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The Physiological Role of Putrescine and Cyanobacteria in the Alleviation of the NaCl-salt Stress on Rice Plants

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Abstract

The existing work proposed to explain alterations in growth and a few metabolic actions in salt-treated rice (cv. Giza-178) plants by salt irrigation (100 mM), and assessing the role of presoaking of seeds by polyamine putrescine (4.0 mM) with soil treating by cyanobacteria (23 mg/pot) interaction to alleviate these changes. At 70-day old both putrescine and cyanobacteria *Azotobacter sp*. had a clear effect on the fresh weight plants stressed by salt compared to the control, and the same result was also observed on the dry weight of stressed and unstressed rice plants with salt. Chl (a) and (b) were stimulated especially with the application of a high concentration of putrescine. However, carotenoids were slightly suppressive. The effect of cyanobacteria alone or interacted with putrescine in a significant increase in the photosynthesis activity compared to the control of stressed and non-saline-stressed rice plants at 70-day old.

The total carbohydrate and protein contents of the roots showed a significant increase. A shortage in proline content was observed in both shoots and roots under the influence of cyanobacteria and putrescine overlapped together in saline-stressed plants compared to the control.

The influence of cyanobacteria and putrescine together show a significant effect on nitrogen, phosphorus, potassium, magnesium, iron and manganese shoot contents compared to the control. Exogenous application of putrescine and cyanobacteria succeeded in mitigating the harmful effect of salt on growth rate and various physiological metabolites.

Keywords: *Cyanobacteria; metabolic activities; NaCl salt; putrescine; Rice.*

1 Introduction

Saline soil affects farming usefulness in a few districts of the world including many countries like Egypt, Iraq, and Thailand (Rengasamy 2010). It is assessed that five percent or less than four million ha of the absolute developed region on the planet (seventy seven million ha) is influenced by NaCl pressure (Sheng, Tang *et al*. 2008). Continuously 2050, salt influenced in soil is anticipated to be expanded up to sixteen million ha, which might bring about food frailty for the total populace (Yadav, Mahatma *et al*. 2017).

Overabundance saltiness in soil is one of the main ecological components that end the development and productivity of a wide assortment of harvests including *Rice* (Tester and Davenport 2003, Hasanuzzaman, Nahar *et al*. 2013, Hasanuzzaman, Nahar *et al.* 2013).

Among the cultivable land, twenty percent of watered soil and two percent of the dry region are influenced by saltiness straightforwardly or by optional saltiness (Munns and Tester 2008).

Plants have productive guard systems to adapt to plenty of ecological burdens, which incorporate dry spell, UV, high-saltiness, cold anxieties, and microorganism attack (Fujita, Fujita *et al*. 2006). Polyamines (PAs), generally present in living creatures, are currently viewed as another class of development substances which incorporates the triamine spermidine, tetramine spermine, and their commit antecedent putrescine

(Put, a diamine) which assume a critical part in the guideline of plant formative and physiological processes (Kusano, Yamaguchi *et al*. 2007). PAs may likewise work as pressure couriers in plant reactions to various pressure signals. (Liu, Fu *et al*. 2000, Liu, Kitashiba *et al*. 2007).

Polyamines are known collectively of regular mixtures with aliphatic nitrogen structure, present in practically all living organic entities, assume significant parts in numerous physiological cycles, like cell development and advancement, and react to ecological burdens. Putrescine, spermidine, and spermine are the most regularly discovered PAs in higher plants and could be available in free, solvent-formed, and insoluble bound forms (Lefevre, Gratia *et al*. 2001).

It has been accounted for that the exogenous use of plyamines is additionally a successful methodology for improving pressure resilience of yields for upgraded crop efficiency. Exogenous use of Put has been effectively used to improve salinity (Chattopadhayay, Tiwari *et al*. 2002, Verma and Mishra 2005, Ndayiragije and Lutts 2006), chilly (Nayyar and Chander 2004, Nayyar 2005), dryness (Zeid and Shedeed 2006), heavy metals (Wang, Shi *et al*. 2007), salt stress (Liu, Dong *et al*. 2004), elevation of temperature(Murkowski 2001), flooding (Arbona, Hossain *et al*. 2008) and flooding resistance of plants(Yiu, Juang *et al*. 2009).

Cultures of microscopic algae have means back been confirmed as an encouraging change in terms of providing varied supplementary merchandise, like cultivation feedstuff, bio-fuels, fresh food pills, antioxidants, pharmaceuticals, etc., to decision but some of (Priyadarshani and Rath 2012, Sun, Zhao *et al.* 2018). The description of cultured varieties is endlessly extended as unique varieties reveal advantages over others inside the assembly yield and conjointly the commercial usefulness of the beneficial processes that are used. A large listing previously exists helping the data wanted thus to maximize the creation of the popular species, however, the abundance of subjects still exist even for the well-established culture procedures, as a conclusion of the entire set of parameters of culture is responsible for development. This is usually considered valid for the least-studied varieties and unique varieties. Among microscopic algae, eubacteria (Pathak, Maurya *et al*. 2018) own correlated outsized part of the prevalent microorganism in the main due to the great growth of the culture of the thread variety *Arhrospira* (Spirulina) (Saranraj and Sivasakthi 2014) that has strongly been confirmed to be the “Holy Grail” for the global result of a stern strong foodstuff (Spolaore, Joannis-Cassan *et al.* 2006). Always considering the effect of blue-green algae was known, a large amount of analysis has been done out to emerge ways and suggests that to definitely employ these organisms as a bio fertilizer (Vaishampayan, Sinha *et al.* 2000). The present work aimed to study the influence of polyamine putrescine and cyanobacteria on growth measures and some physiological metabolites to evaluate the impact of the damaging force of salt stress on the growth and improvement of the rice plant.

2 Materials and Methods

2.1 Plant material

Rice (cv. Giza-178) seeds were collected from Giza Agricultural research Station, Giza, Egypt. The fine grains were determined for likely unity of size and form, and outside cleaned (2.5% sodium hypochlorite for five min.) and soaked totally in pure distilled H_2O.

2.2 Pot experiment

Sand–clay soil ½ v/v as a clay soil sample randomly collected from agricultural lands from the surface layer (0-35 cm) in the middle of the delta, in addition to sand washed with hydrochloric acid and distilled water to remove excess salts to achieve realism in the effect within the study (Electrical Conductivity of 1:5 soil extract at 25ºC = 0.58 m mohs cm^{-1}, pH of 1:5 soil suspension = 7.8) was done, the soil was stirred wholly to ensure full and similar distribution (25 centimeter diameter, 35 centimeter depth, 5.5 Kg soil/pot).

Physiochemical criteria of the used soil sample

P^H	EC	Soil texture	Organic Carbon	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	CO_3^{2+}	HCO_3^-	SO_4^{2-}	$CaCO_3$
	m mohs cm^{-1}		%					ιeq. L^{-1}				%
7.8	0.58	Sand/Clay 31.53%/68.47	1.71	0.21	7.43	1.14	1.34	3.34	1.86	4.26	1.36	2.57

Five seeds were sown to the pots after being divided into two groups (0.0 and 100 mM NaCl equivalent to 5.84 g/L with EC 9.8 ds/m) as 25% of the cultivated lands in the Nile Valley Delta suffer from the problem of soil salinity, which makes the expansion and cultivation of lands less efficient, such as saline lands, where many of their lands are exposed to the problem of high rates of desertification (Desert Research Center of the Ministry of Agriculture). Each group was classified into 2 subdivisions (0.0 and 4.0 mM putrescine treated "+ve" and untreated "-ve" with 23 mg dry wt./pot cyanobacteria *Azotobacter sp.* as recommended dose according to the Egyptian ministry of agriculture and land reclamation). The previous concentrations of putrescine were chosen after a trial experiment and were proven to be more effective in alleviating the inhibitory impact of saltness on plant growth.

Irrigation is carried out in fixed quantities until the beginning of the saturation level for each pot, with changing the places of the pots daily, while comparing the weight of a sample at a depth of 10 centimeters of irrigated soil with the weight of completely dry soil from the same mixture as a control to calculate the percentage of the water content of all pots, directly before the date of irrigation, which is relatively constant

2.3 Estimation of photosynthetic pigments

Fresh weighed leaves of seventy days recent were homogenized forthwith in five milliliter eighty fifth cold dimethyl ketone, centrifuged for quarter-hour at 3000 XG, then putted in nightlong cooler. The acetone extract was thinned to the adequate amount, so its color intensity was estimated at 663, 644, and 452.5 nm (Metzner, Rau *et al*. 1965). Pigment fractions were expressed as µg/g fresh weight.

2.4 Photosynthetic (Hill reaction) activity

Photo-system 2 (PS2) activity of plastids separated from plant leaves of 70-day old displayed as electron-transport rate resolve by dealing 2,6-dichlorophenol indophenol (DCPIP) as e- acceptor (Biswal and Mohanty 1976). Chloroplasts were isolate in the cold as set by (Osman and El-Shentenawy 1988). The concentricity of Chl a+b in the supernatant was designated, according to the equalization of (Arnon 1949). For evaluation the PS2 action, assay sample was ready by mix 1.6 cm3 of ten millimeter DCPIP (dissolved in ninety six percent ethanol) with fifty µg Chl, so the volume was completed to three cm3 by the reaction buffer. The sample was lightened (at right angles) with red electromagnetic x-rays (300 W m-2, ten min) rendered from a projector. The DCPIP image decline was assayed spectrophotometrically compatible with (Ebrahim and Aly 2005).

2.5 Estimation of total carbohydrates and proteins

Aliquots (100 mg) of fine powdered of 70 days old dry shoot and root of rice plant were ground into a fine powder (100 mg), extracted in borate buffer (28.63 g boric acid + 29.8 g potassium chloride + 3.5 g sodium hydroxide in a one liter of filtered water). The pH was adjusted to 8.0 and kept standing for 24 hours at 4ºC before centrifugation for 15 minutes at 3000 xg. The residue was washed several times and dried at 80ºC for polysaccharide estimation. The supernatant and residue washings were collected and used for the estimation of total carbohydrates and proteins. Carbohydrate sugars were extracted in borate buffer pH 8 [0.1 dry mass (10 cm^3 buffer)$^{-1}$].

Carbohydrate sugars were assessed quantitatively handling Nelson (1944) with some differences was done by Naguib (1963) these changes are 10 mg of the dry plant residue once extracting in borate salt buffer was associated with 0.2 ml of 0.1 % (w/v) enzyme and 0.1 milliliter acetate buffer (six milliliter 0.2 N acetic acid + four milliliter 0.2 N Na- acetate), made to three milliliter with water, left for twenty-four hrs at temperature, then centrifuged for fifteen min at 3000 round/min and starch are often regulated quantitatively. Whole proteins were assayed in line with Lowrey, Rosebrough *et al.* (1951).

2.6 Determination of Proline Content

The proline content in the leaves (70 days old) was prepared according to the process putted by (Bates, Waldren *et al.* 1973). Briefly, 0.1 g of rice leaves was crushed with five millileters of three percent sulfosalicylic acid, and consequently the mix was then purified. To a pair of millileters of the purified mixture during tubing, two millileters of acid- ninhydrin and two millileters of glacial carboxylic acid were added. The mixture was mixed with a Vortex mixer and heated at 100˚C for 1 h. The mix was then cooled in ice and merged with 4 ml of toluene, mixed, and then started to stand for 5 - 10 min. Absorbance of the reddish pink up- per phase was registered at 520 nm upon a toluene blank.

2.7 Mineral contents

Plant shoot samples of ninety days old were examined for Ca, Mg and Mn applications by means that of the Atomic Absorption flame Emission photometer (Model Perkin Elmer 2380 Atomic Absorption Spectrophotometer).

Na and K were accountable by the flame photometer as represented by (Johnson and Ulrich 1959) (Corning Scientific Instruments, model 410)

P was measurable by (Allen, Grimshaw *et al*. 1974). 2 ml of the digested plant sample was putted in a tube and added to it 0.7 ml ammonium molybdate solution (Ammonium molybdate 25 g + Conc. H_2SO_4 250 ml then completed to one liter) shake vigorously, add 0.5 ml vitamin C solution(Ascorbic acid 0.3 g + H_2SO_4 20% 50 ml). Complete to 10 ml purified water, shake then place it in water bath for 15 min at 70ºC until give phosphorous color and the intensity of the color was discovered using spectrophotometer device at 650 nm.

Analytical steps of mineral particle concentrations were assigned in agreement with (Allen, Grimshaw *et al*. 1974). Mixed acid digestion method was applied in preparing ready the sample analysis. A best-known weight of the oven dehydrated plant sample was assigned to a hundred and fifty millimeter flask, and then four milliliter of conc. acid was added. The mix was heated mildly till charring. Consequently, two milliliter of thirty percent was added to the remainder, and then heated till the dissolution of fumes and also the whole mixture converts clear flat green solution. This means that each one organic composite was change adequately to CO_2 and hence the far more than perchloric acid was decreased to chlorine ion. the solution was then thinned to an appropriate volume with H_2O.

Fe contents were estimated by using atomic absorption photometer (Perken Elmer) according to procedure given in (Chemists and Horwitz 1975). Firstly, the plant samples were wet digested as set by (Richards 2012). The assimilated samples were transmitted to hundred ml flask and volume was made with filtered water and then filtered. Samples were then analyzed in Atomic Absorption photometer and estimation of Fe element was carried out.

The total N content of shoot system was estimated using the modified micro Kjeldahl method of (Paech and Tracey 2013). A recognized weight of the dry-matter plant tissue sample was weighted into a digestion flask. The sulfate mixture followed by ammonia-free water and ammonia-free sulfuric was added. The plant sample was then incinerated, ammonia distilled off and N was calculated by transmetting the digest quantitatively into the micro Kjeldahl device with the least amount of ammonia free filtered water, then forty percent NaOH solution were added. A vigorous current of steam was then passed and the ammonia was titrated with exactly N/70 HCl, using bromo cresol green-methyl red indicator. After correction for the reagent blanks, the titration figures were converted into milligram nitrogen.

2.8 Statistical Analysis

Results achieved were examined statistically to evaluate the level of significance among treatments. The design of one-ways analysis of variance (ANOVA; factorial) was plated for all outcomes by Fisher's, individual error rate. The least significant difference (LSD) at five percent was used to contrast means. All the experiments were run in triplicates by using Minitab program version 16.

3 Results

3.1 Pot experiment and growth criteria

In Fig 1a, a significant increase in the shoot length of rice plant was observed under the influence of putrescine alone and zero concentration of salt compared to control, as well as the same ether was evident on the interaction of cyanobacteria with putrescine compared with control, and both putrescine and cyanobacteria had a significant effect on saline-stressed plants compared to control. In Fig 1b, a significant increase in root length of unstressed plants were observed under the influence of each of the putrescine and cyanobacteria alone compared to the control, and each of the putrescine and cyanobacteria had a significant effect on the plants stressed by saline compared with control.

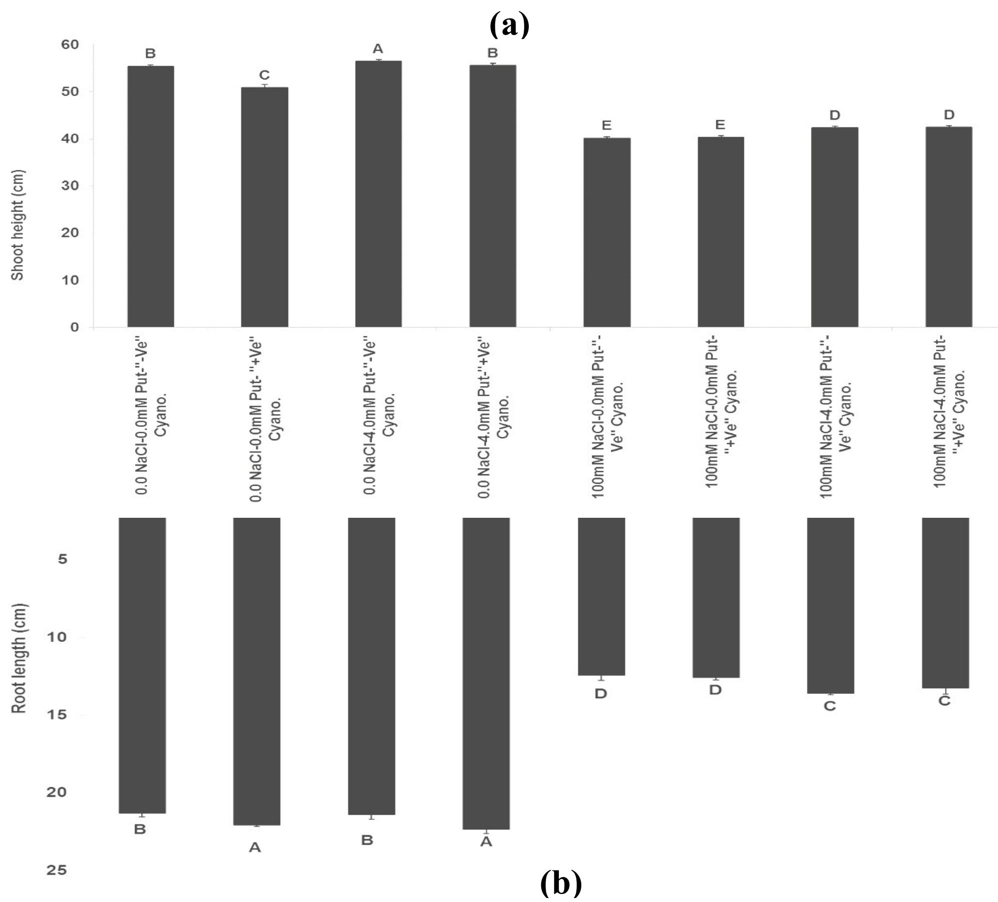
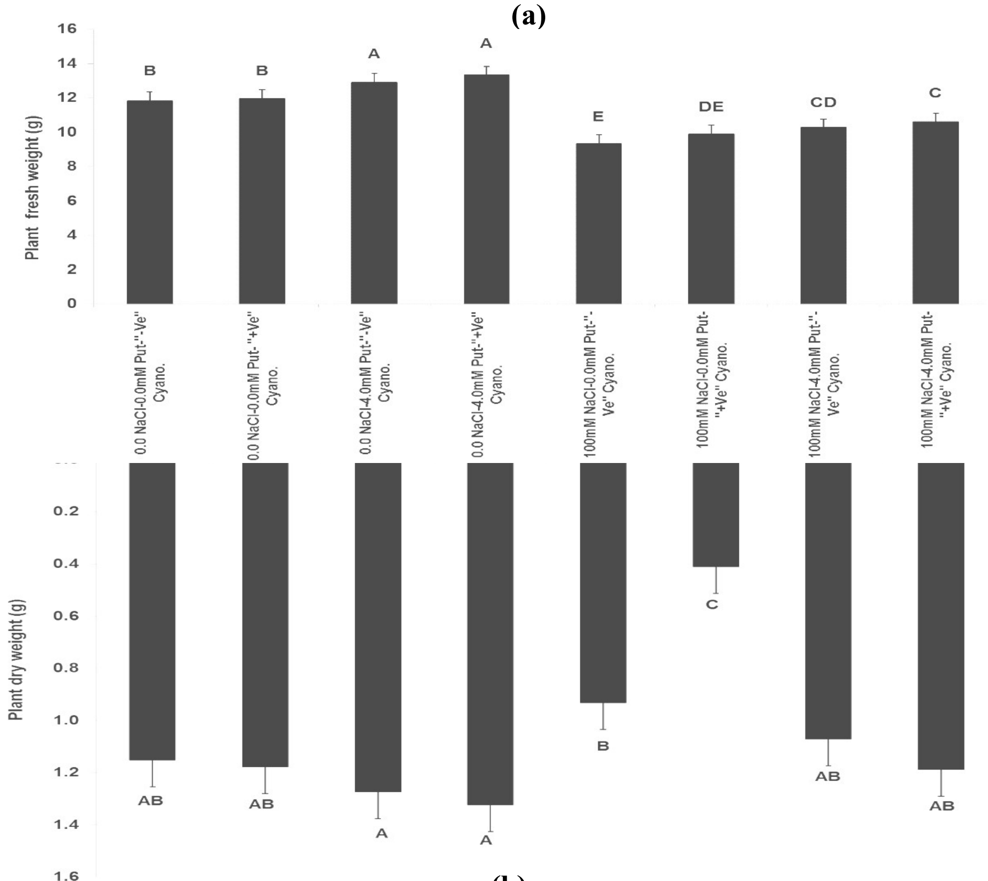


Fig. 1. Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on shoot height and root length of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-E) on the bars indicate significant differences according to the least significant difference (LSD) test ($P \leq 0.05$).

In Fig 2a, a significant raise in the fresh weight of plants not treated with salt was observed, and in the presence of both putrescine and cyanobacteria, and both putrescine and cyanobacteria had a clear effect on the plants stressed by salt compared to the control, and the same result was also observed on the dry weight of stressed and unstressed rice plants with salt (Fig 2b).

(a)



(b)

Fig. 2 Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on fresh and dry weights of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-E) on the bars indicate significant differences according to the least significant difference (LSD) test ($P \leq 0.05$).

3.2 Pigment contents

In Fig 3 the cyanobacteria had a significant increase in chl "a" and "b" for stressed and non-saline stressed plants in the presence or lack of putrescine compared with control. There was a significant increase in the total pigments of plants not stressed by salt in the existence of cyanobacteria compared with control.

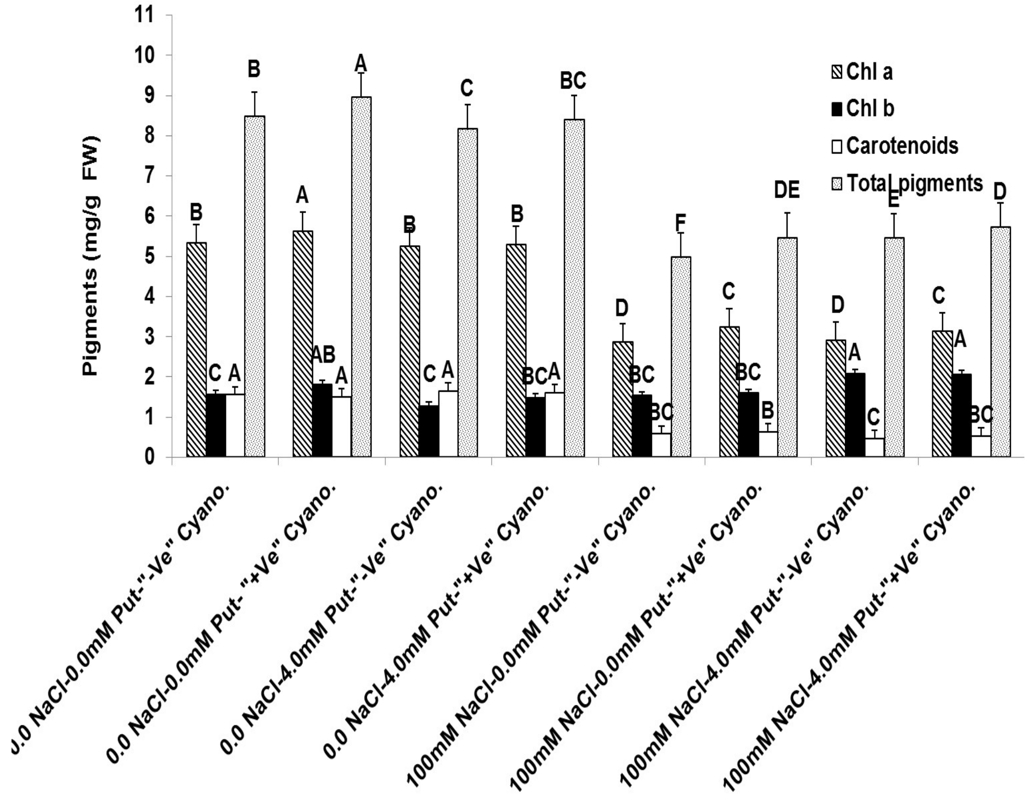


Fig. 3 Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on pigment contents of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-F) on the bars indicate significant differences according to the least significant difference (LSD) test ($P \leq 0.05$).

3.3 Photosynthetic activity

Fig. 4 shows the effect of cyanobacteria alone or interacted with putrescine in a significant increase in the photosynthesis activity compared to the control of stressed and non-saline-stressed rice plants.

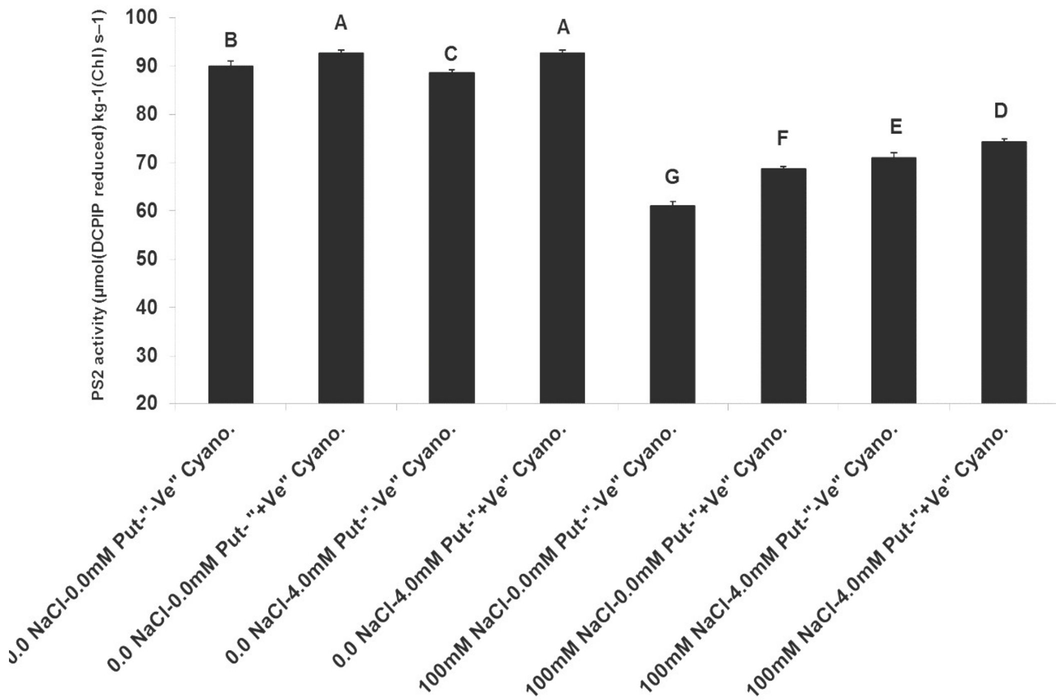


Fig. 4 Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on photosystem Π activity of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-F) on the bars indicate significant differences according to the least significant difference (LSD) test ($P \leq 0.05$).

3.4 Total carbohydrates content

In Fig. 5, the total carbohydrate content of each of the shoots and roots showed a significant increase, especially under the influence of cyanobacteria and putrescine together, compared to the control, whether in stressed or not salt-stressed plants.

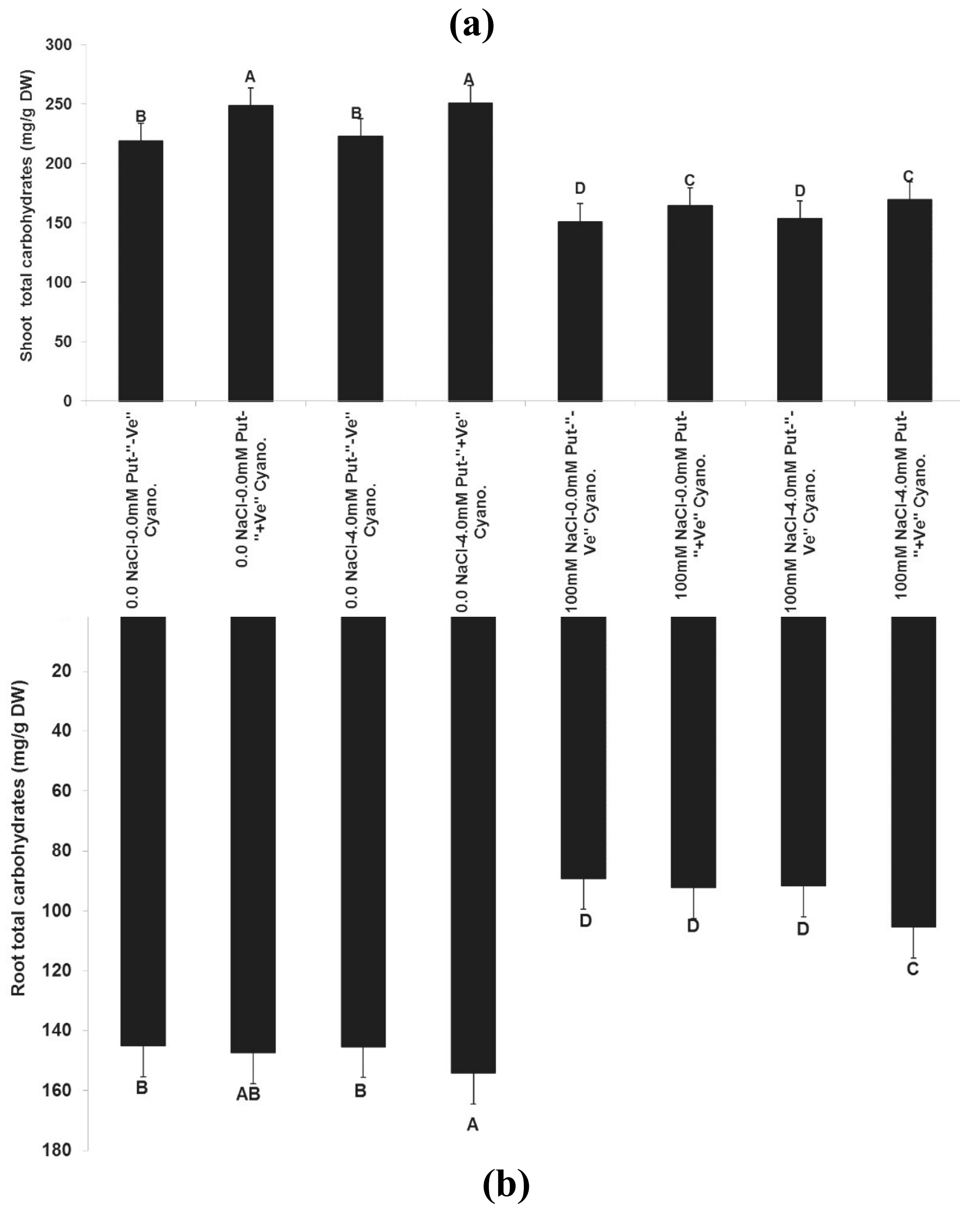


Fig. 5. Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on shoot and root total carbohydrate contents of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-D) on the bars indicate significant differences according to the least significant difference (LSD) test ($P \leq 0.05$).

3.5 Total proteins content

Fig. 6a shows the excess in the total protein content of the shoot under the influence of cyanobacteria and putrescine overlapping together on plants treated with cyanobacteria or putrescine only and not stressed with saline. While Fig. 6b shows an increase in the total proteins content of salt-stressed roots under the influence of both cyanobacteria and putrescine overlapping together, compared with plants treated only with cyanobacteria.

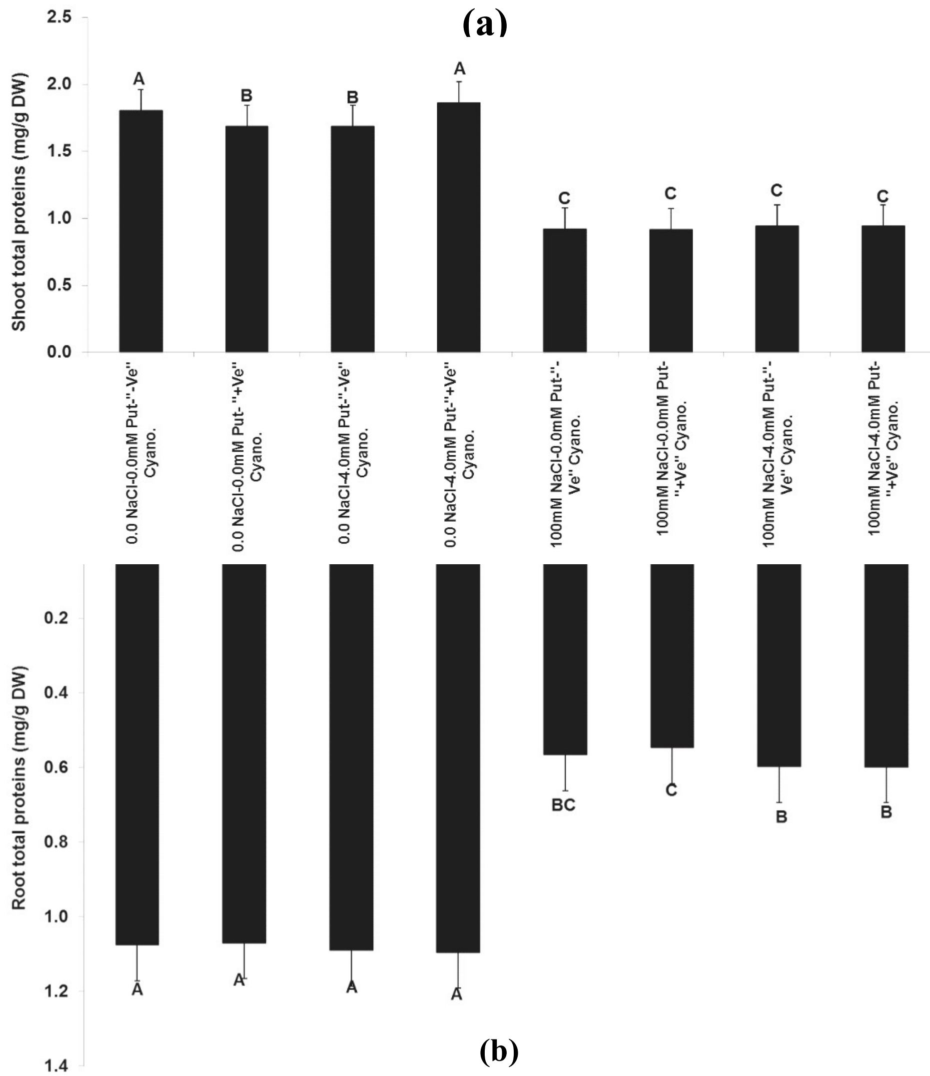


Fig. 6 Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on shoot and root total protein contenets of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-C) on the bars indicate significant differences according to the least significant difference (LSD) test (P ≤ 0.05).

3.6 Determination of Proline Content

In Fig 7a, an increase in the proline content of both shoots and roots was observed in saline-stressed plants compared to non-stressed plants, and a lack in proline content was observed in both shoots and roots under the influence of cyanobacteria and putrescine overlapped together in saline-stressed plants compared with control (Fig 7a-b).

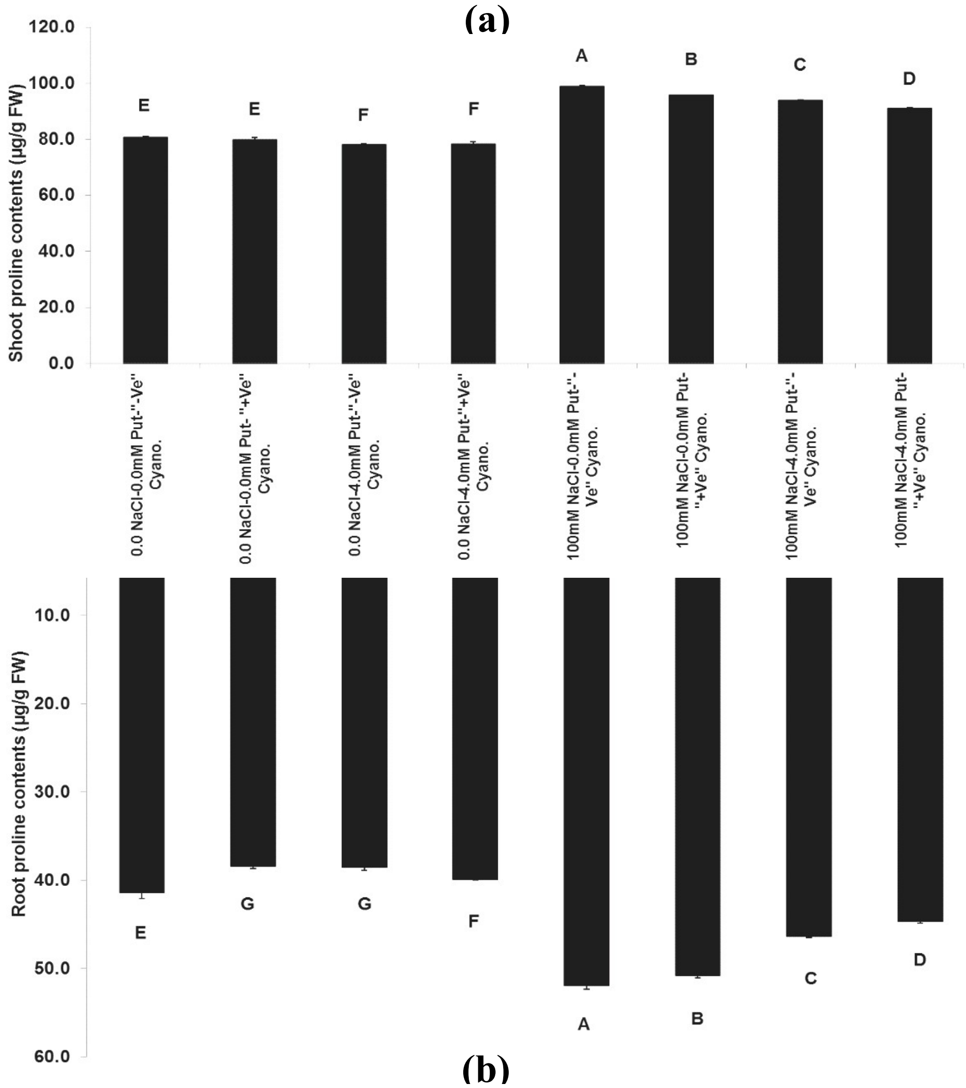


Fig. 7 Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on shoot and root proline contents of stressed *Oryza sativa* plants by NaCl (100 mM) after 70 days. Different letters (A-G) on the bars indicate significant differences according to the least significant difference (LSD) test (P ≤ 0.05).

3.7 Mineral contents

Fig. 8a shows a significant increase in the contents of nitrogen in the shoot of rice that is not salt-stressed under the influence of either cyanobacteria or putrescine alone or overlapping compared to the control. The same effect was also observed on saline-stressed plants, except for the effect of putrescine alone, compared with control.

Fig. 8b shows a significant increase in the contents of phosphorous in the shoot of rice that is not salt-stressed under the influence of cyanobacteria alone or in combination with putrescine compared to control. Also, the same significant effect was very noticeable in saline-stressed plants, whether treated with cyanobacteria and putrescine alone or overlapping together compared to control.

In Fig. 8c, a significant raise of potassium content was observed in the shoots of the brine-stressed and unstressed rice plant under the influence of cyanobacteria alone or in combination with putrescine compared to control.

In Fig. 8d, the effect of putrescine alone or intertwined with cyanobacteria for saline-stressed plants had a significant influence on the amount of calcium in the rice shoot.

In Fig. 8e, each of putrescine alone or combined with cyanobacteria had a significant influence on minimizing the toxicity of shoot sodium content in saline-stressed plants compared to the control.

In Fig. 8f, it was observed that cyanobacteria alone had a clear effect in increasing the iron content in the rice shoot compared to the control for plants that were not brine-stressed, while the effect of both putrescine and cyanobacteria overlapping together had a significant effect on the content of iron for brine-stressed plants compared to the control.

In Fig. 8g, treatment with cyanobacteria alone or intertwined with putrescine resulted in a significant influence in magnesium amount in the shoots of both stressed and unstressed plants.

In Fig. 8h, there was a significant influence in the manganese content of the shoots of stressed and non-saline-stressed rice plants, whether in the presence or absence of cyanobacteria in conjunction with the presence of putrescine.

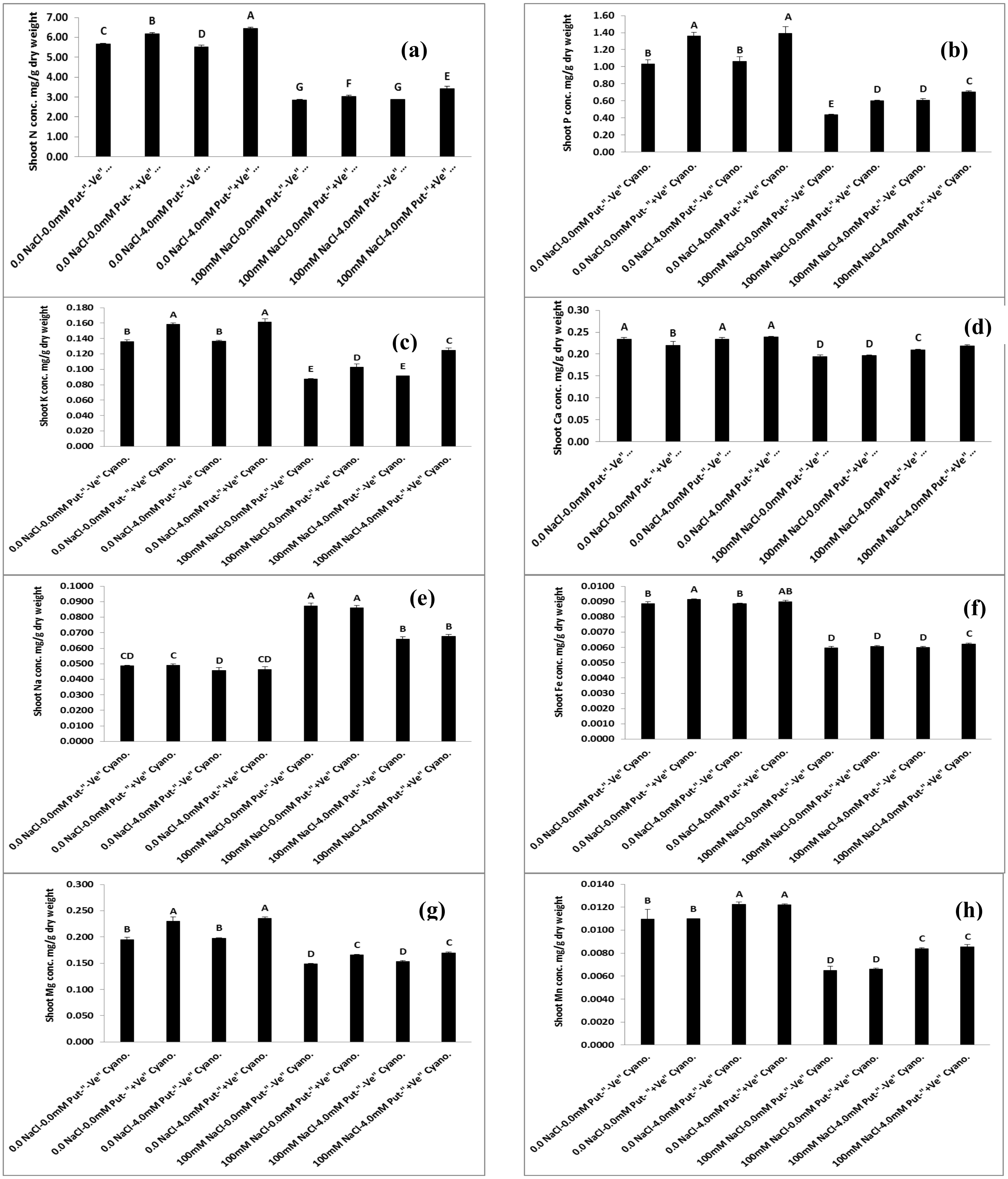


Fig. 8. Effect of putrescine (4.0 mM) and cyanobacteria (23mg/pot**)** on shoot mineral contents of stressed *Oryza sativa* plants by NaCl (100 mM) after 90 days. Different letters (A-G) on the bars indicate significant differences according to the least significant difference (LSD) test (P ≤ 0.05).

4 Discussion

4.1 Pot experiment and growth criteria

A lot of studies published the NaCl salt produced growth reducing in rice. Display of high concentration saltness (150 millimeter NaCl) in rice seedlings lower plant height and mass by salt distribution, ionic and aerobic stresses (Rahman, Hossain *et al*. 2016). (Rahman, Nahar *et al.* 2016) collectively declared that salt (200 mill molar NaCl) lower plant growth by forming ionic and water imbalance, and aerobic stress. Beneath salt stress conditions, growth and water loss were higher inside the unsafe selection compared with a tolerant selection (Islam and Karim 2010, Hasanuzzaman, Nahar *et al.* 2013). (Kumar and Khare 2016) noticed that salt (100 mill molar NaCl ≈10 dS m−1) limited root length, root dry mass, shoot height and shoot dry mass every indifferent and dogmatic selection where growth loss is more promotion in sensitive selection compared with tolerant. On the other hand, the development of rice seedlings is lessened with expanding of salinity (Islam and Karim 2010, Kazemi and Eskandari 2011). (Ologundudu, Adelusi *et al.* 2014) carried Associate in an experiment with eight rice cultivars underneath fully entirely different levels of salinity (0–15 dS m−1) and told that root and shoot length, root and shoot dry weight, and total dry matter product reduced with raising the amount of salinity.

The application of blue-green algae, utilized in many forms on each root and leaves, has been given to definitely have an influence on root improvement and root/shoot quantitative relation in numerous plant varieties, like lettuce (Mógor, Ördög *et al*. 2018), radish (Godlewska, Michalak *et al.* 2019), rice (Singh, Prabha *et al*. 2011), tomato (Elarroussia, Elmernissia *et al.* 2016, Supraja, Behera *et al*. 2020), maize (Ertani, Nardi *et al.* 2019), red beet (Mógor, de Oliveira Amatussi *et al.* 2018), and cucumber (Toribio, Suárez-*Estrella et al*. 2020). A bigger rootage will increase the root expanse and directly enhancement nutrients and water uptake from the soil, thereby improving plant growth and vigor (Mutale-Joan, Redouane *et al.* 2020).

Cyanobacterial polysaccharide sugars may be incorporated within the cyto-membrane, evacuated as definite formations (sheaths, capsules, or stalks), or discharged as mucilage (Rossi and De Philippis 2016). Particularly, exo-polysaccharides are rumored to perform a significant use in soil samples gratitude to their adhering properties (Singh, Kumar *et al.* 2016) and in binding hefty metals (De Philippis, Colica *et al*. 2011) and sodium ions (Ozturk and Aslim 2010), therefore up plant improvement in saline or contaminated soils. (Seifikalhor, Hassani *et al.* 2020) applied *A.platensis* as a maize seed crust, conscious a decrease in Cd uptake due to polysaccharide sugars required of Cd ions, therefore mitigating the lethal influences on plants. The drop in roots Cd content of seed-coated plants was over ninetieth once twelve days from sowing.

4.2 Pigment contents

(Razon 2012) based that photosynthetic eubacteria fix N to ammonia utilizing their own metabolic machinery. The free ammonia into the rhizosphere is obsessed by plants that square measure more utilised by host plants for essential growth processes. in theory, the number of biological process would depend upon the abundance and kinds of class species; but, in apply, variation in biological process may occur as many factors contribute to the method that ranges from ecological to environmental conditions

(Ashraf and Harris 2013) suggested that photosynthesis chemical could be an exceptional process including gas-exchange properties, chemical process pigments, photosystems, portions of the electron transport operation, and performances of various enzymes required in carbon metabolism. Consequently, impairment to any of these parts influences photosynthesis negatively. The speed of photosynthesis decreases under saline states primarily because of diffusion force those end up in stomatal closing and secondarily by the upper quantity of Na^+ and Cl^- which will harm the thylakoid membrane within the plastid (Hasanuzzaman, Nahar *et al.* 2013). In this association, (Abd Elbar, Farag *et al.* 2019) uncovered that the critical diminishing in the Chl a/b proportion of *Thymus vulgaris* submerged deficiency could clarify the defensive job of Chl b for the photosynthetic mechanical assembly under dry spell pressure. Showering with various groupings of putrescine further developed chlorophylls fixation under both contemplated water system levels though no huge contrasts were distinguished in the Chl a/b proportion contrasted with the control. The improvement of chlorophyll fixation and photograph blend measures by exogenous uses of putrescine has been perceived (Zeid, Omer *et al*. 2014).

Exogenous utilization of amino acids is considered to incite atomic number 7 metabolism efficiency and structure of chlorophylls in managed plants (Mógor, de Oliveira Amatussi *et al.* 2018). Phytohormones like cytokinins and gibberelic acids in cyanophyte selections might play a function in diminishing chlorophyll disgrace in the main through restraint of chlorophyllase activity (Whapham, Blunden *et al*. 1993, Martinez, Chaves *et al*. 1996). Between materials with hormone-like action, exogenously supplied polyamines are covalently attached to chlorophyll-bound proteins by plastidial transglutaminases, so increasing chlorophyll resistance throughout leaf decay (Lin and Lin 2019). These findings advisers a job for cyanophyte attention in alleviating the negative results of abiotic forces on yields.

4.3 Photosynthetic activity

From the common importance of saltness stresses in plants is that the stimulated formation of reactive oxygen species (Gill and Tuteja 2010). Exposure of plants to severe salinity causes the close of stomata. As consequence, greenhouse gas advantage similarly as fixing inside the leaf cells become decreased. At an alike time, a diminished decrease of greenhouse gas by carbon cycle and a case of severe excitation energy occurs, of that chloroplasts become displayed, and following, electron transport system becomes harmed. Thus, saltiness produces the extreme organization of ROS as well as superoxide ($O_2^{\bullet-}$), hydroxyl radical radicals ($OH^{\bullet}$), and peroxide (H_2O_2) and ends up in oxidative stress (Ahmad and Umar 2011, Hasanuzzaman, Nahar *et al.* 2013, Hasanuzzaman, Nahar *et al.* 2013).

4.4 Total carbohydrates content

(Nemati, Moradi *et al.* 2011) who described that two cultivars of rice salt-tolerant and salt-sensitive grew below NaCl saltiness stress (100 mmol NaCl), in which the collection of sugars in the root is more distinguished in the shoot total of the tolerant variety compared with the sensitive variety. Improved total soluble carbohydrates have been proposed to adjust osmotic ability and water intake capacity below salt stress. Starch degeneration and sugar collection in salt-affected rice plants were also announced and reported as a strategy to increase the osmotic state and plant durability.

Concerning impact of putrescine on solvent sugar, (Toupchi Khosroshahi, Salehi-Lisar *et al.* 2018) detailed that Putrescine as an osmolyte and receptive oxygen species forager diminished the creation and aggregation of viable osmolytes. In non-focused on safflower plants, the impacts of putrescine treatment on solvent sugars content in shoot and proline content in leaf were distinctively identified with fixations. Insoluble sugars expanded with both grouping of putrescine in shoot and diminished in the root.

4.5 Total proteins content

(Kumar and Khare 2016) noticed the inhibitory impacts of saltiness as grain nature of rice weakened by saltiness through decrease of protein and polysaccharides content of grain. These outcomes are as per those got by (Ayala-Astorga and Alcaraz-Meléndez 2010) expressed that protein content was higher in *Paulownia imperialis* plants filled in the most minimal centralization of sodium chloride (20 mM) at 15 days.

Several abiotic agents (dryness, saltiness, severe temperatures) are displayed in plants as diffusion forces, resulting in the collection of reactive chemical element species (ROS) that hurt polymer, fats, sugars, and proteins and additionally create anomalous cell information (Panda, Pramanik *et al*. 2012). Soil treatment or foliar applying of cyanobacterial-based bio-stimulants are determined to increase the inhibitor action of treated plants, therefore decreasing the results of stress-induced free radicals by nonstop scavenging and blocking ROS production (Abd El-Baky, El-Baz *et al.* 2010, Singh, Prabha *et al*. 2011, Mala, Celsia *et al*. 2017, Ertani, Nardi *et al*. 2019). (Singh, Prabha *et al*. 2011) according to that soil treatment with *Oscillatoria acuta* and *Plectonema boryanum* caused general tolerance upon stress by improving accelerator action of oxidase and primary amino acid ammonia-lyase in rice plant leaves, whereas whole phenolic resin amount arrived at most values when vaccinated with *A. oryzae.*

4.6 Determination of Proline Content

Proline preserves against oxidative destruction by up improving the antioxidant protection and glyoxalase system (Rejeb, Abdelly *et al*. 2014, Hossain, Burritt *et al.* 2016).

The results forces towards cyanobacteria-derived proline as the primary bioactive ingredient as proline buffers upon oxidative damage by diagonally scavenging reactive oxygen species in harmed plants (Rejeb, Abdelly *et al.* 2014, Hossain, Burritt *et al.* 2016). In addition to this, unspecific leakage of proline indoors the transporter releases its unleash by *Nostoc muscorum* cells within the active factor (Picossi, Montesinos *et al.* 2005). Lastly, plants produce three amino-alkanoic acid transporter subfamilies (two general and one proline-specific) that can give proline from their surroundings to plant roots (Lehmann, Funck *et al.* 2010). On the total, this offers an alternate example as to how cyanobacteria-derived proline can prime the root filaments stress response in heat-shocked *Arabidopsis* seedlings.

Rice plants in pots treated with several cyanophyte bacteria strains showed uniform collection in plant leaves of phenolic resin (gallic acids, gentisic acids, caffeic acids, chlorogenic acids, and ferulic acids), flavonoids (rutin and quercetin), phytohormones (indole carboxylic acid and indole fatty acid saturated), proteins, and chlorophyll pigments (Singh, Prabha *et al.* 2011).

4.7 Mineral contents

The nutritional diseases could also be correlated to the influence of salinity on nutrient handiness, competing uptake, disposal, or transport among the plant (Hasanuzzaman, Hasan *et al*. 2012). Commonly, Na^+, K^+, $Ca2^+$, Mg^{2+}, Cl^- and typically and CO_3^{2-} ions responsible for salinization. As Na^+ subjects inside the soils, the general deficiency for crop output is soil sodicity, additionally, sodic soils required as a result of they give very bad soil balance gratefulness to disaggregation of soil components that diminish water flux (Hasanuzzaman, Nahar *et al*. 2013).

Cytokinins improvement in plant roots can produce an enrichment inside the expression of genes coding for root nitrate and sulfur transporters (Ohkama, Takei *et al*. 2002, Collier, Fotelli *et al*. 2003), whereby magnifying plant nutrient uptake. Ultimately this concern, (Mutale-Joan, Redouane *et al*. 2020) covered NPK concentrations in roots to predict the effects on nutrient uptake of eighteen rough extracts acquired from microscopic algae and true bacteria, utilized three terms as soil drench on tomato seedlings.

Micronutrients amount in radish returned the same object, with the more important content of Fe, Cu, B, Mn, Zn, and Ni performed following the utilization of twenty percent *A.platensis* growth medium, while higher consistencies commonly provided a loss in the microelements amount (Godlewska, Michalak *et al*. 2019).

5 Conclusion

The purpose of the current task is to mitigate the harmful impact of salt on an important field crop by exogenous application of putrescine and cyanobacteria, which succeeded in mitigating the harmful effect of salt on growth rate and various physiological metabolites.

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Declaration

Ethical approval: This study was conducted in accordance with the specifications and requirements that serve the Faculty of Science - Damanhur University

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الدور الفسيولوجى للبوترسين والسيانوبكتريا فى تخفيف تأثير الإجهاد الملحى لكلوريد الصوديوم على نبات الأرز

وائل فتحى سعد غرابه

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الملخص

يهدف العمل الحالي إلى تحقيق تغيرات في النمو وبعض الأنشطة الأيضية في نبات الأرز صنف جيزة ١٧٨ المعالج بالملح عن طريق الري بالملح بتركيز (١٠٠ مل مولار)، وتقييم دور النقع المسبق للبذور بواسطة عديد الأمين بوتريسين بتركيز (٤,٠ مل مولار) مع معالجة التربة بواسطة البكتيريا الخضراء المزرقه (٢٣ مليجرام/ أصيص) للتخفيف من هذه التغييرات. في عمر ٧٠ يومًا، حيث كان لكل من البوتريسين والبكتيريا الخضراء المزرقه (أزوتوباكتر) تأثير واضح على النباتات ذات الوزن الطازج المجهدة بالملح مقارنةً بالكنترول، وقد لوحظت نفس النتيجة أيضًا على الوزن الجاف لنباتات الأرز المجهدة وغير المجهدة بالملح. تم تحفيز كلوروفيل "أ" و "ب" بشكل خاص مع تطبيق التركيز العالٍ من البوتريسين. ومع ذلك ، كانت الكاروتينات منخفضة قليلاً. تأثير البكتيريا الخضراء المزرقة وحدها أو متداخلة مع البوتريسين بتؤدى إلى زيادة كبيرة في نشاط التمثيل الضوئي مقارنةً بالكنترول في نباتات الأرز المجهدة وغير المجهدة ملحيا عند عمر ٧٠ يومًا.

أظهر إجمالي محتوى الكربوهيدرات والبروتين فى المجموع الجذرى زيادة معنوية. لوحظ انخفاض في محتوى البرولين في كل من المجموع الخضرى والجذرى تحت تأثير البكتيريا الخضراء المزرقة والبوتريسين المتداخلان معًا في النباتات المجهدة بالملح مقارنةً بالكنترول.

أظهر تأثير البكتيريا الخضراء المزرقة والبوتريسين معًا تأثيرًا معنويًا على محتويات النيتروجين والفوسفور والبوتاسيوم والمغنيسيوم والحديد والمنجنيز مقارنةً بالكنترول. التطبيق الخارجي للبوتريسين والبكتيريا الخضراء المزرقة نجحت في تخفيف التأثير الضار للملح على معدل النمو والنشاطات الفسيولوجية المختلفة.