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# Exploration of modify genes expression in wheat via the enhancement of *Bacillus Subtilis.*

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**ABSTRACT**

*Bacillus subtilis* (BS) is a plant growth promoting rhizobacteria (PGPR) with outstanding abilities to enhance plant growth and strengthen resistance to the environmental stresses. In the present investigation, we sought to determine whether, inoculation with *B. subtilis* could alleviate the adverse effects on promotion wheat plant growth. Two wheat cultivars (*Triticum asitivum* L.) nominated (Beni Suef and Salambo) were grown up to ninety days after germination as *B. subtilis* treated and control. Seven different yield related traits were investigated as plant growth detectors. The traits were, plant height (P.H.), number of tillers (N.T.), shoot fresh weight (S.F.W.), number Leaves of plant (N.L.P.), total root length (T.R.L.), weight of the youngest elongate blade leaf (Y.E.B.) and number spikes of plant (N.S.P). The results indicated that all the aforementioned yield related traits were increased with different proportions as response for root inoculation with *B. subtilis* as compared to the non-inoculated plants of the two cultivars. These results reflected the great effects of root inoculation with *B. subtilis* on wheat plant growth and productivity. The performances of (CDPKs), (PEPCs) and (P5CS) genes expression were evaluated via semi quantitative RT-PCR. The results confirmed that wheat root inoculation with *B. subtilis* led to increasing gene expression compare to the non-inoculated ones. We postulate that the present investigation provides a more robust topology of the root inoculation with *B. subtilis* and its ability to prompting some important genes to improve productivity in wheat plant.

**Keywords**: *Bacillus subtilis*, *Triticum asitivum* L., yield related traits, inoculation, CDPKs, PEPCs, P5CS, genes expression.

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## INTRODUCTION

The most widely grown crop in the world is the wheat (*Triticum* spp.), provides 20% of the food calories and daily protein for 4.5 billion people [1]**.** The microbial based fertilizers has been increasing worldwide due to the deleterious effects which generated by the excessive and/or improper application of chemical on the environment [2]**.** Rhizosphere colonizing bacteria can control soil borne diseases and promote plant growth include plant growth promoting rhizobacteria (PGPR), which are a great importance in applied microbiology. The important member of PGPR is *Bacillus* spp. which has been used commercially as biocontrol agents and biofertilizers [3]. *B. subtilis* has the ability to form a tough, rod-shaped, protective endospore, allowing the organism to tolerate extreme environmental conditions [4]. In the context, [5] discussed ability of some root colonizing bacteria to make the phytate phosphorus in soil available for plant nutrition under phosphate-starvation conditions might contribute to their plant-growth-promoting activity. In principle, phytate is the main form of phosphorus storage in plant seeds. It is improving phosphorus bio-availability, recently they introduced into the cytoplasm of cells a characterized phytase from *Bacillus subtilis* [6]. One of the most important cellular ionic species is calcium ($Ca^{2+}$), which function as second messengers in many biological processes aspects and stimuli in animals and plants [7]. When calcium concentration changes in the cells, calcium dependent protein kinases (CDPKs) play important roles in signaling pathways for various stress responses and disease resistance [8]. The gene family of CDPK comprises Ser/Thr protein kinases organized in four subgroups. CDPKs have a conserved molecular structure, consisting of CDPK activation domain (CAD) and variable N-terminal domain, fused to a Ser/Thr kinase domain, [9]. With respect to PEPC (phosphoenolpyruvate) carboxylase, it is an important enzyme situated at a crucial branch point in plant carbohydrate metabolism. Extensive biochemical and genetic studies of photosynthetic PTPC isozymes of $C_4$ and CAM leaves have elucidated catalytic and regulatory features that are well suited for their function as an atmospheric $CO_2$-concentrating mechanism that reduces photorespiration to improve overall photosynthetic efficiency up to 2 fold relative to C3 leaves [10]. With regards to Proline effect, it is an osmo-protecting molecule that accumulates in many organisms, in response to low water stress and salinity. In plant cells, proline accumulation is increases cell potential, subcellular structures and stabilizes proteins membranes. Therefore, P5CS play an important role in the proline synthesis pathways and is regulated by abscisic acid (ABA) [11]. Actually, P5CS gene expression increased by 1.34 folds under salt stress [12]. Recently, [13] explore on P5CS and show that plants exposed to biotic and abiotic stresses strongly express the gene. Based on all of the foregoing, differences in the transcription levels at stage of reproductive between drought sensitive and drought tolerant genotypes therefore identify genes important in enhancing drought tolerance [14]. The powerful technique which capable of accurately quantitative mRNA expression levels is real-time quantitative RT-PCR (qPCR), which most widely used for studying gene expression. Selecting appropriate controls or normalization factors is an important aspect of qPCR to account for any differences in starting cDNA quantities among samples during expression studies [15].

Microorganisms such as endorhizosphere and rhizosphere are widely used to modification of plant response at gene expression level to help plants tolerate abiotic stresses. [16]. The expressions of three genes with potentially regulatory functions were investigated from the arbuscular mycorrhizal fungus Glomus intraradices in symbiosis with barley [17]. Sandhya *et al*., [18], reported that PGPR strains can be used for crops grown in stressed ecosystems as inoculants. Other studies, such as [19, 20], whose evaluate the gene expression of CDPK, PEPCs and P5CS genes which were increased in the inoculated plants with mycorrhiza as compared to non-inoculated ones in Barley. Recently [21] evaluate the impact of *Bacillus subtilis* gene expression using real-time PCR. The results indicated that it is a sensitive and quantitative technique to measure the expression profiles.

In the present investigation, we sought to determine a new basis for the ongoing discussion about (1) study the effect of *Bacillus subtilis* with wheat root inoculation in some yield related traits (2) discuss the gene expression changes in the genes conferring CDPKs, proline-5 carboxylate synthesize (P-5CS) and phosphoenol pyrovate carboxylases (PEPCs) in response of wheat plants to their inoculation with *Bacillus subtilis* using SqRT-PCR.

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## MATERIALS AND METHODS

### Sandy culture experiment

A greenhouse trial designed as a factorial experiment (completely randomized type) with four replications was conducted in two wheat cultivars (*Triticum aestivum* L.) nominated Beni Suef and Salambo, which were obtained from Agriculture research center. While the inoculants of *Bacillus subtilis* strain was provided by biofertilizers unit, faculty of agriculture, Ain Shams University.

Seeds of wheat cultivars were surface sterilized and bacterized with talc based formulation (108 cells/g) of bacterial strains. Each pot filled with two kg of sterile soil and washed with sterilized water and mixed with equal amounts of *Bacillus subtilis* inoculants for the treated plants. Both inoculated and uninoculated treatments were replicated four times, maintaining three plants per pot. The plants were grown up to 120 days after germination as *Bacillus subtilis* treated and none treated ones (control).

In the end of the experiment several parameter related to plant growth was measured to detected the effect of wheat roots inoculation with *Bacillus subtilis* via plant height (P.H.), number of tillers (N.T.), shoot fresh weight (S.F.W.), number Leaves of plant (N.L.P.), Total root length (T.R.L.), weight of the youngest elongate blade leaf (Y.E.B.) and number spikes of plant (N.S.P). Data were obtained and statistically analyses according to [22].

### Molecular studies:

Samples of leafs wheat plants were collected from *Bacillus subtilis* inoculated and non-inoculated ones (control) of the two cultivars under investigation for the purpose of RNA extraction.

### RNA extraction from Plant Cells and Tissues:

Total RNA of fresh wheat tissue was extracted using the Gene JET Plant RNA Purification Mini Kit (Thermo Scientific, EU) according to the instructions of manufacturer. The cDNA was synthesized from the total RNA using Revert-Aid First Strand cDNA Synthesis Kit (Thermo Scientific).

### Semi-quantitative Reverse transcription-PCR (SqRT-PCR) for (CDPKs), (PEPCs) and (P5CS) genes.

RNA of each of the four aforementioned samples was reverse transcribed (RT), to produce the first strand of cDNA in the presence of 5 mM $MgCl_2$, 1X PCR Buffer, 1 mM dNTPs, 25 u MuLV Reverse Transcriptase, and 4 u RNA-guard Ribonuclease inhibitor, the mixture prepared as described three times in three different PCR tubes and 2.5 µl of 20 P mol of CDPKs reveres primer with the following sequence (AATTGATGGCCATGG CCTGACTTTC) was added to the mixture in one of the three PCR tubes and to the second tube 2.5 µl of 20 P mol of PEPCs reveres primer with the following sequence (GCCGGCTTGCTCGTGTCC AT), finally .5 µl of 20 P mol of P5CS reveres primer with the following sequence (GTAAAGCGTATCCGCACTAACGC) was added in a final reaction volume of 30 µl in each tube. Reactions were carried out at 42 °C for 30 min, followed by a 10 min step at 94 °C to denature the enzyme, then was cooling at 4 °C.

To assay Real time PCR quantification of cDNA encoding for CDPKs, PEPCs and P5CS. One µl of cDNA of the four aforementioned samples was used as template in the reaction mix, in a final volume of 25 µl in all assays. Conventional PCR, using CDPKs, PEPCs and P5CS forward and reverse primers with the following sequences [CDPKs forward (TGAGTAAGGCCGACAAGG AGGATA), reverse (AATTGATGGCCATGGCCTGACTTTC)] and [PEPCs forward (TGGCCCCACTCATCTTGCTATCTT), reverse (GCCGGCTTGCTCGTGTCCAT)] and [P5CS forward (ATTCCGACCTTGTGTAACCGGC), reverse (GTAAAGCGTATCCGCACTAACGC)], respectively, were employed to define the detection limit of the assay.

Cycling was carried out in a Stratagene Mx-3000 Real-time PCR system which allows the detection of most commercially available dyes including FAM, SYBR® Green I, TET, HEX™, JOE™, VIC™, TAMRA™, TexasRed®, ROX™, Cy5™, Cy3™ and ALEXA Fluor® 350. The system supports 96-well plate format and can perform multiple sub experiments up to four dyes in the same well. Bioron product, SYBR® Green I Real Time QPCR (cat No.

119205) master mix for (100 rcs) detection protocol was used in this investigation as described in Bioron manual.

**Quantification of CDPKs, PEPCs and P5CS genes expression**

sqRT-PCR quality and concentration was measure by use of a Quawell Q5000 UV-Vis spectrophotometer (V2.1.4, USA).

## RESULTS AND DISCUSSIONS

**Assessment of *Bacillus subtilis* Effect on Plant Growth**

Much attention has been given to the use of plant growth-promoting rhizobacteria (PGPR) as biofertilizers is of increasing interest due to economic and biological control agents for plant growth. *Bacillus subtilis* causes changes in plant growth and production. The growth of two cultivars under investigation Beni Suef and Salambo were evaluated as response for *Bacillus subtilis* inoculation and control (non-inoculated plants) in three replications.

The following yield related traits were scored and used as detectors for plant growth changes as response for *Bacillus subtilis* inoculation. In general plant growth of the two cultivars under investigation was elevated as response for root inoculation with *Bacillus subtilis*. The plant response for the inoculation with *Bacillus subtilis* was varied on the yield related traits level.

**Yield related traits**

The results of the investigated yield related traits are shown in (Table 1). The results of Plant height and total root length were increased from (50 to 56) and (29.75 to 36) cm in cultivar Beni Suef inoculated plants compare to the control (non-inoculated plants) under *Bacillus subtilis* inoculation with increasing of 12 and 21%, respectively. While in Salambo cultivar the results of plant height and total root length were increased from (29.25 to 42.5) and (8.75 to 13.5) cm in the non-inoculated plants to the *Bacillus subtilis* inoculated plants with percentage of 45.3 and 54.29%, respectively. These results indicated that Salambo plants had a greater response for *Bacillus subtilis* inoculation than the other cultivar Beni Suef in plant height. Whereas, in total root length, both cultivars Beni Suef and Salambo plants have convergent response for *Bacillus subtilis* inoculation.

**Table (1): Seven yield-related traits among two wheat cultivars, where C mean (Control), T (Inoculated) and % the percentage of increase.**

| Cultivars | Plant height | | Total root length | | Number of tillers/plant | | Average number of spikes/plant | | Shoot fresh weight | | Numbers of leaves/plant | | Weight youngest elongate blade leaf | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beni Suef | C | 50 | C | 29.75 | C | 5.75 | C | 3.75 | C | 8.2 | C | 24.25 | C | 0.24 |
| | T | 56 | T | 36 | T | 8.5 | T | 7 | T | 8.77 | T | 38.25 | T | 0.30 |
| | % | 12 | % | 21.01 | % | 47.8 | % | 86.67 | % | 7.01 | % | 57.73 | % | 25 |
| Salambo | C | 29.2 | C | 8.75 | C | 4 | C | 1.5 | C | 2.53 | C | 12.5 | C | 0.1 |
| | T | 42.5 | T | 13.5 | T | 5 | T | 4.5 | T | 4.7 | T | 26.25 | T | 0.1 |
| | % | 45 | % | 54.29 | % | 25 | % | 200 | % | 85.8 | % | 110 | % | 0 |

With respect to the number of tillers/plant and the average number of spikes/plant, the results were increased from (5.75 to 8.50) and for (3.75 to 7) in cultivar Beni Suef inoculated plants compare to the control (non-inoculated plants) under *Bacillus subtilis* inoculation with increasing of 47.82% and 86.67%, respectively. In contrast, Salambo cultivar had less response for *Bacillus subtilis* inoculation than cultivar Beni Suef in number of tillers/plant (4 to 5), respectively, with percentage of 25%. Whereas, the average number of spikes/plant in Salambo cultivar was increase from 1.50 (non-inoculated plants) to 4.50 (inoculated plants) with an average of 200%. These results reflected that the effects of *Bacillus subtilis* inoculation on the number of wheat spicks/plant, led to increase in seed yield production in wheat.

Regards to shoot fresh weight and the numbers of leaves/plant, the results were increased from (8.2 to 8.77 gm) and for (24.25 to 38.25 gm) in cultivar Beni Suef inoculated plants compare to the control (non-inoculated plants) with percentage of 7 and 57.73%, respectively. On the other side, the results of cultivar Salambo in those traits ranged from 2.53 to 4.7 and for 12.50 to 26.25 gm, with percentage of 85.77 and 110%, respectively. These results reflected that the increase in those traits was greater in cultivar Salambo than cultivar Beni Suef.

On behalf to the average of Weight youngest elongate blade leaf, the result was ranged from 0.24 to 0.30gm in cultivar Beni Suef with an increasing of 25%. However, the result of cultivar Salambo was the same in the non-inoculated plants and in the inoculated. These results indicated that the increasing in the trait was occurred only in cultivar Beni Suef as response for the inoculation with *Bacillus subtilis*. Several previous studies confirmed that, inoculation with PGPR has been found effective under drought stress environment to increase productivity [23]. Generally, it possible to conclude that, wheat root inoculation with *Bacillus subtilis* resulted in an increase of plant growth in form of increase the plant height, number of tillers/plant, shoot fresh weight, root dry weight, number of spicks/plant and the weight of youngest elongate blade leaf. Although the inoculation with *Bacillus subtilis* resulted in increase of all the investigated traits, the response was varied from one trait to another. Moreover, the response was varied from cultivar Beni Suef to cultivar Salambo. Commonly, the incensement in the rest of the traits under investigation were greater in cultivar Salambo but some of the traits were greater in cultivar Beni Suef, indicating that the growth increased as response for root inoculation with *Bacillus subtilis* was superior in cultivar Salambo than cultivar Beni Suef. The obtained results are in agreement with the previous results of [24], finding inoculation in wheat leading to better grain yield. Abo-Doma *et al*. [25], reported comparable results in a studying of the effect of mycorrhiza inoculation on wheat plant growth using two wheat cultivars. In subsequent studies, [26] reported that the activity of the bacterial isolate identified as *Bacillus subtilis* NA-108 exerted the greatest influence on strawberry growth. Upadhyay *et al*., [27] confirmed that co-inoculation with *B. subtilis* and Arthrobacter sp. could alleviate the adverse effects of soil salinity on wheat growth. Recently, Pishchik *et al*. [28] reported that the inoculation by bacteria *B. subtilis* in wheat enhances the resistance of the plant microbial system and high agronomic efficiency.

**Molecular Studies**

The recorded changes in plant growth and productivity as response for root inoculation with *Bacillus subtilis* switch the author's mined to study the internal changes in the plant on the molecular level. The bacteria, as detected from the previous studies, effects extended to the whole plant. This fact leads to study the way by which the effects of *Bacillus subtilis* inoculation transferred from roots to different parts of the plant. Cell signaling is the way by which the effects of *Bacillus subtilis* inoculation transferred from roots to different part of the plant.

**Calcium dependent protein kinase (CDPKs)**

Calcium dependent protein kinase (CDPKs) is a membranous protein which acts as secondary messenger. It is activated by receipting calcium and then it is activates different protein molecules by phosphorylation. The activated proteins could affect finally the expression of different genes. The affected genes could be leads to increase photosynthesis rate in the inoculated plants and thus increase plant growth. Other genes could increase the plant tolerance for abiotic stress by increasing the solid substances in plant cells of the inoculated plants. CDPKs have been shown to function in many different aspects of plant biology, from environmental stress signaling upon the perception of abiotic and biotic stress stimuli to hormone-regulated processes in development [9]. Semi quantitative reverse transcriptase-polymerase chain reaction (sqRT-PCR) of (CDPKs) was performed as described previously. The results of sqRT-PCR were recorded in Table (2) and Figure (1), showed that the gene expression of the gene conferring calcium dependent protein kinases exhibited two different lengths 860 and 780 bp. The amplicon with length of 860 bp was newly synthesized amplicon under inoculation in both cultivars under investigation but it was not exhibited in control plants of the two cultivars. The results indicated that this amplicon possess a higher intensity in the treated plants of cultivar Salambo and qRT-PCR results confirm these results where it showed the higher concentration (19.81 pg/µL). The second amplicon with length of 780 bp was almost similar in the control and inoculated plants of the two cultivars indicating no response for root inoculation with *Bacillus subtilis* for this amplicon. In general,

CDPKs gene expression increased in the inoculated plants as compared to non-inoculated ones in both cultivars Beni Suef and Salambo but with a little higher increase in Salambo plants compare to Beni Suef cultivar reflecting a higher response in Salambo than cultivar Beni Suef. Our results were in agreement with the previous findings of [29] who reported that the increase of phosphate in the form of phosphoric acid in the soil resulted decreasing of rhizospher pH and thus increase the availability of different elements specially $Ca^{2+}$. Additionally [30] reported that CDPK1 is a key component of one or more signaling pathways that directly or indirectly modulates cell expansion or cell wall synthesis in *M. truncatula*. Likewise [31] investigated the ability of phosphate-mobilizing bacteria, related to four species of Bacillus genus, to colonize the cucumber root zone. The results indicated that, the four different strains increased the plant growth with different rates. In subsequent study, [18] reported that inoculation maize seeds with Pseudomonas spp. bacteria showed high levels of proline, sugars and free amino acids. Our results were in line with the results of [32] who confirmed that the influence of granulated bacterial preparation of complex action on the growth and yield of barley (*H. distichum* L.). They reported that, the treatment of barley seeds by this preparation has been established to have a very significant effect on the mass of 1000 grains, grain natural weight and to increase the yield of plants. Interestingly that, CDPK genes in wheat play important roles in signaling pathways for disease resistance and various stress responses as indicated by emerging. Among the 20 wheat CDPK genes, 10 were found to respond to drought, salinity and ABA treatments [8]. Recently, [33] study the positively regulates of Ca CDPK15 in pepper and reported that this positive-feedback loop would amplify defense signaling against RSI and efficiently activate strong plant immunity.

**Table (2): Quantification of (CDPKs), (PEPCs) and (P5CS) genes expression among two wheat cultivars under *Bacillus subtilis* (BS) inoculation, where (C) mean Control, (T) mean Quantitative gene with the Inoculated and (Tim.) time of increasing.**

| Cultivars | CDPKs gene | | | PEPCs gene | | P5CS gene | |
|---|---|---|---|---|---|---|---|
| Beni Suef | M.W. | 860 bp | 740 bp | M.W. | 320 bp | M.W. | 570 bp |
| | C | 0.0 | 13.34 | C | 6.17 | C | 8.75 |
| | T | 11.38 | 13.47 | T | 15.62 | T | 17.83 |
| | Tim. | 0.0 | 1.01 | Tim. | 2.5 | Tim. | 2.03 |
| Salambo | M.W. | 860bp | 740 bp | M.W. | 320 bp | M.W. | 570 bp |
| | C | 0.0 | 12.89 | C | 6.82 | C | 7.11 |
| | T | 19.82 | 13.010 | T | 21.15 | T | 23.87 |
| | Tim. | 0.0 | 1.009 | Tim. | 3.1 | Tim. | 3.3 |

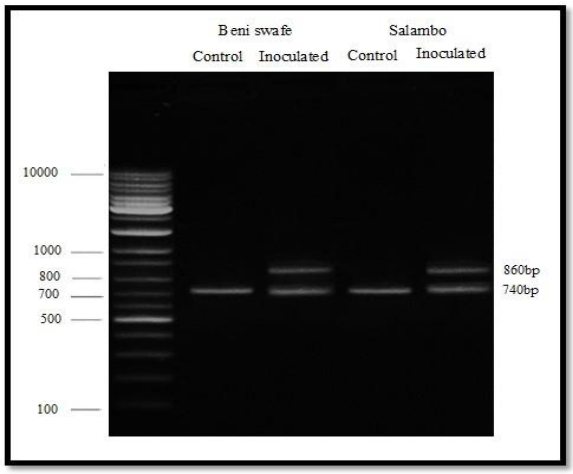


**Figure (1): Quantification of Calcium dependent protein kinase (CDPKs) gene expression among two wheat cultivars under *Bacillus subtilis* (BS) inoculation.**

**Phosphoenol Pyruvate Carboxylase (PEPCs)**

In the case of the Phosphoenolpyruvate carboxylase (PEPC) plays an important role in carbon and nitrogen metabolism of C3 plants. Acting in cytoplasm of plant cells PEPCs show different physiological roles

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depending on the developmental phases of plants [34]. sqRT-PCR amplicon were recorded in Table (2) and Figure (2) with length of 320 bp was resulted in the four samples with different intensities. The results of the gene expression conferring phosphoenol pyruvate carboxilase (PEPCs) increased in the inoculated plants as compared to non-inoculated ones in both cultivars, with greater amplicon density in cultivar Salambo than Beni Suef, reflecting a kind of higher response for *Bacillus subtilis* inoculation in Salambo cultivar than Beni Suef. Quantification of the gene expression of this gene using sqRT-PCR protocol revealed that, concentration of PEPCs gene expression was increased from 6.17 in the control to 15.62 in the treated plants with two and a half folds over of the control in Beni Suef cultivar. While in Salambo cultivar, the concentration was increased from 6.82 in the control to 21.15 in the treated plants with 3.1 folds over its control. These results indicated that *Bacillus subtilis* inoculation stimulates the photosynthesis in form of the electron transmitter compounds which their synthesis conferred by (PEPCs). That's finally reflected on the rate of photosynthesis and thus plant growth. In fact, the DNA, RNA, and protein expression levels, as well as the physiological characteristics, of wheat over-expressing may be related to the insertion site, plasmid construct, and/or gene copy number [35]. Many previous studies of elevated discuss the elevated CO2 concentrations showed a close relationship between enhanced photosynthesis, biomass, and yield. These results suggest a positive correlation between potential leaf photosynthesis and maximal crop growth rate [36]. In view of results obtained by [19] who pointed PEPC activity in wheat inoculation with AM, showed almost enhances the expression of different plant genes including PEPCs genes which improves the plant growth and development. [37], confirmed that (PEPCs) gene is more effective than ppdk at improving wheat photosynthetic performance. Recently, [34] suggested that the involvement of the enzymes fulfilling the initial carboxylation step Rubisco and PEPC in the adaptation mechanisms of wheat genotypes against water and salt stresses.

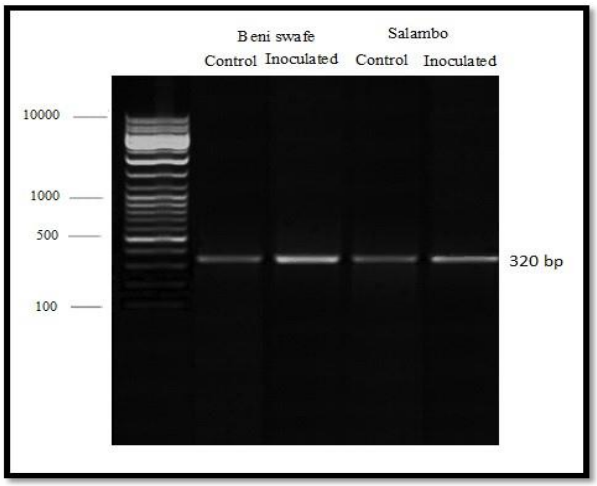


**Figure (2): Quantification of Phosphoenol Pyruvate Carboxylase (PEPCs) gene expression among two wheat cultivars under *Bacillus subtilis* (BS) inoculation.**

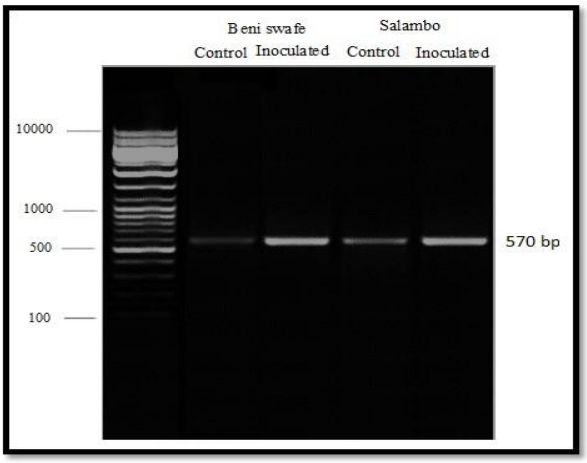


**Figure (3): Quantification of Proline-5 Carboxylate Synthetase (P5CS) gene expression among two wheat cultivars under *Bacillus subtilis* (BS) inoculation.**

**Proline-5 Carboxylate Synthetase (P5CS)**

With respect to sqRT-PCR of Proline-5 Carboxylate Synthetase (P-5CS), analysis of variance showed significant differences between P5CS gene expressions in the two wheat cultivars Beni Suef and Salambo under Inoculation with bacteria.

sqRT-PCR amplicon were recorded in Table (2) and Figure (3) with length of 570 bp was resulted in the four samples with different intensities as response for *Bacillus subtilis* inoculation. This amplicon was presented with highly intense in the inoculated plants compare to non-inoculated ones. These results indicated that the gene expression of the gene conferring P5CS increased in the inoculated plants as compared to non-inoculated ones in both cultivars Beni Suef and Salambo. The results also showed that the increase in gene expression of the gene conferring P5CS was greater in cultivar Salambo than Beni Suef, reflecting a kind of higher response for *Bacillus subtilis* inoculation in Salambo cultivar.

Quantification of the gene expression of this gene using sqRT-PCR protocol revealed that, concentration of P5CS gene expression was increased from 8.75 in the control to 17.83 pg/µl in the treated plants with 2.038 folds over of the control in Beni Suef cultivar. While in Salambo cultivar, the concentration was increased from 7.11 in the control to 23.87 pg/µl in the treated plants with 3.3 folds over its control. Results indicated that *Bacillus subtilis* inoculation stimulates the bio-synthesis pass way of proline in form of P5CS which conferring the production of proline. That's finally reflected on the plant tolerance for abiotic stresses. The results were in agreement with the findings of [38] where they reported the beneficial effect on the defense status is detected in distal leaves, demonstrating a systemic induction of resistance by a root-endophytic fungus. They concluded that the fungus could be exploited to increase salt tolerance and increase in grain yield in barley. On view of the results of [39], they reported that the endophyte significantly elevated the amount of ascorbic acid and increased the activities of antioxidant enzymes in barley roots under salt stress conditions. These findings suggest that antioxidants might play a role in both inherited and endophyte mediated plant tolerance to salinity in barley. Likewise, transcriptome analysis using real time-reverse transcriptase indicated that mycorrhiza treatment resulted in an increase in the gene expression of proline 5 carboxilate synthetase (P5CS) on the transcription level in barley [20].

Previous studies indicate a positive relationship between P5CS, proline accumulation and plant stress tolerance. Proline accumulation during osmotic stress is mainly due to increased synthesis and reduced degradation [40]. Recently, [13] reported that difference in gene expression patterns between (Mahuti) as a salt tolerant cultivar and (Alamut) as a salt sensitive cultivar is due to different signaling pathways that activate P5CS and BADH expressions.

## CONCLUSION

In this manuscript, we have obtained a broad overview of the behavior of the *Bacillus* spp. as one of the inoculation bacteria stimulate plant growth and increase yield. Apparently, it has been demonstrated that the molecular mechanisms of *Bacillus subtilis* elucidating the ability to enhance the phonotypical and physiological changes upon inoculation. However, as a consequence of this study, changes in gene expression on CDPKs, PEPCs and P5CS genes could be involved in the phenotypes that are observed in yield related traits in wheat inoculated plant. This assumption is probably due to the effect of cell signaling which the effects of *Bacillus subtilis* inoculation transferred from roots to different part of the plant. Overall, these findings contribute to a better understanding of wheat plants and beneficial *Bacillus subtilis* interactions, opening new avenues to study these relevant biological associations.

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