate the biochemical alternations, namely, enzymatic and osmoprotectant which are induced by ZnO-NPs in ten populations of *M. peregrina* under drought stress.

Materials and Methods: *Moringa peregrina* seeds were collected from southeast of Iran in 2014. The young plants were exposed to drought stress by withholding irrigation (until 50% filed capacity [FC]) 40 days after germination followed by spraying 0 (as control), 0.05, and 0.1% of the ZnO-NPs.

Results: The alternations of Na/K ratio, enzymes activities, and osmoprotectant content varied under drought stress depending on the *M. peregrina* populations. Generally, drought stress significantly enhanced peroxidase (POD) and polyphenol oxidase (PPO) activities, as well as proline content in untreated plants. Further, the Na/K ratio and carbohydrates content significantly decreased under the drought stress. Furthermore, ZnO-NP treatment significantly enhanced POD and PPO activities, as well as proline and carbohydrates content under both well-watered (100% FC) and drought stress conditions (50% FC), and at the concentration of 0.05% rather than 0.1%, ZnO-NPs was more effective.

Conclusion: Overall, ZnO-NP treatment could effectively improve the drought tolerance by enhancing the enzymes activities and osmoprotectant content in different *M. peregrina* populations under drought stress. Therefore, foliar application of ZnO-NPs at 0.05% concentration could be a recommended treatment for growing different *M. peregrina* populations under drought stress conditions.

Keywords: Antioxidant enzymes, Osmoprotectants, Peroxidase, Proline, ZnO-NPs

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Introduction

Moringa peregrina (Forssk.) Fiori, also known as horseradish tree, is a plant with economic and medicinal importance. The leaves are rich sources of various biomolecules (proteins), nutrients (amino acids), and natural antioxidants (vitamins A and C). In addition, the seed kernel contains high-quality oil with the inflorescences being used to feed the livestock.[1,2] The plant grows in many areas of the world like Northeast Africa and Southwest Asia. The cultivation of medicinal plants has recently increased due to their enormous beneficial effects on health and nutrition industries.[3] Accordingly, cultivation of *M. peregrina* has continuously extended in Iran.[1]

Totally, about one-third of the land on the earth belongs to the arid and semi-arid areas. Further, studies on global climate change predicted that the increasing frequency and duration of drought periods in the world are facing problems with plants cultivation in the drought stress.[4,5]

Drought is major abiotic stress which substantially limits the growth, morphogenesis, nutrients balance, and biochemical and physiological processes of the plants.[5] Furthermore, oxidation of cellular biomolecules by means of various

reactive oxygen species (ROS) like O_2^-, singlet oxygen $^1O_2$, $^\cdot OH$, and H_2O_2 ultimately leads to deranged cellular functions.[6]

To keep a steady ratio between the ROS production and removal, and consequently to relieve the damage related to the drought stress, plants evolved appropriate mechanisms such as stomata regulation, osmotic adjustment, and antioxidative defenses including enzymatic and non-enzymatic antioxidant systems.[7]

Antioxidant enzymes such as peroxidase (POD), polyphenol oxidase (PPO), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) are the "first line of defense" against the ROS. The POD, among others, is a cell-wall located anti-oxidant enzyme which promotes its function through iron-containing heme core. Moreover, it is the main cellular component catalyzing the H_2O_2 detoxification in the cells and a major enzyme scavenging the H_2O_2 in chloroplasts produced from O_2^- catalyzed by the superoxide dismutase.[8] Similarly, the PPO is an enzyme which oxidizes some phenols to chinone. This enzyme is attributed to the biosynthesis of the alkaloids under biotic and abiotic stress conditions.[9] The activity of enzymatic antioxidant defense systems can be variably suppressed when exposed to drought stress depending on the duration and intensity of the stress, genotypic variability, and crop developmental stage.[10]

Accumulation of different active ions, sugars, and organic compounds such as proline and carbohydrates plays a crucial role in cell osmoregulation and osmoprotection during the drought condition. Proline is an essential cytosolute regulating osmotic balance within the cells. In fact, proline executes its function by augmenting cellular anti-oxidative components, as well as preserving membrane and cellular structures and functioning as an oxygen radical scavenger.[11] Additionally, it promotes some actions within the cell cytoplasm without interfering with cellular structure and metabolism.[12]

Drought stress can change the carbohydrate content within the plant tissues. Moderate, intense, and severe water deficits can lead to elevation, preservation, and reduction of soluble sugars in rapeseed (*Brassica napus*), respectively.[13] In addition, based on their primary function as carbon and energy resources, carbohydrates are vital in maintaining the function of photosynthetic machinery within the cells. This is mainly triggered by regulating the cellular osmotic capacity and preserving the intracellular water.[14]

Micronutrient fertilizers are suggested as potential agents which increase the tolerance of plants to drought stress. By conserving membrane integrity and cellular potassium content, zinc (Zn) is essential in regulating the stomatal function by inducing the stomatal closure and preventing the transpiration in plants under water-deficit conditions.[15] The Zn has important functions in the synthesis of auxin, as well as biochemical reactions required for the formation of carbohydrates.[16] Further, it is revealed that Zn reduces the ROS production and increases the activity of enzymatic antioxidant systems such as the POD.[17] Therefore, the Zn has a protective role in plant survival during the drought conditions. The Zn deficiency is a widespread micronutrient dysregulation in plants throughout the arid and semi-arid regions necessitating the Zn supplementation in these regions.[18]

Currently, the use of nanomaterials such as zinc oxide nanoparticles (ZnO-NPs) has expanded. Nanotechnology is now a revolutionary science increasing the application of nanoparticles in every field of science including agriculture, medicine, food, and biological and pharmaceutical applications. The sudden rise in the demand for the ZnO-NPs is largely related to their higher efficiency compared to the conventional ZnO.[19]

To our knowledge, no studies have been conducted to understand the physiological response of medicinal plants including *M. peregrina* to the ZnO-NPs foliar use under the drought condition. Therefore, the present study sought to evaluate the effect of ZnO-NPs on the physiological responses and antioxidant enzyme activities in different *M. peregrina* species under the drought stress.

Materials and Methods

Plant Materials and Growth Conditions

This study was performed in Agricultural Faculty of Sistan and Baluchestan University, Zahedan, Iran (latitude of 29°27′34″ N, longitude of 60°51′10″ E, altitude of 1385 m, mean annual temperature of 18.3°C, and an annual rainfall of 72 mm) in a greenhouse during the growing season of 2014. The seeds of the ten *M. peregrina* species were collected from different regions of Sistan and Baluchestan province located in the southeast of Iran. Damaged parts of the plants and any other impurities (i.e., wood, leaves, stones, and dust) were removed. The seeds were then stored within black plastic bags and labeled. The locations and conditions regarding the arid and semi-arid climate are presented in Table 1.

The surfaces of the seeds were cleaned using 0.5% (v/v) sodium hypochlorite and treated with benomyl solution for 30 minutes at 24°C, and then rinsed three times with sterilized deionized water (DW). At least 20 seeds were placed in each 9-cm petri dish covered by sheets of moistened filter paper and were kept in a germination chamber 12 hours light cycle at 25°C. After germination, the seedlings were translocated to a growth chamber for 2 weeks at room temperature, 70% relative humidity with a 12 hours light cycle and daily access to water. Later, the young plants were sown in plastic pots (30 cm height and 20 cm diameter) filled with sterilized steam containing organic horizon soil (black soil), clay horizon (red soil), and washed sand (3:1:1, v/v/v). They were exposed to drought stress and ZnO-NP treatment 40 days after the germination.

Before the treatments, the field capacity (FC) of the

Table 1. Geographical Location of 10 *Moringa peregrina* Populations in Sistan and Baluchestan Province

Population Number	Positions	Location of Collected Seeds	Latitude	Longitude
1	Nikshahr	Konardan	29°16′69″ N	21°79′56″ E
2	Nikshahr	Keshik	29°13′84″ N	23°27′94″ E
3	Nikshahr	Shegim	29°29′04″ N	21°87′17″ E
4	Nikshahr	Desk	29°17′75″ N	21°92′40″ E
5	Nikshahr	Nesfuran	29°18′55″ N	78°40′95″ E
6	Fanuj	Girls Seven	29°19′72″ N	75°26′06″ E
7	Fanuj	Tange Fanuj Entrance	29°38′89″ N	76°27′30″ E
8	Fanuj	Tange Fanuj	29°35′68″ N	76°29′73″ E
9	Fanuj	Madohi Village	29°29′45″ N	76°26′47″ E
10	Fanuj	Dahan Village	29°15′45″ N	75°39′26″ E

medium was measured by filling 3 pots with water. The posts were covered with plastic to prevent evaporation. This was continued for 24 hours to drain. Then, the moisture content of the soil samples was determined according to Kramer and Boyer.[20] Next, the seeds were exposed to drought stress 40 days post germination. All the pots were kept at 100% FC (well-watered) and 50% FC (drought condition) during the study.

Seven days after the drought stress, plants were sprayed with different concentrations of ZnO-NPs (0 [as control], 0.05, & 0.1%) and a second ZnO-NPs foliar was applied 1 week later. The sprays continued to completely render the leaves soaked with the excess solution ran off the leaves. The ZnO-NPs were purchased from Iranian nanomaterials Pioneers Company. Plants were harvested at 30th day of the ZnO-NPs spray and were further used for other analyses.

Measurements and Analyses

Sodium/Potassium Ratio

To measure sodium (Na) and potassium (K), leaf samples were initially washed thoroughly with tap water and then rinsed in DW. Then, fruit flesh samples were obtained and oven-dried at 70°C for 48 hours. The fruits were powdered and then passed through a forty-mesh sieve. A portion of the fine powder (2 g) was turned to ashes in a furnace at 550°C for 4 hours. Next, 10 mL of 2M hydrochloric acid (HCl) was added to dissolve the ashes. The digested samples were passed through the filter paper (Whatman No. 40). Finally, the purified solution was used for Na and K analyses. The contents of Na and K were determined by flame photometric (Biotech Engineering Management Co. Ltd. the UK) method as described by Waling et al.[21]

Enzymes Assays

Spectrophotometry was employed for determining the POD activity according to Hung and Kao.[22] To this end, fresh leaves (500 mg) were initially frozen (liquid nitrogen) and then transferred into 2 mL extraction buffer (0.1M phosphate buffer, pH = 6.8). After centrifugation for 15 minutes at 13 000 g of the homogenate, the enzyme activity was measured in the obtained supernatant by reading the absorbance at 470 nm (UV/Vis spectrophotometer, Bio-Rad, USA). Furthermore, the extinction coefficient [26.6 (mmol L^{-1})$^{-1}$ cm^{-1}] for tetraguaiacol was applied to calculate the enzyme activity. The result was expressed as μM tetraguaiacol formed per minute per mg protein (μM mg^{-1} protein min^{-1}).

Moreover, the PPO activity was estimated according to Kar and Mishra[23] with slight modifications. Briefly, 100 μL of the enzyme extract was added to 2800 μL sodium phosphate buffer solution (25 mM, pH = 6.8) and 100 μL of 1 mM pyrogallol. The blank consisted of 2900 μL sodium phosphate buffer solution (25 mM, pH = 6.8) and 100 μL of the enzyme extract. Absorbance at the wavelength of 420 nm was considered as corresponding to the enzymatic activity. Finally, the PPO activity was expressed as μM mg^{-1} protein min^{-1}.

Osmoprotectant Capacity of Zn-NPs

Osmoprotectants are small molecules with minimum electrical conductance. At very low concentrations, these molecules are highly water-soluble with no cellular toxicity. Osmoprotectants are particularly important for the plants regarding tolerating the extreme osmotic conditions. Additionally, these osmoprotectants as the membrane stabilizers play a role in regulating the membrane permeability to water and avoiding intracellular dehydration. Proline is one of the main osmoprotectants, the content of which was determined by a colorimetric assay according to Bates et al.[24] Briefly, fresh leaf samples (200 mg) were homogenized in an aqueous solution of (w:v) sulphosalicylic acid 3%. The mixture of the plant extract with an acid ninhydrin and glacial acetic acid (2:2:2 ratios, respectively) was incubated at 100°C for 1 hour. The reaction was terminated on ice. In addition, the mixture was extracted using 4 mm of toluene generating a chromophore-containing toluene phase which was harvested. The absorbance was read at 520 nm using the toluene as blank to calculate the proline content by the

following formula:

Proline (µmoles / g of fresh weight material) = [(µg proline / mL × mL toluene) / 115.5 µg/µmole] / [(g sample) / 5]

Soluble carbohydrates were then measured according to the method reported by Irigoyen et al.[25] In sum, 2 g of fresh leaves were boiled in 10 mL of hot 95% ethanol for 2 hours at 80 °C. After cooling, the mixture was centrifuged at 9000 g for 15 minutes and then the supernatant was decanted. The 5 µL of sulphuric acid (98%) and 1 mL of phenol (0.5%) were added to 1 mL of supernatant. Next, the soluble carbohydrates content was determined using a spectrophotometer (Bio-Rad, USA) at 625 nm. The total soluble carbohydrates content was calculated by creating a standard curve using the glucose and expressed in µg g^{-1} FW.

Statistical Analysis

The statistical methods were conducted according to 3-factors linear model based on a completely randomized design in triplicate. Data were analyzed by the analysis of variance (ANOVA) employing the SAS software, version 9.1). Further, the data were checked for normality and homoscedasticity using the Shapiro-Wilk test. The least significant difference (LSD) test at $P \leq 0.01$ was considered statistically significant.

Results

Changes Under the Drought Stress

The Na/K ratio reduced or enhanced in response to drought stress were dependent on the *M. peregrina* populations. Generally, as shown in Figure 1, the drought stress significantly ($P \leq 0.01$) reduced the Na/K ratio in the control plants (0.00% ZnO-NPs). The population number 10 demonstrated the highest changes in response to drought stress.

Based on the results provided in Table 2, POD and PPO

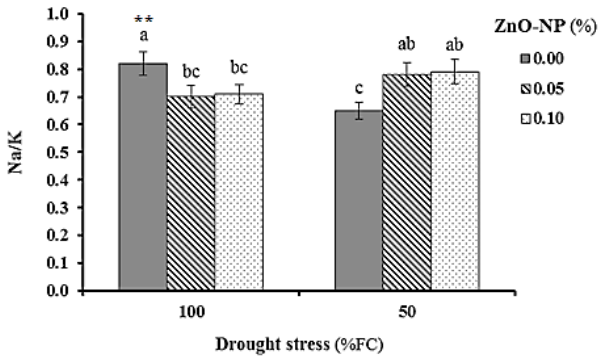

Figure 1. The Na/K Ratio Changes of *Moringa peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) in Response to ZnO-NP Treatments. The values are presented as the means (n = 3) ± standard errors. Different letters indicate a significant difference ($P \leq 0.01$) according to the LSD test.

Table 2. Changes of Some Biochemical Parameters in Different *Moringa peregrina* Populations the Under Well-Watered (100% FC) and Drought Stress (50 s% FC) Conditions

Drought Stress	Populations	Na/K	POD Activity (µmol min^{-1} mg^{-1} Protein)	PPO Activity (µmol min^{-1} mg^{-1} Protein)	Proline (µmol g^{-1} FW)	Carbohydrates (µG g^{-1} FW)
100% FC	1	0.90 abc*	0.054 h	0.039 m	0.604 gh	33.45 gh
	2	0.67 def	0.053 h	0.046 jk	0.576 hi	37.64 cdef
	3	0.77 bcd	0.042 j	0.052 h	0.516 j	32.46 h
	4	0.89 abc	0.054 h	0.052 h	0.583 gh	35.51 fgh
	5	0.91 abc	0.039 kl	0.042 l	0.582 gh	38.37 cdef
	6	0.61 def	0.037 l	0.047 ij	0.610 g	39.23 cd
	7	0.66 def	0.047 i	0.043 kl	0.523 j	35.50 fgh
	8	0.74 cde	0.050 i	0.050 hi	0.527 j	38.93 cde
	9	0.70 def	0.040 jk	0.049 hi	0.618 g	38.81 cde
	10	0.53 f	0.037 kl	0.035 n	0.433 k	35.67 fg
50% FC	1	1.03 a	0.092 a	0.062 f	0.708 def	36.32 cdef
	2	0.62 def	0.084 c	0.068 e	0.72 de	44.15 a
	3	0.73 cde	0.059 g	0.075 d	0.843 a	37.70 cdef
	4	0.94 ab	0.074 d	0.094 b	0.871 b	38.03 cdef
	5	0.60 def	0.067 e	0.062 f	0.765 bc	43.08 ab
	6	0.70 def	0.054 h	0.068 e	0.768 bc	40.76 bc
	7	0. 69 def	0.086 b	0.058 g	0.701 ef	38.01 cdef
	8	0.67 def	0.088 b	0.098 a	0.682 f	37.76 cdef
	9	0.57 ef	0.064 f	0.089 c	0.735 cd	45.13 a
	10	0.75 cde	0.067 e	0.068 e	0.547 ij	35.89 efg

Abbreviations: Na, sodium; K, potassium; POD, peroxidase; PPO, polyphenol oxidase; FC, filed capacity.
*For each column, the values followed by the same letters are not significantly different at $P \leq 0.01$ according to the least significant difference test.

activities were significantly affected by the drought stress depending on the *M. peregrina* population. The highest POD activity was found in population number 1 (0.092 µmol min^{-1} mg^{-1} protein) while the highest PPO activity was related to population number 8 (0.098 µmol min^{-1} mg^{-1} protein). The POD and PPO activities represented a similar pattern in response to the drought stress (Figures 2 and 3). In other words, drought stress significantly enhanced enzymes activities in ZnO-NPs treated and untreated plants. Number 8 and 10 populations had the highest percentage of PPO changes under the drought stress.

Furthermore, proline content significantly increased in all the *M. peregrina* populations under the drought stress. The results indicated that the highest proline content was obtained in population number 3 (0.843 µmol g^{-1} FW). Moreover, drought stress significantly ($P \leq 0.01$) increased proline content in untreated plants. Population number 3 had the highest percentage of proline changes under the drought stress.

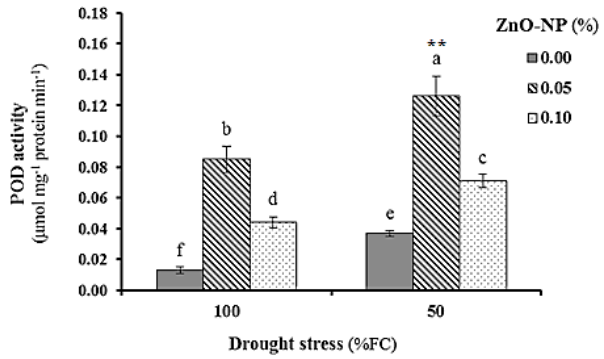

Figure 2. Changes of POD Enzyme Activity Related to the *Moringa peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) in Response to ZnO-NP Treatments. The values are provided as the means (n = 3) ± standard errors and different letters demonstrate a significant difference ($P \leq 0.01$) based on the LSD test.

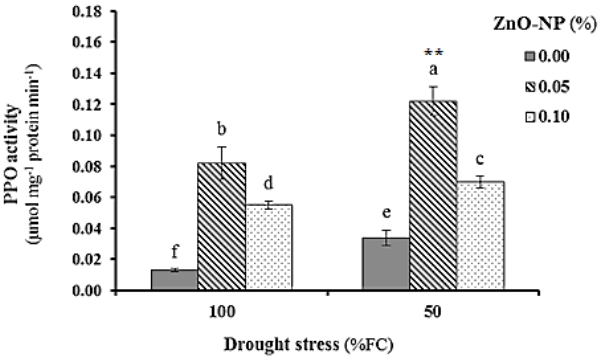

Figure 3. Changes of PPO Enzyme Activity Related to the *Moringa peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) in Response to ZnO-NP Treatments. The values are provided as the means (n = 3) ± standard errors and different letters demonstrate a significant difference ($P \leq 0.01$) based on the LSD test.

Populations 9 and 2 had the highest carbohydrates content (45.13 and 44.15 µg g^{-1} FW, respectively). Additionally, under the drought stress, carbohydrates content significantly reduced in the control plants. As regards the drought stress, population number 2 had the highest percentage changes of carbohydrates content.

ZnO-NP Treatments Induced Responses

As the results demonstrate, ZnO-NPs treated plants had a higher Na/K ratio compared to the untreated plants. Populations 1 (at 0.05% ZnO-NPs) and 4 (at 0.10% ZnO-NPs), among the evaluated populations, had the highest Na/K ratio (Table 3).

Further, the results revealed that POD and PPO activities significantly increased by foliar use of the ZnO-NPs under both well-watered and drought stress since 0.05% ZnO-NP treatment was more effective in enhancing the enzymes activities. The highest POD activity, among others, was found in populations 4 and 1 at 0.05% ZnO-NP treatment. Furthermore, populations 4 and 8 at 0.05% ZnO-NPs demonstrated the highest PPO activity as compared to the other populations. Moreover, in response to ZnO-NP treatments, the highest percentage of POD changes were observed in population number 4 at 0.05% ZnO-NPs while the population number 3 represented the highest percentage of PPO changes at 0.05% ZnO-NPs.

Additionally, ZnO-NP treatments significantly ($P \leq 0.01$) increased the proline content under both well-watered and drought stress conditions (Figure 4). Among the ZnO-NPs treated plants, the dose of 0.05% ZnO-NPs was more effective to enhance the content of proline compared to that of the 0.10% since *M. peregrina* populations had the highest proline content under 50% FC conditions treating with 0.05% ZnO-NPs (1.16 µmol g^{-1} FW). In addition, population number 3 at 0.05% ZnO-NP treatment had the highest proline content among the other populations. Further, in response to ZnO-NP treatments, the highest percentage of proline changes were obtained in population number 3 at 0.05% ZnO-NP treatment (Table 4).

Similarly, the results revealed that under both well-watered and drought stress conditions, carbohydrates content significantly ($P \leq 0.01$) increased in response to ZnO-NP treatment (Figure 5). Furthermore, 0.05% ZnO-NPs, among the ZnO-NPs treated plants, was more effective in enhancing the carbohydrates content as compared to the 0.1% ZnO-NPs. Populations 9 and 2 at 0.05% ZnO-NP treatments demonstrated the highest carbohydrates content as compared to the others. Under ZnO-NP treatments, the highest percentage changes related to the carbohydrates content were found in population number 7 as compared to other populations.

Discussion

The results demonstrated that Na^+/K^+ ratio significantly decreased in control plants under drought stress, which is

Table 3. Changes of Some Biochemical Features in Different *Moringa peregrina* Populations in Response to ZnO-NP Treatments

ZnO-NP Treatments	Populations	Na/K	POD Activity (μmol min^{-1} mg^{-1} protein)	PPO Activity (μmol min^{-1} mg^{-1} protein)	Proline (μmol g^{-1} FW)	Carbohydrates (μg g^{-1} FW)
0.00%	1	0.08 $^{c-i*}$	0.039 ^{n}	0.026 ^{kl}	0.033 ^{lmn}	16.93 ^{j}
	2	0.08 $^{c-i}$	0.026 ^{pqr}	0.022 ^{m}	0.039 ^{l}	17.85 ^{j}
	3	0.05 ^{ij}	0.021 r	0.021 ^{m}	0.025 ^{p}	17.03 ^{j}
	4	0.07 $^{c-j}$	0.013 s	0.035 ^{jk}	0.032 ^{lmn}	16.19 ^{j}
	5	0.07 $^{c-j}$	0.034 ^{op}	0.024 ^{lm}	0.028 ^{no}	19.46 ^{j}
	6	0.07 $^{c-j}$	0.025 ^{pq}	0.022 ^{m}	0.038 ^{l}	16.37 ^{j}
	7	0.07 $^{c-j}$	0.023 ^{qr}	0.024 ^{lm}	0.026 ^{op}	16.04 ^{j}
	8	0.06 $^{f-j}$	0.036 ^{no}	0.038 ^{j}	0.035 ^{lm}	19.52 ^{j}
	9	0.06 $^{f-j}$	0.038 ^{j}	0.024 ^{lm}	0.029 ^{mn}	18.26 ^{j}
	10	0.07 $^{c-j}$	0.039 ^{j}	0.024 ^{lm}	0.026 ^{op}	15.97 ^{j}
0.05%	1	0.11 ^{a}	0.129 ^{a}	0.074 ^{f}	0.107 ^{d}	53.82 ^{f}
	2	0.06 $^{g-j}$	0.114 ^{c}	0.103 ^{d}	0.118 ^{bc}	67.04 ^{ab}
	3	0.09 $^{b-e}$	0.083 ^{e}	0.116 ^{c}	0.129 ^{a}	54.84 ^{ef}
	4	0.09 $^{b-e}$	0.131 ^{a}	0.139 ^{a}	0.124 ^{b}	62.56 ^{c}
	5	0.06 $^{g-j}$	0.087 ^{de}	0.086 ^{e}	0.115 ^{c}	63.67 ^{bc}
	6	0.06 $^{g-j}$	0.078 ^{g}	0.104 ^{d}	0.124 ^{b}	61.88 ^{cd}
	7	0.06 $^{g-j}$	0.121 ^{b}	0.083 ^{f}	0.104 ^{e}	62.93 ^{c}
	8	0.08 $^{c-i}$	0.110 ^{d}	0.128 ^{ab}	0.108 ^{d}	61.50 ^{cd}
	9	0.07 $^{c-j}$	0.080 ^{f}	0.124 b	0.114 ^{c}	68.91 ^{a}
	10	0.06 $^{g-j}$	0.080 ^{f}	0.083 f	0.096 ^{f}	58.57 ^{de}
0.01%	1	0.10 ^{b}	0.067 ^{h}	0.054 ^{i}	0.069 ^{gh}	33.84 ^{i}
	2	0.05 ^{ij}	0.076 ^{g}	0.053 ^{i}	0.056 ^{j}	37.80 ^{h}
	3	0.08 $^{c-i}$	0.050 ^{lm}	0.068 ^{h}	0.062 ^{i}	33.38 ^{i}
	4	0.11 ^{a}	0.058 ^{j}	0.072 ^{gh}	0.061 ^{i}	31.82 ^{i}
	5	0.09 $^{b-e}$	0.057 ^{j}	0.053 ^{i}	0.073 ^{g}	49.06 ^{gh}
	6	0.06 $^{g-j}$	0.046 ^{m}	0.053 ^{i}	0.065 ^{hi}	41.73 ^{g}
	7	0.07 $^{c-j}$	0.063 ^{i}	0.054 ^{i}	0.074 ^{g}	31.38 ^{i}
	8	0.07 $^{c-j}$	0.075 ^{g}	0.075 ^{g}	0.055 ^{j}	33.80 ^{i}
	9	0.06 $^{g-j}$	0.054 ^{jk}	0.069 ^{h}	0.073 ^{g}	48.95 ^{gh}
	10	0.06 $^{g-j}$	0.052 ^{kl}	0.060 ^{i}	0.046 ^{k}	32.67 ^{i}

Abbreviations: Na, sodium; K, potassium; POD, peroxidase; PPO, polyphenol oxidase; ZnO-NPs: Zinc oxide nanoparticles.
*For each column, the values followed by the same letters are not significantly different at $P \leq 0.01$ according to the least significant difference test.

in line with the findings of El-Gendy et al[26] and Talebi et al.[27] The reduction in Na^+/K^+ ratio is due to a decrease or increase in Na^+ or K^+ concentrations, respectively. Talebi et al[27] reported significantly reduced Na while increased K uptakes (correlated with the ion uptake efficiency) in different chickpea (*Cicer arietinum*) species under drought stress.[27]

The mechanisms by which the drought stress inhibits the mineral uptake in plants were previously suggested. This inhibition is probably promoted by limiting the root growth and nutrient mobility in the soil. Moreover, the total nutrient and mineral uptakes and concentrations are reduced in crops encountered with the drought stress.[28] Similarly, such reduced absorption of the nutrients is possibly due to the defects in nutrient uptake and unloading mechanisms, as well as reduced transpiration flow under this condition.[29]

Additionally, the findings of the current study regarding changes in nutrient balance in response to the Zn-NPs spray corroborate with those of the Soliman et al who reported that foliar use of Zn-NPs significantly improved mineral nutrient content in *M. peregrina*.[30]

The plant responses to abiotic stresses depend on various factors such as the involved species, the metabolic rate of the plant, and intensity and duration of the stress.[7,10] Drought stress induces free radicals and ROS leads to alternations in physiological and biochemical properties such as growth, protein degradation, and

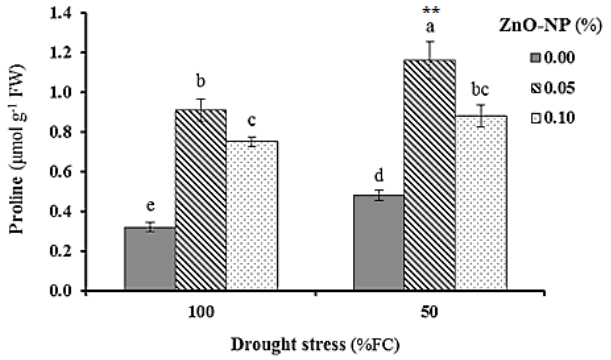

Figure 4. Changes of Proline Content in the *Moringa peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) in Response to ZnO-NP Treatments. The values are provided as the means (n = 3) ± standard errors and different letters demonstrate a significant difference ($P \leq 0.01$) based on the LSD test.

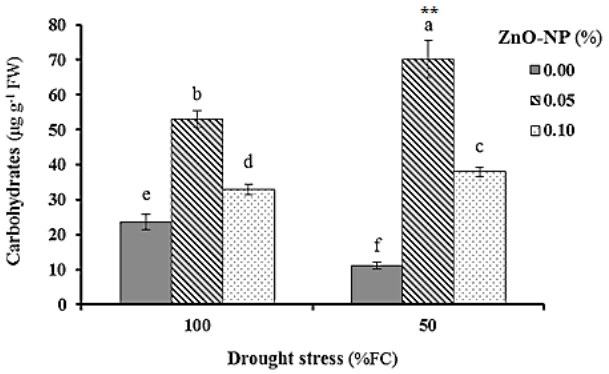

Figure 5. Changes of carbohydrates Content in the *Moringa peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) in Response to ZnO-NP Treatments. The values are provided as the means (n = 3) ± standard errors and different letters demonstrate a significant difference ($P \leq 0.01$) based on the LSD test.

Table 4. The Percentage Changes of Some Biochemistry Traits in 10 *Moringa peregrina* Populations Compared to the Control Conditions in Response to Drought Stress and Different Concentrations of ZnO-NP Treatments

	Populations	Na/K	POD Activity	PPO Activity	Proline	Carbohydrates
			Drought Stress			
50% FC	1	14.44	70.37	58.97	17.22	8.58
	2	-7.46	58.49	47.83	25.00	17.30
	3	-5.19	40.48	44.23	63.37	16.14
	4	5.62	37.04	80.77	49.40	7.10
	5	-34.07	71.79	47.62	31.44	12.28
	6	14.75	45.95	44.68	25.90	3.90
	7	4.55	82.98	34.88	34.03	7.07
	8	-9.46	76.00	96.00	29.41	-3.01
	9	-18.57	60.00	81.63	18.93	16.28
	10	41.51	81.08	94.29	26.33	0.62
			ZnO-NP Treatments			
0.05%	1	37.50	230.77	184.62	224.24	217.90
	2	-25.00	338.46	368.18	202.56	275.57
	3	80.00	295.24	452.38	416.00	222.02
	4	28.57	907.69	297.14	287.50	286.41
	5	-14.29	155.88	258.33	310.71	227.18
	6	-14.29	212.00	372.73	226.32	278.01
	7	-14.29	426.09	245.83	300.00	292.33
	8	33.33	205.56	236.84	208.57	215.06
	9	16.67	110.53	416.67	293.10	277.38
	10	-14.29	105.13	245.83	269.23	266.75
0.10%	1	25.00	71.79	107.69	109.09	99.88
	2	-37.50	192.31	140.91	43.59	111.76
	3	60.00	138.10	223.81	148.00	96.01
	4	57.14	346.15	105.71	90.63	96.54
	5	28.57	67.65	120.83	160.71	152.11
	6	-14.29	84.00	140.91	71.05	154.92
	7	0.00	173.91	125.00	184.62	95.64
	8	16.67	108.33	97.37	57.14	73.16
	9	0.00	42.11	187.50	151.72	168.07
	10	-14.29	33.33	150.00	76.92	104.57

Abbreviations: Na, sodium; K, potassium; POD, peroxidase; PPO, polyphenol oxidase; ZnO-NPs: zinc oxide nanoparticles; FC, field capacity.

lipid peroxidation. Plants augment the antioxidant systems including both non-enzymatic and enzymatic antioxidants such as the POD and PPO in order to cope with the ROS.[31]

The results regarding the enhanced PPO and POD activities by ZnO-NPs are in conformity with the findings of Kheirizadeh Arough et al[32] who found that 0.4 and 0.8 g L^{-1} ZnO-NPs significantly increased the activities of antioxidant enzymes such as PPO and POD in *Triticale* plants under abiotic stress.

Zinc can act as a stabilizing and protective factor for bio-membranes by binding to membrane proteins.[33] In addition, zinc ions can bind to ligands such as sulfur, nitrogen, and oxygen, and therefore alleviate oxidative stress. The balance, production, and removal of the oxidative agents are the main factors in determining the cellular fate. Therefore, Zn-NPs may have survival benefits by scavenging the ROS and boosting the antioxidant systems in plants.[33]

Proline functions as the osmolyte, ROS scavenger, as well as the regulator of the redox potential and intracellular pH. Further, it contributes to the stabilization of protein structures Furthermore, proline is essential for the activity of the pentose phosphate pathway which generates the cellular redox potency needed for maintaining many antioxidants in their reduced state. Similarly, it prevents the oxidative photo-damage removing and/or reducing the production of O_2 radicals in thylakoid membranes.[34]

The synthesis, storage, and catabolism of proline are considered as highly regulated processes in the plants. Accumulation of large quantities of proline represents that the plants adapt to various biotic and abiotic conditions. Proline is accumulated in plants in response to abiotic stress which is related to its increased production or decreased degradation.[35] Studies reported that drought stress induced the proline catabolizing enzymes such as proline dehydrogenase (*pro*-DH) and proline biosynthetic enzymes like glutamate dehydrogenase (GDH), pyrroline-5-carboxylate synthetase (P5CS), and pyrroline-5-carboxylate reductase (P5CR).[36-38]

Altered carbohydrate content is one of the important characteristics of the stress conditions meddling with processes like photosynthesis, transportation, and respiration.[39,40] Moreover, an important osmoregulatory role is noted for soluble carbohydrates in the plants. Carbohydrates further augment the ROS removal and protection of biomolecules under the drought stress. They are necessary for the synthesis of phenolic compounds as secondary messengers in plant adaptative responses under drought and other environmental stimuli. Carbohydrate content of the plants is regulated by the rate of photosynthesis, as well as polysaccharides hydrolysis and cellular export under drought stress therefore may demonstrate either increased or decreased carbohydrate pool which is determined by the extent of the carbohydrate deranged metabolism.[14]

Additionally, drought stress can lead to cell death in part by decreasing the content of the carbohydrates.[41] This may be partly related to the impacts of drought stress on plant physiological processes including reduced photosynthesis which are due to the stomatal constriction, increased metabolic demand for carbohydrates, elevated carbohydrates metabolization, and finally shrinkage of the carbohydrates storages leading to carbon depletion.[42]

The findings are concurrent with those of Mansour et al[43] who indicated that foliar application of Zn (100 ppm) significantly enhanced the total soluble carbohydrates in soybean plants. In addition, El Habbasha et al[44] reported that the carbohydrates content of different wheat populations increased in response to foliar use of 100 and 200 g Zn ha^{-1}.

The positive effect of Zn foliar use on total carbohydrates content is probably related to the role of Zn in regulating the key enzymes of the carbohydrate metabolism.[43,45] It was further pinpointed that the activity of carbonic anhydrase is reduced in conditions of Zn deficiency. This enzyme contains Zn in its structure and is located within the cytosol and chloroplasts. Therefore, carbonic anhydrase can particularly modulate the photosynthesis rate by facilitating the CO_2/HCO_3^- transportation.[46]

Conclusion

Generally, an increment was observed in antioxidant enzyme activities and osmoprotectants content under both well-watered (100% FC) and drought stress (50% FC) conditions in *M. peregrina* plants sprayed with 0.05% ZnO-NPs. This indicates that 0.05% ZnO-NPs could enhance drought tolerance of the plants either in unstressed or stressed conditions. Such information helps further understand the substantial role of the antioxidant defense mechanisms against the drought stress in *M. peregrina*.

Ethical Approval

Not applicable.

Competing Interest

There are no competing interests to declare.

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References

1. Salehi M, MajnunHoseini N, NaghdiBadi H, Mazaheri D. Biochemical and growth responses of *Moringa peregrina* (Forssk.) Fiori to different sources and levels of salinity. J Med Plant. 2012;11(43):54-61.
2. Salaheldeen M, Aroua MK, Mariod AA, Cheng SF, Abdelrahman MA. An evaluation of *Moringa peregrina* seeds as a source for bio-fuel. Ind Crop Prod. 2014;61:49-

61. doi:10.1016/j.indcrop.2014.06.027
3. Salehi A, Tasdighi H, Gholamhoseini M. Evaluation of proline, chlorophyll, soluble sugar content and uptake of nutrients in the German chamomile (*Matricaria chamomilla* L.) under drought stress and organic fertilizer treatments. Asian Pac J Trop Biomed. 2016;6(10):886-891. doi:10.1016/j.apjtb.2016.08.009
4. Soliman AS, El-feky SA, Darwish E. Alleviation of salt stress on *Moringa peregrina* using foliar application of nanofertilizers. J Hortic Forest. 2015;7(2):36-47. doi:10.5897/jhf2014.0379
5. Xiaolu W, Jie Y, Aoxue L, Yu C, Yijun F. Drought stress and re-watering increase secondary metabolites and enzyme activity in dendrobium moniliforme. Ind Crop Prod. 2016;94:385-393. doi:10.1016/j.indcrop.2016.08.041
6. Wang R, Gao M, Ji S, Wang S, Meng Y, Zhou Z. Carbon allocation, osmotic adjustment, antioxidant capacity and growth in cotton under long-term soil drought during flowering and boll-forming period. Plant Physiol Biochem. 2016;107:137-146. doi:10.1016/j.plaphy.2016.05.035
7. Jeena GS, Phukan UJ, Shukla RK. Drought-tolerant plants. In: Dubey SK, Pandey A, Sangwan RS, eds. Current Developments in Biotechnology and Bioengineering: Crop Modification, Nutrition, and Food Production. 2017:101-123. doi:10.1016/B978-0-444-63661-4.00005-0
8. Vardhini BV, Anjum NA. Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. Front Environ Sci. 2015;2:67. 2:1-16. doi:10.3389/fenvs.2014.00067
9. Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Ghassemi-Golezani K. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). Plant Omics J. 2012;5(2):60-67.
10. Fini A, Guidi L, Ferrini F, et al. Drought stress has contrasting effects on antioxidant enzymes activity and phenylpropanoid biosynthesis in *Fraxinus ornus* leaves: An excess light stress affair? J Plant Physiol. 2012;169:929-939. doi:10.1016/j.jplph.2012.02.014
11. Nazar R, Umar S, Khan NA, Sareer O. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. South Afr J Bot. 2015;98:84-94. doi:10.1016/j.sajb.2015.02.005
12. Sun J, Gu J, Zeng J, et al. Changes in leaf morphology, antioxidant activity and photosynthesis capacity in two different drought-tolerant cultivars of *chrysanthemum* during and after water stress. Sci Hortic. 2013;161:249-258. doi:10.1016/j.scienta.2013.07.015
13. Muller T, Lentzsch P, Muller MEH. Carbohydrate dynamics in leaves of rapeseed (*Brassica napus*) under drought. J Agron Crop Sci. 2012;198(3):207-217. doi:10.1111/j.1439-037x.2011.00502.x
14. Hura T, Dziurka M, Hura K, Ostrowska A, Dziurka K. Different allocation of carbohydrates and phenolics in dehydrated leaves of triticale. J Plant Physiol. 2016;202:1-9. doi:10.1016/j.jplph.2016.06.018
15. Seghatoleslami M, Forutani R. Yield and water use efficiency of sunflower as affected by nano ZnO and water stress. J Adv Agric Technol. 2015;2(1):34-37. doi:10.12720/joaat.2.1.34-37
16. Laware SL, Raskar S. Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. Int J Curr Microbiol App Sci. 2014;3(7):874-881.
17. Torabian S, Zahedi M, Khoshgoftarmanesh A. Effect of foliar spray of zinc oxide on some antioxidant enzymes activity of sunflower under salt stress. J Agric Sci Technol. 2016;18:1013-1025.
18. Karami S, ModarresSanavy SAM, Ghanehpoor S, Keshavarz H. Effect of foliar zinc application on yield, physiological traits and seed vigor of two soybean cultivars under water deficit. Not Sci Biol, 2016;8(2):181-191. doi:10.15835/nsb.8.2.9793
19. Swain PS, Rao SBN, Rajendran D, Dominic G, Selvaraju S. Nano zinc, an alternative to conventional zinc as animal feed supplement:A review. Anim Nutr. 2016;2(3):134-141. doi:10.1016/j.aninu.2016.06.003.
20. Kramer P, Boyer JS. Water Relations of Plants and Soils. San Diego, CA, USA: Academic Press; 1995:495.
21. Waling I, Van Vark W, Houba VJG, Van der lee JJ. Soil and plant analysis, a series of syllabi. Part 7. Plant analysis procedures. Wageningen Agriculture University; 1989.
22. Hung KT, Kao CH. Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid. J Plant Physiol. 2003;160 (8):871-879. doi:10.1078/0176-1617-01118
23. Kar M, Mishra D. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 1976;57(2):315-319. doi:10.1104/pp.57.2.315
24. Bates LS, Waldren, SP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205-207. doi:10.1007/bf00018060
25. Irigoyen JJ, Emerrich DW, Sanchez-Diaz M. Water stress induced changes in concentrations of praline and total soluble sugars in nodulated alfalfa plant. J Physiol Plant. 1992;84(1):55-60. doi:10.1034/j.1399-3054.1992.840109.x
26. El-Gendy SA, Abd Elmoniem EM, Al-Abdulla MM, Eisa SS. Morphological and physiological responses of *Acacia saligna* (Labill.) to water stress. Aust J Basic Appl Sci. 2012;6(4):90-97.
27. Talebi R, Ensafi MH, Baghebani N, Karami E, Mohammadi K. Physiological responses of chickpea (*Cicer arietinum*) genotypes to drought stress. Environ Exp Biol. 2013;11:9-15.
28. Gunes A, Cicek N, Inal A, et al. Genotypic response of chickpea (*Cicerarietinum*L.) cultivars to drought stress implemented at pre- and post-anthesis stages and its relations with nutrient uptake and efficiency. Plant Soil Environ. 2006;52(8):368-376. doi:10.17221/3454-pse
29. Garg BK. Nutrient uptake and management under drought:nutrient-moisture interaction. Curr Agric. 2003;27:1-8.
30. Hafeez B, Khanif YM, Saleem M. Role of zinc in plant nutrition-A review. Am J Exp Agric. 2013;3(2):374-391. doi:10.9734/ajea/2013/2746
31. Ahmad P, Jamsheed S, Hameed A, et al. Drought stress induced oxidative damage and antioxidants in plants. In: Ahmad P, ed. Oxidative Damage to Plants. 2014:345-367. doi:10.1016/B978-0-12-799963-0.00011-3
32. KheirizadehArough Y, SeyedSharifi R, Sedghi M, Barmiki M. Effect of zinc and bio fertilizers on antioxidant enzymes

activity, chlorophyll content, soluble sugars and proline in *Triticale* under salinity condition. Not Bot Horti Agrobo. 2016;44(1):116-124. doi:10.15835/nbha44110224

33. Zago MP, Oteiza PI. The antioxidant properties of zinc:Interactions with iron and antioxidants. Free Radic Biol Med. 2001;31:266-274. doi:10.1016/s0891-5849(01)00583-4
34. Hossain MA, Anamul Hoque M, Burritt DJ, Fujita M. Proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. In: Ahmad P, ed. Oxidative Damage to Plants. 2014;477-522. doi:10.1016/B978-0-12-799963-0.00016-2.
35. Oloumi H, Soltaninejad R, Baghizadeh A. The comparative effects of nano and bulk size particles of CuO and ZnO on glycyrrhizin and phenolic compounds contents in *Glycyrrhiza glabra* L. seedlings. Ind J Plant Physiol. 2015;20(2):157. doi:10.1007/s40502-015-0143-x
36. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments. A review. Plant Signal Behav. 2012;7(11):1456-1466. doi:10.4161/psb.21949
37. Chaitanya KV, Rasineni GK, Reddy AR. Biochemical responses to drought stress in mulberry (*Morusalba* L.): evaluation of proline, glycine betaine and abscisic acid accumulation in five cultivars. Acta Physiol Plant. 2009;31(3):437-443. doi:10.1007/s11738-008-0251-6
38. Rao S, Shekhawat GS. Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in *Brassica juncea*. J Environ Chem Eng. 2014;2(1):105-114. doi: 10.1016/j.jece.2013.11.029
39. Disante KB, Fuentes D, Cortina J. Response to drought of Zn-stressed *Quercus suber* L. seedlings. Environ Exp Bot. 2011;70:96-103. doi:10.1016/j.envexpbot.2010.08.008
40. Ghorbanli M, Arbabian S, Taghizadeh Z. The effect of zinc excess on antioxidant enzymes, proline and soluble carbohydrates in *Plantago major* L. Iran J Plant Physiol. 2015;6(1):1551-1556.
41. Piper FI. Drought induces opposite changes in the concentration of non-structural carbohydrates of two evergreen *Nothofagus* species of differential drought resistance. Ann Forest Sci. 2011;68(2):415-424. doi:10.1007/s13595-011-0030-1
42. McDowell NG, Allen CD, Marshall L. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. Glob Chang Biol. 2009;16(1):399-415. doi:10.1111/j.1365-2486.2009.01994.x
43. Mansour MM. Response of soybean plants to exogenously applied with ascorbic acid, zinc sulphate and paclobutrazol. Rep Opin. 2014;6(11):17-25.
44. El Habbasha SF, Badr EA, Abdel Latef E. Effect of zinc foliar application on growth characteristics and grain yield of some wheat varieties under Zn deficient sandy soil condition. Int J ChemTech Res. 2015;8(6):452-458.
45. Saadati S, Moallemi N, Mortazavi SMH, Seyyednejad SM. Effects of zinc and boron foliar application on soluble carbohydrate and oil contents of three olive cultivars during fruit ripening. Sci Horti. 2013;164:30-34. doi:10.1016/j.scienta.2013.08.033
46. Sawan ZM, Mahmoud MH, El-GuibaliA H. Influence of potassium fertilization and foliar application of zinc and phosphorus on growth, yield components, yield and fiber properties of Egyptian cotton (*Gossypiumbar badense* L.). J Plant Ecol. 2008;1(4): 259-270. doi: 10.1093/jpe/rtn021