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Combined Effect of Biofertilizers and Putrescine Amine on Certain Physiological Aspects and Productivity of Date Palm (*Phoenix dactylifera* L.) Grown in Reclaimed-Saline Soil

M. N. Helaly[*] and Hanan A. R. El-Hosieny[**]

[*]*Agriculture Botany Department, Faculty of Agriculture, Mansoura University and* [**]*Horticulture Research Institute, Agricultural Research Centre, Cairo, Egypt.*

AMINES play an important role in the plant response to adverse environmental conditions including salt and osmotic stress. In this investigation, the complemented effect of putrescine amine (put), biofertilizers and mycorhiza on date palm zaghloul genotype irrigated with salinized water and grown in reclaimed salinized soil was studied. The data indicated that the selected plant growth promoting rhizobacteria in the presence of putrescine amine make and/or enable date palm zaghloul genotype to increase its tolerance and adaptation to grow in the reclaimed-salinized soil. The treatments reduced salt-induced oxidative damage, increased productivity of date palm and improved its fruit quality. These results may be due to the increase in the photosynthetic pigments, organic solutes, promoting growth substances (GA, IAA and cytokinins), and activities of oxidant enzymes. Moreover, a decrease in the levels of lipid peroxidation and inhibitor substances (ABA) may be related. The most effective interaction treatments were pronounced at 2.5 mM putresine due to more increase in the activities of ascorbate peroxidase (APOX) glutathione reductase (GR) and superoxide dismutase (SOD) and more decrease in lipid peroxidation. The interaction treatments with put at 5 mM increased disamine oxidase (DAO) and polyamine oxidase (PAO) activities compared to the other interaction treatments. The activates of these two enzymes produce hydrogen peroxide H_2O_2 which may act in structural defense as a signal molecule and decreasing the production of polyamines against salt-induced oxidative damage in date palm. Further investigations are needed to explain the mechanisms develop in date palm grown in stress condition.

Keywords: Biofertilizer, Mycorhiza, Rhizobacteria, Putrescine (put), Date palm (*Phoenix dactylifera* L.), reclaimed saline soil.

Date palm (*Phoenix dactylifera* L.) the dioecious, monocotyledon species belonging to the family Arecaceae is a multipurpose tree having food, medicinal and ornamental importance. With the present uncertainty in the world food supply and the expected increase in demand, the date palm could be a good source of food of high nutritional value (Anonymous.., 2010). From the economical standpoint, date palm soft cultivars grown in Egypt differ in their sensitivity to salinity (El-Khawaga, 2013). Furthermore, growers have

mistakenly believed that date palm does not require much attention, while the successful orchard management practices are the way to high yield of good fruit quality. One of the best tools of horticultural practices is fertilization. The use of fertilizers to increase yield is an important factor in all agricultural systems (Dong *et al*., 2005). Salt stress like other environmental stresses, induces adverse effects on growth, development, survival and productivity of date palm plants like other plant species by producing oxidative damage generation of reactive oxygen species (ROS), (Minura *et al*., 2003). It imposes both an ionic and an osmotic stress (Borsani *et al*., 2001) and becoming ever more prevalent as the intensity of agriculture increments (Zhu, 2002). It is developmentally regulated and stage specific phenomenon in many plant species (Tang and Newton, 2005). Plant cell utilizes three different strategies for coping with ionic and osmotic stress. 1) Osmotic adjustment of the cytoplasm due to the accumulations of compatible solutes such as betaine and proline (Tang and Newton, 2005). 2) Salt extrusion from the cell across the plasma membrane using ion transporters such as the Na^+/ H^+ antiporter (Minura *et al*., 2003) and 3) Salt accumulation in vacuoles using tonoplast transporters (Zhu 2005). Helaly and Hanan El-Hosieny (2011) showed that stress conditions increased lipid peroxidation or induce oxidative (stress) in plants tissues as a high by degree of membrane deterioration. They added that lipid peroxidation can be initiated by ROS which severely affects functionality and integrity of cell membranes. It requires active O^-_2 uptake and involves the production of superoxide radical (O^-_2). The other highly reactive chemical species involving singlet oxygen (O^-_2) hydroxyl free radical (OH^-) and H_2O_2 all of which initiate lipid peroxidation (Dhindsa *et al*., 1981). Hence, constitutive and/or induced activity of SOD and other antioxidants such as POX, APOX, CAT and GR is essential. According to Seckin *et al* (2010) MDA has been frequently described as a suitable biomarker for lipid peroxidation under stress condition. biofertilizers and polyamines (putrescine and others) have been reported to be involved in the plant response to salt and osmotic stress by playing an important role in the ROS mediated damage caused by salt-stress (Zhu 2002 and Rasmia & Darwesh 2013). Moreover, they behave as antioxidants in the protective mechanisms (Tang & Newton, 2005 and Salama *et al*., 2014).

On the other hand, it was found that biological fertilizers play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers (Mohammadi and Sohrabi, 2012). They added that with using the biological and organic fertilizers, a low input system can be carried out and it can help achieving sustainability of farms. Effective Microorganism humic acid and compost enriched with actinomyces agents were very effective in alleviating the adverse effect of salinity on date palm (El-Khawaga, 2013). Similary, the application of microorganisms at the rate of 90ml/ palm/ one year combined with potassium sulphate at 1.5kg/palm/year as a soil application enhanced leaf chlorophyll content, fruit set percentage, retained fruit percentage, yield, fruit quality and leaf minerals content of "Hayany" date palm cv. (Salama *et al*., 2014).

In the most plant species, salinity modify functional polyamine production and its distribution within the plant organs (Ghoulan *et al*., 2001) represented by a decrease in putrescine (Put) and increases in spermidine (Spd) and/or spermine (Spm). Depletion of endogenous polyamine levels due to salinity was previously reported (Groppa *et al*., 2003). In addition, putrescine (Put) is a low molecular weight polyamine compound which has been successfully applied to induce salt tolerance in many plant species (Borsani *et al*., 2001) by enhancing ROS generation during photosynthesis and plant developmental processes. Tang and Newton (2005) reported that putrescine was accumulated to high levels in the roots of the salt-resistant Virginia pine plants compared with the salt-sensitive plants, while an opposite trend was recorded in the shoots. They added that put increased the activities of key enzymes involved in oxidative stress such as ascorbate peroxidase (APOX), glutathione reductase (GR) and superoxide dismutase (SOD) and decreased lipid peroxidation. However, no clear relationship was observed between the mean level of salt resistance and the endogenous levels of putrescine . Moreover, the mechanism of tolerance at specific stage of plant development is a common approache and needed to introduce genetic and/or environmental and physiological improvement to salt stress tolerance.

In order to elucidate the role of biofertilizers and putrescine in reducing salt stress induced oxidation, the present study was undertaken to investigate the combined effect of plant growth promoting rhizobacteria (*mixture of Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus circulans*), mycorhiza (AM) and dressing application with putrascine (put) at 0, 2.5, 5, mM individually or incombination on certain physiological aspects and productivity quality of date palm (*Phoenix dactylifera* L.) zaghloul cv. irrigated with salinized water and grown in reclaimed-salinized soil.

Material and Methods

Two field experiments were carried out in reclaimed-salinized soil at the Agriculture Experimental Station located at Kalabshow and Zayan, Faculty of Agric, Mansoura Univ. Egypt during the two growing seasons of 2013 and 2014. The mechanical and chemical analyses of the experimental soil as well as the chemical analysis of the irrigation water used were estimated (Jackson, 1973 and Black *et al*., 1982) and presented in Table 1.

Micro organisms inoculants

Azospirillum lipoferum was grown on semi-solid N-free malate medium (Doberiner, 1978) whereas *Paenibacillus polymyxa and Bacillus circulans* were individually grown on nutrient broth media (Dowson, 1957). They were separately suspended into sterile water and incubated after 5 days for 2-3 days at 30^{o}C to maintain population of $3x10^{8}$ colony forming unit m^{-1}(CFU/ml). The mixed inoculums of the microbial cells were prepared by mixing equal volumes of the desired cell suspensions. All microbial strains were kindly provided from

the Biofertilizers unit Fac. of Agric. Ain shams Univ. Egypt. Arbuscular mycorhiza fungus (*Glomus mosseae*) was grown on pot cultures containing onion plants for 4 months and the mycorhiza inoculums used were consisted of roots, hyphae, spores and growth media of the pot cultures. The standard inoculums (400 kg/4200m^2) contained about 270 spors/g. They were obtained from Agric. Microbial Dept. Soil, Water and Environment Res. Ins (SWER), Agric. Res. Center (ARC) Egypt. Fungus spores were measured by a wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). The plant growth promoting rhizobacteria (PGPR) and the mycorhiza (My) treatments were used at the rate of 85 ml for each palm.

TABLE 1. Mechanical (a) and chemical (b) analyses as well as the other properties of the experimental soil (soil:water extract 1:20) as well as the chemical analysis of the irrigation water (c) used during the two growing seasons, 2013-2014.

Season	a) Mechanical analysis%				b) Chemical analysis of the soil								
	Coarse+ fine Sand	Silt	Clay	Soil Texture	Cations meq/100g				Anions meq/ 100g				
2013	69.3	20.26	10.44	Sandy Loamy soil		Ca	Mg	Na	K	SO4	Cl		
					6.07	3.64	12.92	0.35	0.18	9.25	13.55		
2014	68.5	21.03	10.51		6.05	3.57	12.53	0.27	0.17	8.63	13.56		

b) Chemical analysis of the soil (cont.)													
Season	EC dsm^{-1}	pH	SP%	CaCO$_3$%	Organic matter%	Micro elemrnts (ppm)					Available (ppm)		
						Fe	Zn	Mn	Cu	B	N	P	K
2013	4.42	8.03	43.21	2.67	0.59	17.14	3.23	10.26	2.24	9.15	24..25	3.78	24.23
2014	4.4	8.31	41.34	2.6	0.47	16.21	3.35	9.38	2.29	9.11	30.21	3.7	19.34

c) Chemical analysis of the irrigation water used meq/l													
Season	EC dsm^{-1}	Anions				Cations							
		CO_3^{-}	HCO_3^{-}	SO_4^{-}	Cl^{-}	Na^{+}	K^{+}	Ca^{++}	Mg^{++}				
2013	5.64	….	1.28	26.49	25.02	50.63	0.18	0.75	1.24				
2014	5.52	….	1.17	25.99	26	51.03	0.17	0.71	1.25				

Putrescine treatments

Concentrations of putrescine (Sigma Aldrich) denoted 2.5 and 5 mM in addition to distilled water as a control were applied to the soil in circle holes around each palm tree with 50 cm depth and 70 cm distance from the plant truck.

Experiment design

108 female palm trees,10 years old, similar vigor, height, pollen source and planted at 7x7 m were selected. The trees were arranged in a randomized

complete block design with 3 replicates (three trees for each) and irrigated using furrow irrigation system.

The experiment included control, plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), PGPR+Mycorhiza (My) with or Without Putresine (Put). The application of all treatments took place at three times with 20 days interval. The 1st time was done at blooming and the beginning of set stage at April 15. Other agricultural practices were applied as recommended by Ministry of Agric., Egypt.

Sampling and data recorded

Leaflets of the 4th full expanded leaf from the plant tip were taken at Kimri stage (30 June) for chemical analysis. Photosynthetic pigments were extracted and determined (Wettestein, 1957). Nitrogen, phosphorus and potassium concentrations were detected according to the methods described by Bremner & Muluaney (1982), Olsen & Sommers (1982) and Chapman & Pratt (1982) respectively. Moreover, certain organic osmolytes components with have possible involvement of the antioxidant system in relation to salt tolerance were analyzed, Total free amino acids (TAA) and total sugars (TS) were extracted from the plant material by 80% ethanol. TAA was determined spectrophotometrically by the methods of Dubey and Rani (1989a, b) and total sugars were determined by phenol-sulphuric acid method as described by Sadasivam and Manickam (1996). Glutathione Reductase (EC 1.8.1.7) (GR) activity was measured according to Foyer and Halliwell (1976). One enzyme unit is defined as μmoL mL^{-1} oxidized Glutathione per min. Ascorbate Peroxidase (EC.1.11.1.11), (APOX) activity was assayed according to Nakano and Asada (1981). One enzyme unit is defined as μmoL mL^{-1} oxidized ascorbate per min. Superoxide Dismutase (EC 1.15.1.1) (SOD) activity was assayed based on the method of Beauchamp and Fridovich (1971) and the specific enzyme activity was expressed as units mg^{-1} protein g FW. Lipid peroxidation was measured as the quantum of thiobarbituric acid reactive susbstances (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by Borsani *et al*., (2001). The concentration of TBARS was calculated (Groppa *et al*., 2001 and 2003). Diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, 1.4.3.4) activtities were prepared as described by (Aribaud *et al*., 1994 and Faive-Rampant *et al*., 2000) and determined using H_2O_2- D method (Nag *et al*., 2000). Endogenous phytohormone quantity determined using high-performance liquid chromatography (HPLE) according to Koshioka *et al*., (1983) for auxins (IAA), gibberelic acid (GA_3) and Abcisic acid (ABA) while cytokinins were detected according to Nicander *et al*., (1993).

The harvesting took place periodically for six times, 15 days intervals from August 15th till the November 1*st*. At the end of harvesting time, cumulative fruit yield was measured during the two growing seasons. At the third harvesting time (Sept. 15*th*) fruit component of total sugars and carbohydrates (Amberger, 1954), total soluble solid, (TSS) using a hand refractometer, Vitamin C (AOAC, 1995) as well as total nutrients of N, P and K (as previously mentioned) were estimated. Total crude protein % was calculated by multiplying total nitrogen x 6.25 (AOAC, 1995).

Statistical analysis

All data were subjected to analysis of variance (Snedecor and Cochran, 1980) using SAS system (2003).

Results and Discussion

Physoligical parameters

Photosynthetic pigments

Table 2 shows that Chl a,b and carotenoids concentrations were increased due to PGPR, My and put application compared to the control during the two growing seasons. The combination treatments between PGPR and My gave the highest values in the presence of put at 2.5 mM compared with the individual application and the control. Increase of Chl(s) and carotenoids concentration may be enhanced photosynthesis efficiency and that is a good explain to the increasing of date palm productivity. In addition, this enhancement could be indication for expectable high fruit yield quality when date palm irrigated with saline water and grown in the reclaimed salinized soil. In this respect, Heidari and Golpayegani (2012) found that inoculation with rhizobacteria could be efficiently used to improve growth, antioxidant status and photosynthesis pigments under water stress. Moreover, Makela *et al*, (2000) used My to protectively photosynthetic machinery of plant organelles by stabilizing the ultrastructure of the chloroplast. PSII reaction centers and maintaining the oxygen – evolving machineries. Similarly, the increase in photosynthetic pigments in the plants treated with exogenous My may be due to its effects on lowering membrane damage, better photosynthesis rate, improved leaf water potential and greater shoot dry weight (Wahid and Shabbir, 2005). Stabilizes pigments as well as prevents water oxidation and photo oxidation were also detected due to My and putresine application (Cha-um *et al*., 2006). They added that stabilization of Chl a,b and carotenoids in light energy capture as required for photosynthesis. Putresine may assume a different role in non-photosynthetic organs vs. photosynthetic ones since it acculated to high levels in roots of the salt-resistant plants compared with the salt sensitive one while an apposite trend was recorded in the shoots (Cowley and Walters, 2002).

NPK minerals and Organic osmolytes

Table 3 show the effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorhiza (My) with or without Putresine amine on concentrations of N,P,K, as well as total sugars, total free amino acids. The data in the table indicated that PGPR, My in the presence or absence of put significantly increased concentrations of N, P and K, total sugars, and total free amino acids in the leaflets of date palm in both seasons. Moreover put at 2.5 mM alone or incombination with other treatments gave the best significantly highest values of total sugars, total free amino acids. Similar results were reported by Bhatti *et al*. (2013) who found that the content of total sugars and some of the bioconstituets may be considered as a direct result for high rate of photosynthesis with great efficiency that was preceded with large photosynthetic area and high content of photosynthetic pigments. Liu and Huang (2000) suggested that

high carbohydrate available (glucose and sucrose) during stress condition are represents of important physiological traits associated with stress tolerance. In addition accumulation of amino acids and sugars are necessary to regulate osmotic activities and protect cellular structures from stress condition by maintaining the cell water balance and membrane stability. Ahmed and Kibret (2014) suggested that PGPR promote plant growth directly by facilitating resource acquisition nitrogen, phosphorus and other essential elements. Moreover, changes induced by salt stress in sugars and amino acids related to synthesis of polyamines (glutamate, arginine and proline) and polyamines putrescine were reported in leaves of a salt-sensitive tomato species (*Lycopersecon esculeatum, Mill*) and its wild salt tolerant reactive species (L. *pennelli Correll*) Darcy in light and dark after short-term exposure (Zapata *et al*., 2004). Therefore polyamines have been suggested to contrast oxidative damage in plants as indicated in our results.

TABLE 2. Effect of plant growth promoting rhizobacteria (PGPR) Mycorhiza (My), Putresine amine alone or in combination on chlorophyll a, b and carotenoids concentrations (mg/gFwt) in the leaflets of the 4th full expanded leaf from the plant tip of date palm (zaghloul cv.) irrigated with saline water and grown in the reclaimed saline soil during the two growing seasons (2013 and 2014) .

Treatments	photosynthetic Pigments mg/100g F.Wt.							
	Growing seasons							
	First season 2013				Second season 2014			
	Chl a	Chl b	Ch a+b	Car.	Chl a	Chl b	Ch a+b	Car.
Control	27.6± 0.63f	9.64± 0.42f	38.54± 0.91f	13.26± 0.44e	27.9± 0.73e	9.95± 0.41f	38.85± 1.65g	13.53± 0.47d
2.5 mM put	35.4± 0.71c	11.62 ± 0.73c	48.02± 1.90c	14.8± 0.53c	35.8± 0.83c	11.62± 0.49c	46.02± 1.78c	14.8± 10.49c
5 mM put	32.7± 0.84e	10.75± 0.42e	43.75± 1.82e	14.2± 0.57c	33.4± 0.61d	10.86± 0.47e	43.75± 1.68f	14.2± 0.51c
PGPR	33.2± 0.72e	11.62± 0.48c	44.46± 1.86e	14.7± 0.56c	33.6± 0.77d	11.92± 0.53c	44.46± 1.73e	14.7± 0.53c
PGPR+ 2.5mMput	38.3± 0.88b	12.81± 0.44b	51.11± 1.84b	16.3± 0.57b	39.4± 0.88b	13.21± 0.56b	51.11± 1.72b	16.3± 0.52b
PGPR+ 5 mM put	34.9± 0.61d	11.92± 0.58c	46.82± 1.87d	15.2± 0.63b	35.8± 0.87c	12.68± 0.57b	46.82± 1.68d	15.2± 0.55b
My	34.1± 0.53d	11.21± 0.43d	45.31± 1.82e	15.3± 0.64b	34.7± 0.79c	11.82± 0.51c	45.31± 1.71e	15.3± 0.56b
My + 2.5 mM put	35.1± 0.75c	12.12± 0.49b	47.32± 1.91d	15.8± 0.61b	35.8± 0.78c	12.14± 0.55b	47.22± 1.70c	15.8± 0.52b
My + 5 mM put	34.3± 0.61d	11.65± 0.53c	45.95± 1.93d	15.1± 0.59b	35.2± 0.87c	12.15± 0.57b	45.95± 1.73d	15.1± 0.57b
PGPR + My	34.8± 0.66c	11.35± 0.54c	46.16± 1.83d	15.3± 0.62b	34.8± 0.85c	11.74± 0.53c	46.16± 1.75d	15.3± 0.54b
PGPR+My+ 2.5mMput	47.6± 0.82a	15.41± 0.62a	63.01± 1.92a	19.7± 0.64a	48.9± 0.84a	15.82± 0.59a	63.01± 1.77a	19.7± 0.61a
PGPR+My+ 5 mM put	35.3± 0.77c	12.24± 0.64	47.54± 1.94d	15.8± 0.65b	35.7± 0.87c	12.76± 0.54b	47.54± 1.69c	15.8± 0.52b

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

TABLE 3. Effects of plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), Putresine amine alone or in combination on concentrations of N,P,K, as well as total sugars and total free amino acids in the leaflets of the 4th leaf from the plant tip of date palm (*Phoenix dactylifera* L.) irrigated with saline water and grown in reclaimed saline soil during the two growing seasons (2013 and 2014).

Treatments	Growing seasons									
	First season 2013					Second season 2014				
	Nutrients mg/g D.wt.			Total sugars mg/g F.wt.	Total free amino acids mg/g F.wt.	Nutrients mg/g D.wt.			Total sugars mg/g F.wt.	Total free amino acids mg/g F.wt.
	N	P	K			N	P	K		
Control	27.2± 1.05d	2.3± 0.51e	31.1± 1.23f	16.2± 0.43f	8.7± 0.68e	27.7± 0.63f	2.4± 1.37d	33.2± 1.21d	16.4± 1.53e	9.3± 1.03d
2.5 mM put	31.4± 1.12b	3.9± 0.42c	40.6± 1.31c	21.4± 0.43c	12.7± 0.87c	31.8± 1.02c	4.0± 0.48b	41.4± 1.01b	21.0± 1.13b	12.7± 0.67c
5 mM put	28.9± 1.21c	3.1± 0.53d	39.1± 1.32c	19.4± 0.51d	11.2± 0.76c	29.8± 1.20d	3.4± 0.57c	39.2± 1.02c	19.9± 1.21c	11.6± 0.68c
PGPR	28.7± 1.32c	3.2± 0.57d	38.4± 1.05d	18.7± 0.78e	10.7± 0.83d	28.9± 1.02e	3.6± 0.59c	38.6± 1.15c	18.6± 0.85d	10.9± 0.86d
PGPR+ 2.5mMput	34.8± 1.15a	4.3± 0.62b	42.1± 1.07b	22.7± 0.45b	13.4± 0.76b	34.2± 1.17b	4.5± 0.72b	42.3± 1.16b	22.1± 1.05b	13.6± 0.78b
PGPR+ 5 mM put	32.4± 1.18b	3.8± 0.54c	38.5± 1.13d	20.3± 0.65c	11.8± 0.92c	32.8± 1.08c	3.9± 0.56b	38.6± 1.03c	20.4± 1.07c	11.8± 0.81c
My	31.5± 1.17b	3.8± 0.74c	37.9± 1.07e	19.8± 0.86d	11.2± 0.83c	31.2± 1.09c	3.9± 0.83b	37.9± 1.17c	19.8± 0.76c	11.5± 0.86c
My + 2.5mM put	32.9± 1.42b	3.8± .75c	40.3± 1.36c	21.2± 0.54c	12.3± 0.94c	31.5± 1.12c	3.7± 0.65c	40.3± 1.36b	21.3± 1.14b	12.6± 0.85c
My + 5 mM put	30.7± 1.32b	3.5± 0.68c	39.2± 1.16c	19.6± 0.68d	12.1± 0.85c	30.7± 1.02d	3.6± 0.78c	39.3± 1.06c	19.7± 0.69c	12.4± 0.65c
PGPR + My	32.5± 1.27b	3.2± 0.74c	38.6± 1.06d	20.2± 0.88c	11.2± 0.93c	30.8± 1.21d	3.4± 0.70c	38.7± 1.08c	20.5± 0.87c	11.3± 0.92c
PGPR+My+ 2.5mMput	35.3± 1.12a	5.0± 0.34a	43.8± 1.08a	23.5± 0.92a	14.1± 0.86a	35.9± 1.01a	5.3± 0.37a	43.9± 1.18a	23.8± 0.52a	14.6± 0.75a
PGPR+My+ 5mMput	31.9± 1.14b	3.4± 0.48c	40.3± 1.12c	21.8± 0.87c	12.2± 0.74c	31.7± 1.34b	3.6± 0.58c	40.2± 1.14b	21.7± 0.87b	12.3± 078c

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

Ascorbate peroxidase (APOX) glutathione reductase (GR) and superoxide dismutase (SOD) activities

The activities of APOX, GR, and SOD were decreased due to the application of PGPR, My and/or Put (Table 4). Put showed an additive effects to that of PGPR and My in combined treatments. The lowest activity levels were recorded in Bio+My in the presence of put at 2.5mM (Table 4). No significant differences were observed in the activates of APOX, GR, and SOD when 5 mM put was used compared to the control. Similar results were reported by Tang and Newton (2005), who reported that amines reduce salt-induced oxidative damage by increasing the activates of antioxidant enzymes and decreasing lipid peroxidation in Uirginia pine. They added that APOX, GR, and SOD were associated with the oxidative damage of the enzymatic defense system caused by salt stress.

TABLE 4. Effects of plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), Putresine amine alone or in combination on the activities of antioxidant enzyme, ascorbate peroxidase (APOX), glutathione reductase (GR) and superoxide dismutase (SOD) in the leaflets of the 4th leaf from the plant tip of date palm (*Phoenix dactylifera* L.) irrigated with saline water and grown in reclaimed saline soil during the two growing seasons (2013 and 2014).

Treatments	Growing seasons					
	First season 2013			Second season 2014		
	APOX	GR	SOD	APOX	GR	SOD
	µ/g Fwt.	µ/100g Fwt.	µ/100g Fwt.	µ/g Fwt.	µ/100g Fwt.	µ/100g Fwt.
Control	18.76±0.571d	13.96±0.41d	13.61±0.57e	18.74±0.71f	14.21±0.81f	12.41±0.73d
2.5 mM put	11.63±0.84b	10.86±0.63b	9.96±0.73b	11.42±0.67b	12.26±0.80c	9.62±0.74b
5 mM put	18.77±0.62d	13.12± 0.583	11.32± 0.72c	18.17±0.81e	13.12± 0.78e	11.62± 0.72c
PGPR	16.30±0.54d	13.37± 0.51c	12.38± 0.81d	16.01±0.72d	13.48± 0.75e	12.32± 0.91d
PGPR+2.5 mMput	10.76±0.61a	10.1± 0.41b	9.21± 0.68a	10.25±0.78a	11.26± 0.82b	9.31± 0.90b
PGPR+ 5 mM put	16.34±0.77d	13.39± 0.35c	12.46± 0.81d	16.95±0.28c	13.53± 0.81e	11.52± 0.85c
My	16.53±0.64c	13.57± 0.59c	12.48± 0.75d	15.83±0.81c	13.57± 0.89e	12.26± 0.82d
My + 2.5 mM put	15.43±0.58c	13.48± 0.46c	12.06± 0.87c	15.33±0.64c	13.18± 0.74e	11.86± 0.86c
My + 5 mM put	16.87±0.54c	13.26± 0.64c	12.64± 0.81d	15.20±0.53c	13.16± 0.74c	12.14± 0.91c
PGPR + My	17.54±1.65d	13.58± 0.43c	13.26± 0.74d	17.04±0.74e	12.88± 0.83c	9.46± 0.93b
PGPR+My+2.5mM	10.10±0.68a	10.02± 0.58a	9.03± 0.86a	10.02±0.78a	9.42± 0.84a	8.01± 0.94a
PGPR+My+ 5mM	16.26±0.78c	14.26± 0.58d	13.04±0.82d	16.14±0.88d	12.56± 0.93c	10.74±0.82b

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

Lipid peroxidation as well as Activates of diamine oxidase (DAO) and polyamine oxidase (PAO)

Table 5 shows that lipid peroxidation was significantly decreased due to the application of PGPR, My and/or putresine. Highest decreasing levels were detected in Bio + My +2.5 put treatment. However this parameter was not significantly decreased when 5 mM put was used compared to the control. Therefore putresine amine protection on cell membranes was evidenced by the reduction on lipid peroxidation

The same table shows that different exogenously added PGPR, My and/or Put increased the activates of DAO and PAO in the leaflets of date palm irrigated with salinizied water and grown in the reclaimed-salinized soil. The highest activates levels were found with put (5mM) interacted with PGPR+My. Higher PAO activity from date palm leaflets treated with putresine amine may be resulted in liberating more hydrogen peroxidase, which in turn exert powerful physiological effects on productivity. Similar results were reported by Tang and Netwton (2005) with Viriginia pine plants.

TABLE 5. Effects of plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), Putresine amine alone or in combination on Lipid peroxidation. (Mmol/g.Fwt) as well as the activities of diamine oxidase (DAO) and amine oxidase (PAO) and activates (mMH_2O_2/30min/gFwt.) in the leaflets of the 4th full expanded leaf from the plant tip of date palm (zaghloul cv.) irrigated with saline water and grown in the reclaimed saline soil during the two growing seasons (2013 and 2014).

Ttreatments	Growing seasons					
	First season 2013			Second season 2014		
	Lipid peroxidation Mmol/gF.Wt	DAO µ/g Fwt.	PAO µ/g Fwt.	Lipid peroxidation Mmol/gFWt	DAO µ/g Fwt.	PAO µ/g Fwt.
Control	160.4±1.06f	1.25±0.49d	1.40 ±0.85d	162.1±1.08f	1.41±0.35e	1.56±0.32f
2.5 mM put	153.3±1.25c	2.64±0.43b	2.82±0.68b	153.2±1.12c	2.79±0.28c	3.24±0.23c
5 mM put	156.3±1.14e	2.21±0.57c	2.43±0.48c	154.3±1.27d	2.23±0.62d	2.46±0.57d
PGPR	157.6±1.03e	2.46±0.43b	2.69±0.09b	156.6±1.24e	2.46±0.43d	2.76±0.09d
PGPR+ 2.5 mM put	150.3±1.32b	3.48±0.48a	4.13±0.45a	150.1±1.41b	3.48±0.69b	4.23±0.48b
PGPR+ 5 mM put	156.7±1.86e	3.04±0.50a	3.43±0.75c	155.4±1.75d	3.05±0.42c	3.48±0.66c
My	155.6±1.95d	2.74±0.55b	2.86±0.64b	155.1±1.62d	2.76±0.72c	2.92±0.53c
My + 2.5 mM put	152.7±1.87c	2.64±0.48b	2.42±0.61c	152.2±1.73c	2.67±0.74c	2.71±0.53d
My + 5 mM put	153.8±1.65c	2.14±0.54c	2.02±0.26c	153.2±1.57c	2.16±0.54d	2.12±0.46e
PGPR + My	154.3±1.85c	2.01±0.53c	2.43±0.74c	154.0±1.74d	2.01±0.63d	2.43±0.74e
PGPR+My+2.5mMput	148.7±1.96a	3.89±0.52a	4.28±0.46a	148.2±1.36a	4.01±0.81a	4.38±0.06a
PGPR+My+ 5mM put	153.9±1.93c	2.86±0.43a	2.94±0.85b	153.1±1.41c	2.88±0.42c	2.97±0.64c

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

Endogenous phytohormones

Regarding the effects of Bio, My and/or put on endogenous phytohormones, the data tabulated in Table 6 showed that all pronounces (GA, Auxin, cytokinin) were increased whereas Abscisic acid was decreased. The most effective treatment was found with Bio + My + put at 2.5 mM. These data could be of great influence upon different vegetative and reproduction growth. Moreover, increasing cytokinin level on the account of auxin could be in favor of increasing productivity of date palm and improving its fruit quality. Larkindale *et al*, (2005) found that several phytohormones including ABA and ethylene were increased under stress condition while others decreased such as GA, auxins and cytokinin.

TABLE 6. Effects of plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), Putresine amine alone or in combination on endogenous phytohormones, (GA, Auxin, cytokinin) and Abscisic acid (mMH_2O_2/30min/gFwt.) in leaflets of the 4th full expanded leaf from the plant tip of date palm (zaghloul cv.) irrigated with saline water and grown in the reclaimed saline soil during the two growing seasons (2013 and 2014).

Treatments	Growing seasons							
	First season 2013				Second season 2014			
	Gibberellin	Auxins	cytokinins	Abscicicacid	Gibberellin	Auxins	cytokinins	Abscicicacid
Control	30.5± 1.03g	18.7± 1.13e	5.3± 0.64f	0.85± 0.04h	30.7± 1.23e	18.9± 1.11e	5.8± 0.56e	0.83± 0.04gf
2.5 mM put	34.1± 1.01c	25.6± 1.12c	10.7± 0.78c	0.64± 0.08c	34.7± 1.21c	27.1± 1.23b	11.8± 0.88b	0.63± 0.07c
5 mM put	31.3± 1.04f	22.7± 0.83d	8.5± 0.64e	0.72± 0.12f	31.9± 1.13d	23.6± 1.43d	8.9± 0.67c	0.68± 0.08e
PGPR	32.5± 1.03e	23.9± 1.13d	7.9± 0.68e	0.76± 0.16g	33.1± 1.02c	24.2± 1.23d	8.9± 0.63c	0.72± 0.14f
PGPR+2.5mM put	35.2± 1.21b	27.3± 1.11b	12.9± 0.64b	0.61± 0.13b	36.1± 1.24b	29.1± 1.34b	13.9± 0.53a	0.61± 0.06b
PGPR+ 5 mM put	33.0± 1.06c	24.5± 1.03c	8.6± 0.68e	0.69± 0.17c	33.2± 1.14c	25.3± 1.13c	11.6± 0.68b	0.67± 0.13e
My	33.5± 1.13c	25.4± 1.02c	8.3± 0.58e	0.65± 0.04c	31.1± 1.23d	25.9± 1.02c	8.8± 0.58d	0.64± 0.12 c
My + 2.5 mM put	34.2± 1.15b	24.5± 1.13c	9.8± 0.68d	0.66± 0.21d	34.6± 1.10c	26.2± 1.13c	10.8± 0.68c	0.63± 0.21c
My + 5 mM put	32.5± 1.02e	23.7± 0.74d	8.4± 0.67d	0.68± 0.24e	33.2± 1.02c	25.7± 0.74b	9.0± 0.67c	0.67± 0.20e
PGPR + My	33.4± 1.01c	25.5± 1.11c	10.9± 0.68c	0.66± 0.18c	33.2± 1.021c	26.8± 1.31b	11.1± 0.68b	0.65± 0.15d
PGPR+My+2.5 mM put	36.5± 1.23a	29.8± 1.30a	13.8± 0.68a	0.58± 0.06a	36.9± 1.13a	31.8± 1.30a	14.2± 0.68a	0.57± 0.03a
PGPR+My+5m Mput	33.9± 1.32b	27.5± 1.12b	10.5± 1.03c	059± 0.03a	33.7± 1.42c	27.5± 1.12b	11.1± 1.03b	059± 0.05b

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

Yield and its components as well as fruit quality

Table 7 shows that all tested treatments gave significant increase on total palm yield, fruit weight and flesh weight in both seasons compared with the control. Soil application of either PGPR or Mycorhiza, both at the rate of 85 ml/tree increased total yield/tree in the presence or absence of putrescine. Conjugation of both PGPR and Mycorhiza gave an additive effects in this respect. These data are being more evident when related to the control.

As for the effect of putrscine amine, the data demontested that it is more effective in increasing total yield/tree. Putrscine at the rate of 2.5 mM was the best compared to the concentration of 0 (control) and 5 mM. The complemented treatments gave the highest values in this respect.

Improving date palm yield and its components with putrescine application may be due to its effects on increasing photosynthates production and decreasing photosynthates consumption and injury to the membrane, thereby increasing crop productivity under reclaimed salinizied soils (salt stress). In this context, Tang and Newton (2005) attributed the positive effect of putrscine on increasing total yield under salinity to its effects on reducing salt induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation, similarly to that found in the present investigation.

TABLE 7. Effects of plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), Putresine amine alone or in combination on yield and its components of date palm (*Phoenix dactylifera* L,) irrigated with saline water and grown in reclaimed saline soil during the two growing seasons (2013 and 2014).

Treatments	Growing seasons					
	First season 2013			Second season 2014		
	Yield/tree (kg)	Fresh Wt./ fruit (g)	Flesh Wt (g)	Yield/tree (kg)	Fresh Wt./ fruit (g)	Flesh Wt (g)
Control	126.1± 2.01h	13.36± 1.11d	10.31± 1.17e	123.7± 1.11j	13.64± 1.24c	10.45± 1.42d
2.5 mM put	150.4± 2.71c	15.72± 1.24b	14.20± 1.05b	154.4± 2.42c	15.95± 1.35b	14.70±1.28 b
5 mM put	130.1± 2.05g	14.35± 1.54c	13.42± 1.43c	132.6± 1.85h	14.95± 1.14c	13.85± 1.30c
PGPR	135.6± 1.85	13.90± 1.25c	13.02± 1.25c	136.6± 1.25g	13.67± 1.25c	12.44± 1.15c
PGPR+2.5mMput	160.4± 1.73b	16.45± 1.43b	15.14± 1.01b	164.4± 1.70b	16.75± 1.43b	15.36± 1.14b
PGPR+ 5 mM put	140.1± 1.75e	14.26± 1.25b	14.54± 1.71b	143.4± 1.45f	14.46± 1.25c	14.85± 1.21b
My	126.7± 1.75	14.14± 1.01c	13.51± 1.05c	130.7± 1.78i	14.36± 1.01c	13.94± 1.45b
My + 2.5 mM put	157.8± 1.90d	15.14± 1.71b	14.16± 1.85b	159.2± 1.65e	15.75± 1.71b	14.76± 1.75b
My + 5 m M put	135.1± 2.04f	14.32± 1.05c	13.24± 1.33c	137.1± 1.25g	14.21± 1.05c	13.54± 1.39c
PGPR + My	160.4± 1.72e	14.16± 1.85c	12.14±1.52 c	163.4± 1.05e	14.48± 1.85c	12.34± 1.42c
PGPR+ My + 2.5m Mput	166.4± 1.70a	17.54± 1.33a	16.15± 1.33a	180.5.4± 1.58a	18.12± 1.55a	16.45± 1.63a
PGPR+ My + 5 mM put	137.2± 1.50d	16.14± 1.52b	15.01± 1.33b	139.6± 1.30d	16.58± 1.52b	15.34± 1.75b

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

Regarding fruit quality, data presented in Table 8 shows that application of PGPR, and/or My with or without Putresine to the date palm trees irrigated with salinized water and grown in the reclaimed saline soil increased concentrations of N,P,K, crude protein (data not presented) and total carbohydrate in the date palm fruits. PGPR + My in the presence of Putresine at 2.5 mM gave highest levels in this respect. The percentage of vitamin C, total soluble solids in date palm fruits were also increased due to all treatments used in the two growing

seasons. Although recovery of fruit production and their quality was observed from all three exogenously addition of PGPR, My and/or put., put is the most detective treatments. Among different concentration of put, 2.5 mM resulted highest increase in date fruits productivity and their quality compared to other treatments. The additive effect of put was more pronounced incombination with PGPR+My. The highest values of Vit C and TSS were existed with PGPR, Mycorhiza and Putresine at 2.5 mM. These data are being important from the view of fruit quality since, that could prolong the shelf time. Therefore, put would be the best candidate for not only recovering productivity of date palm but also improving fruit quality. In this respect, Mohamed and Tarpley (2009) found that abiotic stress can decrease crop yields by decreasing crop growth duration, suppressing floral bud development and decreasing pollen viability. The same authors (2011) attributed the suppressing of crop yield under stress condition to a shortage of photosynthetic assimilates supplied to the floral buds and/or inability of floral buds to mobilize carbohydrates. Increased respiration and decreased photosynthesis and stability were also recorded (Freire *et al*., 2009)

TABLE 8. Effects of plant growth promoting rhizobacteria (PGPR), Mycorhiza (My), Putresine amine alone or in combination on fruit quality of date palm (*Phoenix dactylifera* L.) zaghloul irrigated with saline water and grown in reclaimed saline soil (Means of the two growing seasons 2013 and 2014).

Treatments	Total sugars mg/g F.Wt.	Total Carbohy-drate %	Total Soluble Solids %TSS	Vit. C mg/100g F.Wt.	Mg/g D. Wt.		
					N	P	K
Control	18.8± 1.56d	53..7± 1.23e	23.11± 0.32d	43..6± 1.01i	10.6± 1.05e	2.2± 0.37d	20.02± 1.21d
2.5 mM put	23.3± 1.33b	60.9± 1.26c	24.03±0. 41c	67.9± 1.21c	14.4± 1.24c	3.8± 0.74b	22.9± 1.23b
5 mM put	22.2± 1.13b	57.3± 1.15d	23.78± 0.35c	54.3± 1.13g	13.1± 1.11c	3.5± 0.75b	21.3± 1.15c
PGPR	21.7± 1.11c	56.8± 1.14d	23.87± 0.35c	57.8± 1.16f	12.6± 1.32c	3.0± 0.45b	20.8± 1.14c
PGPR+ 2mM put	25.1± 1.43a	63.8± 1.02b	24.18± 0.35b	71.8± 1.09b	17.3± 1.27b	4.2± 0.75a	23.1± 1.02b
PGPR+ 5mM put	23.5± 1.24b	58.2± 1.24c	23.94± 0.26c	59.2± 1.20e	14.6± 1.15c	3.7± 0.55a	22.2± 1.24b
My	21.7± 1.20c	54.4± 1.22	23.76± 0.32c	61.4± 1.27e	12.2± 1.28d	2.9± 0.42c	21.4± 1.22c
My + 2.5 mM put	24.1± 1.20a	59.3± 1.13c	24.16± 0.31b	62.3± 1.11d	15.2± 1.35b	3.6± 0.58b	22.3± 1.13b
My + 5 mM put	23.4± 1.45b	57.1± 1.16d	23.54± 0.25c	60.1± 1.13e	12.6± 1.02d	3.0± 0.45b	22.1± 1.16b
PGPR + My	22.7± 1.21b	58.7± 1.30c	23.72± 0.25c	63.7± 1.34c	13.1± 1.17c	3.7± 1.05a	21.7± 1.34c
PGPR+My+ 2.5mMput	25.9± 1.21a	66.8± 1.18a	24.83± 0.32a	76.8± 1.15a	19.8± 1.23a	4.6± 0.75a	24.8± 1.15a
PGPR+My+5 mM put	23.8± 1.61b	60.2± 1.05c	23.31± 0.28c	63.9± 1.15c	15.3± 1.27b	4.0± 0.68a	22.9± 1.15b

Mean values ± SD followed by different letter are significantly different by ANOVA at 0.05 level.

The important role of PGPR and My on salinized soil may exhibit through increment in nutrient elements availability by reducing soil pH, increasing the exchangeable capacity and reducing their losses by leaching as well as the ability of organic chelating agents to protect the nutrient elements against the conversion to unavailable forms (Abada *et al*., 2010 and Kassem, 2012). Most plants adapted to dry stress environments have mycorrhizal symbiosis, which improves water and nutrient supply but is also a sink for carbohydrates and many consume 5-10% of total photosynthate (Koch & Johnson, 1984 and Fogel, 1985). Results of Kumar *et al*. (2014) indicated that the nutritional status might be attribute to enhance inorganic and organic nutrient absorption by biofertilizers which in turn make the essential nutrient available to the promoting growth and increase nutrient content in leaves. The factor leads to increase photosynthetic surface area there by indicating the sufficient utilization of solar radiation ultimately leading to production of assimilates.

It could be concluded that putresine, some microbial species strains could play an important role for explaining how date palm can be tolerant and adapt to stress condition. The selected plant growth promoting rhizobbacteria and mycrorhiza in the presence of Putrescine enable date palm to increase their tolerance and adaptation to reclaimed- salinizied areas as one of many abiotic stresses. Moreover, the interaction treatments between PGPR and Putresine under salt and osmotic stress condition could affect not only the productivity of date palm but also the properties of soil. Further investigation are needed to explain the mechanisms those develop in date palm under stress condition. Thereby, selection of certain microorganisms from stressed ecosystems would insert in to the concept of biotechnology application in agriculture mangements. The physiological behavior mechanism of the plants under these conditions is also needed in order to understand the role of Put in reducing salt-stress induced oxidative.

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التأثير التوافقى بين الاسمدة الحيوية والبيوتراسين امين على بعض المظاهر الفسيولوجية و إنتاجية نخيل البلح المنزرع فى اراضى ملحية مستصلحة

محمد نصر الدين هلالى* و حنان أحمد محمد رشاد الحسينى**
*قسم النبات الزراعى – كلية الزراعة – جامعة المنصورة – المنصورة و**مركز البحوث الزراعية – معهد بحوث البساتين – القاهرة – مصر .

تلعب مركبات الأمين دورا هاما في استجابة النبات للظروف البيئية الضارة بما في ذلك الاجهاد الملحى وارتفاع الضغوط الاسموزية فى البيئة ويهدف هذا البحث الى دراسة الأثر التوافقى بين الاسمدة الحيوية والبيوتراسين امين للتغلب على نقص محصول أشجار نخيل البلح المنزرعة فى اراضى ملحية مستصلحة و تروى بمياه ملحية ، ولهذا الغرض درس التأثير التوافقى لبعض معاملات من الأسمدة الحيوية و البيوتراسين أمين على بعض المظاهر الفسيولوجية المؤثرة على إنتاجية وصفات الثمار الناتجة، وقد اوضحت النتائج أن معاملات التسميد الحيوى المستخدمة فى وجود البيوتراسين أمين أدت الى زيادة مقاومة نخيل البلح (صنف زغلول) للظروف الملحية، حيث حفزت المعاملات من خفض التلف التاكسدى كما زادت من انتاجية المحصول و حسنت من صفات الثمار النوعية وقد أوضحت النتائج أن ذلك يرجع لإرتفاع درجة تركيز الصبغات النباتية و المستوى العنصرى و المواد الإسموزية العضوية فى وريقات الورقة الرابعة التى تم تحليلها فسيولوجيا إضافة إلى زيادة درجة تركيز المنشطات الهرمونية وإنخفاض المثبطات مع إرتفاع درجة النشاط الانزيمى لإنزيمات مضادات الاكسدة المختبرة وهى أسكوربات البيروكسيديز والجلوتاثيون ريدكتيز والسوبر اكسيد ديسميوتيز وخاصة عند تركيز ٢,٥ مللى مول من البيوتراسين أمين ومن ناحية اخرى فقد أدت المعاملات فى وجود البيوتراسين عند تركيز ٥ مللى مول إلى انخفاض درجة النشاط الانزيمى لكل من ديس أمين أوكسيديز والبولى أمين أكسيديز مسببة ارتفاع فى تركيز فوق اكسيد الهيدروجين وتوصى الدراسة بإجراء المزيد من الدراسة لمعرفة آلية تاثير مركبات الامين فى تحسين مقاومة الظروف البيئية الغير ملائمة.