Romanian Biotechnological Letters *Vol. 22, No. 6, 2016*

ORIGINAL PAPER

Overexpression of *Synechocystis glutaredoxin-2* improves the growth of *Pseudomonas fluorescens* under salt stress

Received for publication, October 14th, 2014
Accepted, February 29th, 2015

AHMED GABER[1,2*], MOHAMED EL-AWADY[1,2], NAGWA I. ELARABI[2], SALAH A. MOUSTAFA[3,4]
[1]Scientific Research Center, Biotechnology and Genetic Engineering Unit, Taif University, KSA [2]Department of Genetics, Faculty of Agriculture, Cairo University, El-Gamaa street P.O. Box 12613 Giza, Egypt
[3]Agriculture Genetic Engineering Research institute (AGERI), Agricultural Research Center (ARC), Giza 12619
[4]Jazan University, faculty of medicine, Department of medical biology
***Address correspondence to**: Scientific Research Center, Biotechnology and Genetic Engineering Unit, Taif University, P.O. Box 689, Zip code 21944, Taif, KSA.*
Tel.: 0966530741541; Email: agaber60@yahoo.com
Ahmed Gaber and Mohamed El-Awady contributed equally to this work.

Abstract

Plant Growth Promoting Rhizobacteria (PGPR) play an important role in agricultural systems, especially as biofertilizer. Previously, we screened one bacterial isolate as indole acetic acid (IAA) producer on the basis of IAA colorimetric assay. Based on morphological, physiological and biochemical characteristics presented in Bergey's Manual of Systematic Bacteriology and on 16SrRNA homology, this isolate identified as Pseudomonas fluorescens (accession # JQ809429). Pseudomonas Fluorescens, like other PGPR isolates, produced IAA in the presence of tryptophan, thus genes for IAA production (iaaM and iaaH) were detected in the present study. Moreover, the overexpression of Synechocystis PCC 6803 glutaredoxin- 2 (ssr2061) in Pseudomonas fluorescens cells significantly enhance the growth of the recombinant cells on LB media supplemented with different concentration of NaCl. The recombinant strain was able to produce IAA under 2% of NaCl in the medium. While, the wild type strain failed to produce IAA under same condition. Additionally, effect of the salt on the growth of recombinant and wild type cells was studied by pot culture experiments by using sterilized air dried soil supplemented with 0.5 M or 1 M NaCl. Interestingly, the cell density of the recombinant cells markedly increased even after 6 days from the irrigation with the salt water over the wild type cells. These results suggest that theglutaredoxin-2proteincould play a major role in regulating abiotic tolerance against salt stress in Pseudomonas fluorescens cell.

Keywords: Luria-Bertani (LB), salt stress, Plant growth promoting rhizobacteria Glutaredoxins (GRXs), *Synechocystis* PCC 6803, recombinant enzyme.

1. Introduction

Excess use of chemical fertilizer is undesirable because (i) production of chemical fertilizer is a costly process, (ii) most of the energy is provided by consumption of non-renewable fossil fuel, (iii) considerable pollution is caused through both the production and use of mineral N- fertilizer (MAI & al. [1]; DESHWAL & al. [2]). Therefore, scientists are searching new alternative of chemical fertilizer. Internationally, biofertilizer has been accepted as an alternative source of chemical fertilizer. Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which actively colonize in plant root and increase plant growth by production of various plant growth hormones, P-solubilizing activity, N_2 fixation and biological activity (DESHWAL & al.[3]; DESHWAL & al. [4]).

Various species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia*have been

reported to enhance the plant growth (KLOEPPER & al. [5]; GLICK [6]; RODRIGUEZ & FRAGA [7]; MISKO & GERMIDA [8]; JOSEPH & al. [9]). Very recently, SHEN & al. [10] reported that *Pseudomonas chlororaphis*, *P. fluorescens*, *P. aeruginosa*, *P. stutzeri*strains were PGPRs. Also, NOORI & SAUD [11] mentioned that *Pseudomonas fluorescens* bacteria encourage the plant growth through their diverse mechanisms. During the past thirty years, the use of PGPR for sustainable agriculture has increased greatly in various parts of the world. Extraordinary remarkable raises in growth and yield of agronomical important crops in response to inoculation with PGPR have been repeatedly reported (KLOEPPER & al. [12]; CHEN & al. [13]; AMARA & DAHDOH [14]; CHANWAY [15]; BISWAS & al. [16]; ASGHAR & al. [17]; VESSEY [18]; GRAY & SMITH [19]; FIGUEIREDO & al. [20]; BHARUCHA & al. [21]; DESHWAL& KUMAR [22].

Indole-3-acetic acid (IAA), one of the most physiologically active auxins, has been involved in regulating a variety of developmental and cellular processes in plants, such as cell division, shoot growth, apical dominance and roots develop (DAVIES [23]). IAA is produced through L-tryptophan metabolism by plants and many of soil micro-organisms such as bacteria, fungus and algae (SARWAR & KREMER [24]). Indole-3-Pyruvic acid, Indole-3-Acetaldehyde and Indole-3-Acetonirile are pre-substances of IAA which have a feeble virtue of auxines. There are five observed paths to produce IAA from tryptophan but the bacteria use only three ways of IAA metabolic synthesize (LAMBRECHT & al. [25]; COSTACURTA & al. [26]). One of them is Indole-3-acetamideIn (IAM) path. In IAM path, two enzymes are engaged in IAA production. Tyrptophan-2-mono oxygen is the first enzyme, encoded by *iaaM* gene, which catalyzes the changing L-tryptophan to indole-acetamides. The second enzyme is indole-acetamidehydrolyase, encoded by *iaaH* gene, which change IAM to IAA (BEYELER & al. [27]). The second path is using indole-3-pyrovic acid (IPYA). This path is depending only on indolepyruvate decarboxylase enzyme, encoded by *ipdc* gene, which catalyze the indolepyruvic acid to produce IAA (ARSHAD & FRANKENBERGER [28]).

Many microbes produce IAA. Recent literature suggested that *Pseudomonas* strains produced plant growth hormones. DESHWAL& al. [29] reported that 75% *Pseudomonas* strains produced IAA and improved plant growth in soybean. BHARUCHA & al. [21] isolated nine IAA producing rhizobacteria from the rhizospheric soil of alfalfa (*Medicago sativa*). DESHWAL& al. [2] demonstrated that IAA generating from *Pseudomonas aeruginosa* MR-9 enhanced maximum plant dry weight, plant height, nodule per plant, nodule fresh weight of *Mucunaprurien*splant by 184, 124, 139, 180% respectively as compared to control plants. PAUL & NAIR [30] stated that the saline areas under agriculture are increasing every year across the world. Additionally, TANK & SARAF [31] demonstrated salinity as one of the main anthropogenic as well as environmental stresses that reduced plant growth.

Salinity badly affects plant growth and development because rising salinity in the soil reduced plant growth, photosynthesis, stomatal conductance, chlorophyll content and mineral uptake compared to soil without salinity (HAN & LEE [32]). SHUKLA & al. [33] detected that salinity adversely affects plant growth and development. Similarly, AHMAD & al. [34] reported that salinity stress remarkably reduced plant growth, while that inoculation with PGPR containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase and rhizobia enhanced plant growth, thus reducing the inhibitory effect of salinity. Moreover, UPADHYAY & al. [35] supported that salt-tolerant PGPR can participate an important role in reducing soil salinity stress during plant growth.

Glutaredoxins (Grxs) are ubiquitous small heat-stable disulfide oxidoreductases that play an important role in cell development and response to oxidative and abiotic stresses. Previously, we demonstrated that the over-expression of the protein of *Synechocystis* PCC 6803 Grx2 or Grx1, encoded by *ssr2061* or *slr1562* respectively, in *E. coli* cells showed high

tolerance to NaCl compared to cells transformed with the vector alone (GABER & al. [36]; GABER & EL-ASSAL [37]). Moreover, tomato glutaredoxin gene *SIGRX1* was found to be induced by oxidative, drought and salt stresses and the over-expression of *SIGRX1* in *Arabidopsis* plants significantly increased resistance of plants to oxidative, drought and saltstresses (GUO& al. [38]).All the above literature suggested that salt is one of the major problems in agriculture and soil micro-flora.

Here we have evaluated the effect of different salt concentration on the growth and IAA production of the new *P. fluorescens*strain (accession # JQ809429). These have showed that the growth level of *P. fluorescens*strain was depleted by the addition of 3%, 4% or 5% of NaCl in the medium. Whereas, the production of IAA was more sensitive towards 2% NaCl in the medium.Furthermore, over-expression of *Synechocystis* PCC 6803 *glutaredoxin- 2* (*ssr2061*) gene in *P. fluorescens*strain was investigated. Changes in the growth levels and IAA synthesis as a consequence of the addition of salt in the medium with the recombinant cells were documented and compared to the effects of the wild-type bacterium.

2. Materials and Methods

P. fluorescens(accession # JQ809429)was previously isolated from the soil rhizosphere samples collected from different agricultural areas of Giza, Egypt (unpublished data). The isolate was characterized based on the 16S rRNA partial sequencing using specific primers set 16F (5'-GGTCTGAGAGGATGATCAGT-3') and 16R (5'-TTAGCTCCACCTCGCGGC-3') designed by WIDMER& al. [39] according to the convention of the Oligonucleotide Probe Database (OPD), and the expected size is about 1000 bp.Amplification was performed in thermo-cycler with following PCR conditions: 35 cycles of 95°C for 1 min, 55°C for 1 min, and72°C for 1 min with initial denaturation at 95°C for 5 min and final extension at 72 °C for 10 min. The isolate was identified as *P. fluorescens. P. fluorescens*was routinely grown at 28°C in LB agar medium (Difco). To check the effect of salt on the growth of *P. fluorescens*strain, NaCl incorporated into LB agar medium and various concentrations of NaCl (0 to 865 mM) were added to the medium and the test bacterial strains were streaked.Other*Pseudomonas spp*was obtained from Microbial Molecular Biology Laboratory, Faculty of Agriculture, and Cairo University. *Escherichia coli* JM109 strainhave been used for the transformation (GABER& al. [36]) and were grown at 37°C LB broth (Difco).

For the measurement of IAA production, wild-type and recombinant *P. fluorescens* strains were propagated overnight in 5 ml of LB media, and then 20µl aliquots were transferred into 5 ml of LB media supplemented with the following concentrations of 2.5 mML-tryptophan (from a filter-sterilized 2-mg/ml stock prepared in warm water; Sigma). After incubation for 45 h, the density of each culture was measured spectrophotometrically at 600 nm, and then the bacterial cells were removed from the culture medium by centrifugation (5,500 *Xg*, 10 min). A 1-ml aliquot of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled H_2O, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$ [GORDON & WEBER [40]) and allowed to stand at room temperature for 30 min before the absorbance at 535 nm was measured. The development of a cherry red color indicated a positive reaction. The concentration of IAA in each culture medium was determined by comparison with a standard curve.Authentic IAA (Sigma- Aldrich, St. Louis, MO) was used as a standard.

For theexpression of *ssr2061*gene in *P. fluorescens*, thechromosomal DNA was isolated from *Synechocystis* PCC 6803 according to the method described by WILLIAMS [41]. One DNA fragment containing the open reading frame of *ssr2061*was amplified by

PCR. The forward primer (5'- AAGCGTTCATATGGCTGTCT -3') and reverse primer (5'- TACGAATAAGATTAGCCAGC -3') were deduced from the nucleotide sequence of *ssr2061*of *Synechocystis* PCC 6803. Amplified DNA fragment was cloned into a pT7Blue-T vector (Novagen, Madison, WI, USA) and sequenced with an automated DNA sequencer (ABI310A, Applied Biosystems, Japan). For the construction of the plasmid to express *ssr2061*gene, the plasmid was digested with *Nde*Iand *Bam*H1 and the resultant 0.5-kb DNA fragment was cloned into a pET3a vector (Novagen, Madison, NI, USA) digested with the same restriction enzymes. The resulting constructs, designated *pET:Grx2*, was introduced into the competent cells of*P. fluorescens* strainaccording to HANAHAN[42] with a slight modification as follows:Five µl of *pET:Grx2*(about 5 ng/µl) was added to 50 µl of the competent cells in sterile Eppendorf tube. The content of the tube was mixed by gently swirling and store in ice for 30 min. The mixture was incubated for exactly 40 seconds in water bath, which was preheated to 42˚C, without shaking. Rapidly, the tubes were transferred to ice bath. After 2 min, 500 µl of LB broth was added to the tubes. Then, the culture was incubated at 37˚C for 60 min with shaking. A hundred and fifty µl of the transformed competent cells were spread sing a sterile bent glass rodonLB solid medium supplemented with 50 µg/ml ampicillin. The plate was left at room temperature until the liquid was absorbed and incubated in inverted position at 37˚C. Transformed *P. fluorescens* colonies appeared after 16 hours.

Different oligonucleotide primers (**Table 1**) specific to IAA production related genes in *P. fluorescens (iaaM and iaaH*genes*)* andin *P. putida*(*ipdc*gene)weredesignedto span the entire open reading frame using Applied Biosystem 380A Automated DNA Synthesizer with reagent and conditions supplied by the manufacturer.PCR reactions were carried out in a total volume of 50µlcontaining 1X PCR buffer, dNTPs, 2.5 units of Taq DNA polymerases, 100 pmol of each primer and the DNA template, which released from the bacterial cells by boiling in a water bath for 5 min to lyses the cells and then the tubes were spun briefly to collect the condensate (CAROZZI& al. [43]). PCR conditions was: 94ºC for 3 min; 35 cycles of denaturation at 94ºC; annealing at 52ºC; and extension at 72ºC for 2 min each, followed by 7 min extension at 72ºC. Amplification products (10 µl) were analyzed on 1.5% agarose gels, detected by ethidium bromide staining and photographed under UV light.PCR products were sequenced with an automated DNA sequencer (ABI, Gene line DNA sequencing, New York) and the sequence was analyzed by using the algorithms BLAST (National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov]) and ClustalW (EMBL [http://www.ebi.ac.uk]).

Table 1. Oligonucleotides used in this

Gene	Encoding for	Primer Sequence	Expected size (bp*)*	IAA pathway
iaaM	tryptophan monooxygenase	F:5'-ATGGATGACCATTTTAATTCACCCAC'-3 R: 5'-CTAATAGCGATAGGAGGCGTTAA-'3	1670	Indole-3-acetamideIn (IAM)
iaaH	indole-3-acetamide hydrolase	F:5'-ATGCGCGAAATGATCACACTTGAATCG'3 R:5'-TTAATACTTTCTACTATAAGACTCACG-'3	1340	Indole-3-acetamideIn (IAM)
ipdc	indole-3-pyruvate decarboxylase	F: 5'-ATGGATGACCATTTTAAATTTTGTACG-'3 R: 5'-CTAATAGCGATAGGAGGCGGGTTG-'3	1650	indole-3-pyrovic acid (IPYA)

To determine whether *P. fluorescens*can produce IAA in saline soli or not, cultures of recombinant *P. fluorescens* and wild type isolates were grown overnight from single colonies in 5 ml of LB media without tryptophan. After approximately 24 h, 50 µl of each culture was

transferred to 5 ml of LB media medium and incubated for an additional 6 h. When the OD_{600} of the cells reached 0.4 – 0.8, the bacterial cells were sprayed in sterilized soli pouch (0.5 gram). Three soil pouch were placed in aseptically place and were irrigated day by day for six days with distilled water supplemented with 0, 0.5 and 1 M of NaCl.The pouches were placed upright in metal racks, with one treatment per rack to prevent cross-contamination and covered loosely with plastic wrap to prevent dehydration. At the end of each day the soil samples were diluted three time with distilled water and the turbidities of the cultures were measured spectrophotometrically at 600 nm.

3. Results and Conclusions

Pseudomonas is a well-known plant growth promoting bacteria.Our new isolate of *P.fluorescens*, tested for their IAA production showed a significant amount of IAA production in tryptophan-supplemented medium, whereas a little amount of IAA production was observed in the medium devoid of tryptophan(data not shown). The production of IAA by the isolates only in the presence of L-tryptophan indicates that the tested strains utilize L-tryptophan as a precursor for IAA production during their growth in the medium. To approve this hypothesis, degenerate gene-specific primers targeted toward the three genes *iaaM*,*iaaH* and *ipdc* (Table 1),were used to amplify thesegenes from *P. fluorescens*strain. Electrophoresis of the PCR products obtained by using *P.fluorescens*genomic DNA as templates revealed single band (1670 bp)as expected for *iaaM* gene and 1340 bpfor*iaaH* gene (data not shown). Identification was based on the size of the amplified product from *P.fluorescens* and was visualized on agarose gels. The fragment weighing 1670 bp and 1340 bp are of interest because the *iaaM* and *iaaH* specified primers were expected to amplify a product of 1670 bp and 1340 bp, respectively (**Table 1**). On the other hand, the amplification product of *ipdc* gene, 1650 bp, was not observedby using *P.fluorescens*genomic DNA as templates (data not shown). These resultsvalidatethat this strain is *P.fluorescens* and the PCR primers for *iaaM* and *iaaH* genes are specific for *P.fluorescens* and does not amplify non-specifically other genes.To confirm these results, we quantify the production of IAA in the presence of tryptophan. In the absence of tryptophan supplements, the*P.fluorescens*strain produced very low levels of IAA (around 0.38 mg/ml). However, when the strain was grown in the presence of 2 mg/mlof tryptophan for approximately 45 h, *P.fluorescens* strain reacted by producing higher levels of IAA (around 7 mg/ml). As the concentration of tryptophan in the growth medium was increased, IAA production by *P.fluorescens*strain increased (data not shown).

Literature suggests that all *Pseudomonas* strains tolerated up to 1.25% NaCl in medium. But plant growth activity was more affected as compared to growth (DESHWAL& Kumar [22]).Our results showed that the *P. fluorescens* strain produced optimum IAA in NaCl concentration from 0 to 0.75% (data not shown). While, as concentration of NaCl increased to 2% NaCl in LB liquid medium, *Pseudomonas* failed to produce IAA (data not shown). These results was in agreement with the DESHWAL& Kumar [22]. They studied the effect of NaCl on the growth and activity of *P. aeruginosa*, *P. putida*, *P. cepacia*and*P. fluorescens.* Their results suggested that above 1.75% NaCl concentration in medium, the survival number of *Pseudomonas* was gradually reduced. All strains produced optimum IAA in NaCl concentration from 0 to 0.75% but higher concentration of NaCl delayed IAA production in *Pseudomonas* strains (DESHWAL& Kumar [22]).

Glutaredoxins (Grxs) are found to be involved in cellular responses to various abiotic stresses especially oxidative and osmotic stresses (COTGREAVE&GERDES [44]; LUIKENHUIS & al. [45]; RODRIGUEZ-MANZANEQUE& al. [46]; ROUHIER& al. [47];

KIM& al. [48]). Previously, we reported that by the treatment of the *Synechocystis* PCC 6803 cells with 200 mMNaCl, the *ssr2061* mRNA level was steadily increased with time till 2 hr (GABER& al. [36]). Moreover, we documented that the over-expression of*Synechocystis* PCC 6803*Grx1* or *Grx2*in *E. coli* cells enhanced the growth levels over than the empty vector transformed cells in the presence of different concentrations of NaCl (GABER& al. [36]; GABER& EL-ASSAL [37]).

In the present study, *P. fluorescens* was used as a model systemto test whether the *Synechocystis* PCC6803 *ssr2061* genecan circumvent salt stress(Figure1). Such salt stress was generated *in-vitro* by NaCl (0-5%) which is known as salt stress inducer. As expected, overexpressing cells with*ssr2061*grew better than the empty vector transformed cells in the presence of 3%, 4% and 5% of NaCl concentrations on the LB solid medium (Figure2).The results clearly illustrated that the growth level of the wild type *P. fluorescens*strain gradually reducedin LB solid medium supplemented with 3% NaCl concentration. While,no strains of *P. fluorescens*wild type survived on medium supplemented with 4% or 5%NaCl (Figure2). Interestingly,we found that the growth rate and production of IAA in the transformed *P. fluorescens* were higher than that of the cells transformed with empty vector cells under 2% of NaCl in LB liquid medium(Figure 3). Surprisingly, the*P. fluorescens* transformed cells was able to grow in saline clay soil, up to 6 days,supplemented with 0.5 M or 1 M of NaCl (Figure4). It may be noted that both the wild type and the transformed *P. fluorescens* strains had endogenous glutaredoxingenes in their chromosomes. However, the normal level of Grx was not sufficient in the empty wild typestrainto protect the cells against NaCl mediated toxicity and the increased level of tolerance observed in the transformed strain might be due to the overexpressed of Grx2 protein.These data were in agreement with EGAMBERDIEVA [49]. Hestated that *Paureantiaca* TSAU22, *P. extremorientalis* TSAU6 and *P. extremorientalis*TSAU20 significantly produced IAA under salt conditions.Also, KHARE& al. [50]elucidated the capability of fluorescent *Pseudomonas* strain EKi, in production of bio-control and plant growth promontory metabolites under saline stress.

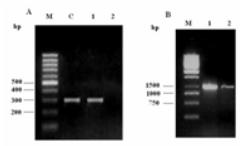

Figure 1. (A) PCR amplification of *ssr2061*gene from the recombinant *Pseudomonas*, lane 1 is the plasmid harboring *ssr2061*gene, lane 2 is the transformed *Pseudomonas*, lane 3 is wild type *Pseudomonas* and M is 100 bp ladder marker.(B)PCR amplification of*16s rRNA*, lane1 is transformed *Pseudomonas*, lane 2 is wild type *Pseudomonas* andM is 1Kb ladder marker.

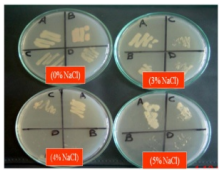

Figure 2. Evaluation of salt-tolerance in transformed *Pseudomonasfluorescens* expressing *ssr2061* gene, A is the transformed *Pseudomonas*, B is wild type*Pseudomonas*, C is the transformed *E. coli* expressing *ssr2061* gene and D is the *E. coli* wild type.

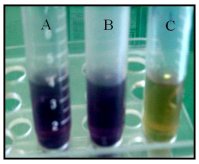

Figure 3. Effect of NaCl on the production of IAA by wild type and transformed *Pseudomonasfluorescens*. A is transformed *Pseudomonasfluorescens* growing in LB medium containing zero gNaCl, B is transformed *Pseudomonasfluorescens* growing in LB medium containing 20 g /LNaCl andC is WT *Pseudomonas* that could not able to grow in LB medium containing 20 g /LNaCl.

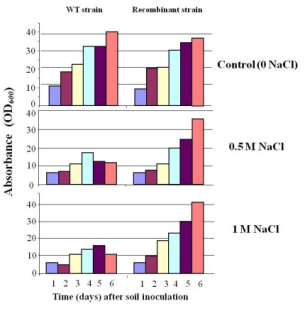

Figure 4. Effect of NaCl on the growth of wild type and transformed *Pseudomonas fluorescens*in soli during 6 days.

In conclusion, the plant growth promoting rhizobacteria (PGPR) isolate *Pseudomonas fluorescens*(accession # JQ809429) have the capacity to produce IAA as a plant growth promoting substances. The wild type strain of *P. fluorescens* was able to survive and produce IAA up to 0.7 % NaCl concentration in the medium while that the production of IAA is more sensitive towards 2% NaCl in the medium. Interestingly, the expression of the *Synechocystis* sp. PCC 6803 *ssr2061*gene in the cytoplasm of *P. fluorescens*plays an important physiological role in protection of *P. fluorescens* cells against salt stress. Surprisingly, *P. fluorescens* cells survived in medium supplemented by 2, 3, 4 or 5% NaCl and showed production of IAA under 2% NaCl. The results suggest that a *ssr2061* gene/protein is essential for adaptation of *P. fluorescens*cells to salt stress.

4. Acknowledgements

We thank Dr. Shigeru Shigeoka, Department of Advanced Bioscience, Faculty of Agriculture, Kinki University, 3327-204 Nakamachi, Nara, Japan, for the generous gift of the construct of the pET:Grx-2.

References

1- M.A.B. MAI, Z.H. SHAMSUDDIN, Z. WAHAB, M. MARZIAH, Effect of plant growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen free hydroponics condition.*Aus. J. Crop. Sci*.4:85(2010).
2- V.K. DESHWAL, M.S. DEVI, N. BHAJANKA, J. MISTRI, A. BOSE, N. SAINI,*Pseudomonas aeruginosa* strains and their role in plant growth promotion in medicinal plant. *Global J. Appl. Agr. Res.*1:49 (2011).
3- V.K. DESHWAL, P. PANDEY, S.C. KANG, D.K. MAHESHWARI, Rhizobia as a biological control agent against soil borne plant pathogenic fungi. *Ind. J. Exp. Biol.*41: 1160 (2003).
4- V.K. DESHWAL, S.B. SINGH, K. NILMANI, T. RAZA, F.A. ANSARI, A. JHA, A.D. KUMAR,Plant growth and nodulation of Mucuna (*Mucunapruriens*) in response to Rhizobium inoculation. *J. Plant Devel. Sci.*2: 103 (2010).
5- J.W. KLOEPPER, R. LIFSHITZ, R.M. ZABLOTWICZ,Free-living bacterial inocula for enhancing crop productivity. *Trend. Biotechnol.*7: 39 (1989).
6- B.R. GLICK, The enhancement of plant growth by free living bacteria. *Can. J. Plant Microbiol.*41 (Suppl 2): 109 (1995).
7- H. RODRIGUEZ, R. FRAGA, Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotech. Adv.*17 (Suppl 4-5): 319 (1999).
8- A.L. MISKO, J.J. GERMIDA, Taxonomic and functionaldiversity of pseudomonads isolated from the roots of field growncanola. *FEMS Microbiol. Ecol.*42: 399 (2002).
9- B. JOSEPH, R.R. PATRA, R. LAWRENCE, Characterization of plant growth promoting Rhizobacteria associated with chickpea(*Cicerarietinum*L). *Int. J. of Plant Prod.*1(Suppl 2): 141 (2007).
10- X. SHEN, H. HU, H. PENG, W. WANG, X. ZHANG, Comparative genomic analysis of four representative plant growthpromoting rhizobacteria in *Pseudomonas*. *BMC Genomics.*14: 271 (2013).
11- M.S.S. NOORI, H.M. SAUD, PotentialPlant Growth-Promoting Activity ofPseudomonas sp Isolated from Paddy Soilin Malaysia as Biocontrol Agent. *J. Plant Pathol. Microb.*3(2): 120 (2012).
12- J.W. KLOEPPER, M.N. SCHROTH, T.D. MILLER, Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology*70: 1078(1980).
13- C. CHEN, E.M. BAUSKE, G. MUSSON, R. RODRIGUEZ-KABAÑA, J.W. KLOEPPER, Biological control of*Fusarium* on cotton by use of endophytic bacteria. *Biol. Control*5: 83, 91, (1994).
14- M.A.T. AMARA, M.S.A. DAHDOH, Effect of inoculation with plant growth-promoting rhizobacteria (PGPR) on yield and uptake of nutrients by wheat grown on sandy soil. *Egypt. J. Soil*.37: 467 (1997).
15- C.P. CHANWAY, Bacterial endophytes: ecological and practical implications. *Sydowia*. 50: 149(1998).
16- J.C. BISWAS, J.K. LADHA, F.B. DAZZO, Rhizobial inoculation influences seedling vigor and yieldof rice. *Agron. J.*92: 880 (2000).
17- H.N. ASGHAR, ZA ZAHIR, M ARSHAD, A KHALIG, Plant growth regulating substances in therhizosphere: microbial production and functions. *Adv. Agron.*62: 146 (2002).
18- J.K. VESSEY, Plant growth-promoting rhizobacteriaasbiofertilizers. *Plant Soil* 255: 571 (2003).

19- E.J. GRAY, D.L. SMITH, Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol. Biochem*.37: 395 (2005).
20- M.V.B. FIGUEIREDO, H.A. BURITY, C.R. MARTINEZ, C.P. CHANWAY, Alleviation of water stress effectsin common bean (*Phaseolus vulgaris* L.) by co-inoculation *Paenibacillus* x *Rhizobium tropici*. *Applied Soil Ecol.*40: 182 (2008).
21- U. BHARUCHA,K. PATEL,U.B. TRIVEDI, Optimization of Indole Acetic Acid Production by *Pseudomonas putida* UB1 and its Effect as Plant Growth-PromotingRhizobacteria on Mustard (*Brassica nigra*). *Agric. Res.*2(3): 215 (2013).
22- V.K. DESHWAL, P. KUMAR, Plant growth promoting activity of Pseudomonads in Rice crop. *Int. J. Curr. Microbiol. App. Sci.* 2(11): 152 (2013).
23- P.J. DAVIES, The plant hormone concept: concentration,sensitivity and transport. In: Davies PJ (ed) Plant hormones:physiology, biochemistry, and molecular biology. Kluwer Academic Publishers, Dordrecht, pp. 13-18, (1995).
24- M. SARWAR, R.J. KREMER, Determination of bacterially derived auxins using a micro plate method. *Lett. Appl. Microbiol.*147: 282 (1995).
25- M. LAMBRECHT, Y. OKON, A.V. BROEK, J. VANDERLEYDEN, IAA: a reciprocal signaling molecule in bacteria-plant interaction. *Tren. Microbiol.*8: No. 7 (2000).
26- A. COSTACURTA, P. MAZZAFERA, Y.B. ROSATO, Indole-3-acetic acid biosynthesis by *Xanthomonasaxonopodis*pv. *Citri*is increased in the presenceof plant leaf extracts. *FEMS Microbiol. Lett.* 159: 215 (1998).
27- M. BEYELER, C. KEEL, P. MICHAUX, D. HASS, Enhanced production of IAA by a geneticallymodified strain of *Pseudomonas fluorescens*CHAOeffects root rot. *FEMS Microbiol. Ecol.* 28: 225(1999).
28- M. ARSHAD, W.T. Jr. FRANKENBERGER, Microbial production of plant hormones. *Plant Soil*133: 1(1991).
29- V.K. DESHWAL, K. VIG, S.B. SINGH, N. GUPTA, S. AGRWAL, S. PATIL, ANKITA, Influence of the Co-inoculation *Rhizobium* SR-9 and *Pseudomonas* SP-8 on Growth of Soybean Crop. *Develop. Microbiol. Mol. Biol.* 2(1): 67 (2011).
30- D. PAUL, S. NAIR, Stress adaptations in a Plant GrowthPromoting Rhizobacterium (PGPR) with increasing salinity in thecoastal agricultural soils. *J. Basic. Microbiol.* 48(5): 378(2008).
31- N. TANK, M. SARAF, Salinity-resistant plant growthpromoting rhizobacteria ameliorates sodium chloride stress on tomato plants. *J. Plant Interact.* 5(1): 51(2010).
32- H.S. HAN, K.D. LEE, Plant Growth PromotingRhizobacteria effect on antioxidant status, photosynthesis, mineraluptake and growth of lettuce under soil salinity. *Res. J. Agri. Biol. Sci.*1(3): 210, 215,(2005).
33- P.S. SHUKLA, P.K. AGRWAL, B. JHA, Improved salinitytolerance of *Arachishypogaea*(L.) by the Interaction of halotolerantplant-growth-promoting rhizobacteria. *J. Plant Growth Regul.* 31(2): 195(2012).
34- M. AHMAD, Z.A. ZAHIR, H.N. ASGHAR, M. ASGHAR, Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. *Can. J. Microbiol.* 57(7): 578(2011).
35- S.K. UPADHYAY, J.S. SINGH, D.P. SINGH,Exopolysaccharide-Producing plant growth-promoting rhizobacteriaunder salinity condition. *Pedosphere*21(2): 214(2011).
36- A. GABER, M. EL-AWADY, N.I. ELARABI, M.H. SOLIMAN, Overexpression of glutaredoxin-2 from Cyanobacterium*Synechocystis* PCC 6803 in *Escherichia coli* conferring enhanced salt stress tolerance.*Arab J. Biotech.*10(1): 13(2007).
37- A. GABER, S.E. EL-ASSAL, A Cyanobacterium*Synechocystis* sp. PCC 6803 GlutaredoxinGene (*slr1562*) Protects *Escherichia coli* against Abiotic Stresses. *Am. J. Agri. & Biol. Sci.*7(1): 88(2012).
38- Y. GUO, C. HUANG, Y. XIE, F. SOMG, X. ZHOU, A tomato glutaredoxin gene SlGRX1 regulates plant responses to oxidative, drought and salt stresses. *Planta*232: 1499(2010).
39- F. WIDMER, R.J. SEIDLER, P.M. GILLEVET, L.S. WATRUD, G.D. DI GIOVANNI, A highly selective PCR protocol for detecting 16S rRNA genesof the genus *Pseudomonas* (sensustricto) in environmental samples.*Appl. Environ. Microbiol*. 64(7): 2545(1998).
40- S.A. GORDON, R.P. WEBER, Colorimetric estimation of indoleaceticacid. *Plant Physiol.*26: 192(1951).
41- J.G.K. WILLIAMS, Construction of specific mutants in photosystem II photosynthetic reaction center by genetic engineering methods in Synechocystis 6803. *Methods Enzymol.*167: 766 (1988).

42- D. HANAHAN, Studies on transformation of*Escherichia coli* with plasmids.*J. Mol. Biol.*166(4): 557 (1983).
43- N.B. CAROZZI, V.C. KRAMER, G.W. WARREN, S. EVOLA, M.G. KOZIEL, Prediction of insecticidal activity of *Bacillus thuringiensis* strains by Polymerase Chain Reaction product profiles. *Appl. Environ. Microbiol.* 57: 3057(1991).
44- I.A. COTGREAVE, R.G. GERDES, Recent trends in glutathione biochemistry glutathione–protein interactions: a molecular link between oxidative stress and cell proliferation. *Biochem. Biophys. Res. Commun.* 242: 1 (1998).
45- S. LUIKENHUIS, G. PERRONE, I.W. DAWES,C.M. GRANT, The yeast *Saccharomyces cerevisiae* contains two glutaredoxin genes that are required for protection against reactive oxygen species. *Mol. Biol. Cell.*9: 1081(1998).
46- M.T. RODRIGUES-MANZANEQUE, J. ROS, E. CABISCOL, A. SORRIBA, E. HERRERO,Grx5 glutaredoxin plays a central role in protection against protein oxidative damage in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 19: 8180 (1999)**.**
47- N. ROUHIER, A. VLAMIS-GARDIKAS, C.H. LILLIG, C. BERNDT, J.D. SCHWENN, A. Holmgren, J.P.Jacquot, Characterization of the redox properties of poplar Glutaredoxin.*Antioxid. Redox Signal.*5: 15 (2003).
48- H. KIM, E. PARK, C. LIM,The Fission Yeast Gene Encoding MonothiolGlutaredoxin 5 Is Regulated by Nitrosative and Osmotic Stresses. *Mol. Cells*20: 43(2005).
49- D. EGAMBERDIEVE, Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol. Planta.*31(4): 861 (2009).
50- E. KHARE, S. SINGH, D.K. MAHESHWARI, N.K. ARORA, Suppression of Charcoal Rot of Chickpea by Fluorescent *Pseudomonas* under saline stress condition. *Curr. Microbiol.*62(5): 1548(2011).