Journal of Crop Science and Biotechnology (2021) 24:103–116
https://doi.org/10.1007/s12892-020-00064-3

ORIGINAL RESEARCH

Alleviation of salt stress in *Triticum aestivum* by biopriming with *Phanerochaete chrysosporium*

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Accepted: 31 July 2020 / Published online: 10 August 2020

Abstract

Wheat is one of the most important cereals, vital basic crop in Egypt, covering approximately 32.6% of the total winter land. However, sensitivity to abiotic stress especially salinity is one the major current hurdles that reduce the global yield of this crop. Plant biopriming with fungi is the recent technology that might alleviate the salt stress effect. Thus, the objective of this work was to examine the effect of *Triticum aestivum* grains biopriming with *Phanerochaete chrysosporium* on the response of wheat to salt stress. From the physiological and biochemical parameters, biopriming of wheat grains with *P. chrysosporium* significantly alleviates the salt stress and markedly increases the growth parameters, photosynthetic pigments and osmolytes (soluble sugars, soluble protein and proline) contents. In addition, it alleviates the oxidative damage, as indicated by the lower accumulation of malondealdehyde and increasing the activity of antioxidant enzymes; superoxide dismutase, catalase, and ascorbate peroxidase in wheat seedlings. Results indicate the potential of using *P. chrysosporium* biopriming for reducing the deteriorating effects of salinity.

Keywords Biopriming · *Phanerochaete chrysosporium* · *Triticum aestivum* · Salt stress

Introduction

Wheat (*Triticum aestivum* L.) is one of the essential grown cereal crop, bread wheat is the main food crop in most of the countries including Egypt (Maha et al. 2017). Egypt is one of the countries that suffer severe salinity problems, 33% of the cultivated land which comprises only 3% of total land area in Egypt is already salinized (El-Hendawy 2004). Salinity affects water and nutrient absorption as well as membrane permeability, reflecting on water relations and nutrient balance, and in turn affecting the plant metabolism, hormonal balance, gas exchange, and reactive oxygen species (ROS) (Nawaz et al. 2010; Gheyi et al. 2016). Salinity causes reduction in plant growth through four predicted pathways (1) Osmotically induced water stress, (2) Specific ion toxicity owing to high sodium and chloride concentrations, (3) Nutrient ion imbalance, because of high level of Na^+ and Cl^- which reduces the absorption of K^+, NO^-, and PO_4^{3-}, (4) Increased production of ROS which damages the macromolecules (Nawaz et al. 2010). Visual symptoms of salt injury in plant growth appear progressively such as wilting, yellowed leaves and stunted growth, then followed by chlorosis of green parts, leaf tip burning, and necrosis of leaves and the old leaves appear scorched (Machado and Serralheiro 2017).

Salinity diminishes net photosynthesis due to decrease in stomatal conductance, intercellular partial pressure of CO_2 in leaves, and chlorophyll content, altered ultrastructure of chloroplasts, decreased photochemical and carboxylation reactions, and increase in the level of soluble sugars in the tissues (Iqbal et al. 2014). Under salinity, plants accumulate osmotically active compounds called osmolytes like proline, protein, mannitol, sorbitol, glycine betaine, so forth so as to bring down the osmotic potential. These are referred to as compatible metabolites that maintain cell turgor and thus providing the driving gradient for water uptake (Rasool et al. 2013). Plants possess complex antioxidative defence system comprising ascorbate and glutathione (γ-glutamyl-cysteinyl-glycine, tocopherol, carotenoids, and phenolic compounds, in addition to enzymatic components such as superoxide dismutase, catalase, guaiacol peroxidase, ascorbate glutathione ascorbate peroxidase, monodehydroascorbate reductase, and glutathione reductase to scavenge ROS (Sharma et al. 2012).

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Increase in lipid peroxidation under environmental stresses like salinity and drought parallels with increased production of ROS (Anjum et al. 2015).

Seed priming theory was firstly reported by Heydecker et al. (1973) to alleviate the environmental stresses. Several priming techniques have been reported including hydropriming, osmopriming, hormopriming, solid matrix, thermopriming, nutripriming, chemopriming, and biological priming (Lutts et al. 2016). A combination of seed hydration and inoculation of the seeds with advantageous microbes is referred to as biopriming or biological priming. It improves the seed viability, germination, vigour indices, plant growth and subsequent protection against diseases (Afzal et al. 2016; Lutts et al. 2016; Prasad et al. 2016).

Endophytic fungi can interact with several plant species and in that way significantly participate in the tolerance of these plants to a number to environmental stresses. These conditions include salinity, drought, heat, pathogens, herbivores, or limiting nutrients to the extent that some plants cannot endure stress conditions in the absence of their associated microbes (De Zelicourt et al. 2013; El-Sayed et al. 2017a). Symbiotically -mediated stress alleviation imparted to plants involves two mechanisms; activation of host stress response systems, and biosynthesis of anti-stress compounds by endophytes such as phytohormones that promote the growth of root hairs and increase total root area, facilitating the nutrients uptake, in addition to increasing the activity of 1-aminocyclopropane-1-carboxylate deaminase (ACC) to reduce the level of ethylene and osmolytes as antioxidative defence (El-Sayed and Shindia 2011; Singh et al. 2011; Milošević et al. 2012; Prasad et al. 2016; Vardharajula et al. 2017; Lata et al. 2018). Thus, the objective of the present study was to evaluate the effect of wheat grains biopriming with different endophytic fungal isolates on the amelioration of salt stress through exploring the growth attributes and biochemical mechanisms of the plant in response to salinity stress.

Materials and methods

Fungal inocula, plant materials and biopriming conditions

Five endophytic fungal isolates were selected from our Lab Fungal cultural stock (El-Sayed et al. 2018a, b, 2019a, b, c), to assess their biopriming effect with the tested plant. These fungal isolates were maintained on potato dextrose broth, incubated for 10 days at 30 °C, the entire fungal cultures were collected and washed thoroughly with sterile saline solution (El-Sayed and Ali 2020). The fungal biomass was dried overnight at 40 °C, then the mycelia were powdered by mechanical mortar, and the fine powdered fungal biomass was used for further biopriming experiments with wheat grains.

Wheat grains (*Triticum aestivum* L.) were purchased from Wheat Research Department, Field Crops Research Institute (FCRI), Agriculture Research Centre, Egypt, and this study was conducted from 30th November 2017 to 19th January 2018 in the greenhouse of Botany Department, Zagazig University, Egypt. The grains of wheat were surface sterilized with 1% sodium hypochlorite for 3 min, rinsed with sterile water and re-dried under shade for 10 min (Afzal et al. 2008). The grains were presoaked in sterilized distilled water (1:5 *w*/*v*) for 12 h at room temperature (Farooq et al. 2006), and surface-dried on air to their approximately original weight (Basra et al. 2002). The powdered mycelial were mixed with the prepared grains at ratio 1:0.1 (*w*/*w*) (10 g powdered mycelia and 1 kg plant grains) in presence of 1% carboxy methylcellulose as adhesive agent. The treated grains were put as a heap, covered with a moist jute sack to maintain high humidity for 48 h at 28 °C, under moist conditions until pre-radical emergence. The primed grains were stored at 4 °C until use. Non-primed seeds were used as control. Four salt levels (0, 50, 100 and 150 mM NaCl) were applied to soil in plastic pots (23 × 17 cm), filled with 5 kg of air-dried soil disinfected with 4% formalin (Cardona and Rodriguez 2006) and passed through 2 mm sieve. Leaf samples were harvested at 15 days interval until the end of the experiment (45th day) for estimation of various growth and biochemical parameters.

Molecular identification of the fungal endophyte

Fungal genomic DNA (gDNA) was extracted according to our previous studies (El-Sayed et al. 2015a, b), and used as a template for PCR amplification with the primer set ITS4 5′-GGAAGT AAAAGTCGTAACAAGG-3′ and ITS5 5′-TCCTCCGCTTATTGATATGC-3′ (Schoch et al. 2012). The PCR amplicons were analysed by 1.5% agarose gel in 1 × TBE buffer (Ambion Cat# AM9864) comparing to DNA ladder (Thermo Scientific™ GeneRuler™ 50). Negative control PCR reactions without fungal gDNA were used. The amplicons were visualized by gel documentation system (Vilber Lourmat, France), purified and sequenced by Applied Biosystems Sequencer, HiSQV Bases, Version 6.0 using the same primer sets. The obtained ITS sequences were non-redundantly BLAST searched with the NCBI database sequences. The quality of retrieved sequences was visually inspected from the sequence chromatograms. For the multiple sequences alignments, FASTA sequences were imported into MEGA 6.0 software and aligned with Clustal W muscle algorithm (Edgar 2004). The phylogenetic tree of the target sequences was constructed with neighbour-joining method of MEGA 6.0 with 1000 bootstrap replication (Tamura et al. 2007).

Determination of various plant growth parameters in response to fungal priming

Plant growth assessments

Soil was washed off from the roots, then lengths and fresh weights (FW) of shoots and roots were determined. Whole plant samples were oven dried at 70 °C for 72 h for dry weight (DW) calculations.

Photosynthetic pigments, total soluble sugar, free proline and total protein contents

Fifty mg fresh weight of leaves were homogenised with 5 ml DMF (*N*, *N*-dimethylformamide). After centrifugation, the supernatants were separated and the pigment contents were determined by measuring the absorbance of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) at 663.8, 646.8, and 470 nm, respectively (Vicas et al. 2010).

Total soluble sugars were estimated by the method of Dey (1990). Half gram of fresh leaf material was kept in 10 ml of 90% ethanol for 1 h at 60 °C. The extract was then decanted into a 25 mL volumetric flask and the residue was re-extracted. Final volume was made up to 25 mL by adding 90% ethanol. An aliquot (1 mL) was then transferred to a thick-walled test tube and 1.0 mL of 5% phenol was added and mixed thoroughly and 5 mL of analytical grade sulfuric acid (97–99%) was then added and mixed thoroughly by vertical agitation with a glass rod. For exothermic reaction, the test tube was cooled in the air and absorbance was recorded at 485 nm.

Proline content was determined using dry leaf material, according to the ninhydrin-acetic acid method (Bates et al. 1973). Extracts were prepared in 3% aqueous sulfosalicylic acid, mixed with acid ninhydrin solution, incubated for 1 h at 100 °C, cooled on ice and then extracted with toluene. After centrifugation, the absorbance of the organic phase was read at 520 nm using toluene as a blank.

Fresh leaf tissue was collected from stressed and well-watered plants of both transgenic and control lines. Approximately 1 g of leaf tissue was weighed and ground to a fine powder in liquid nitrogen using a precooled mortar and pestle. The exact weight of each powdered sample was determined before it was thoroughly homogenized in 6 mL of 0.2 M potassium phosphate buffer (pH 7.8) with 0.1 mM ethylenediaminetetraacetic acid (EDTA). The samples were centrifuged at 15,000×*g* for 20 min at 4 °C. The supernatant was removed, the pellet resuspended in 4 mL of the same buffer, and the suspension centrifuged for another 15 min at 15,000×*g*. The combined supernatants were stored on ice and used to determine different antioxidant enzyme activities (Elavarthi and Martin 2010). Protein estimation was done by following the method of (Lowry et al. 1951).

Determination of antioxidative enzyme activities and lipid peroxidation

The activity of superoxide dismutase (SOD) was estimated by the method of Giannopolitis and Ries (1977). Reaction mixture contains 50 mM potassium phosphate buffer, 12 mM methionine, 75 µM nitro-blue tetrazolium (NBT) and 50 mM Na_2CO_3 and enzyme extract of 50 µg protein. At the end, 30 µl of 1 µM riboflavin was added to the mixture. The enzyme extracts were then placed under fluorescent light and removed after 15 min, the absorbance was recorded at 560 nm. One unit of SOD activity was represented by the amount of enzyme inhibiting 50% of the photochemical reaction.

The activity of catalase was assayed (Aebi 1983; Kebeish et al. 2016), with slight modifications. Briefly, the rate of decomposition of H_2O_2 was followed by a decrease in absorbance at 240 nm for 3 min in a reaction mixture containing 50 mM phosphate buffer (PH 7), 10 mM hydrogen peroxide and enzyme extract containing 50 µg protein. The enzyme activity was calculated using the H_2O_2 extinction coefficient of (39.4 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX) activity was determined spectrophotometrically according to the method of Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM sodium ascorbate and enzyme extract containing 50 µg protein. The reaction was started by the addition of 0.5 mM H_2O_2. The decrease in the concentration of ascorbate was recorded at 290 nm for 3 min. The enzyme activity was calculated using the extinction coefficient of ascorbate (2.8 mM^{-1} cm^{-1}) (El-Sayed et al. 2017b).

Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobar bituric acid reaction as described by (Heath and Packer 1968).

Fungal deposition

The potential fungal isolate *P. chrysosporium* was deposited at Assiut University Mycology Centre AUMC14235, as well as on genbank with accession # MN736179.1.

Statistical analysis

Statistical analysis was carried out using the personal computer software packages SPSS version 16 using two-way ANOVA, post hoc comparisons were made using the Tukey HSD test. Differences were considered to be significant at $P < 0.05$.

Results

Screening for the potent biopriming fungal isolates with wheat

Five endophytic fungal isolates namely *P. chrysosporium, Fusarium* sp., *Penicillium* sp., *Pestalotia* sp *and Trichoderma* sp were bio-primed with the seeds of wheat at different concentrations of NaCl. The percentage of seeds germination and shoot and root lengths of seedlings were measured. From the obtained results (Table 1), the highest germination percentage of wheat seeds was reported in presence of *P. chrysosporium* at the experimented salt concentrations (50, 100, 150 mM), followed by *Penicillium* sp EFBL1 and *Trichoderma* sp, *Fusarium* sp, and *Pestalotia* sp (Figs. 1, 2). Based on the higher germination percentage of wheat seeds, physiological parameters of wheat seeds bio-primed with *P. chrysosporium* and *Penicillium* sp EFBL1 were for further assessed based on the root and shoot systems lengths. From the results (Table 2), *P. chrysosporium* displayed the highest enhancing effect on the lengths of the shoot and root systems of wheat seedlings along with the experimented salt concentrations, comparing to controls. Thus, *P. chrysosporium* has been selected for biopriming with wheat seeds, for further physiological analyses.

Growth parameters

One goal of seed biopriming research is to improve growth parameters under adverse conditions such as salinity. In light of these results, the influence of *P. chrysosporium* priming on plant growth markers such as root and shoot lengths, fresh and dry weights of wheat plants were measured. Root growth was found to be less sensitive to salt stress than shoot growth. The results showed that root length significantly declined with the increase in salinity concentration. However, EFB28 biopriming increased root growth by 10% after 45 days of stress. This increment was found as 8, 20, and 23% for plants pre-treated with *P. chrysosporium* then exposed to NaCl when compared to 50, 100, and 150 mM NaCl irrigated the only group, respectively, at the end of the experiment (Fig. 3a).

In addition to that, shoot elongation reduced with the increment in salt dosage. In *P. chrysosporium* biopriming, only plants, an increment in shoot growth at a rate of 5% was recorded as compared to those untreated (control) plants, in the last harvest of plant material. However, when EFB28 seed biopriming was performed before NaCl expose, the adverse effects of salinity were alleviated, showing an increase in shoot length by 11, 13, and 21% in comparison to the non-bioprimed ones under 50, 100, and 150 mM NaCl stress, respectively, after 45 days of stress (Fig. 3b).

Accordingly, fresh and dry weights of the whole plant also significantly decreased in plants under salt stress with the increase in salinity level. *P. chrysosporium* bioprimed plants induced an increase in FW at a rate of 59% as compared to control plants, after 45 days of stress. Seed biopriming of *P. chrysosporium*, however, significantly prevented the deleterious effects of NaCl on fresh weight by 32, 77, and 58% as compared to 50, 100, and 150 mM NaCl-stressed only plants, respectively, after 45 days of salinity imposition (Fig. 4a).

The exposure of wheat plants to salt resulted also in a progressive, concentration-dependent inhibition of growth, in relation to the non-stressed controls, as shown by a relative reduction in dry weight with increasing external NaCl concentrations. Dry weight was enhanced up to 11% in *P. chrysosporium* bioprimed plants relative to the control group of plants, after 45 days of treatment. Again, EFB28 biopriming caused amelioration of salt-induced adverse impact on dry weight via elevating it up to 6, 47, and 51% when compared to those 50, 100, and 150 mM NaCl irrigated only plants at the age of 45 days after stress imposition (Fig. 4b).

Photosynthetic pigments

A decline in the content of photosynthetic pigments under abiotic stress like salinity is an obvious outcome. Taking into consideration, the mitigation effects of *P. chrysosporium* priming on the content of chlorophyll a, Chlorophyll b, and carotenoid were quantified. With one exception (50 mM

Table 1 Effect of seed fungal bio-priming with different five isolates

	Control	*Phanerochaete chrysosporium*	*Fusarium* sp	*Penicillium* sp	*Pestalotia* sp	*Trichoderma* sp
Zero salt	90	98 ± 11.2	25 ± 5.2	92 ± 12.2	32 ± 5.3	38 ± 4.2
50 mM NaCl	89	95 ± 13.2	22 ± 3.2	62 ± 9.2	24 ± 4.2	36 ± 6.2
100 mM NaCl	78	83 ± 12.1	19 ± 4.2	51 ± 8.6	22 ± 3.8	28 ± 4.4
150 mM NaCl	73	81 ± 11.2	14 ± 3.2	43 ± 9.3	20 ± 3.5	16 ± 4.3

F1, *Phanerochaete chrysosporium,* F2: *Fusarium* sp., F3: *Penicillium* sp., F4: *Pestalotia* sp., F5: *Trichoderma* sp. on percentage of germination at 10 days after sowing in wheat seedlings

Table 2 Effect of fungal biopriming with F1: *Phanerochaete chrysosporium* on root length (cm/ plant) and shot length of wheat plants after 15, 30 and 45 days of salt treatment (DAT)

Growth Parameter	Root length (cm/plant)						Shoot length (cm/plant)					
NaCl Treatments	15 DAT		30 DAT		45 DAT		15 DAT		30DAT		45 DAT	
	Control	F1	Control	F1	Control	F1	Control	F1	Control	F1	Control	F1
Zero	22.4 ± 3.2	29.8 ± 2.2	35.25 ± 2.0	38.2 ± 3.0	43.5 ± 1.8	48.08 ± 1.9	20.7 ± 1.8	23.4 ± 1.2	30 ± 2.2	33.9 ± 1.1	39.08 ± 1.2	40.75 ± 1.7
50 mM	19.3 ± 1.3	23.8 ± 1.9	31.25 ± 1.9	36 ± 1.8	39.75 ± 3.2	42.87 ± 3.2	17.4 ± 1.2	21.1 ± 2.7	29.0 ± 2.9	32.8 ± 2.1	34.33 ± 2	38.17 ± 2.2
100 mM	15.2 ± 2.2	20.4 ± 3.2	28.4 ± 3.2	35.0 ± 3.2	31.75 ± 3.2	38.17 ± 3.2	17 ± 3.2	20.5 ± 2.3	28.0 ± 2.2	32.3 ± 3.2	31.75 ± 0.8	35.75 ± 1.0
150 mM	14.8 ± 1.3	19.8 ± 2.2	26.6 ± 1.9	30.9 ± 1.6	29.62 ± 1.9	34.3 ± 1.1	16.7 ± 2.5	20.0 ± 2.3	26.4 ± 3.2	30.8 ± 2.8	28.83 ± 2.2	34.92 ± 1.2

NaCl after 15 days of stress), all photosynthetic pigments content declined steadily in the dose and age-dependent manner in plants subjected to NaCl only, compared to normal condition (0 mM). After 45 days of stress, compared to the control, the values of chlorophyll a (chl a) content were enhanced with the application of *P. chrysosporium* without salt stress at a rate of 44%. However, in wheat plants pre-inoculated with *P. chrysosporium* then exposed to NaCl chl an increased by 211, 295, and 320%, relative to those treated with 50, 100, and 150 mM NaCl alone after 45 days of treatment (Fig. 5a).

Chlorophyll b (chl b) contents were found to be displaying a similar trend for *P. chrysosporium* bioprimed plants, as the increase was 72% comparing to control group. Beyond that, EFB28 seed biopriming notably increased the chl b content of 50, 100, and 150 mM NaCl stressed plants (50 mM, 100, and 150 mM) in presence of *P. chrysosporium* in compared with 50, 100 and 150 mM NaCl treated the only group of plants by 279, 517, and 425% at the end of the experiment (Fig. 5b).

After 45 days of stress, 65% was recorded as the increment rate of the carotenoid (car) content in the *P. chrysosporium* primed plants as compared to the control (untreated) ones. *P. chrysosporium* seed biopriming increased carotenoid content in the seedlings treated with 50, 100, and 150 mM NaCl by 40, 51, and 35%, respectively, regarding the non-primed but saltstressed ones at the end of the experiment (Fig. 5c).

Osmolytes contents

Accumulation of osmotically active compounds gives rise to osmotic adjustment in plants subjected to salt stress. In view of this, ameliorating effects of biopriming under salinity were further confirmed by analysing total soluble sugars, proline, and protein.

When we analysed the total soluble sugars (TSS) data, the content of TSS at the end of the experiment decreased down to 44, and 54% after the 100 and 150 mM NaCl treatment alone, respectively, compared to the control, while no significant change was detected under lower dose (50 mM). TSS content increased by 7.5% when treated with *P. chrysosporium* alone, and increased by 66, and 88%, respectively, when treated with *P. chrysosporium* in combination with 100, and 150 mM of NaCl, compare to the NaCl-stressed plants, while no significant change was observed under 50 mM concentration (Fig. 6a).

The results regarding the outcome of salt stress and *P. chrysosporium* priming on proline content that serves also as osmotic marker of salinity stress are demonstrated in (Fig. 6b). Proline content significantly increased in the seedlings exposed to the NaCl. However, it is obvious that the seed biopriming of *P. chrysosporium* under salt stress leads to a remarkable increase in proline content in comparison

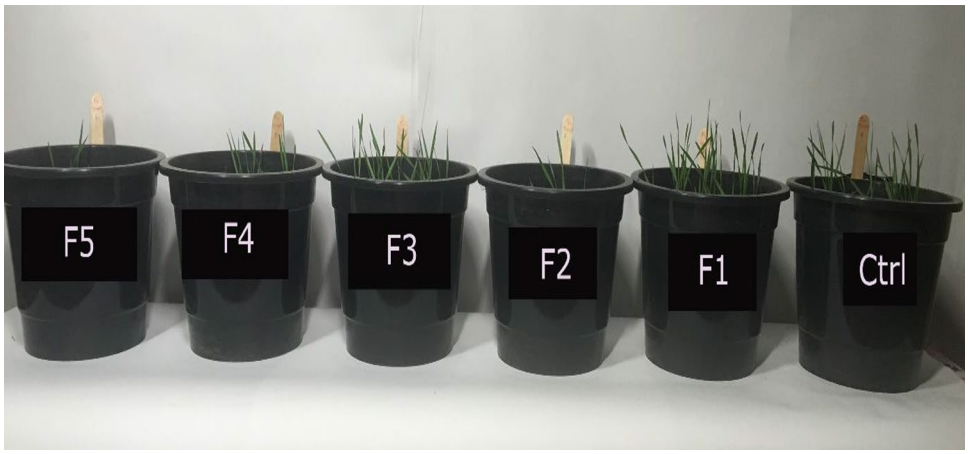

Fig. 1 Effect of seed biopriming using different endophytic fungal isolates on salt tolerance under 150 mM NaCl concentration on the growth of wheat seedlings. Photograph was taken at 15th day of salt treatment. Ctrl: control, F1: *Phanerochaete chrysosporium,* F2: *Fusarium* sp.*,* F3: *Penicillium* sp.*,* F4: *Pestalotia* sp.*,* F5: *Trichoderma.* sp

Fig. 2 Effect of *Phanerochaete chrysosporium* biopriming under various concentrations of NaCl (0, 50, 100, 150) mM on root and shoot length of wheat plants. Photographs were taken at the end of salt treatment (45 days): **a** normal condition (left), fungal biopriming only (right), **b** 50 mM NaCl treatment (left), fungal biopriming + 50 mM (right), **c** 100 mM NaCl (left), fungal biopriming + 100 mM (right), and **d** 150 mM NaCl (left), fungal biopriming + 150 mM (right)

to the salt stress alone. The increment of proline content in the seedlings obtained from *P. chrysosporium* bioprimed seeds and stressed with 50, 100, and 150 mM NaCl was 41, 31 and 26% with regard to NaCl stressed only plants after 45 days of treatment.

Concerning the content of protein, it significantly decreased after the treatment of NaCl alone. However, the application of *P. chrysosporium* increased the protein content in the wheat seedlings grown under salt stress or non-stress compared to the unbioprimed. After 45 days of salt processing, the content of protein was increased by 16.5% upon treatment with *P. chrysosporium* alone compared to the control and increased by 62, 117, and 168%, respectively, when treated with *P. chrysosporium* in combination

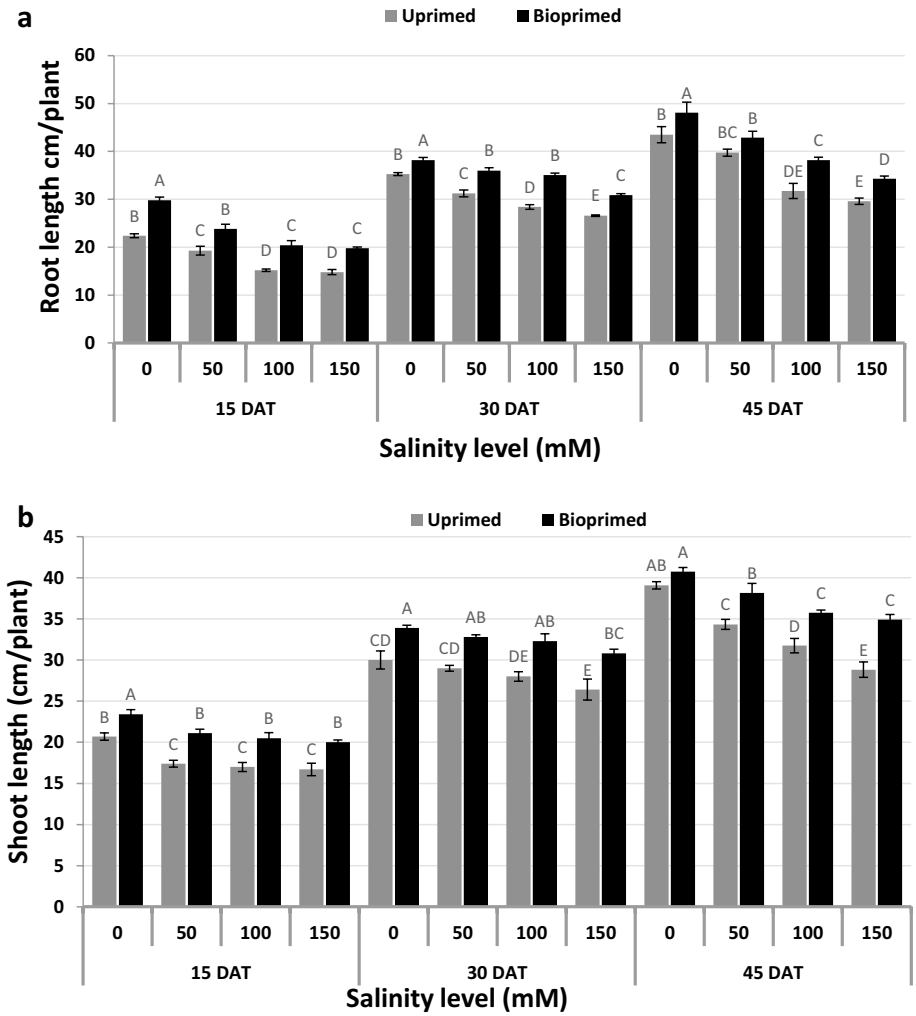

Fig. 3 Effect of *Phanerochaete chrysosporium* biopriming on **a** root and **b** shoot length (cm/plant) after 15, 30 and 45 days of salt treatment (DAT) in wheat plants. Values are shown in (mean ± SE, $n = 6$). Different letters are significantly different from each other according to the Tukey test ($P < 0.05$)

with 50 mM, 100 mM, and 150 mM of NaCl, over the NaCl-stressed plants, in the third harvest of plant material (Fig. 6c).

Lipid peroxidation

Malondialdehyde (MDA) is a product of membrane lipid peroxidation and a reliable biomarker of oxidative stress. On account of this, MDA content was analysed to prove the important role played by *P. chrysosporium* priming against damage caused by salt stress. A significant increase in MDA levels was detected in plants watered with saline solutions alone in parallel with external salinity and in a time-dependent manner. However, when *P. chrysosporium* seed biopriming exist in advance, the level of MDA production decreased down to 11.4% of the corresponding controls at 45 days of treatment. *P. chrysosporium* biopriming substantially modified the lipid oxidation percentage by decreasing the production of MDA at a rate of 23, 28.6, and 31% with respect to the 50 mM, 100 mM, and 150 mM NaCl only irrigated plants, respectively, in the third harvest at 45 days (Fig. 7).

Antioxidant enzyme activity

Plants have evolved an efficient defense system by which the ROS is scavenged by antioxida nt enzymes. Thereby, correlations between salt tolerance via biopriming and activity of superoxide dismutase, calatase, and ascorbate peroxidase have been examined. There was a remarkable and gradual increase in the activities of all antioxidant enzymes measured in the leaves of the *P. chrysosporium* bioprimed plants under either dose of salinity stress compared to the control conditions. Maximum activity increments were commonly found in a higher dose of salt concentrations after 45 days, our last experimental period in this study.

SOD activity elevated in the dose and age-dependent manner in both NaCl-stressed only and *P. chrysosporium* treated plants. *P. chrysosporium* seed biopriming right before salt stress for each dose showed a rise at a rate of 4%

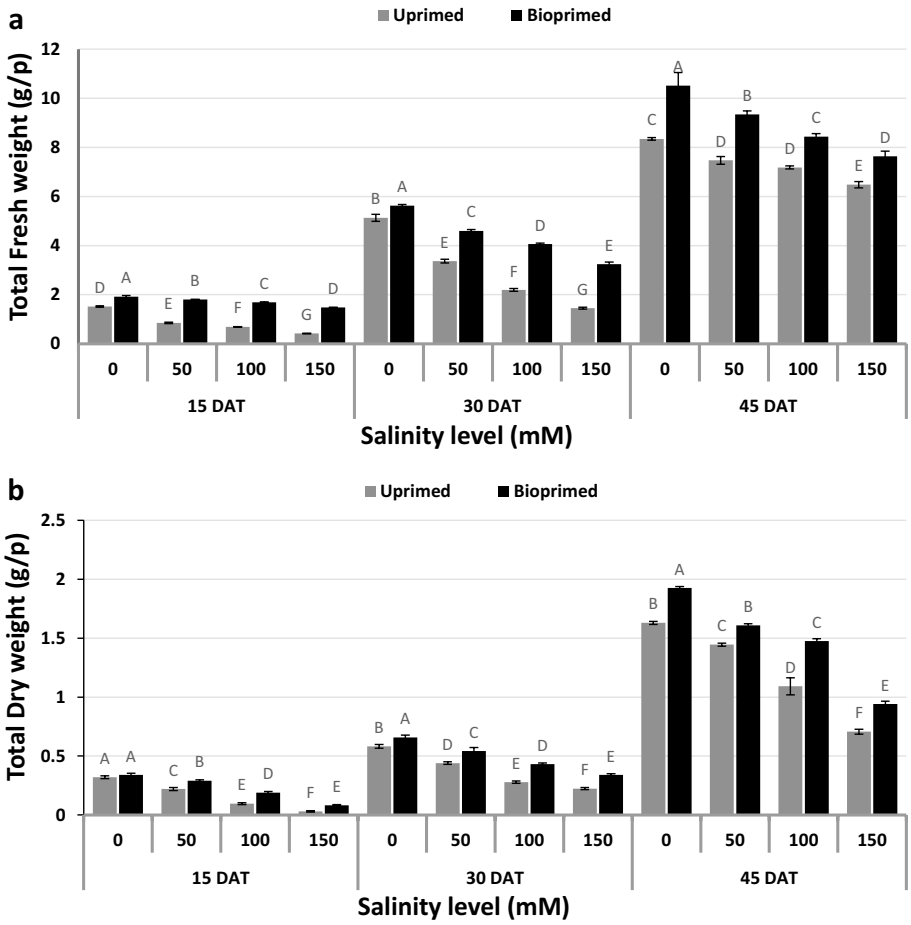

Fig. 4 Effect of *Phanerochaete chrysosporium* biopriming on **a** total plant fresh and **b** dry weight (g/p) after 15, 30 and 45 days of salt treatment in wheat plants. Values are shown in (mean ± SE, $n = 6$). Different letters are significantly different from each other according to the Tukey test ($P < 0.05$)

when compared to non-inoculated controls. Plants inoculated with *P. chrysosporium* under salt stress had higher efficiency in increasing the activity of SOD at rates of 12.6, 7, and 9% in comparison with 50, 100, and 150 mM NaCl-stressed plants, respectively (Fig. 8a).

In parallel, compared with the control plants, catalase (CAT) activity increased linearly with increasing levels of salt treatment alone and age of the plant. CAT activity was measured higher in the *P. chrysosporium* bioprimed plants with regard to the control group at a rate of 12.5% after 45 days of stress. Also, CAT activity rose 40, 28.5, and 62.5% in EFB28 bioprimed plus NaCl stressed plants as compared to the non-bioprimed but salt-stressed only ones (50, 100, 150 mM NaCl), consecutively, at 45 days after salt supplementation (Fig. 8b).

At last, we had the identical dose and age-dependent increment trend in the activity of ascorbate peroxidase (APOX) in the NaCl treated only and EFB28 bioprimed groups in comparison with the control group. EFB28 biopriming alone (without NaCl) resulted in an increase in APOX activity is compared with the control group at a rate of 35% after 45 days of stress. The increment in the leaves of the plants stressed with 50, 100, and 150 mM NaCl right after bioprimed with EFB28 was detected as 58, 62, and 74%, respectively, as compared to the non-bioprimed but salt-stressed only ones after 45 days of stress (Fig. 8c).

Discussion

In the current investigation, it was intriguing to find that although the fact that the roots were directly exposed to the Na^+-rich environments it appeared significant that shoot growth was influenced more than the root growth. In agreement with our results, Sharma (2015) observed that the wheat shoot growth was frequently inhibited more than the root growth under saline stress. In parallel, (Agarwal et al. 2015) found that soybean shoot growth was affected more adversely than root growth. Djanaguiraman et al. (2006) has also reported that rice shoots had higher sensitivity than the roots to a lethal dosage of salinity. This might be ascribed to the fact that root growth is commonly sensitive to a lesser extent to saline stress than shoot growth, an augmented root/shoot ratio is repeatedly witnessed when plants are confronted to saline conditions. The escalation in root/shoot proportion lessens the need for element provision to

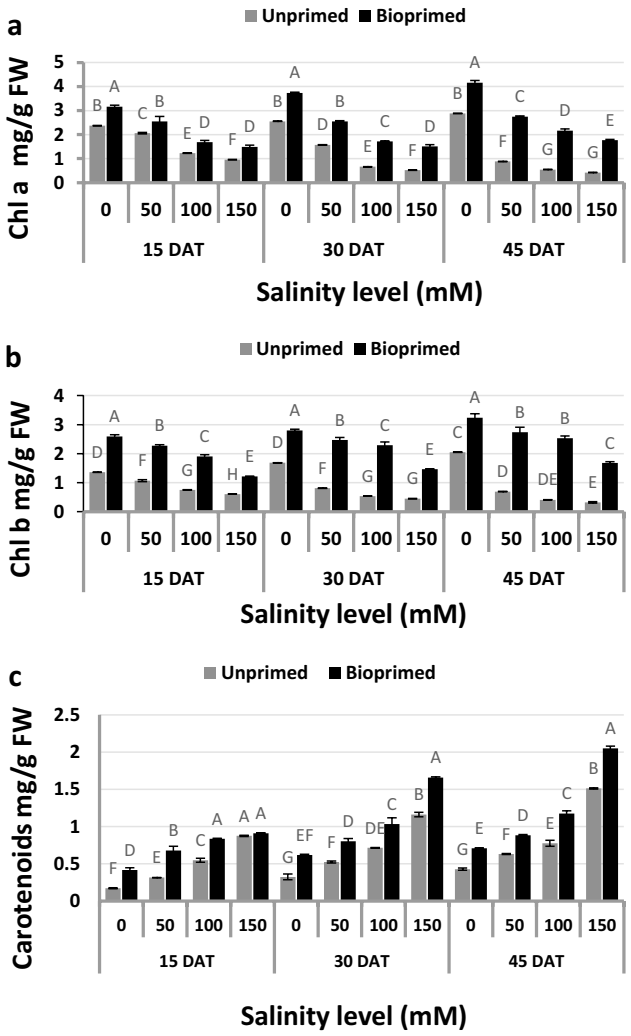

Fig. 5 Effect of *Phanerochaete chrysosporium* biopriming on levels of chlorophyll a (**a**), chlorophyll b (**b**) and total carotenoids (**c**) after 15, 30 and 45 days of salt treatment in wheat plants. Values are shown in (mean ± SE, $n = 3$). Different letters are significantly different from each other according to the Tukey test ($P < 0.05$)

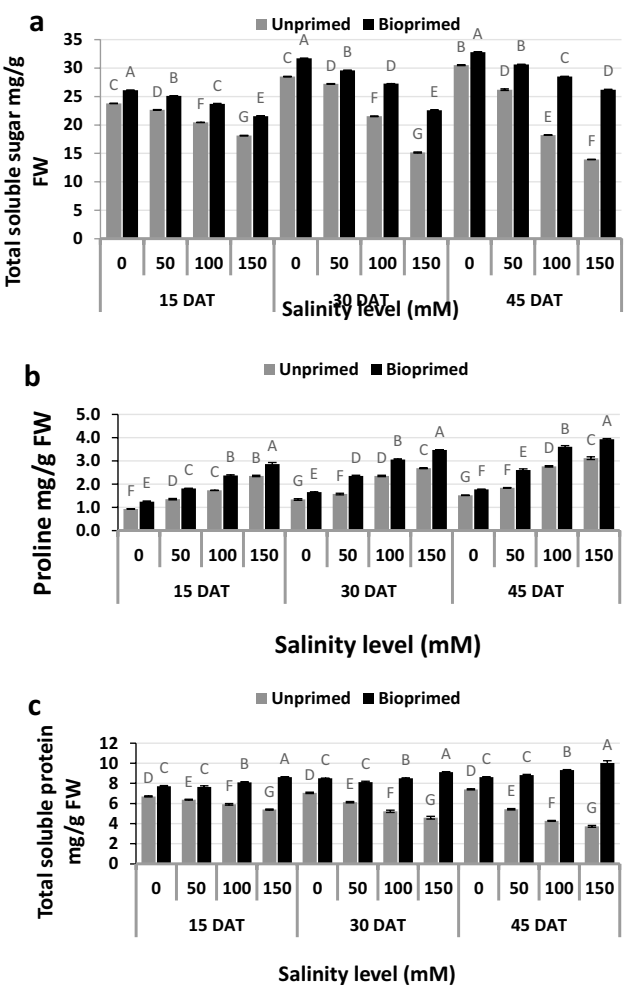

Fig. 6 Effect of *Phanerochaete chrysosporium* biopriming on soluble sugars (**a**), proline (**b**) and soluble protein (**c**) after 15, 30 and 45 days of salt treatment in wheat plants. Values are shown in (mean ± SE, $n = 3$). Different letters are significantly different from each other according to the Tukey test ($P < 0.05$)

the shoot and by this means has a capacity to gain the root aptitude to provide those elements and present an adaptive benefit. A possibly undesirable outcome of such an alteration is the reduced ability of the shoot to provide the root and the growing tissues with assimilates, that is conceivably under the long-term salinity to influence plant development and subsistence (Bernstein et al. 2013). Although the whole plant growth is withdrawn in many agriculturally key species throughout water shortage, but the root system is more resilient than shoots and grows continually under low water potentials which are entirely retardants for shoot growth (Ali et al. 2015; Sánchez-Calderón et al. 2013). Maximum growth parameters of wheat plants were observed in plants issued from seeds bioprimed with EFB28. Faster growth of plants produced from bioprimed seeds may be analyzed by

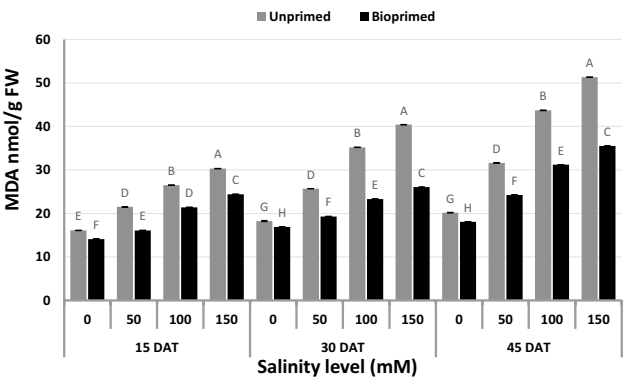

Fig. 7 Effect of *Phanerochaete chrysosporium* biopriming on levels of malondialdehyde (MDA) after 15, 30 and 45 days of salt treatment in wheat plants. Values are shown in (mean ± SE, $n = 3$). Different letters are significantly different from each other according to Tukey test ($P < 0.05$)

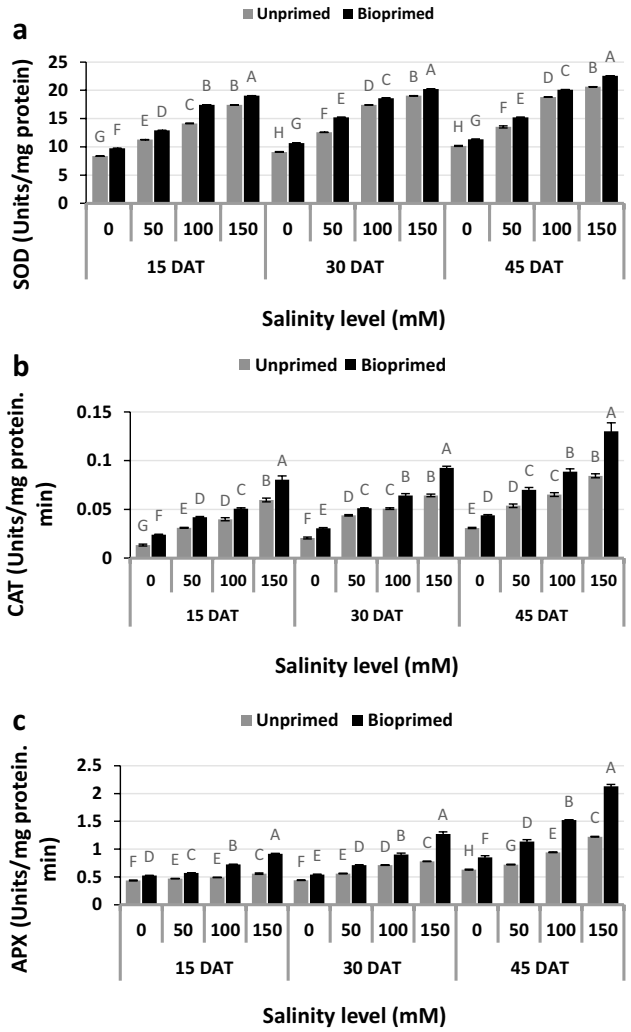

Fig. 8 Effect of *Phanerochaete chrysosporium* (EFB28) biopriming on antioxidant enzymes SOD (**a**), CAT (**b**), and APOX (**c**) after 15, 30 and 45 days of salt treatment in wheat plants. Values are shown in (mean ± SE, $n = 3$). Different letters are significantly different from each other according to the Tukey test ($P < 0.05$)

an enhanced nutrient use efficiency and cell cycle regulation and cell elongation processes (Debbarma and Priyadarshinee Das 2017).

As anticipated, salt stress caused a decrease in chlorophyll content which agrees with research done on rice (Jamil et al. 2012), fenugreek (Kapoor and Pande 2015), and sorghum (Sayyad-Amin et al. 2016). It seems to be a common impact of the suppression of chlorophyll biosynthesis enzymes like Rubisco and PEP carboxylase (Zorrig et al. 2013), and the activation of chlorophyllase, an enzyme involved in chlorophyll degradation by cleaving the phytol tail of chlorophyll (Santos 2004). In the present work, EFB28 seed bioprimed plants were capable of ensuring the chlorophyll and carotenoids better. These results are in good agreement with a previous report showing that when *Trichoderma atroviride* seed biopriming was conducted before NaCl expose, the chlorophyll and carotenoid contents increased in maize seedlings (Durmus et al. 2017). Although *Trichoderma citrinoviride* biopriming resulted in a dose-dependent decline in both chlorophyll and carotenoids contents in salt-stressed maize plants but their concentrations were still much higher than in unbioprimed ones (Yesilyurt et al. 2018).

Our results of an increase in carotenoids content with increasing salt concentration corroborated with the findings of Khosravinejad et al. (2008) who reported an augmentation in carotenoids content in barely. Similarly, Chaparzadeh and Hosseinzad-Behboud (2015) noticed also an increase in the content of carotenoids in *Raphanus sativus* under saline conditions. Findings of the current research indicate also that accumulation of carotenoids content in EFB28 primed plants was higher than unbioprimed plants, thereby they performed better than the latter. This is supported by the fact that carotenoids are nonenzymatic antioxidants play multifunctional roles in oxidative stress tolerance by protecting the photosynthetic machinery through reacting with lipid peroxidation products to end the chain reactions, scavenging $^1O_2$ and generating heat as a byproducts, preventing the formation of singlet oxygen ($^1O_2$) by reacting with triplet chlorophyll ($^3Chl^*$) and excited chlorophyll (Chl^*), and dissipating the excess excitation energy, via the xanthophyll cycle (Gill and Tuteja 2010; Sharma et al. 2012; Das and Roychoudhury 2014).

Strengthened plant growth under stress arises from released soluble sugars which lead to turgor maintenance, stabilizing proteins and cell structures, carbon storage, osmoprotection, osmotic homeostasis as well as scavenging of free radicals (Bartels and Sunkar 2005; Sami et al. 2016; Thalmann and Santelia 2017). This trend is confirmed in our results which proved an increase in soluble sugars content in wheat seedlings from primed seeds under salt conditions. This same favourable effect was found on wheat (Zhang et al. 2016), and pea (Ghezal et al. 2016), and due possibly to an enhancement of α-amylase and/or β-amylase activity in primed seeds which gives rise to boosted starch degradation and sugar accumulation, which in turn results in higher respiration rates, seed viability, germination rates, and seedling establishment than for non-primed seeds (Savvides et al. 2016).

Exposure of wheat plants to salinity stress resulted in a drastic decline in protein content. This result is supporting the fact that high Na^+ affects plants by disrupting protein synthesis (Batool et al. 2014). Build-up of osmolytes for instance proline, glycine betaine (GB), soluble proteins and soluble sugars, is another tactic to overcome osmotic stress induced by salinity (Rasool et al. 2013; Ahmad et al. 2016). In our case results showed that, EFB28 seed biopriming increased the contents of total soluble proteins that may play a major role in plant salt tolerance, where the proteins

may function as a reservoir of energy or may be adjuster of osmotic potential in plants undergoing salinity (Torabi 2014). Our results were supported by Zhang et al. (2016) who reported that application of *Trichoderma longibrachiatum* increased the protein contents in the wheat seedlings grown under salt stress or non-saline stress, compared to the control.

Our results pointed out that proline accumulation in wheat seedlings obtained from EFB28 bioprimed seeds was comparatively higher as compared to the NaCl irrigated only plants as expected. In harmony with these findings, earlier studies in maize (Yesilyurt et al. 2018), rice (Rawat et al. 2012), and pea (Ghezal et al. 2016) reported that proline is augmented in bioprimed plants with *Trichoderma citrinoviride*, *Trichoderma harzianum,* and *Typha angustifolia* leaves aqueous extract, respectively, compared to salt-stressed only plants. Reportedly proline serves as an osmolyte for osmotic adjustment, contributes to stabilizing subcellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions primarily drought and salinity stress (Chinnusamy et al. 2005; Carillo et al. 2011; Chakraborty et al. 2013; Rasool et al. 2013; Batool et al. 2014; Gupta and Huang, 2014; Hasanuzzaman et al. 2017).

Stress-prone environments disturb the balance and enhance the production of ROS (Pang and Wang, 2008). Hence, it appears that a concerted action of ROS detoxifying enzymes including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) is required for scavenging process (Abogadallah 2010). Earlier studies on oleander (Kumar et al. 2017), maize and rice (Yasmeen and Siddiqui 2017) have suggested that response of plants to salt stress is associated with the induction of antioxidant enzymes. Nevertheless, the use of EFB28 additionally increased the free radical scavenging enzymes SOD, APOX, and CAT activity in wheat seedlings as compared to that without EFB28 priming, which was in accordance with the findings of Zhang et al. (2016) who demonstrated that the role of *Trichoderma longibrachiatum* in wheat was to mitigate NaCl stress by significant upregulation of the relative expression of SOD, POD, and CAT genes in these wheat seedlings. Baltruschat et al. (2008) demonstrated that a high-saline environment is well tolerated by salt-sensitive barley when previously inoculated with the mutualistic basidiomycete *Piriformospora indica.*

One of the final products of peroxidation of unsaturated fatty acids in phospholipids is malondialdehyde (MDA) that is responsible for cell membrane damage (Sharma et al. 2012). In this regard, salinity induced increase in lipid peroxidation as reported by Kumar et al. (2017) and Abdelgawad et al. (2016). Priming used in this study reduced significantly this accumulation. Similar results were observed in wheat (Rawat et al. 2011; Zhang et al. 2016), soybean (Khomari and Davari 2017) and pea plants (Ghezal et al. 2016). Cconsequently, a decrease in MDA content in primed seedlings in normal or salt conditions, suggesting a membrane protection.

Phanerochaete chrysosporium belongs to wood-decay fungi which possess the efficaciouscapacity to break down lignin to CO_2. In part, this ability is due to the fact that they secrete H_2O_2 and a family of peroxidases which catalyse the free-radical dependent oxidation and depolymerization of lignin (Aust 1990). No reports on the potential utility of *P. chrysosporium* in seed biopriming for reducing the effects of salt stress on plants have been found. Yet, a recent study Yan et al. (2018), reported that plants engineered to accumulate less lignin or xylan are more tolerant to drought and activate drought responses faster than control plants. This finding is an important because it demonstrates that modification of secondary cell walls does not inescapably render the plants robustness less in the environment. Gallego-Giraldo et al. (2011) also postulated that HCT (hydroxycinnamoyl transferase) downregulated alfalfa plants exhibit reduced lignin levels, massive upregulation of pathogenesis and abiotic stress-related genes, and enhanced tolerance to fungal infection and drought, might be explained by taking into account that as a result of impaired secondary cell wall integrity release of bioactive cell wall fragments and production of hydrogen peroxide take place.

Translating these results, one can conclude that the present research offers a new approach to alleviate salinity stress in wheat through seed biopriming with the salinity tolerant white-rot fungus *P. chrysosporium*.

Conclusion

This is the first report regarding the role of fungal biopriming by the salinity tolerant white-rot fungus *P. chrysosporium* (EFB28). Microbial seed priming helped in alleviating the inhibitory impact of salt stress on growth and physiological processes of the salt-sensitive wheat line Gemmeza 12. Bioprimed grains were better capable to develop into seedlings under salt stress by moderating photosynthetic pigments, osmolytes and antioxidant enzymes. Translating these findings, one can conclude that the present research opens a new way to ameliorate abiotic stress in wheat through seed biopriming with *P. chrysosporium*. In perspective, it is essential to persist iiin the study by examining seed bio-priming with *P. chrysosporium* influence on alteration of genes related to stress and seed yield.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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