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SEED BIOPRIMING WITH *PHANEROCHAETE CHRYSOSPORIUM* ENHANCES TOLERANCE OF WHEAT TO SALT STRESS THROUGH IMPROVEMENT OF *TAEXPB23* EXPRESSION

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ABSTRACT

Salt stress is one of the major abiotic stresses limiting the wheat productivity especially in arid and semi-arid regions. In our previous work, biopriming of wheat seeds with the fungus *Phanerochaete chrysosporium* strongly alleviate the salt stress response and improve the overall morphological and biochemical criteria of the plant. Thus, the objective of this study was to explore the mechanisms of salt tolerance of wheat in response to the fungal biopriming. The molecular expression of expansin proteins and the phytohormones levels of wheat under salt stress in response to fungal biopriming were estimated. The expression of expansin gene *TaEXPB23*, in wheat treated with 50 and 100 mM NaCl was increased by about 1.4 and 4fold, respectively, in response to biopriming with *P. chrysosporium* (EFB28) after 45 days of salt imposition. The levels of kinetin (KT), salicylic acid (SA), methyl jasmonate (MeJA), indole-3-acetic acid (IAA), and gibberellic acid (GA_3) in wheat grown at 150 mM NaCl, was increased by about 4, 6, 8, 12, and 24-fold, respectively, in response to biopriming with *P. chrysosporium,* comparing to control plants (without fungal priming). As well as, the level of abscisic acid (ABA) in wheat grown at 150 mM NaCl was increased by about 10-fold with the fungal biopriming comparing to control plants (without fungal priming). Thus, from the metabolic and molecular analyses, the expression of expansin gene *TaEXPB23* and concentration of phytohormones in wheat under high salt concentration, were strongly increased in response to fungal biopriming, that overall correlated with the tolerance of wheat to salt stress.

Key words: *Phanerochaete chrysosporium*, Phytohormones, Expansins, Salt tolerance.

Abbreviations: ABA, abscisic acid; CK, cytokinin; GA_3, gibberellic acid; MeJA, methyl Jasmonate**,** SA, salicylic acid; R_t, retention time; ND, not detected.

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INTRODUCTION

Seed priming, a re-echo of the ancient soaking technique, facilitates fast and even seedling emergence which is a fundamental demand for crop production particularly when undergoing stressful environments (Pál, 2018). Preconditioning of seeds with hydration followed by incubating with an expedient microorganism provided preventing the emergence of the radicle is referred to as 'biopriming'. Stimulation of phytohormones production, variation in secondary metabolites, gene expression alteration, resistance to stress, are all fingerprints in plants derived from bioprimed seeds (Sukanya *et al.*, 2018). Phytosymbiosis mediated stress relief involves both stimulation of host stress response systems, and biosynthesis of anti-stress compounds like phytohormones and 1-aminocyclopropane-1-carboxylate deaminase (ACC) by endophytes (Ali *et al.*, 2015). The former promote the growth of root hairs and increase total root area, easing the nutrients uptake (Singh *et al.*, 2011; Prasad *et al.*, 2016; Lata *et al.*, 2018). Plant hormones serve as vital integrators that link and reprogram the complex developmental and stress adaptive signalling transduction pathways (Golldack *et al.*, 2014). Faster regulation of hormones metabolism in a salt-tolerant rice variety led to specific morpho-physiological responses and growth recovery under salt stress was to faster regulation of hormones metabolism in the tolerant variety (Formentin *et al.*, 2018). Microbial phytohormones influence the metabolism of endogenous plant growth regulators and control root morphology upon exposure to abiotic stresses (Chakraborty *et al.*, 2015; Egamberdieva *et al.*, 2017). Regarding application of *P. chrysoporium* for improving plant's salinity tolerance, information is non-existent. However, *P. chrysoporium* proved to have an increased tolerance to salt stress over time due to its higher capacity for the production and/or retention of solutes (Venâncio *et al.*, 2017).

Cell wall proteins assume responsibility in controlling cell wall extensibility, which in turn facilitates cell enlargement and expansion. Expansin is a superfamily of plant proteins includes 32 and 53 genes in *Arabidopsis* and rice respectively. It composed of four

subfamilies termed as α-expansin (EXPA), ß- expansin (EXPB), expansin like A (EXPLA) and expansin like B (EXPLB). The precise function of both expansins like A and B is still unknown, while α-expansin and ß-expansin proteins are implicated in the cell wall enlargement through their role in wall stress relaxation (Cosgrove, 2005; Choi, 2006). Expansins are pH-dependent proteins that are well-known in loosening cell walls without hydrolysis via a non-enzymatic mechanism known as acid induced growth that results in sliding of the cell wall polymers or the aforementioned polymer creep in the plant cell wall (AbuQamar, 2014; Kuluev *et al.*, 2017). Interestingly, expansins and acid induced growth not only do relate to cell elongation, but they also play roles during various developmental and non-developmental processes. Plant responses to external stimuli such as light, submergence, drought, and salt stress have been found to be in large part mediated by expansin action and acid growth phenomenon (Choi, 2006; Marowa *et al.*, 2016). Most of hormone-regulated genes are involved in the fine-tuning of plants to withstand stressful conditions, for that reason earlier studies proposed that expansin expression is also controlled by phytohormones (Han *et al.*, 2015). In our previous work, biopriming of wheat grains with *P. chrysosporium* (EFB28) significantly alleviated the salt stress (Dief *et al.*, 2021). Thus, in an extension to our previous work, the main objective of this study was to explore the different metabolic and molecular responses of plants under salt stress bioprimed with the fungal isolate comparing to negative and positive control plants. The effect of the fungal biopriming on the concentration of the endogenous phytohormone and expression of the β-expansin gene "*TaEXPB23"* of wheat under various levels of salt stress were assessed.

MATERIALS AND METHODS

Experimental set up and stress application: A pot experiment was conducted from 30th November 2017 to 19th January 2018 in the greenhouse of the Department of Botany, College of Science, Zagazig University (Egypt). Grains of salt-sensitive bread wheat (*Triticum aestivum* L. cv. Gemmeza 12) were purchased from Wheat Research Department, Field Crops Research Institute (FCRI), Agriculture Research Center, Egypt. Loamy soil was collected from the soil surface (Sharkia governorate, Egypt). Seven treatments were used as follows, Salinity treatments; low salinity: 50 mM, moderate salinity: 100 mM and high salinity: 150 mM NaCl. Plant biopriming treatments: *P. chrysosporium* with zero salt, *P. chrysosporium* with 50 mM NaCl, *P. chrysosporium* with 100 mM NaCl, *P. chrysosporium* with150 mM NaCl. Negative control with zero salt and zero fungus were used**.** The plant samples were collected after 45 days. The experiment was laid out in Completely Randomized Design (CRD). A total of thirty two pots (four pots per treatment) were used in the current experiment.

Fungal inoculum and biopriming conditions were described in a previous study (Dief *et al.*, 2021). Soil with initial 10 % gravimetric water content was thoroughly mixed by hand with saline water (50, 100 and 150 mM NaCl) and the pots (23 × 17 cm in size) were then filled with this saline soil without leaching possibility. Twenty-five grains were sown in each pot. Ten days after sowing, the seedlings were thinned to twenty per pot. The weight of the pots was recorded before start of experiment and during it. The pots were weighed daily, and the water loss was replaced by adding water to achieve final water content (25% on dry soil basis). The weight of pots was fixed from first day of experiment till the end of the experiment (El-Hendawy *et al.*, 2005a; El-Hendawy *et al.*, 2005b). Samples of five leaves (0.1 g) from each replicate were harvested at the end of the experimental period (after 45 days), and used for biochemical and growth parameters analyses.

Two-step sequential extraction for determination of endogenous hormone concentrations: Indole-3-acetic acid (IAA), gibberellic acid (GA_3), kinetin (KT), abscisic acid (ABA), methyl jasmonate **(**MeJA) and salicylic acid (SA) were all of standard purity (Sigma) were used without further purification.

Extraction: Quantification of IAA, GA_3, KT, and ABA contents was performed in the same sample for both control and stress-grown plants. After 45 days, 0.5 g of leaf sample was crushed in liquid nitrogen, homogenized and extracted for 12 h with 10 mL 80% cold methanol in absence of light at 4°C. The extract was centrifuged at 5,000 rpm and 4°C for 15 min and the supernatant was collected. Then, fresh, cold methanol was poured into the residue, that was extracted again with the same aforementioned steps (Chen and Yang, 2005, El-Sayed 2009, El-Sayed *et al*., 2015a,b,c, d, 2016).

Total methanolic extract was dried under forced air and dissolved in 10 mL methanol. Determination of methyl jasmonate **(**MeJA) and salicylic acid (SA) contents was conducted as following: a half gram of leaf tissues (after 45 days) was ground in liquid nitrogen, homogenized, then extracted for 12 hours with 10 mL 80% cold aqueous methanol. Post-centrifugation (15 min at 1,400 rpm), extraction for the residue was repeated again but with 0.5 mL 100% methanol containing 10% ethyl acetate and 1% acetic acid (V/V). The two extracts were combined and used for quantification of free SA and MeJA (Mouekouba *et al.*, 2014, El-Sayed *et al*., 2010).

Chromatographic procedure: All previously mentioned phytohormones were separated at residue analysis unit, reference lab for veterinary quality control on poultry production, Animal health research institute, Egypt.

Separation was conducted using HPLC chromatography with a wavelength of UV-254 nm, a 250 mm x 4.6 mm, 5 µm, C18 Agilent 1200, 100% methanol and a flow rate of 1.0 mL/min, a column temperature of 25°C and sample volume 50 µl. Whereas chromatographic separation of ABA was carried using 0.6% acetic acid and a flow rate of 0.8 mL/min (El-Sayed *et al*., 2020a, b).

Analysis of Gene Expression: 100 mg frozen leaves were collected from 45-day-old unprimed and bioprimed wheat seedlings. The leaves were pulverized to a fine powder and the total RNA was extracted using Trizol (invitrogen) following the manufacturer's instructions. RNA concentration was measured by Quantus™ Fluorometer. Expression of the selected gene *TaEXPB23* (accession number in GenBank: AY260547) in leaves was analyzed by real-time quantitative reverse transcriptase (qRT)-PCR using SYBRGreen in a detection system (Agilent technologies stratagene MX3005p). The transcript of tublin gene (AK331512) of wheat was used as an internal control for normalization in the qRT-PCR reactions **(Table 1)**.

qRT-PCR was performed at Scientific and medical research centre 'ZSMRC' of Zagazig Faculty of medicine using one step RealMOD ™ Green qRT-PCR mix (iNtRON). Reactions of 2 µl of total RNA (10 ng), 2 µL of 300 nM forward and reverse primers, 7.5 µl of RealMOD™ Green qRT-PCR mix (1X), 0.3 µl of qRT-PCR Enzyme Mix (1X) and 1.2 µl of nuclease free water for a final reaction volume of 15 µl. Reactions were under the following conditions: 42°C for 15 min, 95°C for 10 min, then 40 cycles of 15 s at 95°C for denaturation and 60 s at 55°C for annealing / extension and plate read. Relative expression of *TaEXPB23* was calculated using delta delta Ct method (Khan *et al*., 2017, El-Sayed *et al*., 2016).

Statistical analysis: The obtained results were represented by the means ± SD. The statistical analyses were conducted by one-way ANOVA, and Tukey's HSD test was determined using CoStat software (CoStat 2005) program. When the ANOVA F statistic was significant, means by Duncan's multiple range test were compared at a p value ≤ 0.01 (Cochran, 1941).

Table 1: Sequences of primers used to measure wheat gene expression by qRT- PCR.

Gene Name	Sequence (5′ -3′)
TaEXPB23	**Forward: CATGCGCATCACCAACGAGT**
Reverse:	**TGGACGATGGAGCGGTAGAAG**
Tubulin	**Forward: ATCTGTGCCTTGACCGTATCAGG**
Reverse:	**GACATCAACATTCAGGACACCATC**

RESULTS AND DISCUSSION

Changes in Hormonal Contents: Phytohormones play key roles in helping the plants to cope with adverse environmental conditions. To study the role of seed biopriming in plant response to salt stress we analysed the levels of indole-3-acetic acid, gibberellic acid, kinetin, abscisic acid, methyl jasmonate and salicylic acid. The data related to the effect of seed biopriming with *P. chrysosporium* plus different concentrations of NaCl on phytohormone profile was depicted in **Figure 5**. In comparison to control (without fungal biopriming), the levels of IAA, GA_3 and KT increased linearly in EFB28 bioprimed plus NaCl stressed plants with increasing levels of salt treatment. In a similar fashion, a sharp and dose dependently increase in the production of stress related hormones (ABA, SA and MeJA) was observed in EFB28 bioprimed seedlings exposed to salt stress.

Results showed that salt stress along with seed biopriming influenced the IAA synthesis. In EFB28 treated seedlings exposed to 50 mM NaCl, a three-fold increase in IAA synthesis was observed compared with control plants. In response to salt stress (100 and 150 mM NaCl), endophyte-primed seedlings showed production of IAA up to 5 and 12-fold, respectively, when compared with control. Our results of linear increase in IAA level in bioprimed salt-stressed plants with increasing doses of salt support the view that inoculation of plants by endophytic microorganisms stimulate auxins production which has been implicated in an abiotic stress mitigation. Contreras-Cornejo *et al.* (2014) suggested that *Trichoderma virens* and *T. atroviride* may aid arabidopsis better withstand salt stress by enhancing the plant IAA level. Further, shoot and root growth and dry matter increased significantly when salt grown cotton plants treated with root-associated IAA-producing bacterial strains *Pseudomonas putida* and *Pseudomonas chlororaphis,* suggesting that they play a role in induction of salt stress tolerance (Egamberdieva *et al.*, 2015).

In the absence of NaCl, GA_3 contents in seedlings bioprimed with fungal endophyte and untreated seedlings were almost similar. At 50, 100 and 150 mM NaCl, endophyte-primed seedlings showed GA_3 production up to 5, 10 and 24-fold, respectively, when compared with control. Gibberellins function to break seed dormancy, form floral organs, and to induce lateral shoot growth (Egamberdieva *et al.*, 2017). Results supporting what has been shown here, are those by Hamayun *et al.* (2017), who reported mitigation of salt stress by gibberellins (GAs) producing basidiomycetous endophytic fungus *Porostereum spadiceum* in soybean seedlings. In another work, Ahmad *et al.* (2010) showed also that gibberellin-producing endophytic fungi, *Penicillium* sp. and *Aspergillus* sp. significantly promoted the plant height of a GA-deficient rice mutant, and *Echinocloa crusgalli.* Additionally, other researchers

have reported that *Fusarium verticillioides* pre-treatment of soybean seeds is a potent strategy to boost soybean plant growth under salinity stress conditions via secretion of wide assortment and varied concentrations of gibberellins (GAs) (Radhakrishnan *et al.*, 2013).

EFB28 biopriming caused induction of KT synthesis in salt affected plant groups. At 50, 100 and 150 mM NaCl, priming with the endophytic fungus *P. chrysosporium* exhibited a 0.5, 2 and 4-fold increase in synthesis of KT when compared to control **(Figure 2)**. The results of gradual increase in kinetin level in bioprimed salt-stressed plants with increasing level of salt, proposed that cytokinins (CKs) contribute to organizing responses to environmental stresses. In a study conducted by Iqbal and Ashraf (2005), the beneficial effects of kinetin priming on water use efficiency and photosynthetic rate under salt stress have been reported. In another work, researchers demonstrated that salt-induced leaf senescence could be alleviated in ryegrass by exogenous application of 6-benzylaminopurine (Ma *et al.*, 2016). Golan *et al.* (2016) suggested that growth of *Arabidopsis thaliana* plants can be sustained by CK under salinity and drought stresses by activating growth-related gene expression while preventing the expression of premature senescence related ones.

Salt treatments (50, 100 and 150 mM) NaCl caused MeJA production up to 3, 5 and 8-fold rise, respectively, in bioprimed wheat seedlings when compared with bioprimed plants without salt treatment. Likewise, a 3, 5 and 6-fold increase in contents of endogenous SA were recorded in EFB28 bioprimed seedlings exposed to 50, 100 and 150 mM NaCl respectively when compared with the control **(Figure 3)**. Biopriming treatment again affected positively MeJA level. Transgenic expression of the wheat *TaAOC1,* a gene encoding an allene oxide cyclase enzyme, in arabidopsis lifted JA levels up and promoted salt tolerance, implying that JAs regulate salt endurance in a positive way (Zhao *et al.*, 2014). Foliar application of methyl Jasmonate (MeJA) significantly increased fitness of tomato plants by significantly increasing the contents of free proline, total soluble protein, free amino acids and by enhancing the enzymatic activity of both catalase (CAT) and peroxidase (POX) (Manan *et al.*, 2016). Similarly, while working with soybean, MeJA abolished the undesirable outcomes of salt constrain on growth, chlorophyll and proline content, leaf photosynthetic and transpiration rate (Yoon *et al.*, 2009).

In response to salt stress (100 and 150 mM NaCl), bioprimed seedlings showed production of ABA at a level of 7 and 10-fold, respectively, compared to control. It was obvious that salt stress positively regulated the synthesis of ABA in a dose-proportional manner inseedlings grown from both bioprimed and untreated seeds. At 100 and 150 mM NaCl production of ABA was 2-fold higher in seedlings bioprimed with EFB28 than that of the corresponding unprimed ones but salt subjected **(Figure 4).** In support of our results, enhanced ABA accumulation can regulate the plasma membrane Na^+/H^+ antiporter (H^+-ATPase type vacuolar pump and vacuolar pyrophosphatase) and water uptake during salt stress. Hence, ABA helps to enhance the cell ion selectivity and promotes vacuolar Na^+ import and export (Zheng *et al.*, 2020). It has been shown that ABA-deficient mutants performed poorly under salinity stress (Xiong *et al.*, 2001). For example, *OsNCED5* induction takes place in rice as a result of exposure to salt stress. Accordingly, *nced5* mutant exhibited diminished ABA levels, concomitantly with a drop in tolerance to salt stress. On the other hand, overexpression of *NCED5* augmented ABA levels and improved salt tolerance (Huang *et al.*, 2019).

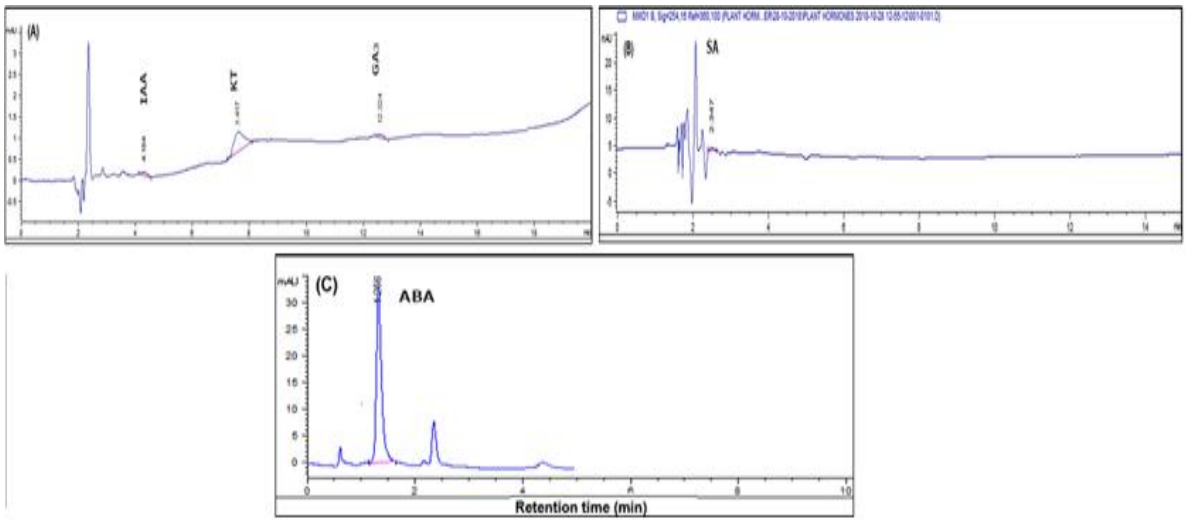

Figure 1: HPLC chromatogram showing the effect of biopriming on endogenous phytohormone content (IAA, Rt =4.109; KT, Rt=7.356; GA_3, Rt=12.326; SA, Rt =2.475; ABA, Rt =1.253) of 45-day old control wheat seedlings.

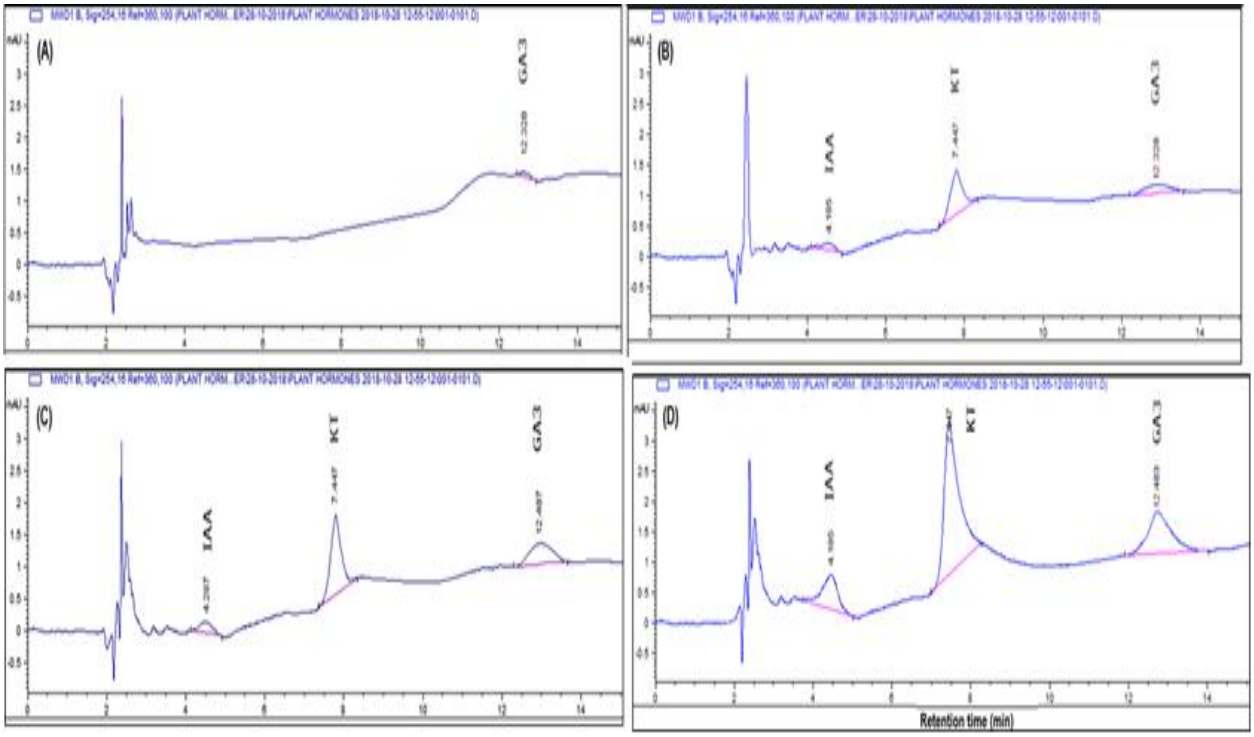

Figure 2: HPLC chromatogram showing the effect of biopriming on endogenous phytohormone content (IAA, Rt =4.109; KT, Rt=7.356 and GA_3, Rt=12.326) of 45-day old wheat seedlings. (A) Seed bioprimed plants, (B) Seed bioprimed + 50 mM NaCl, (C) Seed bioprimed + 100 mM NaCl, (D) Seed bioprimed + 150 mM NaCl. Note. 50 mM NaCl, 100 mM NaCl, 150 mM NaCl; ND (results not shown).

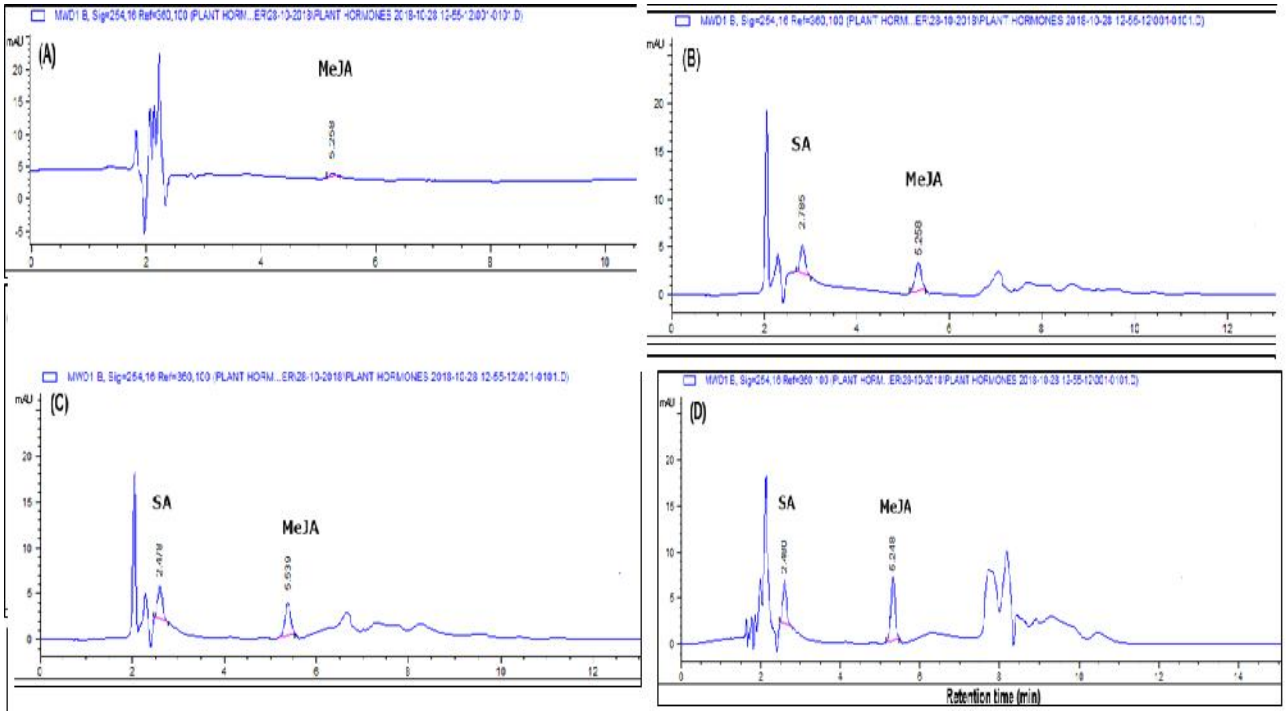

Figure 3: HPLC chromatogram showing the effect of biopriming on endogenous phytohormone content (SA, Rt =2.475 and MeJA, Rt =5.317) of 45-day old wheat seedlings. (A) Seed bioprimed plants, (B) Seed bioprimed + 50 mM NaCl, (C) Seed bioprimed + 100 mM NaCl, (D) Seed bioprimed + 150 mM NaCl. Note. 50 mM NaCl, 100 mM NaCl and 150 mM NaCl; ND (results not shown).

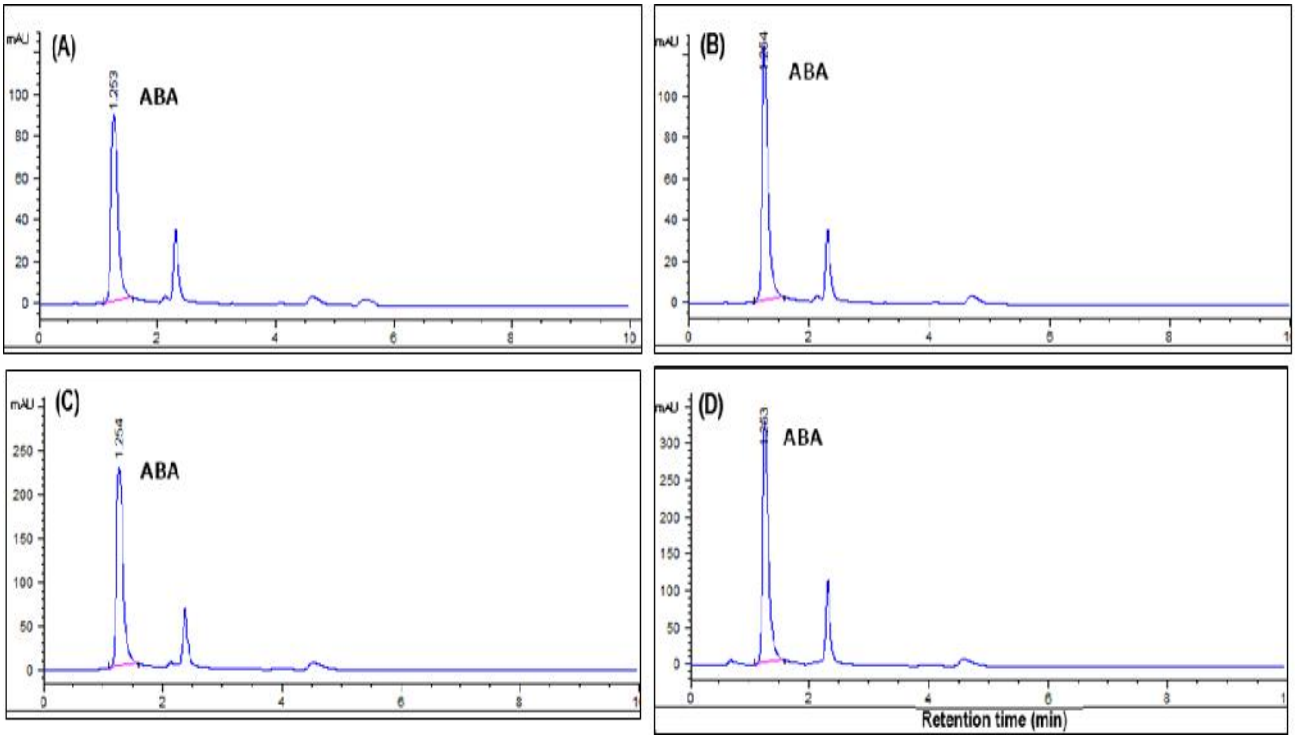

Figure 4: HPLC chromatogram showing the effect of biopriming on endogenous phytohormone content (ABA, Rt =1.253) of 45-day old wheat seedlings. (A) 100 mM NaCl (B) 150 mM NaCl, (C) bioprimed + 100 mM NaCl, (D) bioprimed + 150 mM NaCl. Note. 50 mM NaCl and bioprimed + 150 mM NaCl; ND (results not shown).

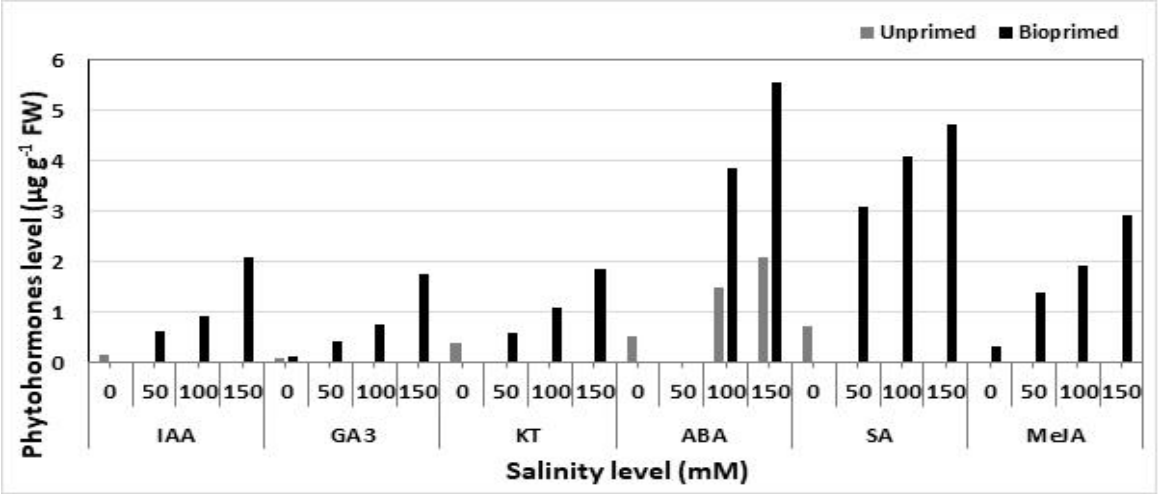

Figure 5: Effect of seed biopriming under various concentrations of NaCl (mM) on leaf concentrations (µg g^{-1} FW) of endogenous indoleacetic acid, gibberellic acid, kinetin, abscisic acid, salicylic acid, methyl jasmonate in wheat plants either derived from seeds unprimed or bioprimed with *P. chrysosporium* (EFB28).

Expression pattern of *TaEXPB23* in wheat: Notably, changes in the levels of *TaEXPB23* expression were dose-dependent under NaCl stress. After 45 days of stress, compared to the control, transcript levels of *TaEXPB23* were enhanced with the application of EFB28 without salt stress at a rate of 50%. In wheat plants pre-inoculated with EFB28 then exposed to NaCl, *TaEXPB23* expression increased by 1.4 and 4-fold, relative to those treated with 50 and 100 mM NaCl alone, respectively, after 45 days of treatment. Although, at 150 mM in the EFB28-primed plants, *TaEXPB23* expression recorded

the highest level it was not detectable in plants subjected to 150 mM NaCl only **(Figure 6)**.

The results in figure 6 suggest that the upregulation of the *TaEXPB23* gene may be partly responsible for the increased tolerance in wheat plants derived from EFB28-bioprimed plants. One of the early comebacks that plants incite towards adaptability to saline environments is regulation of both growth and expression of key genes differentially, resulting in either growing away from hostile conditions or towards more favourable conditions. On one hand, cell walls are sensors for environmental stresses and serve to communicate with other parts of the cell. Expansins (EXPs), cell wall-loosening proteins, take charge of all phytohormone-controlled responses in which the cell wall is altered so as to make it more extensible (Choe and Cosgrove, 2010).

As our findings highlight**,** fungal biopriming altered endogenous level of plant growth regulators which orchestrate increased salt tolerance in host plants. From our results, one can also notice a connection between the increase in levels of plant growth regulators and the increase in the expression level of *TaEXPB23* in wheat seedlings derived from bioprimed grains. Auxin, for instance, is responsible for apoplast acidification through pumping out protons (H^+) to the wall matrix via stimulating the activity of plasma membrane H^+-ATPase proton pumps. This auxin-stimulated acidic pH is prerequisite to trigger EXPs which dissociate polysaccharide networks by means of severing and loosening links among cellulose microfibrils (CMFs) and non-cellulosic polysaccharides such as xyloglucans (XyGs). Accordingly, CMFs glide and split up, boosting wall loosening, hydration and swelling (Majda and Robert, 2018). *PtrEXPA3* and *PnEXPA3* encoding cytokinin- and auxin-regulated expansins are engaged in promoting cell expansion in the leaves of poplar (Kuluev *et al.*, 2017). Additionally, exogenous ethylene and auxin stimulated the expression of two Arabidopsis expansin genes (*AtEXP7* and *AtEXP18*) (Cho and Cosgrove, 2002, El-Sayed *et al.*, 2021). Exogenous application of 2,4-dichlorophenoxy-acetic acid showed a substantial stimulation of LeExp2 mRNA (up to 15-fold more abundant) in etiolated tomato hypocotyls (Caderas *et al.*, 2000). Upon addition of cytokinin, a soybean β-expansin gene (*GmCim1*) accumulated 20-60-fold in cytokinin-starved soybean suspension cultures, suggesting that its protein governs cell wall dynamics in response to cytokinin. Cytokinin has long been known to stimulate plant cell division and promote shoot organogenesis. Changes in the cell wall are critically involved in cytokinesis and cell plate formation and control the size and shape of plant cells. Cytokinin regulates the expression of genes involved in cell wall extensibility (Downes and Crowell, 1998, Patel *et al*., 2016). Lee *et al.* (2008) showed that *MaEXPA1* mRNAs accumulated in wild-type *Melilotus alba* (white sweetclover) and *Masym3* mutant roots in response to inoculation with the non-nodulating Nod^-/$pTZS^+$ *Sinorhizobium meliloti* strain (pTZS is a plasmid carrying a constitutive trans-zeatin secretion (*tzs*) gene from *Agrobacterium tumefaciens*).

GA prompts apoplastic acidification, which activates in turn expansin-facilitated wall relaxation and subsequent stem elongation (Cho and Kende, 1997a; b; c). Ochiai *et al.* (2013) stated that MeJA raised the expression of *EgEXPA2*, *EgEXPA3* and *EgXTH1* mRNA and the accrual of the cell wall loosing proteins: expansin and xyloglucan endotrans-glycosylase/ hydrolase (XTH) in petals of *Eustoma grandiflorum* which resulted in early flower opening. In a study, researchers observed that both osmotic stress and ABA amplified the expression of expansin protein in both drought sensitive 921842 and resistant HF9703 wheat lines and that ABA content was improved under osmotic stress, suggesting that the increase of expansin activity urged by osmotic stress was initially related to ABA build-up (Zhao *et al.*, 2012).

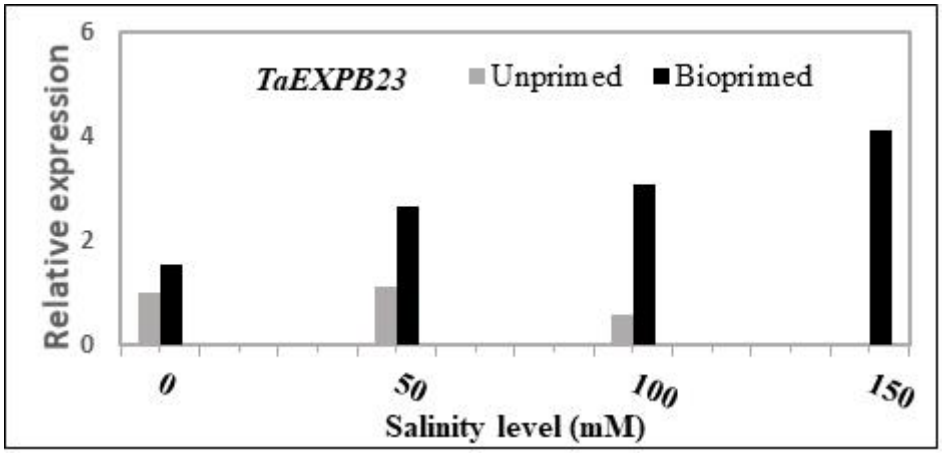

Figure 6: Effect of EFB28 seed biopriming under various concentrations of NaCl (0, 50, 100, 150) mM on relative expression of *TaEXPB23* after 45 days of salt treatment (DAT) in wheat plants.

In a study conducted by Han *et al.* (2015), it has been reported that tolerance to salt stress conferred via overexpression of *TaEXPB23* in tobacco through boosting water retention ability and declining osmotic potential. In a study, on tobacco, drought tolerance has also been achieved through overexpression of *TaEXPB23* (Li *et al.*, 2011). Another study further showed that over-expression of *TaEXPB23* improves oxidative stress tolerance in transgenic tobacco plants (Han *et al.*, 2015). In consonance with those findings, we conclude that expression pattern of *TaEXPB23* depend to a large extent to phytohormones.

Conclusion: In a previous study, we have showed that the salinity tolerant white rot fungus *P. chrysosporium* assisted via fungal biopriming in alleviating the inhibitory impact of salt stress on the salt-sensitive wheat line Gemmeza 12. Bioprimed grains were better capable to develop into healthy seedlings under salt stress (up to 150 mM NaCl) by moderating photosynthetic pigments, osmolytes and antioxidants. Here, involvement of *TaEXPB23* in increased fitness of wheat plants to salt stress through seed biopriming with *P. chrysosporium* was mediated by phytohormones. One can infer that the present research opens a new way to ameliorate abiotic stress in plants by means of seed biopriming with *P. chrysosporium*. In perspective, seed bio-priming with *P. chrysosporium* is still require in-depth study to investigate its influence on alteration of stress-related genes and seed yield.

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