Effect of priming with proline on the performance of two wheat cultivars

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Abstract: Amino acids (bio-stimulants) are considered as cheap and biodegradable chemicals that increased yield and agronomic performance of several crops. A field experiment was conducted at the experimental station of National Research Centre, Nubaria district El-Behera Governorate-Egypt, during two winter seasons 2017/2018 and 2018/2019. Grains of Misr 3 and Sids 12 cultivars were soaked with proline at 5 or 10 mM for 12 hrs and dried at room temperature before sowing to study the effect of proline treatments on the performance and quality of wheat plants grown under sandy soil conditions. Data show that performance of Misr 3 cv grown under the conditions of sandy soil was more better than Sids 12 cv. Moreover, proline treatments at both concentrations caused significant increases in most parameters measured in this study as plant fresh and dry weight; total photosynthetic pigments; grains weight/fedan. Proline treatment at 5 mM caused the highest significant increase in grains yield and yield components of Misr 3 cv. Where, grains yield/feddan was increased by 21.32%. Meanwhile, the highest significant increase in grains yield of Sids 12 cv was achieved by proline at 10 mM by 16.90%. It was noted that Misr 3cv responded effectively to 5 mM proline, while response of Sids 12 cv was more effective to 10 mM proline. It could be concluded that proline treatments showed promotive effect on quality and quantity of both wheat cultivars grown under sandy soil conditions.

Keywords: *Triticum aestivum* L.,amino acid, proline, grains quality, productivity, priming

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1 Introduction

In Egypt, wheat (*Triticumaestivum* L) is considered the strategic food crop. The increase in cultivated area of wheat should be done in the new land due to the limited areas of the Nile Valley and the competition with main crops. Both wheat exploiting and increasing wheat production of the cultivated area are necessary to overcome the gap between production and consumption. So, improving both quantitative and qualitative characteristics of wheat was still the aim of many investigators. Wheat cultivar, nitrogen fertilizer and amino acids application could play an important role in determining wheat grain productivity.

Amino acids (bio-stimulants) are considered as cheap and biodegradable chemicals that increased yield and agronomic performance of several crops. Moreover, an importance of amino acids came from the ability to the biosynthesis of a large variety of non-proteinic nitrogenous

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materials, such as pigments, vitamins, coenzymes, pyrimidine bases and purine. Amino acids treatments may be reduced the application of fertilizers and improved the quality of some plants via increasing plant mineral uptake and improving the nutrients use efficiency (Vernieri et al., 2005).

Proline amino acid has been proposed to act as a source of carbon and nitrogen (Santos et al., 1996) osmoprotectant, inhibitor of lipid membrane peroxidation, metal chelator, protein stabilizer, maintaining the membrane integrity, maintaining the cytoplasmic pH and ROS scavenger(Trovato et al., 2008).The effect of proline application on plants depends on the type of plant species, plant developmental stage, time of application and concentration. Proline at low concentrations has been shown to simulate growth and yield of different crops(Serraj and Sinclair, 2002; Ali et al., 2008; Dawood et al., 2014).

Despite the beneficial effects of proline application, it imparts toxic effects as well if over-accumulated or applied at excessive concentrations. Proline application at a low concentration (e.g., 30 mM) ameliorated the adverse effects of salinity on early seedling growth in rice, whereas at higher concentrations (40–50 mM) proline resulted in toxic effects and poor plant growth as mentioned by Roy et al. (1993).Hare (1998) stated that proline treatments at lower concentrations activated a cycle of cytosolic proline synthesis from glutamate and mitochondrial proline degradation, which simultaneously provided $NADP^+$ to drive cytosolic purine biosynthesis and reducing equivalents for mitochondrial ADP phosphorylation. The exogenous application of proline at the rate of 80 mM resulted in the reduction of generation of reactive oxygen species and enhanced accumulation of proline and protein contents in wheat varieties under copper stress environment (Noreen et al., 2018).Dawood et al. (2014) reported that exogenous application of proline at a concentration of 25 mM partially mitigated the toxicity of diluted seawater on faba bean plants, whereas the 50 mMproline treatment was toxic. Where, the toxic effect of proline treatments at high concentrations may be attributed to destabilize the DNA helix, lower the DNA melting point, and increase susceptibility to S1 nuclease and increase insensitivity to DNAase1as mentioned byRajendrakumar(1997).Moreover, Sadak and Mostafa (2015); El-Awadi et al.(2016);Elewa et al.(2017) reported that proline treatments have promotive effect on different vegetative growth parameters ; photosynthetic pigments, seed quality and quantity of sunflower; faba bean; quinoa respectively under normal or stressed conditions.

Seed priming is a simple, safe, economic, and effective approach for enhancement of seed germination, early seedling growth, and yield under stressed and non-stressed conditions (Sadeghi et al., 2011). Seed priming is a form of seed preparation in which seeds are presoaked before planting with a certain solution that allows partial hydration but not germination and re-dried to original moisture content (Ahmad et al., 2012). This work aimed to investigate the physiological role of proline as grain priming in improving quality and productivity of two wheat cultivars grown under sandy soil conditions.

2 Materials and methods

A field experiment was carried out at the experimental station of National Research Centre, Nubaria district El-Behera Governorate-Egypt, during two winter seasons2017/2018-2018/2019. Two wheat cultivars (Misr 3 and Sids 12) were obtained from Agricultural Research Centre, Giza, Egypt. The experimental design was a complete randomized block design with four replicates. Wheat grains were soaked with proline (Pyrrolidine-2-carboxylic acid; C5H9NO2; 115.132g·mol^{-1}) at 5 or 10 mM for 12 hours and dried at room temperature. Then, wheat grains were sown on the middle of November in both season in rows 3.5 meters long, and the distance between rows was 20cm apart, Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length).The soil texture of the experimental site was sandy soil and having the following characters: sand 85.3%, silt 10.7%, clay 4%, pH 7.84, $CaCO_3$, 1.0%, EC 3.95 dsm^{-1} and the available total N, P and K were 8.0, 3.0 and 19.8 ppm, respectively at 30 cm depth according to the method described by Chapman and Pratt (1978).

The recommended agricultural practices of growing wheat grain were applied according to Agricultural Research Centre, Giza, Egypt. The seeding rate was (60 Kg grains fed^{-1}). Pre-sowing, 150 kgfed^{-1} calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 75 kgfed^{-1} at five equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulphate (48.52% K_2O) was added at two equal doses of 50 kgfed^{-1}, before the 1st and 3rd irrigations. Plant samples were taken after 60 days from sowing for measurements of some growth parameters (shoot height, number of branches and leaves/plant as well as plant fresh and dry weight). Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in fresh leaf tissues were determined as method recommended byMoran(1982).

At harvest, the following characters were recorded on random samples of plants in each treatment: spike length; spike weight; number and weight of grains/spike; spikelets number/spike; number of tillers and spikes per 1/4 meter; weight of straw yield per 1/4 meter and grains yield/fadan (Kg). The yielded grains were cleaned and used for determination of soluble protein, proline and soluble sugars contents. Soluble protein content was determined according to Bradford (1976) method. Proline was estimated according to Bates et al. (1973). Soluble sugar content was determined using the colorimetric method described by Dubois et al. (1956**)**.Data were statistically analyzed using the least significant difference at 5% level of probability according to Snedecor and Cochran (1980).

3 Results and discussion

3.1 Cultivars variation

It was noted that Misr 3 cv was characterized by higher some growth parameters (Number of branches and leaves/plant, plant fresh and plant dry weight) than Sids 12 cv when grown under sandy soil conditions (Table 1). Moreover, growing Misr 3 cv. under sandy soil condition shows more adaptable to such conditions than growing Sids 12 cv. Where, Misr 3 cv. was higher vegetative growth parameters (Table 1), the photosynthetic pigments (Table 2), grains yield and yield components (Table 3 A and B); soluble sugar than Sids 12 cv. (Table 4). Meanwhile, Sids 12 cv has the higher content of soluble protein and proline. These differences in wheat cultivars may be explained according to Sultan et al.(2000) who stated that wheat cultivars differed in their yield and yield components. Sharaan et al.(2000) mentioned that wheat cultivars were significantly different in plant height, spike length, number of spikes, number of grains per spike and grain weight per spike. In addition Zakiet al.(2004)established that, grain, straw and biological yields and its components were significantly differed owing to cultivar differences. Further, there were significant differences in all the growth characters of wheat cultivars (Sakha93, Gemiza7 and Gemiza9) as reported by Zakiet al. (2007).

3.2 Vegetative growth parameters

Proline treatments at 5 mM and 10 mM significantly increased some of growth parameters under investigation in two wheat cultivars relative to corresponding control. Regarding Misr 3 cv, proline treatment at 5 mM was more pronounced treatment. Since, plant fresh weight significantly increased by 55.37% and plant dry weight significantly increased by 112.34% relative to corresponding controls. While in case of Sids 12 cv, proline treatment at 10 mM was more pronounced treatment, where plant fresh and dry weight were increased by 38.94 and 25.65% respectively relative to corresponding controls. These results indicate that response of Misr 3cv to proline treatment at 5 mM was more effective than response ofSids 12cvto proline treatment at10mM.

Amino acids application can influence the physiological activities in plant growth. According to Nikiforova et al. (2006), amino acids promote development of the root system and activate the growth of the aboveground plant part. Amino acids may be play main role in metabolism and protein assimilation of plant which necessary for plant cells and consequently increase fresh and dry weight of plant (Abo Sedera et al., 2010). Further, amino acids can play different roles in plants; they can act as stress-reducing agents, source of nitrogen and hormone precursors (Zhao, 2010; DeLille et al., 2011; Maeda and Dudareva, 2012) thereby inducing growth parameters. The increases in growth characters as shown in Table 1 caused by proline treatments might be due to the role of proline in protecting enzymes, 3D structures of proteins and organelle membranes and also supplies energy for growth and survival thereby helping the plant to tolerate stress (Hoque et al., 2007; Ashraf and Foolad, 2007; Dawood et al., 2014).

Proline treatments at 100 and 200 mg L^{-1} increased plant height, number of leaves, fresh and dry weight of leaves and roots, root/shoot ratio, and root diameter and length while a higher concentration (400 mg L^{-1}) increased some parameters but decreased others(EL-Sherbeny and DA Silva, 2013). The obtained results are in good agreement with those reported by Sadak and Mostafa (2015),El-Awadi et al.(2016),Elewa et al.(2017) on different plant species.

Table 1 Effect of proline treatments on vegetative growth parameters of two wheat cultivars

Cultivars	Treatments	Shoot height (cm)	Number of branches/plant	Number of leaves/plant	Plant fresh weight (g)	Plant dry weight(g)
Misr 3	Control	83.60	1.50	8.16	18.33	6.24
	proline at 5 mM	93.16	2.83	11.5	28.48	13.25
	proline at 10 mM	85.75	2.33	8.66	18.48	9.57
Sids 12	Control	70.55	1.00	6.16	13.02	4.21
	proline at 5 mM	73.82	1.83	6.33	15.20	4.69
	proline at 10 mM	74.66	2.73	8.30	18.09	5.29
LSD at 5%		5.88	0.75	2.04	2.73	0.74

3.2 Photosynthetic pigments

Data in Table 2 show that Misr 3 cv was characterized by significant increases in total photosynthetic pigments than Sids 12 cv under control conditions by 14.23%. Regarding effect of proline, it is clear that the concentration of proline significantly increased all content of photosynthetic pigments of two wheat cultivars. Proline treatment at 5 mM caused significant increases in photosynthetic pigments of Misr 3 cv by 25.98%. Whereas in Sids 12 cv proline treatment at 10 mM was more effective (32.78%).

The increments of chlorophyll values (Table 2) by proline application may be due to the role of proline in chloroplast protection from oxidation damage (Smirnoff and Cumbes, 1989). Since, this amino acid has an important role in protecting enzymes as well as membranes (Solomon et al., 1994)or elevating the level of Mg^{+2}ions which needed for chlorophyll synthesis (Shaddad, 1990). Ali et al.(2007)attributed the beneficial effect of 30 mM proline treatment on photosynthetic pigments of maize plants to stimulating chlorophyll biosynthesis and/or inhibiting its degradation, improved efficiency of photosynthetic pigments and stabilizing photosynthetic reactions by proline. The exogenous application of proline at 10 mM improved the content and efficiency of photosynthetic pigments of wheat plant (Rady and Mohamed, 2018).

Table 2 Effect of proline treatments on photosynthetic pigments of two wheat cultivars

Cultivars	Treatments	Chlorophyll a	Chlorophyll b	Carotenoids	Total photosynthetic pigments
		mgg^{-1} fresh leaf tissues			
Misr3	Control	1.50	0.91	0.40	2.81
	proline at 5 mM	1.90	1.09	0.55	3.54
	proline at 10 mM	1.76	0.99	0.50	3.25
Sids 12	Control	1.33	0.78	0.30	2.41
	proline at 5 mM	1.53	0.90	0.39	2.82
	proline at 10 mM	1.80	1.00	0.40	3.20
LSD at 5%		0.24	0.06	0.03	0.24

3.3 Grain yield and yield components

The obtained data indicate that Misr 3 cv was characterized by more grains yield and yield components than Sids 12 cv as shown in Table 3. In addition, data show that proline treatments have enhancement effect of on grains yield and yield components (spike length, spike weight, number of grains/spike, weight of grains\spike, number of spiklets/spike, number of spikes per ¼m^2, number of tillers per ¼ m^2, weight of straw per ¼ m^2, straw yield and grains yield (Kgfeddan^{-1}) of two wheat cultivars when grown under sandy soil conditions. Prolinetreatment at 5 mM caused the highest significant increase in grains yield and yield components of Misr 3 cv.Where, grains yield/feddan was increased by 21.32%. Meanwhile, the highest significant increase in grains yield of Sids 12 cv was achieved by proline at 10 mM by 16.90%. Hence, response of Misr 3cv to proline treatments at lower concentration (5 mM) was more pronounce than the response of Sids 12 cv to proline treatment at higher concentration (10 mM).

Grain yield of wheat is affected by many factors, of which cultivar, nitrogen fertilizer and amino acids application may be played an important role in determining productivity of wheat. Azimiet al.(2013) pointed out that foliar application of amino acids caused the highest significant increase in plant height, number of tillers/m^2, number of grains/spike, 1000-grains weight and grain yield of barley. Hammad and Ali (2014) revealed that foliar spraying of amino acids induced significant increases in plant height, number of leaves/plant, number of tillers/plant, flag leaf area, number of spikes/m^2, number of grains/spike, 1000-grain weight, grain yield (tonfed^{-1}), straw yield (tonfed^{-1}), protein and carbohydrate percentage in grain.

The improvement of wheat yield by applying proline could be a reflection of increments of vegetative growth parameters and photosynthetic pigment which in turn led to an increase of photosynthetic assimilates production that translocated to the reproduction organs during maturation and ripening stages. Moreover, this amino acid can feed energy to support plant metabolism of production process in plants (Munoz et al., 1988). Likewise, proline amino acid can regulate some needs of cell plant such as acceleration of organ growth, meristem stimulation and cell division thus promoted plant growth and yield formation (Mattioli et al., 2008).

Table 3 Effect of proline treatments on grain yield and yield components of two wheat cultivars

Cultivers	Treatments	Spike length (cm)	Spike weight (g)	Number of grains/spike	weight of grains\spike (g)	Number of spikletes/spike	Number of spikes ¼ m^2	Number of tillers	Weight of straw ¼ m^2	weight /faddan (Kg)
Misr 3	Control	9.61	2.57	48.16	1.98	19.66	85.33	89.00	123.28	1226.97
	proline at 5 mM	11.77	4.03	74.66	2.94	22.16	124.33	106.30	172.19	1488.65
	proline at 10 mM	12.16	4.33	78.33	3.33	21.55	96.00	148.00	134.46	1437.10
Sids 12	Control	8.31	2.25	42.5	1.74	18.99	85.33	73.00	10341	1185.25
	proline at 5 mM	10.48	3.60	62.50	2.47	20.70	97.66	97.3	155.00	1385.61
	proline at 10 mM	10.08	3.24	55.03	2.34	21.00	97.50	103.00	154.37	1369.00
LSD at 5%		0.88	0.48	4.56	0.34	1.23	22.62	37.28	38.66	118.03

3.4 Chemical composition of the yielded grains

It was note that Misr 3 cv was characterized by higher proline and soluble sugar than Sids 12 cv when grown under sandy soil conditions (Table 4). Meanwhile, Sids 12 cv has the higher content of soluble protein and production. Proline treatments caused significant increases in soluble protein and proline content in two wheat cultivars under investigation accompanied by significant decreases in soluble sugars relative to corresponding controls.

The increase of soluble proteins with grains soaking with proline might be a result of osmotin-like or structural protein synthesis, especially of those proteins that play a role in cell wall modification (Amini and Ehsanpour, 2005) or due to the result of enhancing de novo synthesis of proteins for cell protection (Teixeira et al., 2005).In addition to the beneficial effect of the exogenous proline in stabilizing membranes and the protein synthesis machinery (Khedr et al., 2003) it reduced membrane lipid oxidation (Okuma et al., 2004). The accumulation of greater quantum of proline in the plant system resulted in enhancing the photosynthetic system and soluble protein (Shahid et al., 2014).

Regarding endogenous proline, there were many of reports showed that proline application increased its endogenous levels in plant tissues under the stress conditions (Ali et al., 2007; Ashraf and Foolad, 2007; Hogue et al., 2007).The decrease in soluble sugars values due to proline treatment was previously reported by Gadallah (1999) and Đogić et al. (2017). It is worthy to mention that the effect of proline is dependent on its concentration, as mentioned by Dawood et al. (2014), because an excessive amount of free proline has the negative effects on protein functions and cell growth. The over-accumulation of intracellular proline significantly represses several genes involved in the synthesis of other amino acids or normal morphogenesis in Arabidopsis plant**s** (Nanjo et al., 2003).

Table 4 Effect of proline treatments on some chemical composition of two wheat cultivars

Cultivars	Treatments	Soluble protein	Proline	Soluble sugar
		mg g^{-1}		
Misr 3	Control	70.46	1.26	22.97
	proline at 5 mM	73.07	1.61	22.81
	proline at 10 mM	75.58	1.78	21.37
Sids 12	Control	78.09	1.54	20.65
	proline at 5 mM	80.99	1.94	19.60
	proline at 10 mM	81.78	2.10	19.46
LSD at 5%		2.70	0.15	1.03

4 Conclusion

It could be concluded that performance of Misr 3 cv was more pronounced than Sids 12cv when grown under sandy soil conditions. Proline treatments showed promotive effect on quality and quantity of two wheat cultivars. Generally, Misr 3 cv responded effectively to proline treatment at 5 mM, while Sids 12 cv responded effectively to proline treatment at 10 mM.

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