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Research paper

Improvement of yield-related traits of spring rapeseed in response to nano-superabsorbent and bio-fertilizers under water deficit conditions

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Abstract
A two-year experiment was performed to evaluate the efficacies of nano-superabsorbent and bio-fertilizers on the field performance of rapeseed under different levels of irrigation in 2018 and 2019. The experiment was arranged as the split-plot factorial based on a randomized complete block design with three replications. Three irrigation levels (I_1, I_2, I_3: irrigation after 70, 120, and 170 mm evaporation from class A pan, respectively) were arranged in main plots and factorial combination of two levels of nano-superabsorbent (0 and 45 kg ha^{-1}) and four levels of bio-fertilizers (control, *Azotobacter* and *Enterobacter*, chitosan, and bacteria + chitosan) in sub-plots. The activities of antioxidant enzymes, hydrogen peroxide, osmolytes, and malondialdehyde content were increased under I_2 and I_3. This reaction led to a decline in leaf water content, membrane stability index, leaf protein content, and yield-related traits. Application of bio-fertilizers especially chitosan + plant growth-promoting bacteria (PGPR) with and without nano-superabsorbent increased antioxidant enzymes activities. Utilization of nano-superabsorbent decreased the activity of these enzymes. The lack of reduction in these traits by application of nano-superabsorbent + bio-fertilizers indicates that the additive effect of chitosan + bacteria is more than the reduction effect of nano-superabsorbent on these enzymes' activity. The utilization of nano-superabsorbent with bio-fertilizers increased these enzymes' activities through higher nitrogen retention in the soil and increased fertilizer effect. The utilization of chitosan, PGPR, and nano-superabsorbent, especially chitosan + PGPR + nano-superabsorbent, decreased proline content, however, increased soluble sugars, protein, chlorophyll, leaf water contents, and membrane stability index, and consequently, these treatments affected yield-related traits of rapeseed under water stress conditions.

Keywords: Bacteria; Chitosan; Chlorophyll; Membrane stability; Proline

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Introduction

Due to the increase in population and per capita consumption of vegetable oil, the cultivation, and processing of oilseeds in the world has grown dramatically. Rapeseed is known as one of the most important oil crops due to the suitable fatty acids and high oil content of grains (Jian *et al*. 2019). The field performance of this important oil crop could be limited by adverse conditions such as water deficit (Ghassemi-Golezani *et al.* 2019). Drought stress causes physiological and biochemical changes, including decreased photosynthesis, stomata conduction, and damage to cellular membranes (Azarpanah *et al.* 2013). Water deficiency causes the production of reactive oxygen species (ROS) and the reduction and decomposition of chlorophyll. During stress, chlorophyll decomposes in chloroplasts, and

thylakoid structures disappear (Choudhary *et al.* 2017). ROS can increase the content of malondialdehyde through the oxidation of lipids, thereby disrupting the cell's natural mechanism (Erdogan *et al.* 2016). Decreased photosynthetic pigments and increased lipid peroxidation under gradual drought in rapeseed was reported by Ghasemi Golzani *et al.* (2019). Plants by several mechanisms including the synthesis of antioxidant enzymes such as superoxide dismutase (SOD), peroxidases (POX), catalase (CAT), and polyphenol oxidases (PPO) can reduce reactive oxygen species to water and oxygen molecules and prevent oxidative stress (Ghassemi-Golezani *et al.* 2019). The accumulation of solutions is another type of plant's defensive response to water deficiency (Emami Bistgani *et al.* 2017). During water scarcity, the plant's access to water and nitrogen absorption capacity is reduced, resulting in reduced yield (Arve *et al.* 2011).

Researchers are looking for new ways to reduce performance loss due to water scarcity. The use of moisture-absorbing materials such as superabsorbents is one of the suitable solutions (Shahram *et al.* 2013). Superabsorbent polymers are non-toxic and no trace of them remains in nature (Zohuriaan-Mehr *et al.* 2010). Superabsorbents absorb water and after the drying of the environment, the water inside the polymer is gradually drained (Abdullah 2019). These polymers, by gradually placing water, can ensure proper plant growth under water scarcity (Shekari *et al.* 2015). The application of nano-suppressants increases plant available water thus increasing plant tolerance under water stress (Abdallah 2019). Superabsorbent polymer, by improving soil structure, provides suitable conditions for root development (Sawut *et al.* 2014). These materials increased the efficacy of fertilizers and nutrients for the plant and reduced phosphorus loss by 84% and nitrogen by 83% (Seyed-Doraji *et al.* 2011). Superabsorbent increased relative water content and decreased CAT and SOD activities and membrane stability index (MSI) under water-deficiency (Afkari 2018). Li *et al.* (2014) stated that superabsorbent enhanced yield by increasing pigment production, photosynthetic material transfer, and reducing seed loss by providing water at the critical stage of seed formation. Afkari (2018) reported that superabsorbent increased the sugar content of the solution by increasing the leaf water.

More attention has recently been paid to the ability of microorganisms to respond to water deficiency (Liu and Zhang 2015). Plant growth-promoting bacteria (PGPR) through biofilm production, exo-polysaccharide secretion, enhancing antioxidant responses, and the accumulation of osmolytes, can ensure proper plant growth under water scarcity (Chen *et al.* 2000). These bacteria affect plant nutrition by providing some essential macro elements such as nitrogen and phosphorus and microelements for plant growth in the rhizosphere. PGPR affects root structure by altering gene transcription and biosynthesis of metabolites under drought conditions (Vacheron *et al.* 2013). The application of PGPR reduces the harmful effects of water deficit on strawberries by increasing CAT, POX, and PPO activities and decreasing malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) (Erdogen *et al.* 2016). *Azotobacter* can

enhance the activity of some important nitrogen metabolizing enzymes such as nitrate reductase in plant organs, thereby improving the nitrogen content under water stress (Ansari and Ahmad 2019). Mondal *et al.* (2017) showed that combined fertilization of the phosphate-solubilizing micro-organism and *Azotobacter* increased the soluble sugar content (Ram Rao *et al.* 2007).

Chitosan is a non-toxic organic material that can reduce the damage of drought stress (Sharif *et al.* 2018). Chitosan, as a carbon source, stimulates the soil microorganisms and thereby enhances plant performance (Cho *et al.* 2008). The application of chitosan as a bio-fertilizer is beneficial due to its degradability with no environmental pollution (Escudero *et al.* 2017). Chitosan increases plant resistance to drought by increasing water and nitrogen uptake (Limpanavech *et al.* 2007), the content of chlorophyll, protein, and osmolytes (Khordadi-Varamin *et al.* 2018), cell membrane stability, antioxidant enzyme activity (Mondal *et al.* 2012), nitrogen fixation and effect on plant gene expression (Li *et al.* 2017), and by reducing the malondialdehyde content (Emami Bistgani *et al.* 2017). Chitosan application led to reduced drought damage in basil (*Ocimum basilicum* L.) by increasing antioxidant enzymes and chlorophyll content (Pirbaloti *et al.* 2017). Due to the importance of oilseed plants and because of the lack of sufficient information about the interaction of nano-superabsorbent and bio-fertilizers on drought stress, this study was undertaken to understand how rapeseed reacts to the use of these drought modifiers at different irrigation levels.

Materials and Methods

Field experiment To investigate physiological changes of the rapeseed under irrigation at different levels in response to nano-superabsorbent, growth-promoting bacteria, and chitosan, two field experiments were conducted as split-plot factorial based on the randomized complete block design with three replications during 2018 and 2019 at the Research Station of the University of Tabriz, Iran. Irrigation levels (I_1, I_2, I_3: irrigation after 70, 120, and 170 mm evaporation from class A pan, respectively) was assigned in the main plots, and factorial combination of nano-superabsorbent (0 and 45 kg ha^{-1}) and bio-fertilizers (control, *Azoto* + *Enterobacter*, chitosan, and bacteria + chitosan) were applied in sub-plots. Urea (46% nitrogen) and triple superphosphate fertilizers (150 and 100 kg ha^{-1}, respectively) were applied by the strip method before seed planting based on the plant requirements and soil analysis (Table 1). Nano-superabsorbent (with a particle size of 200 microns and ion-free water uptake of 500 g) was also placed at a depth of 10 cm before planting. Seeds (cv. Delgan) were inoculated with chitosan 0.4 % and bacteria with the population of 2×10^7 CFU ml^{-1} before planting and sown on a flatbed in 1 cm depth with a density of 80 seeds per m^2 based on the weather conditions. Each experimental plot consisted of nine rows of 1 m long spaced 20 cm apart. Immediately after sowing, the plots were irrigated regularly, and

Table 1. Some soil characteristics of the experimental site

Texture	EC	pH	CaCO3	OC	N	P	K
Sandy Loam	0.77	7.75	14.8	0.7	0.077	13	3.82
	dS/m		%	%	%	mg/Kg	mg/Kg

after the seedling establishment, irrigation treatments were performed. Weeding of the field was implemented as required.

Measurements

Soil-moisture: Soil sampling was performed with a steel cylinder (diameter of 5 cm) from a depth of 0-30 cm in the soil near the harvest time. Field capacity (FC) at 33 k Pa suction and permanent wilting point (PWP) at 1500 k Pa suction were determined using the compression plate method (Dane and Hopmans 2002). Available water content (AWC) was calculated using the following formula:

AWC: FC – PWP

Soil nitrogen and phosphorus: After harvesting the plants, composite soil sampling was done to determine the percentage of nitrogen (Cottenie *et al*. 1982) and available phosphorus concentration (Keeney and Nelson 1982) from the depth of rapeseed root development (0-30 cm) in each plot.

Antioxidant enzymes: For measuring the activity of antioxidant enzymes, 1 g of the leaves harvested at the flowering stage was completely homogenized in the liquid nitrogen, then 10 ml of sodium phosphate buffer (50 mM with pH= 6.8) was added. The samples were centrifuged at 4 ˚C for 20 min at 12000g. The resulting supernatants were used to measure the activities of POX (Gueta-Dahan *et al.* 1997), ascorbate peroxidase (APX) (Nakano and Asada 1981), SOD, CAT (Singh *et al.* 2010), and PPO (Ug^{-1} FW) (Kumar and Khan 1982).

Lipid peroxidation: To measure MDA by the method of Janero (1999), a certain amount of fresh leaf samples (about 0.5 g) were prepared and chopped in 5 ml of 5% trichloroacetic acid. These samples were centrifuged for 20 min at 1000 g. Then, 1 ml of 2% thiobarbituric acid solution was added to 1 ml of supernatant and heated for 25 min at 95 ˚C and afterward, cooled immediately at 0-2 ˚C. The absorption was noted at 532 and 600 nm. About 1 g of leaf sample from each experimental plot was homogenized in 5 mL of 0.1% trichloroacetic acid and then centrifuged at 16000g for approximately 15 min. After this time, 0.5 mL of the supernatant was mixed with 0.5 mL of buffer (Potassium phosphate 10 mM pH 7), and 1 mL of 1 M potassium iodide, and the absorbance rate was noted at 390 nm (Velikova *et al.* 2000).

***Measurement of osmolytes*:** To measure the proline content (Bates *et al.* 1973), about 0.5 g of leaf sample was homogenized in 10 ml of 3% sulfosalicylic acid. About 2 ml of glacial acetic

acid + 2 ml of ninhydrin were mixed. Samples were placed in a Bain-marie (at 100 °C for 1 h) and then cooled in 0-5 °C. Then, 4 ml of toluene was added to each sample, and the absorption was noted at 520 nm. Finally, the amount of proline (mg g^{-1} FW) was calculated according to the standard curve obtained from different concentrations of proline. Kochert (1978) method was used to measure the soluble sugar (mg g^{-1} DW) by using the standard curve obtained from varying levels of pure glucose.

Chlorophyll a and b content: To determine the pigment content, 400 mg of fresh leaf samples were digested with 10 ml of 80% acetone and centrifuged at 6000 g for 10 minutes. The absorption rate was noted by a spectrophotometer at 645 and 663 nm (Arnon 1949).

Leaf protein content (LPC): To measure the leaf protein content, 1 g of the leaves harvested at the flowering stage, was completely homogenized in the liquid nitrogen, then 10 ml of sodium phosphate buffer (50 mM with pH= 6.8) was added. The samples were centrifuged at 4°C for 20 min at 12000 g. The resulting supernatants were utilized to designate LPC in leaf samples by the Bradford (1976) method.

***MSI*:** After washing the leaves with distilled water, a sample (0.1 g) of leaf tissue was removed and placed in the double-distilled water (10 ml) at a temperature of 40 °C for 30 min. Electrical conductivity was measured after reaching 20-24 °C (C_1). The electrical conductivity of the samples was measured after placing at 100 ° C for 10 min (C_2) (Ghassemi-Golezani *et al.* 2016):

$$MSI= (EC1/EC2) \times 100$$

Leaf water content (LWC): The specimens were transferred to the laboratory in an ice flask during the flowering stage to measure LWC. In the laboratory, leaf samples were weighed with scales and recorded. Then, the samples were dried at 75 ° C for 50 h, and afterward, their dry weight was recorded. The LWC was determined as:

$$LWC (\%) = [(FW-DW)/FW)] \times 100$$

where FW and DW are the fresh weight and dry weight of the leaf samples, respectively.

Grain and oil yield: Plants were harvested individually within 1 m^2 of the middle part of each experimental plot after removing the marginal effect at the time of maturity. At this time, the moisture content of the seeds was about 16%. The percentage of oil for the seeds of each plot was determined using a Soxhlet, and the oil yield was determined as:

$$\text{Oil yield} = \text{seed yield} \times \text{oil percentage}$$

Statistical analysis

Data analysis including the comparison of means (by Duncan's multiple range test at $p \leq 0.05$) was performed by the MSTATC software.

Results

Soil moisture

Mixing the nano-superabsorbent with soil significantly increased the AWC. The FC values were 15.62 and 39.24% for 0 and 45 kg h-1 nano-

superabsorbent, respectively. The application of 45 kg of nano-superabsorbent increased the PWP and AWC by 1.80 and 2.49 folds, respectively (Table 2).

Soil nitrogen and phosphorus

The application of chitosan and PGPR, especially chitosan + PGPR, resulted in the increased nitrogen content and phosphorus concentration in the soil. Application of nano-superabsorbent also improved soil nitrogen content at all fertilizer levels, especially the combined application of nano-superabsorbent + chitosan + bio-superphosphate (Table 3).

Enzyme activity and H_2O_2 and MDA content

The interaction of drought stress × nano-superabsorbent × bio-fertilizers was significant for POX, SOD, CAT, PPO, and APX activities, and also for MDA and H_2O_2 ($p \leq 0.01$). The activities of enzymes and the H_2O_2 and MDA content were increased by increasing the drought stress. Nano-superabsorbent and bio-fertilizers did not affect the antioxidant enzymes activities and H_2O_2 content under I_1. Bio-fertilizers and especially bio-fertilizer + nano-superabsorbent increased the antioxidant enzymes activities under I_2 and I_3. The application of all these treatments decreased the content of MDA and H_2O_2 under moderate to severe stress (Table 4).

Osmolytes

The interaction of drought stress × nano-superabsorbent × bio-fertilizers was also significant for proline and soluble sugars. The content of osmolytes increased by increasing drought stress. Application of nano-superabsorbent, chitosan, and PGPR reduced the content of proline, despite the increased soluble sugars content under I_2 and I_3. The application of these treatments did not affect the content of osmolytes under normal irrigation. The effect of nano-superabsorbent + bio-fertilizers on osmolytes was more than other treatments (Figure 1A, 1B).

MSI and LWC

The effect of drought stress × nano-superabsorbent × bio-fertilizers was significant for the MSI. It decreased by increasing drought stress. Application of nano-superabsorbent, chitosan, and PGPR increased MSI under different levels of irrigation. The application of chitosan and PGPR with and without nano-superabsorbent showed the highest MSI (Table 5).

The interaction of drought stress × nano-superabsorbent × bio-fertilizers was significant for the water content of rapeseed leaves. The water content of rapeseed leaves was reduced under I_2 and I_3. The highest LWC was achieved by nano-superabsorbent + bio-fertilizers treated plants, followed by bio-fertilizers treated plants. Untreated plants under severe stress had the lowest LWC (Table 5).

Chlorophylls a and b content

The effect of drought stress × nano-superabsorbent × bio-fertilizers was significant for chlorophyll a and chlorophyll b. These traits declined with enhancing drought stress.

Table 2. Changes in FC, PWP, and AWC of the soil in the experimental site

Nano-superabsorbent (kg h^{-1})	FC	PWP (%)	AWC
0	15.62b	7.82b	7.8b
45	33.24a	13.84a	19.4a

Means with different letters in each column indicate a significant difference at $p \leq 0.05$; FC: field capacity; PWP: permanent wilting point, AWC: available water capacity

Table 3. The effect of nano-superabsorbent, chitosan, and PGPR on soil nitrogen and phosphorus

Nano-superabsorbent (kg h^{-1})	Bio-fertilizer	N (%)	P (mg kg^{-1})
0	F1	0.092ef	15.20c
	F2	0.107d	17.31b
	F3	0.108d	18.85b
	F4	0.143b	22.24a
45	F1	0.095e	15.21c
	F2	0.116c	17.52b
	F3	0.119c	18.90b
	F4	0.157a	23.89a

Means with different letters in each column indicate a significant difference at $p \leq 0.05$; F_1, F_2, F_3, F_4: control, chitosan, bacteria, and chitosan + bacteria, respectively; PGPR: plant growth-promoting bacteria

Chlorophyll a and Chlorophyll b content of plants with or without nano-superabsorbent was increased by fertilization, particularly by chitosan + PGPR. The application of bio-fertilizers and especially bio-fertilizer + nano-superabsorbent led to a synergistic increase in chlorophyll content (Table 5).

LPC

Interaction of drought × nano-superabsorbent × bio-fertilizers was significant for LPC. This trait diminished as a consequence of enhancing drought stress. Application of nano-superabsorbent and bio-fertilizers, especially their combined use, significantly increased the LWC under I2 and I3. Nano-superabsorbent enhanced LWC under I_2 and I_3, but this enhancement was considerable when nano-superabsorbent was applied with bio-fertilizers. The use of bio-fertilizers also increased LPC under normal irrigation, but the nano-superabsorbent did not alter the LPC at these conditions (Figure 1C).

Grain yield, oil percentage, and oil yield

The effect of drought stress × nano-superabsorbent × bio-fertilizers was significant for

Table 4. Changes in POX, APX, SOD, CAT, and PPO activities, and the H_2O_2 and MDA content in rapeseed leaves affected by nano-superabsorbent and bio-fertilizers under different levels of irrigation

Irrigation	Nano-superabsorbent	Bio-fertilizers	POX	APX	SOD	CAT	PPO	H_2O_2	MDA
			(U g^{-1} FW)				(mmol g^{-1} FW)		
I_1	S_1	F1	0.250op	0.250n	0.240n	0.280l	1.550o	0.105p	2.450rq
		F2	0.264op	0.300mn	0.280mn	0.298l	1.580no	0.103p	2.330rst
		F3	0.260op	0.289mn	0.269mn	0.291l	1.570no	0.103p	2.350rs
		F4	0.280no	0.330m	0.310m	0.310l	1.670n	0.102p	2.200u
	S_2	F1	0.247p	0.230n	0.219n	0.279l	1.530o	0.104p	2.400qr
		F2	0.261op	0.298mn	0.271mn	0.302l	1.570no	0.103p	2.300stu
		F3	0.262op	0.287mn	0.267mn	0.301l	1.580no	0.103p	2.280stu
		F4	0.282no	0.338m	0.319m	0.314l	1.680n	0.102p	2.100tu
I_2	S_1	F1	1.700l	2.400k	1.300k	2.170j	2.610l	0.220i	5.500i
		F2	2.000i	2.860i	1.600i	2.520i	2.990k	0.200j	5.l50l
		F3	1.800k	2.770ij	1.500j	2.389i	2.990k	0.209jk	5.250k
		F4	2.994g	3.440h	2.330gh	4.000e	3.900h	0.190l	4.350o
	S_2	F1	1.500m	2.163l	1.100l	1.700k	1.800m	0.205jok	5.357j
		F2	1.850k	2.850i	1.630i	2.580i	3.060jk	0.192l	4.950m
		F3	1.905j	2.798i	1.523j	2.440i	3.093j	0.196l	4.820n
		F4	3.010g	3.460gh	2.350g	4.300c	4.159f	0.175m	3.990p
I_3	S_1	F1	3.410f	3.900e	3.197e	3.600g	3.990g	0.390a	8.900a
		F2	3.900c	4.490c	3.490c	4.185d	4.510c	0.320c	7.600d
		F3	3.700e	4.390d	3.317d	3.856f	4.451e	0.340bc	7.800c
		F4	4.997ab	5.420a	4.180a	5.431a	5.400ab	0.290d	6.207g
	S_2	F1	2.900h	3.700f	2.700f	2.900h	3.200i	0.340b	8.020b
		F2	3.850d	4.521c	3.423c	4.100de	4.500c	0.300cd	7.300e
		F3	3.839d	4.470c	3.400c	3.910ef	4.480d	0.3044cd	6.900f
		F4	5.030a	5.398ab	4.100ab	5.416ab	5.457a	0.260e	5.900h
I×S×F[+]			**	**	**	**	**	**	**

[+]From the analysis of variance table; [**]significant at $p \leq 0.01$; Means with different letters in each column indicate a significant difference at $p \leq 0.05$; POX: peroxidase, APX: ascorbate peroxidase, SOD: superoxide dismutase, CAT: catalase, PPO: polyphenol oxidase, H_2O_2: hydrogen peroxide, MDA: malondialdehyde; I_1, I_2, I_3: irrigation after 70,120, and 170 mm evaporation from Class A pan; S_1, S_2: nano-superabsorbent at 0 and 45 kg h^{-1}; F_1, F_2, F_3, F_4: control, chitosan, bacteria, and chitosan + bacteria, respectively

grain yield, oil percentage, and yield. These traits decreased by increasing drought stress. Grains obtained from plants treated with bio-fertilizers, especially bio-fertilizer + nano-superabsorbent had the lowest oil percentage and highest grain yield and oil yield, while separate application of nano-superabsorbent increased only the oil percentage. The control plants (F1) under I_3 had the lowest grain yield. Application of nano-superabsorbent and chitosan with PGPR resulted in increased grain yield compared to the bacteria inoculation alone under I_3. The application of bio-fertilizers and especially bio-fertilizer + nano-superabsorbent led to a synergistic increase in grain yield (Table 5).

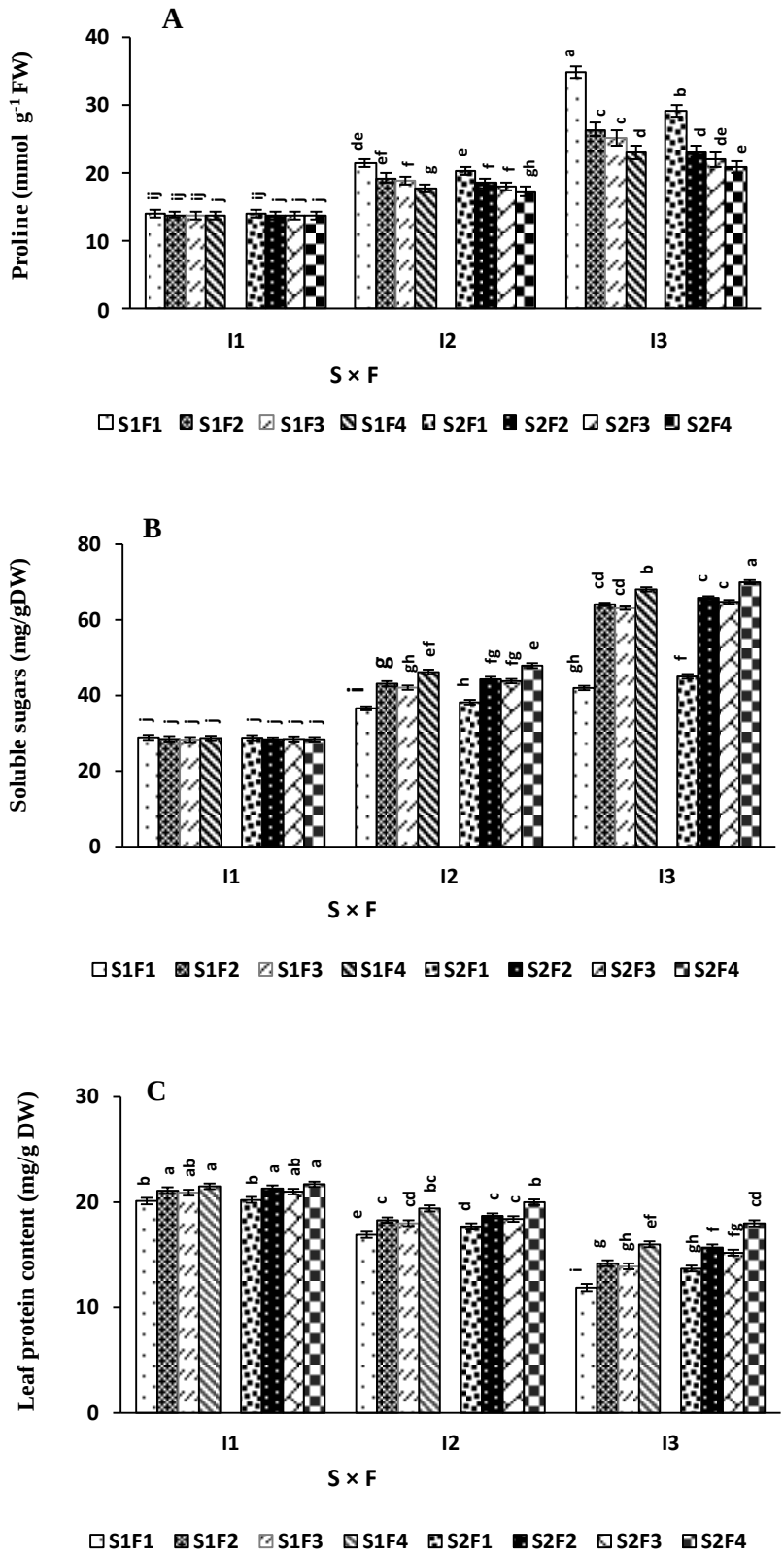

Figure 1. Changes in proline (A), soluble sugars (B), and protein (C) content of rapeseed leaves under different levels of drought, nano-superabsorbent, and bio-fertilizers; Different letters indicate a significant difference at $p \leq 0.05$; S_1, S_2: 0 and 45 kg h-1 nano-superabsorbent, respectively; F_1, F_2, F_3, F_4: control, chitosan, bio-superphosphate, and bio-superphosphate + chitosan, respectively

Table 5. Changes in MSI, LWC, Chl a, and Chl b of rapeseed leaves, grain yield, oil content, and oil yield affected by nano-superabsorbent and bio-fertilizers under different levels of drought stress

Irrigation	Nano Superabsorbent	Bio Fertilizers	MSI (%)	LWC (%)	Chl a (mg g^{-1} DW)	Chl b (mg g^{-1} DW)	Grain yield (g/m^2)	Oil (%)	Oil yield (g/m^2)
I_1	S_1	F1	81.00c	72.11d	1.17e	0.73g	182.15de	42.99a	78.30c
		F2	82.60b	74.13bc	1.59cd	0.87ef	209.21bc	42.09b	88.04ab
		F3	82.20b	75.85c	1.57d	0.84fg	206.33bc	42.00b	86.62ab
		F4	84.00a	81.94ab	3.161a	1.88b	240.91ab	41.15c	99.13a
	S_2	F1	81.40c	73.99c	1.50d	0.80f	190.53cd	43.05a	82.02bc
		F2	82.99b	75.21b	1.61cd	1.08cd	210.97bc	42.04b	88.69ab
		F3	83.00b	76.71b	1.60cd	1.10cd	212.95bc	41.97b	89.37ab
		F4	85.00a	83.90a	3.210a	2.459a	250.84a	41.10c	103.09a
I_2	S_1	F1	73.91h	62.10j	0.72fg	0.50jk	147.11gh	38.99e	55.01h
		F2	76.90ef	65.21g	0.95ef	0.69gh	167.17ef	37.80gh	63.19fg
		F3	76.30ef	63.03h	0.87ef	0.60hi	157.28fg	38.00g	56.76gh
		F4	79.50d	71.01de	1.95c	1.30c	211.47bc	37.19j	78.64cd
	S_2	F1	75.40g	64.48i	0.85ef	0.57ij	153.19fg	39.70d	60.81gh
		F2	77.90e	67.00ef	1.40d	0.86ef	181.14de	38.30f	69.37de
		F3	77.75e	68.43ef	1.80c	0.97de	178.97de	37.75ij	67.56ef
		F4	81.70c	77.51c	2.73b	1.96b	222.15abc	37.59i	83.50bc
I_3	S_1	F1	61.90mn	48.18qr	0.30i	0.25m	50.18j	34.09l	16.10m
		F2	66.90k	52.29n	0.56gh	0.39kl	76.41ij	32.01n	24.63kl
		F3	64.12l	50.09o	0.44hi	0.29mn	61.28j	33.00m	20.23l
		F4	70.90i	59.43k	1.03e	0.70gh	138.87h	29.99q	41.6ij
	S_2	F1	65.15kl	51.11p	0.50hi	0.32lm	59.45j	35.92k	21.35l
		F2	67.90jk	54.20m	0.91ef	0.52jk	98.44i	32.90m	32.38jk
		F3	68.20j	54.68lm	0.92ef	0.59ij	100.2i	31.40o	31.40jk
		F4	73.90h	64.97ij	1.59cd	1.08cd	199cde	30.70p	61.40hi
I×S×F+			**	**	**	**	**	**	**

+From the analysis of variance table; **significant at $p \leq 0.01$; Means with different letters in each column indicate a significant difference at $p \leq 0.05$; MSI: membrane stability index, LWC: leaf water content, Chl a: chlorophyll a, Chl b: chlorophyll b. I_1, I_2, I_3: irrigation after 70,120, and 170 mm evaporation from Class A pan; S_1, S_2: nano-superabsorbent at 0 and 45 kg h^{-1}; F_1, F_2, F_3, F_4: control, chitosan, bacteria, and chitosan + bacteria, respectively

Discussion

The application of nano-suppressants increased plant water availability (Table 2), which indicates their better tolerance to water stress (Abdallah 2019). The enhancement in soil PWP indicates that the water stored in the nano-superabsorbent is not fully available to the plant (Table 2). Sultana *et al.* (2016) stated that superabsorbent gains water retention in the soil. Increasing nitrogen by the application of nano-superabsorbent with bio-fertilizers (Table 3) can be due to more nitrogen retention in the soil and prevention of leaching (Seyed-Doraji *et al.* 2011). An increase in soil nitrogen and phosphorus due to the application of growth-promoting bacteria (Table 3) can be related to their activity in nitrogen fixation and insoluble phosphorus dissolution. PGPR dissolves insoluble phosphorus in the soil by producing organic acids and increasing the mobility of these elements in the soil (Scervin *et al.* 2010). Chitosan also improves soil nitrogen content (Table 3) and the activity of microorganisms due to the presence of nitrogen and carbon in its structure (Cho *et al.* 2008).

Increasing CAT, APX, SOD, POX, and PPO activities under I_2 and I_3 (Table 4) indicate an enhancement in ROS (Çakmakçi *et al.* 2012). Despite the increase in the activity of antioxidant

enzymes under drought stress, the increase in H_2O_2 production led to an increase in MDA and a decrease in MSI (Table 4). Nano-superabsorbent decreased oxidative stress in plants (Table 5) by reducing ROS (Table 2), gradually providing water to the plant (Tongo *et al*. 2014). Bio-fertilizers (particularly chitosan + PGPR) decreased H_2O_2 and MDA contents and increased MSI of the rapeseed leaves by increasing antioxidant enzymes activities (Table 2). Chitosan and PGPR may increase the activity of antioxidant enzymes (Table 4) by gene modification (Li *et al.* 2017) and improving root growth and accessing nutrients (Ibrahim *et al.* 2013). Chitosan inhibits lipid oxidation (Table 4) by combining with lipids and free radical scavengers or by antioxidant enzymes (Hidangmayum and Dwivedi 2018). Studies have shown that the inoculation of seeds with PGPR reduced the content of MDA by increasing the antioxidant capacity (Erdogan *et al.* 2016) and producing the ACC deaminase enzyme (Glick 2014). PGPR also induces the expression of genes responsible for drought stress such as SAMS1, APX1, and HSP17.8 (Kasim *et al.* 2013). The lack of reduction in these traits by application of nano-superabsorbent + bio-fertilizers indicates that the additive effect of bio-fertilizers is more than the reduction effect of nano-superabsorbent on the activity of these enzymes (Table 4). Kumar *et al.* (2019) stated that the combination of chitosan with PGPR led to an additive improvement in the activity of PPO and POX enzymes. Application of nano-superabsorbent with bio-fertilizers also increased the effect of fertilizers and nutrients by reducing nutrient leaching (Table 3), thereby improving the activity of antioxidant enzymes compared to the separate application of nano-superabsorbent (Table 4). Habibi *et al.* (2010) stated that superabsorbent + PGPR treatment reduced the MDI content and oxidative damage due to dehydration.

The accumulation of osmo-regulators in rapeseed leaves under drought (Figure 1A, 1B) is a mechanism for preventing peroxidation of membrane lipids and maintaining water potential in plant tissues (Emami Bistgani *et al.* 2017). Increased proline content under I_2 and I_3 (Figure 1A) is due to the stimulation of synthesis through glutamate and the prevention of its degradation in protein synthesis (Lutts *et al.* 1996). Therefore, increasing proline is a prerequisite for reducing protein synthesis (Figure 1C). Also, the decrease in protein synthesis can be attributed to the changes in polysomes (Aghaei *et al.* 2008). Water deficiency leads to protein degradation by the production of ROS (Chen *et al.* 2000). The increase in soluble sugars during drought stress (Figure 1B) can be attributed to altered amylase and invertase activity, hydrolysis of starch to simple sugars, and synthesis of these compounds by non-photosynthetic pathways (Hsiao 1973). Despite the effect of these osmolytes on improving the water content of the plant, the reduction in available water by the roots due to drought (Caser *et al.* 2017) was led to a decrease in LWC (Table 5).

Decreased proline as a result of the use of drought stress moderators (Figure 1A) is associated with increased photosynthetic pigments (Table 5) and protein synthesis (Figure 1A). The

increase in soluble sugar in rapeseed leaves due to the use of bio-fertilizers and nano-superabsorbent, especially their combined use, maybe due to the increase in LWC (Table 5). Chitosan degrades to ammonia, an oligosaccharide, and a monosaccharide and leading to an increase in soluble carbohydrates (Khordadi-Varamin *et al.* 2018). Afkari (2018) stated that superabsorbent increased the sugar content of the solution by increasing the leaf water. Increased sugar content and stress tolerance due to the use of bio-fertilizer have been reported by Khajeeyan *et al.* (2019). Increased LPC due to the application of bio-fertilizer, especially bio-fertilizer + nano-superabsorbent (Figure 1C) may be related to the retention of water by nano-superabsorbent and nitrogen supply by bio-fertilizers in the soil (Tables 2 and 3), increased activity of antioxidant enzymes (Table 4), and reduction of H_2O_2 (Table 4). Studies have shown that chitosan and PGPR also stabilize nitrogen, which is a key ingredient in protein formation (Agbodjato *et al.* 2016). The accumulation of soluble sugars (Figure 1B), and enhancing chlorophyll content (Table 5) by the treatments, particularly by nano-superabsorbent + bio-fertilizers under drought stress, resulted in higher LWC (Table 5).

The decrease in photosynthetic pigments with increasing drought stress (Table 5) can be due to decreased absorption of essential nutrients, chlorophyll degradation by chlorophyllase, and increased oxidative stress (Goldani 2012). Under drought stress, the chlorophyll-protein complex in the plant becomes unstable, thereby reducing the formation of new plastids and chlorophyll a and chlorophyll b content (Sharifa and Muriefah 2015). Increased activity of enzymes involved in the proline synthesis during drought stress from the glutamate pathway can lead to decreased chlorophyll synthesis (Girija *et al.* 2002). The synergistic effect of all these factors with each other on enhancing chlorophyll a and chlorophyll b content (Table 5) may be the result of a large increase in the supply of the N and P (Table 2 and 3), and the activity of antioxidant enzymes against ROS (Table 4) by bio-fertilizers, water supply and prevention of nitrogen leaching by nano-superabsorbent (Tables 2 and 3), and reduced H_2O_2 (Table 4) and proline (Figure 1A), and LWC by all these treatments (Table 5). PGPR can also induce siderophores synthesis (Raymond *et al.* 2004) which leads to iron mobilization and chlorophyll synthesis (Shilev 2020).

The decreased grain yield of spring rapeseed under drought stress was associated with a decrease in MSI, LWC, photosynthetic pigments (Table 5), LPC (Figure 1C), and increase in MDA (Table 4) and their effect on the production of photo-assimilates (Xu *et al.* 2018). Water scarcity reduces the plant's access to water and nitrogen, closes the stomata, and reduces the flow of carbon dioxide into the mesophilic cells (Arve *et al.* 2011). Yield loss by the declined pigments content, MSI, and increased MDA during drought stress was reported by Ghassemi-Golezani *et al.* (2019). The increase in grain yield by application of bio-fertilizers and especially bio-fertilizer + nano-superabsorbent under drought stress is the result of the increase in the activity of antioxidant enzymes (Table 4), MSI, chlorophyll content,

LWC (Table 5), water, and N availability to the plant (Table 2 and 3), and decreasing H_2O_2 (Table 4). This increase in plant growth is the result of increasing nitrogen uptake (Agbodjato *et al.* 2016) and delaying senescence (Togay *et al.* 2008). Moslemi *et al.* (2012) stated that the application of superabsorbent + PGPR increased grain yield compared to their separate application.

A decrease in oil yield under drought stress was related to a loss in grain yield (Table 5). Application of nano-superabsorbent by gradually providing water (Table 3) and bio-fertilizers by enhancing grain protein content due to increasing nitrogen uptake (Dzung 2005; Narolia *et al.* 2013) and LPC (Figure 1C), resulted in an increase and a decrease in oil percentage of grains, respectively (Table 5). The increase in oil yield with the use of drought stress moderators is due to the increased grain yield (Table 5). The effect of nano-superabsorbent, chitosan, and PGPR in improving oil yield was due mainly to the increase in soluble sugar accumulation (Figure 1B) and reduction of the lipid peroxidase level (Table 5) under I_2 and I_3. Reports showed that the use of chitosan in thyme (*Thymus vulgaris* L.) (Emami Bistgani *et al.* 2017) and PGPR in lemon balm (*Melissa officinalis* L.) (Kazemi Nasab *et al.* 2015) increased grain and oil yield by increasing the content of osmolytes and chlorophyll and reducing the peroxidation of lipids. The high grain and oil production of rapeseed by application of chitosan + PGPR, especially chitosan + PGPR + nano-superabsorbent under water stress, indicates a synergistic effect between these factors (Table 5). The application of nano-superabsorbent with bio-fertilizers increased the effect of fertilization on soil (Table 3) through the reduction of nutrients waste (Seyed-Doraji *et al.* 2011). In this study, chitosan and nano-superabsorbent increased the effect of PGPR on the rapeseed yield. Nano-superabsorbent, as a water absorber (Rafiei *et al.* 2013), and chitosan as nitrogen and carbon source also elevate the performance of soil microorganisms (Cho *et al.* 2008).

Conclusions

Our results revealed that drought stress increased H_2O_2, MDA, and osmolytes content, despite enhancing antioxidant enzymes activities. Application of bio-fertilizers with and without nano-superabsorbent, especially chitosan + PGPR increased POX, SOD, CAT, PPO, and APX activities, while separate application of nano-superabsorbent decreased the activity of these enzymes as compared to the combination nano-superabsorbent with bio-fertilizers. This indicates that the additive effect of bio-fertilizers is more than the reduction effect of nano-superabsorbent on the activity of these enzymes. Also, nano-superabsorbent + chitosan + bio-superphosphate resulted in the improved activity of these enzymes through higher nitrogen retention in the soil and increased fertilizer effect. Exacerbation of the oxidative damage resulted in declined MSI, LWC, chlorophyll, LPC, and yield-related traits of rapeseed under I_2 and I_3. All these treatments increased LWC, MSI, chlorophyll a, chlorophyll b, and LPC under drought stress.

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Conflict of Interest

There is no conflict of interest between authors

References

Abdallah AM, 2019. The effect of hydrogel particle size on water retention properties and availability under water stress. International Soil and Water Conservation Research 7(3): 275-285.

Afkari A, 2018. Impact of super absorbent polymer on physiological traits and activity of antioxidant enzymes in wheat (*Triticum aestivum* L. cv. Mihan) affected by drought stress conditions. Journal of Crop Nutrition Science 4(4): 1-14.

Agbodjato NA, Noumavo PA, Adjanohoun A, Agbessi L, and Baba-Moussa L, 2016. Synergistic effects of plant growth promoting rhizobacteria and chitosan on in vitro seeds germination, greenhouse growth, and nutrient uptake of maize (*Zea mays* L.). Biotechnology Research International 2016: 7830182.

Aghaei K, Ehsanpour AA, and Komatsu S, 2008. Proteome analysis of potato under salt stress. Journal of Proteome Research 7(11): 4858-4868.

Ansari FA and Ahmad I, 2019. Alleviating drought stress of crops. Through PGPR: mechanism and application. In: Singh D *et al*. (eds). Microbial Interventions in Agriculture and Environment. Pp. 341-358. Springer, Singapore.

Arnon DI, 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology 24(1): 1-15.

Arve LE, Torre S, Olsen JE, and Tanino K, 2011. Stomatal responses to drought stress and air humidity. In: Shanker A and Venkateswarlu B (eds.). Abiotic Stress in Plants- Mechanisms and Adaptations. Intechopen, 267-280.

Azarpanah A, Alizadeh O, and Dehghanzadeh H, 2013. Investigation on proline and carbohydrates accumulation in maize (*Zea mays* L.) under water stress condition. Extreme life, Biospeology and Asterobiology- International Journal of the Bioflux Society 5: 47-54.

Bates LS, Waldren RP, and Teare ID, 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207.

Bradford MM, 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2): 248-254.

Çakmakçi R, Erat M, Oral B, Erdogan Ü, and Şahin F, 2009. Enzyme activities and growth promotion of spinach by indole-3-acetic acid-producing rhizobacteria. The Journal of Horticultural Science and Biotechnology 84(4): 375-380.

Caser M, Lovisolo C, and Scariot V, 2017. The influence of water stress on growth, ecophysiology and ornamental quality of potted *Primula vulgaris* 'Heidy' plants. New insights to increase water use efficiency in plant production. Plant Growth Regulation 83: 361-373.

Chen WP, Li PH, and Chen TH, 2000. Glycinebetaine increases chilling tolerance and reduces chilling-induced lipid peroxidation in *Zea mays* L. Plant, Cell and Environment 23: 609-618.

Cho MH, No HK, and Prinyawiwatkul W, 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. Journal of Food Science 73(1): S70-S77.

Choudhary RC, Kumaraswamy RV, Kumari S, Sharma SS, Pal A, Raliya R, Biswas P, and Saharan V, 2017. Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). Scientific Reports 7: 9754.

Cottenie A, Verloo M, Kiekens L, Velghe G, and Camerlynck R, 1982. Chemical analysis of plant and soil. Laboratory of Analytical and Agrochemistry, State University, Gent, Belgium.

Dane JH and Hopmans JW, 2002. Soil water retention and storage - Introduction. In: Dane JH and Topp GC (eds.). Methods of Soil Analysis. Part 4. Physical Methods. Pp. 671-674. Soil Science Society of America Book Series No. 5, USA.

Dzung NA, 2005. Application of chitin, chitosan and their derivatives for agriculture in Vietnam. Journal of Chitin and Chitosan 10(3): 109-113.

Emami Bistgani Z, Siadat SA, Bakhshandeh A, Ghasemi Pirbalouti A, and Hashemic M, 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. The Crop Journal 5(5): 407-415.

Erdogan Ü, Çakmakçi R, Varmazyari A, Turan M, Erdogan Y, and Kitir N, 2016. Role of inoculation with multi-trait rhizobacteria on strawberries under water deficit stress. Zemdirbyste-Agriculture 103(1): 67-76.

Escudero N, Lopez-Moya F, Ghahremani Z, Zavala-Gonzalez EA, and Alaguero-Cordovilla A, Ros-Ibañez C, Lacasa A, Sorribas FJ, and Lopez-Llorca LV, 2017. Chitosan increases tomato root colonization by *Pochonia chlamydosporia* and their combination reduces root-knot nematode damage. Frontiers in Plant Science 8: 1415.

Girija C, Smith BN, and Swamy PM, 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environmental and Experimental Botany 47(1): 1-10.

Ghassemi-Golezani K, Ghassemi S, and Yaghoubian I, 2016. Salicylic acid regulate physiological performance of milk thistle (*Silybum marianum* L.) under water stress. Advances in Bioresearch 7(4): 34-40.

Ghassemi-Golezani K, Mabudi-Bilasvar H, and Dabbagh-Mohammadi-Nassab A, 2019. Improving rapeseed (*Brassica napus* L.) plant performance by exogenous salicylic acid and putrescine under gradual water deficit. Acta Physiologiae Plantarum 41: 12.

Glick BR, 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research 169(1): 30-39.

Goldani M, 2012. Effect of irrigation intervals on some growth indices of basil (*Ocimum basilicum* L.) ecotypes. Iranian Agricultural Research 10(2): 412-420 (In Persian with English abstract).

Gueta-Dahan Y, Yaniv Z, Zilinskas BA, and Ben-Hayyim G, 1997. Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in citrus. Planta 203(4): 460-469.

Habibi D, Moslemi Z, Ardakani MR, Mohammadi A, and Asgharzadeh A, 2010. Effects of super absorbent polymer and plant growth promoting rhizobacteria (PGPR) on yield and oxidative damage of maize under drought stress. 2010 International Conference on Chemistry and Chemical Engineering 1-3 August, Kyoto, Japan, pp. 253-257.

Hidangmayum A and Dwivedi P, 2018. Plant responses to *Trichoderma* spp. and their tolerance to abiotic stresses: a review. Journal of Pharmacognosy and Phytochemistry 7(1): 758-766.

Hsiao TC, 1973. Plant responses to water stress. Annual Review of Plant Physiology 24: 519-570.

Ibrahim MH, Jaafar HZE, Karimi E, and Ghasemzadeh A, 2013. Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth). Molecules 18(9): 10973-10988.

Janero DR, 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine 9(6): 515-540.

Kasim WA, Osman ME, Omar MN, Abd El-Daim IA, Bejai S, and Meijer J, 2013. Control of drought stress in wheat using plant-growth- promoting bacteria. Journal of Plant Growth Regulation 32: 122-130.

Kazemi-Nasab A, Yarnia M, Lebaschy MH, Mirshekari B, and Rejali F, 2015. The response of drought stressed lemon balm (*Melissa officinalis* L.) to vermicompost and PGPR. Biological Forum 7(1): 1336-1344.

Keeney DR and Nelson DW, 1982. Nitrogen-Inorganic Forms. In Page AL (ed.). Methods of Soil Analysis. Agronomy Monograph 9, Part 2 (2nd ed.), pp. 643-698. ASA, SSSA, Madison, WI, USA.

Khajeeyan R, Salehi A, Movahhedi Dehnavi M, Farajee H, and Kohanmoo MA, 2019. Physiological and yield responses of *Aloe vera* plant to biofertilizers under different irrigation regimes. Agricultural Water Management 225: 105768.

Khordadi Varamin J, Fanoodi F, Masuod Sinaki J, Rezvan Sh, and Damavandi A, 2018. Physiological response of sesame (*Sesamum indicum* L.) to application of chitosan and magnesium-nano fertilizers under irrigation cut-off in a sustainable agriculture system. Iranian Journal of Plant Physiology 9(1): 2629-2639.

Kochert G, 1978. Carbohydrate determination by the phenol-sulfuric acid method. In: Hellebust JA and Craigie JS (eds.). Handbook of Phycology Methods: Physiological and Biochemical Methods. Pp. 95-97. Cambridge University Press, London, UK.

Kumar KB and Khan PA, 1982. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. Indian Journal of Experimental Biology 20(5): 412-416.

Kumar GP, Desai S, Moerschbacher BM, and Gueddari NEE, 2019. Seed treatment with chitosan synergizes plant growth promoting ability of *Pseudomonas aeruginosa*-P17 in sorghum (*Sorghum bicolor* L.). BioRxiv 601328, 1-43.

Li X, He JZ, Hughes J, Liu YR, and Zheng YM, 2014. Effects of super-absorbent polymers on a soil- wheat (*Triticum aestivum L.*) system in the field. Applied Soil Ecology 73: 58-63.

Li Z, Zhang Y, Zhang X, Merewitz E, Peng Y, Ma X, Huang L, and Yan Y, 2017. Metabolic pathways regulated by chitosan contributing to drought resistance in white clover. Journal of Proteome Research 16(8): 3039-3052.

Limpanavech P, Chaiyasuta S, Vongpromek R, Pichyangkura R, Khunwasi C, Chadchawan S, Lotrakul P, Bunjongrat R, Chaidee A, and Bangyeekhun T, 2007. Chitosan effects on floral production, gene expression, and anatomical changes in the *Dendrobium* orchid. Scientia Horticulturae 116(1): 65-72.

Liu XM and Zhang H, 2015. The effects of bacterial volatile emissions on plant abiotic stress tolerance. Frontiers in Plant Science 6: 774.

Lutts S, Kinet J, and Bouharmont J, 1996. Effect of various salts and of mannitol on ion and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) callus cultures. Journal of Plant Physiology 149(1-2): 186-195.

Mohammadi M, Modarres-Sanavy SAM, Pirdashti H, Zand B, and Tahmasebi-Sarvestani Z, 2019. Arbuscular mycorrhizae alleviate water deficit stress and improve antioxidant response, more than nitrogen fixing bacteria or chemical fertilizer in the evening primrose. Rhizosphere 9: 76-89.

Mondal T, Datta JK, and Mondal NK, 2017. Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop. Journal of the Saudi Society of Agricultural Sciences16(2): 135-144.

Mondal MMA, Malek MA, Puteh AB, Ismail MR, Ashrafuzzaman M, and Naher L, 2012. Effect of foliar application of chitosan on growth and yield in okra. Australian Journal of Crop Science 6(5): 918-921.

Moslemi Z, Habibi D, Asgharzadeh A, Ardakani MR, Mohammadi A, and Sakari A, 2011. Effects of super absorbent polymer and plant growth promoting rhizobacteria on yield and yield components of maize under drought stress and normal conditions. African Journal of Agricultural Research 6(19): 4471-4476.

Nakano Y and Asada K, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22(5): 867-880.

Narolia GP, Shivran AC, and Reager MI, 2013. Growth and quality of isabgol (*Plantago ovate* Forsk.) influenced by phosphorus, PSB and zinc. International Journal of Plant Sciences 8(1): 160-162.

Pirbalouti AG, Malekpoor F, Salimi A, and Golparvar A, 2017. Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. Scientia Horticulturae 217: 114-122.

Rafiei F, Nourmohammadi G, Chokan R, Kashani A, and Haidari Sharif Abad H, 2013. Investigation of superabsorbent polymer usage on maize) under water stress. Global Journal of Medicinal Plant Research 1(1): 82-87.

Ram Rao DM, Kodandaramaiah J, Reddy MP, Katiyar RS, and Rahmathulla VK, 2007. Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characteristics under semiarid condition. Caspian Journal of Environmental Sciences 5(2): 111-117.

Raymond KN and Dertz EA, 2004. Biochemical and physical properties of siderophores. In: Crosa JH *et al.* (eds.). Iron Transport in Bacteria, pp. 3-17. ASM Press, Washington DC, USA.

Sawut A, Yimit M, Sun W, and Nurulla I, 2014. Photopolymerisation and characterization of maleylatedcellulose-g-poly(acrylic acid) superabsorbent polymer. Carbohydrate Polymers 101: 231-239.

Scervino JM, Mesa MP, Mónica ID, Recchi M, Moreno NS, and Godeas A, 2010. Soil fungal isolates produce different organic acid patterns involved in phosphate salts solubilization. Biology and Fertility of Soils 46: 755-763.

Seyed-Doraji S, Gholchin A, and Ahmadi Sh, 2010. The effects of different levels of superabsorbent polymer and soil salinity on water holding capacity with three textures of sandy, loamy and clay. Journal of Water and Soil 24(2): 306-316 (In Persian with English abstract).

Shahram M, Fazeli Rostampoor M, and Ansari MH, 2013. The effect of different levels of superabsorbent on efficiency of the photosynthetic matter, the remobilization and portion of remobilization in seed yield of maize (*Zea mays* L.) under drought stress. Annals of Biological Research 4(2): 170-176.

Sharif R, Mujtaba M, Ur Rahman M, Shalmani A, Ahmad H, Anwar T, Tianchan D, and Wang X, 2018. The multifunctional role of chitosan in horticultural crops: a review. Molecules 23(4): 872.

Sharifa S and Muriefah A, 2015. Effects of paclobutrazol on growth and physiological attributes of soybean (*Glycine max* L.) plants grown under water stress conditions. International Journal of Advanced Research in Biological Sciences 2(7): 81-93.

Shekari F, Javanmard A, and Abbasi A, 2015. Effects of super-absorbent polymer application on yield and yield components of rapeseed (*Brassica napus* L.). Notulae Scientia Biologicae 7(3): 361-366.

Shilev S, 2020. Plant-growth-promoting bacteria mitigating soil salinity stress in plants. Applied Sciences 10(20): 7326.

Singh BK, Sharma SR, and Singh B, 2010. Antioxidant enzymes in cabbage: variability and inheritance of superoxide dismutase, peroxidase and catalase. Scientia Horticulturae 124(1): 9-13.

Sultana S, Shariff MA, Hossain MA, Khatun A, and Huque R, 2016. Effect of super water absorbent (SWA) hydrogel on productivity and quality of Tomato. Archives of Applied Science Research 8(10): 5-9.

Tongo A, Mahdavi A, and Sayad E, 2014. Effect of superabsorbent polymer Aquasorbon on chlorophyll, antioxidant enzymes and some growth characteristics of *Acacia victoriae* seedlings under drought stress. Ecopersia 2(2): 571-583

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, and Prigent-Combaret C, 2013. Plant growth-promoting rhizobacteria and root system functioning. Frontiers in Plant Science 4: 356.

Velikova V, Yordanov I, and Edreva A, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science 151(1): 59-66.

Xu Q, Chen S, Yunjuan R, Chen S, and Liesche J, 2018. Regulation of sucrose transporters and phloem loading in response to environmental cues. Plant Physiology 176(1): 930-945.

Yusoff MM, Gordon MH, and Niranjan K, 2014. Aqueous enzyme assisted oil extraction from oilseeds and emulsion de-emulsifying methods: a review. Trends in Food Science and Technology 41(1): 60-82.

Zohuriaan-Mehr MJ, Omidian H, Doroudiani S, and Kabiri K, 2010. Advances in non-hygienic applications of superabsorbent hydrogel materials. Journal of Materials Science 45: 5711-5735.

بهبود صفات مرتبط با عملکرد کلزای بهاره در پاسخ به نانو سوپرجاذب و کود زیستی در شرایط کم آبی

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چکیده

آزمایشی دو ساله به منظور بررسی اثر نانو سوپرجاذب و کود زیستی بر عملکرد کلزا تحت سطوح مختلف آبیاری در سال‌های ۱۳۹۷ و ۱۳۹۸ انجام شد. آزمایش‌ها به‌صورت اسپلیت‌پلات-فاکتوریل بر پایه‌ی طرح بلوک‌های کامل تصادفی در سه تکرار با سه سطح آبیاری (I_1، I_2 و I_3: به‌ترتیب آبیاری پس از ۷۰، ۱۲۰ و ۱۷۰ میلی‌متر تبخیر از تشتک کلاس A) در کرت‌های اصلی و دو سطح نانوسوپرجاذب (۰ و ۴۵ کیلوگرم در هکتار) و چهار سطح کود زیستی (شاهد، ازتوباکتر و انتروباکتر، کیتوزان و باکتری + کیتوزان) به صورت فاکتوریل در کرت‌های فرعی ارزیابی شد. فعالیت آنزیم‌های آنتی اکسیدان، پراکسید هیدروژن، محتوای اسمولیت ها و مالون دی آلدئید تحت I_2 و I_3 افزایش یافت. این واکنش منجر به کاهش محتوای آب برگ، شاخص پایداری غشا، محتوای پروتئین برگ و صفات مربوط به عملکرد شد. کاربرد کودهای زیستی به ویژه کیتوزان + باکتری‌های محرک رشد گیاه (PGPR) با و بدون نانو سوپرجاذب سبب افزایش فعالیت آنزیم-های آنتی اکسیدان شد. استفاده از نانو سوپرجاذب باعث کاهش فعالیت این آنزیم‌ها شد. عدم کاهش این صفات با کاربرد نانو سوپرجاذب + کودهای زیستی نشان می‌دهد که اثر افزایشی کیتوزان + باکتری بیش از اثر کاهشی نانو سوپرجاذب بر فعالیت این آنزیم‌ها بود. کاربرد نانوسوپرجاذب با کودهای زیستی سبب افزایش فعالیت این آنزیم‌ها از طریق حفظ نیتروژن بیشتر در خاک و افزایش اثر کود شد. استفاده از کیتوزان، PGPR و نانو سوپرجاذب، به ویژه کیتوزان + PGPR + نانو سوپرجاذب، محتوای پرولین را کاهش داد، ولی قندهای محلول، پروتئین، کلروفیل، محتوای آب برگ و شاخص پایداری غشاء را افزایش داد و در نتیجه این تیمارها صفات مرتبط با عملکرد کلزا را در شرایط تنش کم‌آبی را تحت تأثیر قرار داد.

واژه‌های کلیدی: باکتری؛ پایداری غشاء؛ پرولین؛ کیتوزان؛ کلروفیل