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ORIGINAL ARTICLE

Effect of biofertilizers and putrescine amine on the physiological features and productivity of date palm (*Phoenix dactylifera,* L.) grown on reclaimed-salinized soil

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Abstract

Key message **Plant growth promoting rhizobia bacteria and mycorrhizae in the presence of putrescine allows and/or enables date palm to increase its adaptation to reclaimed/salinized areas.**

Abstract Amines and biofertilizers play an important role in a plant's response to adverse environmental conditions including salt and osmotic stress. This study investigates the integration effect of putrescine amine (Put), biofertilizers, and mycorrhizae (My) on the date palm zaghloul genotype irrigated by saline water and grown in reclaimed saline soil. The data collected indicates that selected plant growth promoting rhizobacteria, in the presence of Put, enables the date palm zaghloul genotype to increase its tolerance and adapt to stress conditions in the reclaimed saline soil. Overall, treatments reduced salt-induced oxidative damage in the date palm, resulting in increased productivity and improved fruit quality. The results observed may be a consequence of the increase in photosynthetic pigments, activities of antioxidant enzymes, organic solutes and/or promoting growth substances such as gibberellic acid (GA_3), auxins (IAA) and cytokinin. Moreover, a decrease in the levels of lipid peroxidation and inhibitor substances such as abscisic acid (ABA) may be related. The most effective interaction treatments were seen at 2.5 mM Put due to an increase in the activities of ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD) while a decrease in lipid peroxidation was noticed. The combination of Put with plant growth promoting rhizobacteria (PGPR) at a 5 mM concentration as biofertilizer increased diamine oxidase (DAO) and polyamine oxidase (PAO) activities when compared to the other treatments. The activity of these two enzymes can produce hydrogen peroxide (H_2O_2), which may act in structural defense as a signal molecule and decrease the production of polyamines against salt-induced oxidative damage in date palm. We believe that further investigation is needed to understand the tolerance/adaptation mechanisms in date palm grown under stress condition.

Keywords Biofertilizers · Mycorrhiza · Rhizobacteria · Putrescine · Date palm (*Phoenix dactylifera* L.) · Reclaimed salinized soil

Abbreviations

ABA	Abscisic acid
APX	Ascorbate peroxidase
CAT	Catalase
DAO	Diamine oxidase
EC	Enzyme entry
GA_3	Gibberellic acid
GR	Glutathione reductase
GR	Glutathione reductase
IAA	Auxins
MDA	Malondialdehyde
My	Mycorrhizae

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PAO	Polyamine oxidase
PGPR	Plant growth promoting rhizobacteria
Put	Putrescine
ROS	Reactive oxygen species
SOD	Superoxide dismutase
Spd	Spermidine
Spm	Spermine
TAA	Total free amino acids
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TS	Total sugars
TSS	Total soluble solid

Introduction

Date palm (*Phoenix dactylifera* L.), a monocotyledon species belonging to the family Arecaceae, is a multipurpose tree of nutritional, medicinal and ornamental importance. In fact, a global demand for food is expected to increase which could promote date palm as a good source for food of high nutritional value (Anonymous 2010). In Egypt, date palm and soft cultivars differ in their sensitivity to salinity (El-Khawaga 2013). Furthermore, horticultures have mistakenly believed that date palm does not require much attention for successful orchard management practices which produce high yields of good fruit quality. One of the best applications for horticultural practice is fertilization. The use of fertilizers to increase yield is an important factor in all agricultural systems (Dong et al. 2005).

Salt stress can induce both ionic and osmotic stress (Alrasbi et al. 2010), which can become ever more prevalent as the intensity of agriculture increases (Qurashi and Sabri 2011). It is a developmentally regulated phenomenon in many plant species (Tang and Newton 2005) that is stage specific. Meanwhile, plant cells have three different strategies for coping with ionic and osmotic stress. First, osmotic adjustment of the cytoplasm due to the accumulations of compatible solutes, such as betaine and proline (Tang and Newton 2005). Second, the salt extrusion from the cell across the plasma membrane uses ion transporters, such as the Na^+/H^+ antiporter (Parvin et al. 2014). Third, salt accumulated in the vacuoles using tonoplast transporters (Zhu et al. 2005). Salt stress, like other environmental types of stress, induce adverse effects on date palm plants, which can be similarly observed in other plant species. Growth, development, survival and the productivity of date palm plants are effected by mechanisms which produce oxidative damage generated from reactive oxygen species (ROS) (Parvin et al. 2014). Helaly and El-Hosieny (2011) found that under stress conditions, the lipid peroxidation increased or induced oxidative (stress) in plant tissues caused by a high degree of membrane deterioration (Pottosin and Shabala 2014). Hence, constitutive and/or induced activity of SOD and other antioxidants such as APX, CAT and GR is essential. According to Seckin et al. (2010), malondialdehyde (MDA) has been frequently described as a suitable biomarker for lipid peroxidation under stress condition.

In most plant species, salinity has been able to modify the functional polyamine production and its distribution within the plant organs represented by a decrease in putrescine (Put) and increments in spermidine (Spd) and/or spermine (Spm). Depletion of endogenous polyamine levels due to salinity has been previously reported (Zhang et al. 2014). Put is a low molecular weight polyamine compound which has been successfully applied to induce salt tolerance in many plant species (Alrasbi et al. 2010) by stimulating the ROS generation during photosynthesis and plant developmental processes. Tang and Newton (2005) reported that Put was highly concentrated 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. Furthermore, Put increased the activities of key enzymes involved in oxidative stress such as APX, GR and SOD, and it decreased lipid peroxidation. However, little information is known about the relationship between the mean level of salt resistance and the endogenous levels of Put. Moreover, the mechanism of tolerance at a specific stage of plant development is a common approach and needs to introduce genetic and/or environmental and physiological improvement to salt stress tolerances.

Biofertilizers and polyamines (Put, 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 (Rasmia and Darwesh 2013). Moreover, they act as antioxidants in the protective mechanisms (Tang and Newton 2005; Salama et al. 2014). On the other hand, it has been found that biological fertilizers play a key role in the productivity and sustainability of soil and can also protect the environment as eco-friendly and cost effective inputs for farmers (Mohammadi and Sohrabi 2012). Moreover, using biological and organic fertilizers can help achieve the sustainability of farms. The soil microorganisms after using humic acid agents, compost enriched with actinomyces, is reported to have been very effective in alleviating the adverse effect of salinity on date palm (El-Khawaga 2013). Similarly, the application of Effective microorganisms (EM) at the rate of 90 ml/palm/year (with about 104 cells/ml) combined with potassium sulfate at 1.5 kg/palm/year as a soil application has and 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).

The present study was undertaken for the purpose of elucidating the physiological role of biofertilizers and Put in reducing salt-stress-induced oxidation. An investigation of the complementary effects of plant growth promoting rhizobacteria (mixture of *Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus circulans*), mycorrhiza (My) and a dressing application with Put at 0, 2.5, 5, mM individually or in combination on certain physiological aspects and the productivity quality of zaghloul cultivar date palm (*Phoenix dactylifera* L.) was irrigated with saline water and grown in reclaimed saline soil.

Materials and methods

Two field experiments were carried out in reclaimed saline soil at the Agricultural Experimental Station, located at Kalabshow and Zayan, Faculty of Agriculture, Mansoura University, 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 (Black et al. 1982) and are presented in Table 1.

Microorganisms' inoculants

Azospirillum lipoferum was grown on semi-solid *N*-free malate medium (Doberiner 1978) whereas *Paenibacillus polymyxa* and *Bacillus ciraulans* were individually grown on nutrient broth media (Dowson 1957). Five (5) days later they were separately suspended in sterile water and incubated for 2–3 days more at 30 °C to maintain a population of 3×10^8 colony forming units (cfu/ml). The mixed inoculums of the microbial cells were prepared by mixing equal volumes of the desired cell suspensions. All microbial strains were provided thanks to the Biofertilizers Unit, Faculty of Agricultural Ain Shams University, Egypt. Arbuscular My fungus (*Glomus mosseae*) was grown in pot cultures containing onion plants for 4 months and the My inoculums used consisted of roots, hyphae, spores and growth media of the pot cultures. The standard inoculums (400 kg/4200 m^2) contained about 270 spores/g. They were obtained from Agricultural Microbial Department of Soil, Water and Environment Res. Ins. (SWER), Agricultural Resources Center (ARC), Egypt. Fungus spores were measured by a wet-sieving and decanting technique, as suggested by Gerdemann and Nicolson (1963). PGPR and My treatments were used at the rate of 85 ml (108 cells ml^{-1}) for each palm.

Putrescine treatments

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

Table 1 Mechanical (a), chemical analyses (b) of the experimental soil (soil: water extract 1:20), and the chemical analysis (c) of the irrigation water used during the two growing seasons of 2013–2014

(a) Mechanical analysis of the soil %

Season	Coarse + fine Sand	Silt	Clay	Soil Texture
2013	69.3	20.26	10.44	Sandy
2014	68.5	21.03	10.51	Loamy soil

b) Chemical analysis of the soil

Season	Cations meq/100 g				Anions meq/100 g		
	Ca	Mg	Na	K	HCO_3	SO_4	Cl
2013	6.07	3.64	12.92	0.35	0.18	9.25	13.55
2014	6.05	3.57	12.53	0.27	0.17	8.63	13.56

(b) Chemical analysis of the soil (cont.)

Season	Electrical conductivity dsm^{-1}	pH	Saturation percentage %	$CaCO_3$ %	Organic matter %	Micro elements (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	Electrical Conductivity 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

Experiment design

Female palm trees (10 years old) of similar vigor, height, pollen source and planted at 7 × 7 m were selected. The trees were arranged in a randomized complete block design with three replicates, and irrigated with saline water (about 5.5 dsm^{-1} as presented in Table 1) using a furrow irrigation system. The trees were arranged in a randomized complete block design with three replicates.

The experiment included the control, PGPR, My, PGPR + My both with and without Put. The application of all treatments took place at three different times, at 20-day intervals. The 1st time was at blooming with the beginning of the set stage on April 15. Other agricultural practices were applied as recommended (Ministry of Agriculture, Egypt).

Sampling and data recorded

Leaflets of the 4th fully expanded leaf from the plant tip were taken at the Kamri 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 and Muluaney (1982), Olsen and Sommers (1982) and Chapman and Pratt (1982) respectively. Moreover, certain organic osmolyte components that were possibly involved in the antioxidant system in relation to salt tolerance were analyzed; total free amino acids (TAA) and total sugars (TS) were extracted from the plant materials 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). GR (EC: 1.8.1.7) activity was measured according to Foyer and Halliwell (1976). One enzyme unit defined as µmol mL^{-1} oxidized Glutathione per min. APX (EC: 1.11.1.11) activity was assayed according to Nakano and Asada (1981). One enzyme unit defined as µmol mL^{-1} oxidized ascorbate per min. SOD (EC: 1.15.1.1) activity was assayed based on the method of Beauchamp and Fridovich (1971) and the specific enzyme activity was expressed as mg^{-1} protein g FW. Lipid peroxidation was measured as the quantum of thiobarbituric acid reactive substances (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by Borsani et al. (2001). The concentration of TBARS was calculated using methods suggested by Groppa et al. (2001). DAO (EC: 1.4.3.6) and PAO (EC: 1.4.3.4) activities were prepared as described by Aribaud et al. 1994; Faive-Rampant et al. 2000 and determined using the H_2O_2- D method (Nag et al. 2000). Endogenous phytohormones were quantitatively determined using high-performance liquid chromatography (HPLC) according to Koshioka et al. (1983) for IAA, GA_3 and ABA. Cytokinin was detected according to Nicander et al. (1993). Harvesting took place periodically six times, at 15-day intervals from August 15th till the first of November. At the end of harvest time, cumulative fruit yield was measured during the two growing seasons. At the third harvest time (Sept. 15th) the fruit component of total sugars and carbohydrates (Amberger 1954), total soluble solid (TSS) using a hand refractometer, Vitamin C (A.O.A.C 1995) as well as total nutrients of N, P and K (as previously mentioned) were estimated. Total cured protein % was calculated by multiplying total nitrogen ×6.25 (A.O.A.C 1995).

Statistical analysis

All data was subjected to an analysis of variance (Snedecor and Cochran 1980) using the SAS (2003).

Results and discussion

Photosynthetic pigments

Chlorophylls (Chl) a, b and carotenoids concentrations were increased due to PGPR, My and Put application compared to the control during the two growing seasons (Fig. 1). The combination treatments between PGPR and My have the highest values in the presence of Put at 2.5 mM compared with the individual application and the control. An increase in Chl(s) and carotenoid concentration can enhance the photosynthesis efficiency and increase date palm productivity. In addition, this enhancement could be an indication of expected high fruit yield quality for date palm irrigated with saline water and grown in the reclaimed saline soil. In this respect, Heidari and Golpayegani (2012) found that inoculation with rhizobacteria could be efficiently used to improve the growth, antioxidant status and photosynthetic pigments under water stress. Moreover, Selvakumar et al. (2014) used My to protect the photosynthetic machinery of plant organelles by stabilizing the ultrastructure of the chloroplast, PSΠ reaction centers and maintaining their oxygen-evolving machineries.

Similarly, the increase in photosynthetic pigments in the plants treated with exogenous My may be due to its effects on reducing membrane damage, a better photosynthesis rate, improved leaf water potential and greater shoot dry weight (Wahid and Shabbir 2005). Pigment stabilizations for water oxidation and photo-oxidation were also detected due to My and Put applications (Cha-um et al. 2006). They showed that the stabilization of Chl a, Chl b, and carotenoids during light energy capture is required for photosynthesis. Put may assume a different role in non-

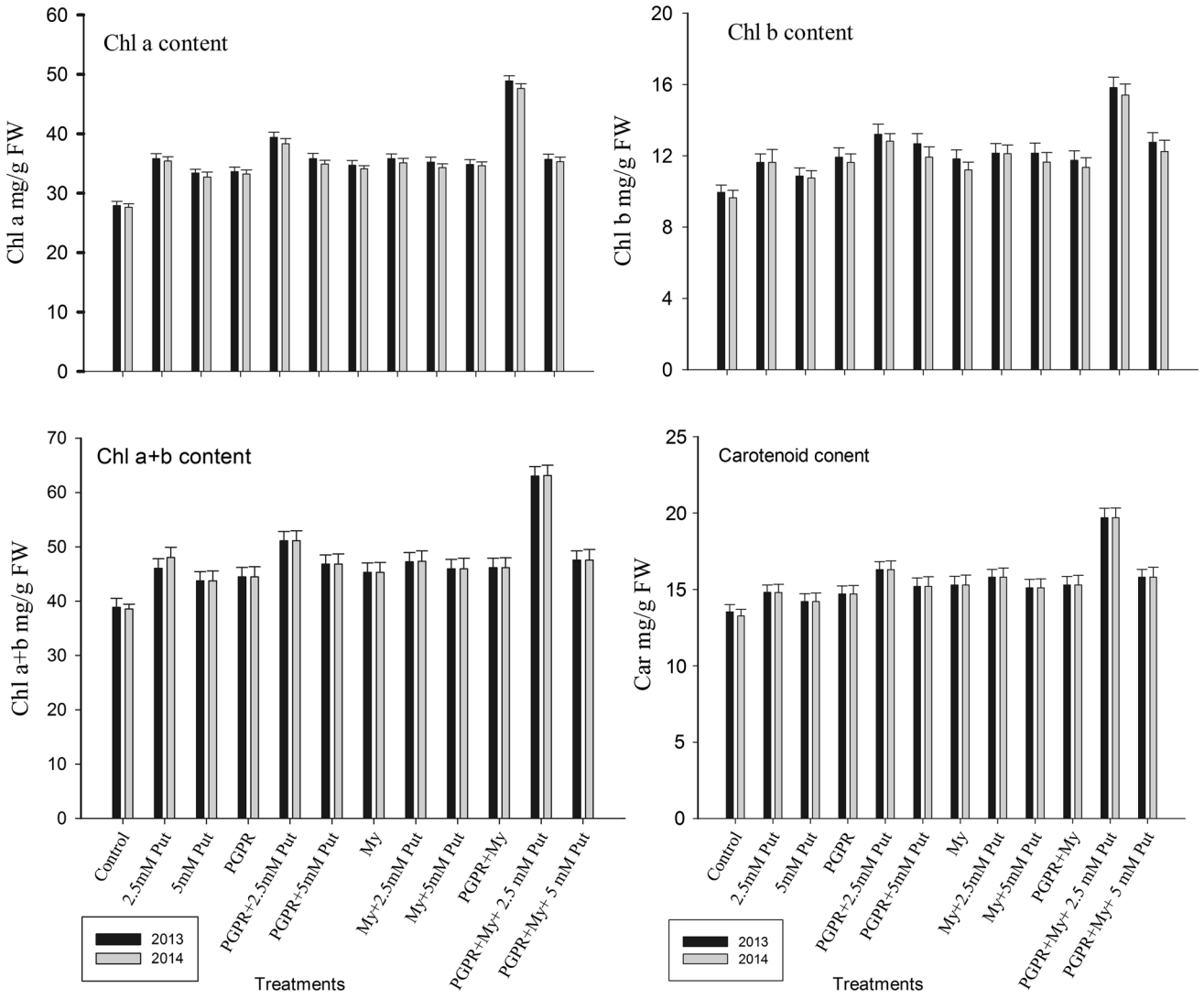

Fig. 1 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on chlorophyll *a*, *b* and carotenoids concentrations (mg/g FW) in 4th full expanded leaf from the plant tip of date palm (zaghloul cv.) irrigation with salinized water and grown in the reclaimed salinized soils during the two growing seasons of 2013–2014

photosynthetic organs vs. photosynthetic ones since it accumulated at a high rate in the roots of the salt-resistant plants compared with the salt-sensitive ones, while the opposite trend was recorded in the shoots (Selvakumar et al. 2014).

NPK minerals and organic osmolytes

The effects of PGPR, and/or My both with and without Put on concentrations of N, P, K, as well as total sugars and total free amino acid, are presented in Fig. 2. In both seasons, PGPR and My in the presence or absence of Put significantly increased concentrations of N, P,K, total sugars, and total free amino acids in the leaflets of date palm. Furthermore, Put at 2.5 mM alone or in combination with other treatments gave the best significant values of total sugars and total free amino acids. Similar results were reported by Bhatti et al. (2013).

The high content of total sugars and some bio-constitutes may be considered a direct result of the high rate of highly efficient photosynthesis of the large photosynthetic area and the high content of photosynthetic pigments. High carbohydrate levels (glucose and sucrose) available during stress conditions are important as a physiological trait associated with stress tolerance (Qurashi and Sabri 2011). In addition, the accumulation of amino acids and sugars is necessary to regulate osmotic activities and to protect cellular structure from stress conditions by maintaining cell water balance and membrane stability. PGPR promotes plant growth directly by facilitating the acquisition of nitrogen, phosphorus and other essential elements (Ahmed and Kibret 2014). Likewise, the changes in sugars and

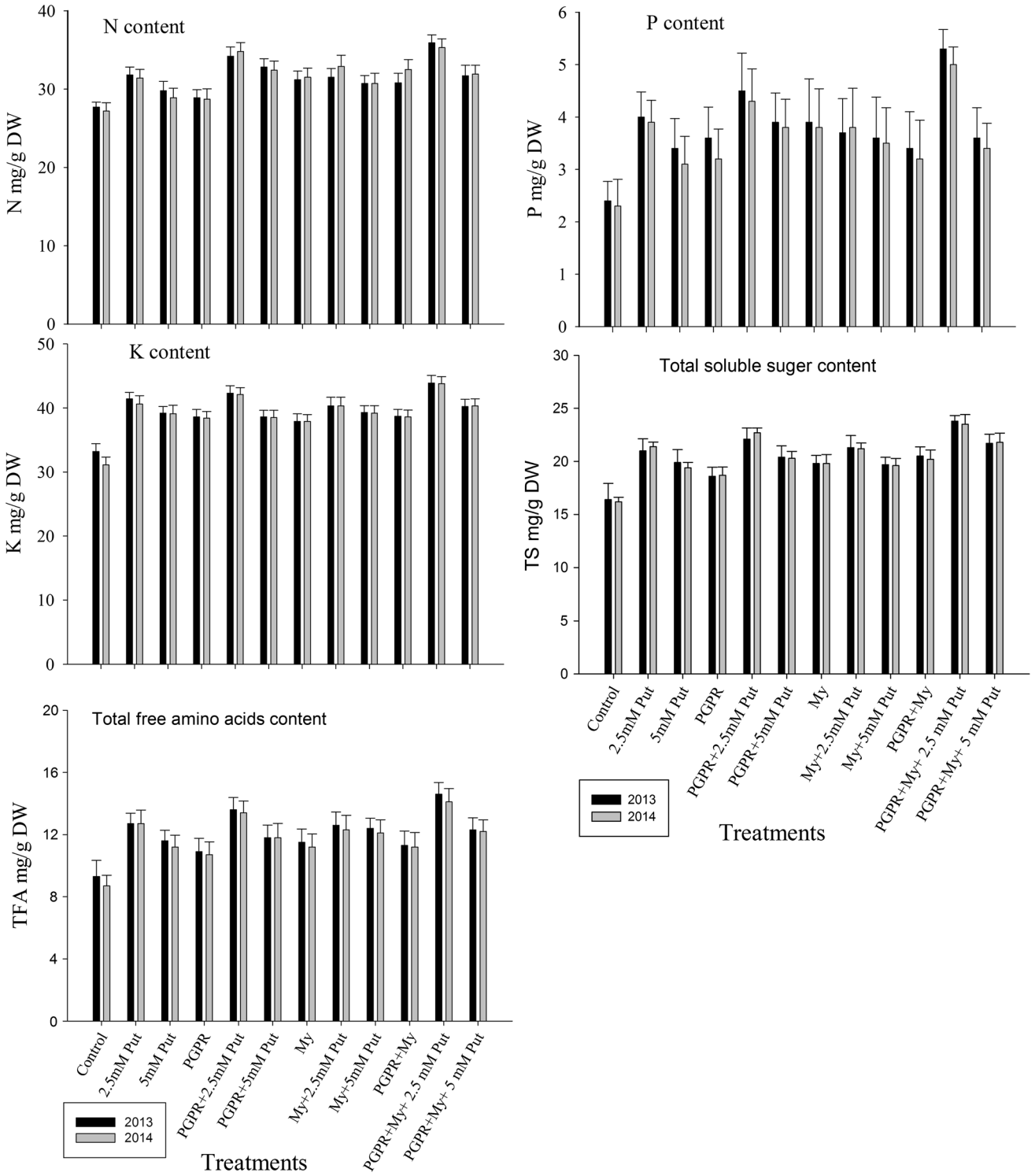

Fig. 2 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on concentrations of N, P, K, as well as total sugars and total free amino acid of date palm (*Phoenix dactylifera* L.) irrigated with saline water (about 5 dsm^{-1}) in the 4th leaf from the plant tip and grown in reclaimed salinized soil during the two growing seasons of 2013–2014

amino acids induced by salt stress related to the synthesis of polyamines (glutamate, arginine and proline) and Put have been reported in leaves of a salt-sensitive tomato species (*Lycopersicon esculentum,* Mill) and its wild salt-tolerant reactive species Darcy (*L. pennelli,* Correll) in light and dark after short-term exposure (Qurashi and Sabri

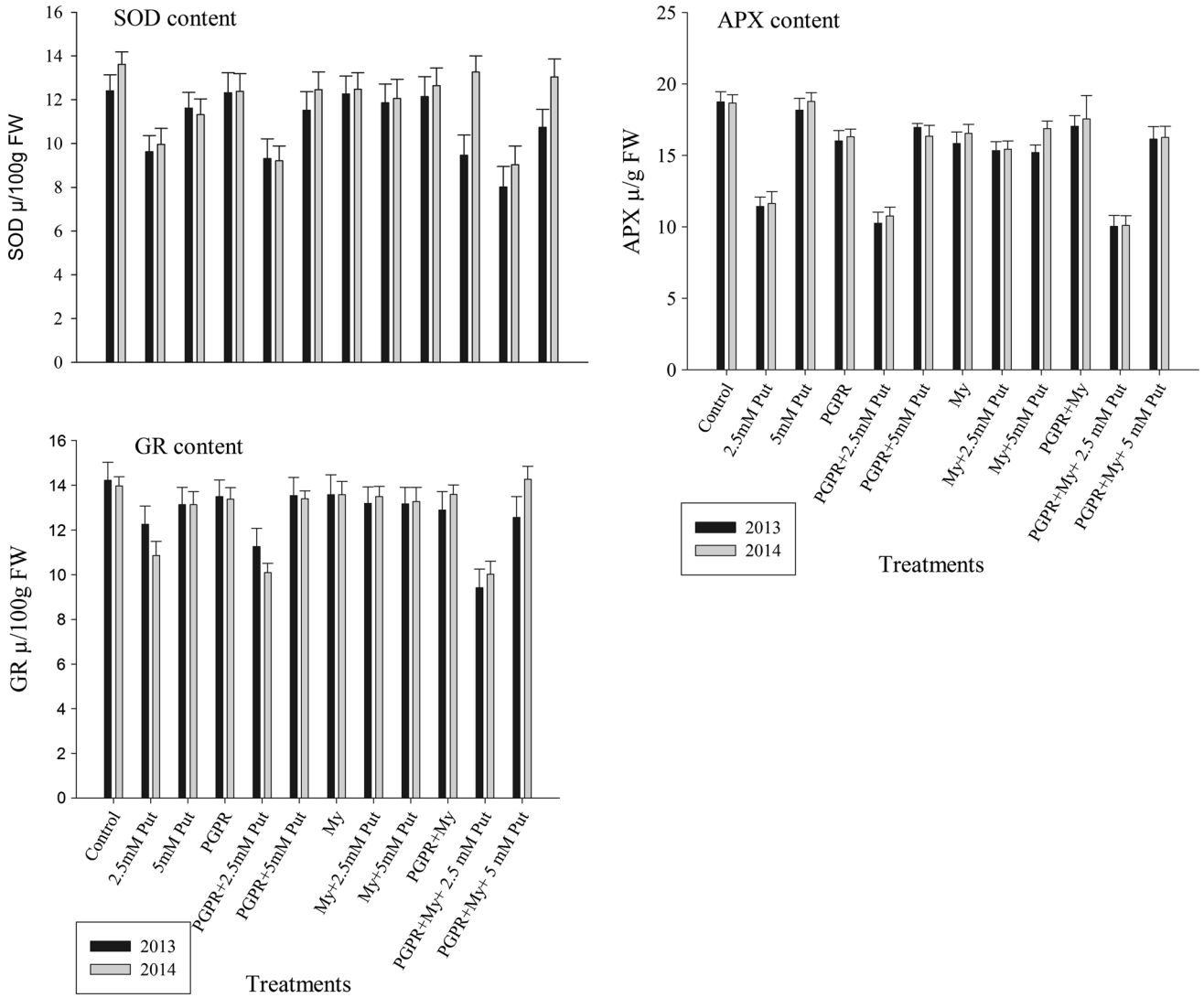

Fig. 3 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on the activities of antioxidant enzyme; ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD) of date palm (*Phoenix dactylifera* L.) irrigated with saline water (about 5 dsm^{-1}) in 4th leaf from the plant tip and grown in reclaimed salinized soil during the two growing seasons of 2013–2014

2011). Therefore polyamines have been suggested to contrast oxidative damage in plants as indicated in our results.

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

The activities of APX, GR, and SOD were decreased due to the application of PGPR, My and/or Put (Fig. 3). Put showed additive effects to that of PGPR, and My in combined treatments. The lowest activity levels were recorded in PGPR + My in the presence of Put at 2.5 mM (Fig. 3). No significant differences were observed in the activities of APX, 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 activities of antioxidant enzymes and decreasing lipid peroxidation in Uirginia pine. They added that APX, GR, and SOD are associated with the oxidative damage of the enzymatic defense system caused by salt stress.

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

Lipid peroxidation was significantly decreased due to the application of PGPR, My and/or Put (Fig. 4). The highest decreasing levels were detected in PGPR + My + 2.5 Put treatment. Differently exogenous added PGPR, My and/or Put increased the activation of DAO and PAO in the leaflets of date palm irrigated with salinized water and grown in the

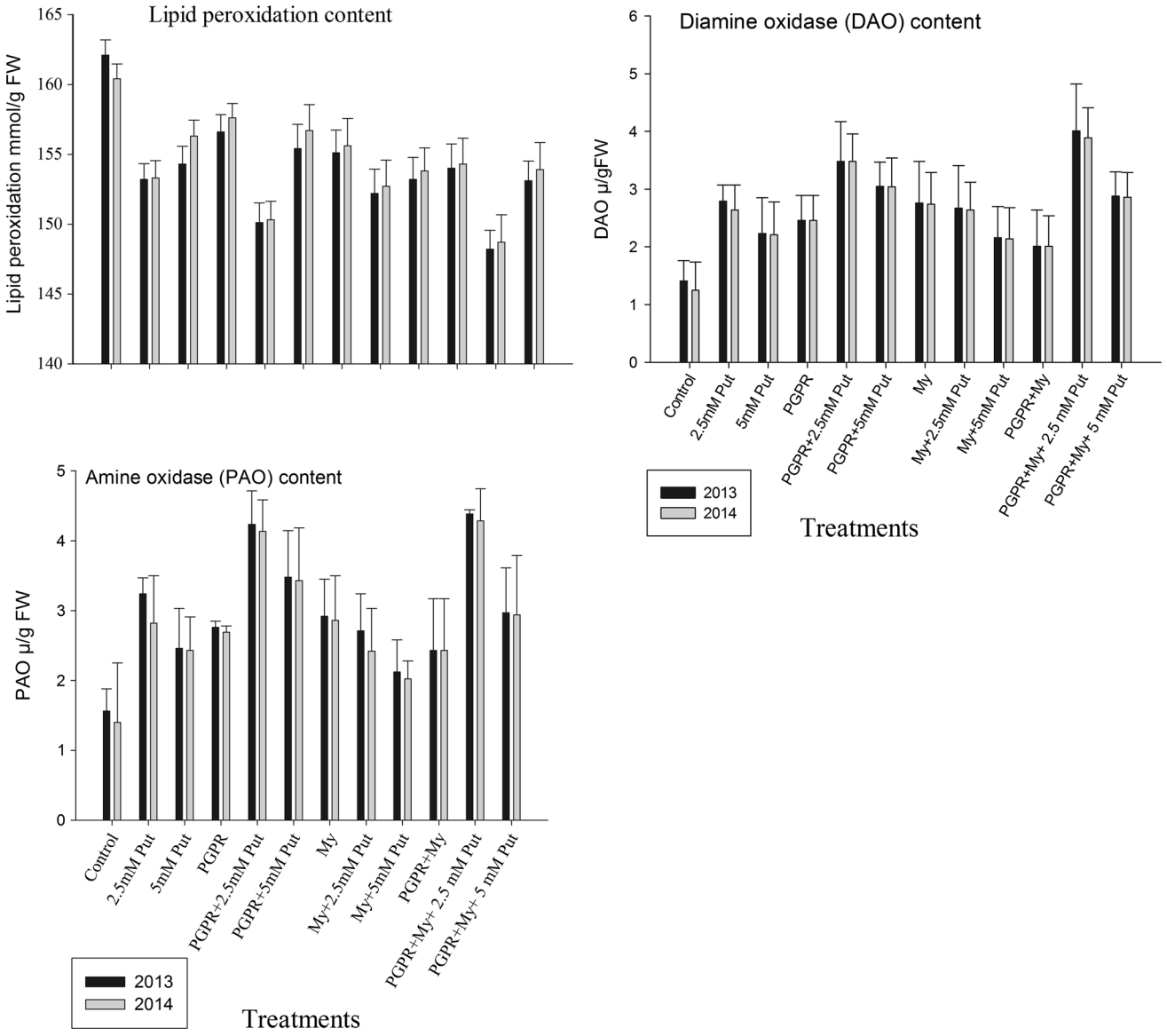

Fig. 4 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on lipid peroxidation (mmol/g FW) as well as the activities of diamine oxidase (DAO), amine oxidase (PAO), and activates (mMH_2O_2/30 min/g FW) of the 4th fully expended leaf from the plant tip of date palm (zaghloul cv.) irrigated with saline water and grown in the reclaimed salinized soils during the two growing seasons of 2013–2014

reclaimed saline soil. The highest activity levels were found with 2.5 mM Put interacting with PGPR + My. A higher PAO activity from date palm leaflets treated with Put may result in liberating more hydrogen peroxidase, which in turn exerts powerful physiological effects on productivity. Similar results were reported by Tang and Newton (2005) with Virginia pine plants.

Endogenous phytohormones

The application of PGPR, My and/or Put on endogenous phytohormones (see Table 2) showed that all pronounces (gibberellins, auxin, and cytokinin) increased whereas abscisic acid decreased (Table 2). The most effective treatment was found with PGPR + My + Put at 2.5 mM. These data could have great influence on different vegetative and reproduction growth. Additionally, increasing the cytokinin level on the account of auxin could result in the increasing productivity of date palm and improvement in 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_3, auxins and cytokinin.

Table 2 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on endogenous phytohormones, (gibberellic acid, auxin, cytokinin) and abscisic acid (mMH_2O_2/30 min/g FW) of 4th full expended leaf from the plant tip of date palm (zaghloul cv.) irrigated with saline water and grown in the reclaimed salinized soils during the two growing seasons of 2013–2014

Treatments	Growing seasons							
	First season 2013				Second season 2014			
	Gibberellin	Auxins	Cytokinin	Abscisic acid	Gibberellin	Auxins	Cytokinin	Abscisic acid
Control	30.5 ± 1.03g	18.7 ± 1.13e	5.3 ± 0.64f	0.85 ± 0.04 h	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 ± 0.16 g	33.1 ± 1.02c	24.2 ± 1.23d	8.9 ± 0.63c	0.72 ± 0.14f
PGPR + 2.5 mM 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 + 5 mM Put	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

All tested treatments resulted in a significant increase in total palm yield, fruit weight and fresh weight in both seasons as compared to the control (Table 3). Soil application of either PGPR or My, both at the rate of 85 ml/tree increased total yield/tree in the presence or absence of Put. Conjugation of both PGPR and My produced additive effects in this respect. These data are more evident when related to the control. It was also observed that Put increased the total yield/tree. Put at the rate of 2.5 mM was the best compared to the concentration of 0 (control) and 5 mM. The complemented treatments produced the highest values in this respect. Increasing the yield of date palm under reclaimed saline soils (salt stress) by the application of Put may be due to its effects on improving the photosynthetic productivity and decreasing photosynthetic consumption, and injury to the membrane. In this context, Tang and Newton (2005) attributed the positive effect of Put on increasing total yield under salinity stress by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation, similarly to that found in the present investigation, then reducing salt-induced oxidative damage.

Regarding the fruit quality, data presented in Table 4 show that the application of PGPR, and/or My both with and without Put to the date palm trees irrigated with salinized water and grown in the reclaimed salinized soil led to increased concentrations of N, P, K, crude protein (data not shown) and total carbohydrate in the date palm fruits. PGPR + My in the presence of Put at 2.5 My resulted in the highest levels in this respect. The percentage of vitamin C and TSS in date palm fruits also increased due to all treatments used in the two growing seasons, although recovery of fruit production and their quality were observed in all three exogenously additions of PGPR, My and/or Put. Among different concentrations of Put, 2.5 mM resulted in the highest increase in date fruit productivity and quality compared to other treatments. The additive effect of Put was more pronounced in combination with PGPR + My. The highest values of Vitamin C and TSS existed with PGPR, My and Put at 2.5 mM. The data is important with regards to fruit quality since it could, with different application treatments, prolong shelf life, especially that of PGPR, My and Put at 2.5 mM. Therefore, Put would be the best candidate for not only recovering productivity of date palm, but also improving fruit quality.

In this respect, Mohammed and Tarpley (2011) found that biotic 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 conditions 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).

The important role of PGPR and My on salinized soil may be exhibited through an 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

Table 3 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on yield and its components of date palm (*Phoenix dactylifera* L.) irrigated with salinized water and grown in reclaimed salinized soil during the two growing seasons of 2013–2014

Treatments	Growing seasons					
	First season 2013			Second season 2014		
	Yield/tree (kg)	Fresh W/fruit (g)	Flesh W (g)	Yield/tree (kg)	Fresh W/fruit (g)	Flesh W (g)
Control	126.1 ± 2.01 h	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 ± 1.28b
5 mM Put	130.1 ± 2.05 g	14.35 ± 1.54c	13.42 ± 1.43c	132.6 ± 1.85 h	14.95 ± 1.14c	13.85 ± 1.30c
PGPR	135.6 ± 1.85f	13.90 ± 1.25c	13.02 ± 1.25c	136.6 ± 1.25 g	13.67 ± 1.25c	12.44 ± 1.15c
PGPR + 2.5 mM Put	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 h	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 mM Put	135.1 ± 2.04f	14.32 ± 1.05c	13.24 ± 1.33c	137.1 ± 1.25 g	14.21 ± 1.05c	13.54 ± 1.39c
PGPR + My	160.4 ± 1.72b	14.16 ± 1.85c	12.14 ± 1.52c	163.4 ± 1.05e	14.48 ± 1.85c	12.34 ± 1.42c
PGPR + My + 2.5 mM Put	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

Table 4 Effects of plant growth promoting rhizobacteria (PGPR), and/or Mycorrhiza (My) with or without putrescine amine (Put) on fruit quality of date palm (*Phoenix dactylifera* L.) zaghloul irrigated with salinized water and grown in reclaimed salinized soil means of the two growing seasons of 2013 and 2014

Treatments	Total sugars mg/g FW	Total Carbohydrate %	Total Soluble Solids %TSS	Vitamin C mg/ 100 g FW	mg/g DW		
					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 ± 1.13 g	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 + 2.5 mM 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 + 5 mM 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 + 2.5 mM Put	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

nutrient elements against conversion into unavailable forms (Abada et al. 2010; Kassem 2012). Most plants adapted to dry stress environments have My symbiosis, which improves water and nutrient supply (Selvakumar et al. 2014). Results of Kumar et al. (2014) indicated that the nutritional status might attribute to enhancing inorganic and organic nutrient absorption by biofertilizers, which, in turn, make the essential nutrient available for promoting growth and nutrient content increase in leaves.

The results obtained clearly show that the application of the selected PGPR, in the presence of Put, enables the date palm zaghloul genotype to increase its tolerance and adapt to grow on reclaimed saline soil. The treatments reduced salt-induced oxidative damage, increased productivity of date palm, and improved fruit quality. Also, more effective interaction treatments were pronounced at 2.5 mmol Put. These results may be due to the increase in the photosynthetic pigments, organic salts, promoting growth substances (GA_3, IAA, cytokines), and the activities of antioxidant enzymes (APX, GR and SOD). Moreover, a decrease in leaflets lipid peroxidation and inhibitor substances (ABA) may be related.

Conclusions

Our results suggest that Put, some microbial species, and strains could play an important role in explaining how date palm can tolerate and adapt to salinity stress conditions. The selected PGPR bacteria and My in the presence of Put allow and/or enable date palm to increase its adaptation to reclaimed/salinized areas. Furthermore, the interaction treatments between PGPR and Put under salt and osmotic stress conditions could affect not only the productivity of date palm, but also the properties of the soil. Further investigations are needed to explain the mechanisms in date palm under stress conditions. Thus, a selection of certain microorganisms from stressed ecosystems can be used as a biotechnology application in agriculture management. The physiological behavior mechanisms of the plants under these conditions is also needed in order to understand the role of Put polyamine in reducing salt-stress-induced oxidative stress.

Author contribution statement The authors confirm the work contribution on our manuscript entitled "Effect of biofertilizers and putrescine amine on physiological features and productivity of date palm (*Phoenix dactylifera*, L.) grown on reclaimed-salinized soil" is EQUAL and as the followings: Helaly M. Naser: discussing the idea, doing the experiment, measurements of NPK content. El-Hosieny Hanan: discussing the idea, ding experiment, measurements of plant hormones. Nabil I. Elsheery: discussing the idea, measurements of enzyme activity. Hazem M. Kalaji: Writing the paper and preparing for submission.

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