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Effects of plant growth promoting rhizobacteria in plant resistance to environmental stress

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Abstract
Plant Growth Promoting Rhizobacteria (PGPR) is a beneficial microbe that occupies the rhizosphere of the plant, and is able to enhance crop productivity and offers an attractive way to replace chemical fertilizers, pesticides and supplements.
Plants are immobile living organisms, so they cannot move to search for better conditions. PGPR helps the plant tolerate many types of environmental stresses, one of these stresses is drought stress.
The studies reveal the important role of PGPR in agriculture and confirm that PGPR are very beneficial to the environment by increasing the crop yields under drought stress, in the presence of pathogens and in poor soils.

Keywords: plant, rhizobacteria, beneficial, organisms, conditions, yields, drought

Introduction

Plant Growth Promoting Rhizobacteria (PGPR) is a beneficial microbe that occupies the rhizosphere of the plant, and is able to enhance crop productivity and offers an attractive way to replace chemical fertilizers, pesticides and supplements.
Plants are immobile living organisms, so they cannot move to search for better conditions. PGPR helps the plant tolerate many types of environmental stresses, one of these stresses is drought stress.
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1. Induced systemic tolerance (IST) to environmental stresses by PGPR

Plants have developed ways to face changing circumstances, these changing circumstances may be biotic or abiotic and in both cases it causes a stress to the plants. Abiotic stresses are greatly diverse including drought, high salt, heavy metals, cold and heat shock (Wang, 2012) [12]. One of the most important abiotic stresses facing the plants these days is the drought stress. Drought stress greatly affects the growth and productivity of crops because it is able to reduce many biochemical and physiological reactions, especially in arid and semiarid areas. A beneficial microorganism termed Plant Growth Promoting Rhizobacteria (PGPR) lives in the rhizosphere of the plant, and helps the plant by various ways to tolerate to abiotic stresses like drought resistance (Lim, 2013) [7].
PGPR induces tolerance in plants to abiotic stresses known as induced systemic tolerance (IST), by causing physical and chemical changes in plants. PGPR confers IST to drought stress in plants by a variety of mechanisms. For example 1-aminocyclopropane-1-carboxylate (ACC) deaminase produced by some PGPR strains (Wang, 2012) [12]. PGPR strains that contain ACC deaminases are able to increase the weight of fresh and dry drought-treated tomato and pepper seedlings (Lim, 2013) [7].
Another example of mechanisms by which PGPR confers IST to drought stress in plants is BBS, association of three PGPR strains (*Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia*sp. XY21). BBS, without involving ACC deaminase, conferred IST to drought in cucumber plants (Wang, 2012) [12].

1.1. BBS inducing tolerance to drought stress in cucumber plants

An experiment was done by implanting cucumber seeds and withholding watering for 13 days, some plants were treated with BBS and the others were not treated with anything and kept as control. Leaves of cucumber plants that were treated with BBS showed darken green color and lighter wilt symptoms (which mean that it is still able to rehydrate at night or in an early morning) (Figure 1) (Wang, 2012) [12].

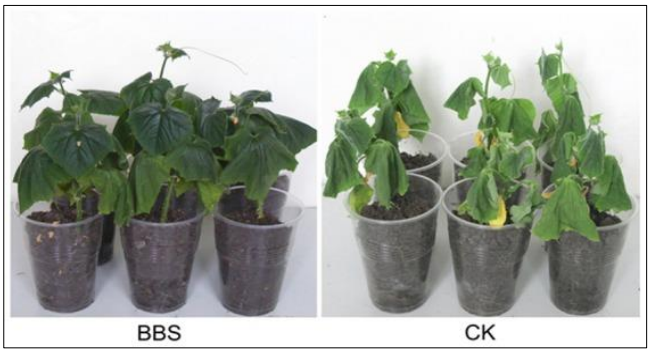

Fig 1: BBS treated cucumber plants show green darken leafs and lighter wilt symptoms than control cucumber plants (CK) (Wang, 2012) [12].

To understand how BBS induces drought tolerance to cucumber plants, physiological indicators of drought tolerance (activities of antioxidant enzymes, expression profiles of ribulose 1,5-biphosphate carboxy/oxygenase (Rubisco)) large and small subunits (rbcL and rbcS) and the activity of antioxidant enzyme SOD) were studied in BBS treated cucumber plants and in control cucumber plants (Wang, 2012) [12].

Comparing chlorophyll content in cucumber plants shows that contents of leaf chlorophylls a, b and a+b in BBS treated cucumber plants was higher than that in the control plants (Figure 2). The difference in chlorophyll content suggests that under drought stress BBS is able to maintain chlorophyll contents. This explains the above observation that leaves of BBS treated plants were much darker than that in the control plants. High leaf chlorophyll contents indicate high photosynthetic performance in the plant, also high transcriptional levels of rbcL and rbcS genes indicates high photosynthetic performance (Wang, 2012) [12].

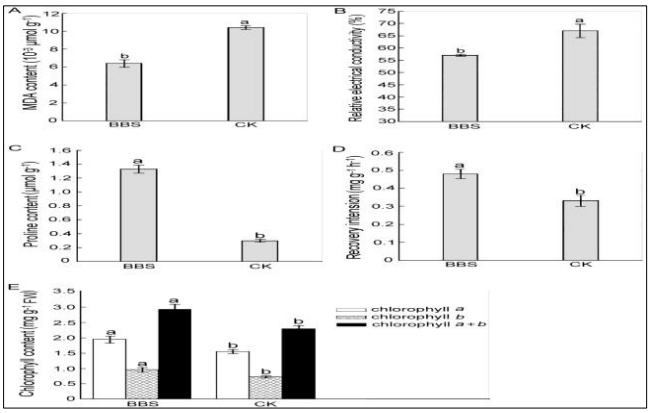

Fig 2: Chlorophyll content in the BBS treated plants was higher than that in the control plants (Wang, 2012) [12].

Rubiscoenzyme catalases photosynthetic CO_2 fixation which leads to form ribulose-1,5-biphosphate (RuBP). Transcriptional levels of large and small subunits of Rubisco enzyme, rbcL and rbcS respectively, were compared (Figure 3). Transcriptional levels of rbcS gene were down regulated to a large extent in the control plants than in the BBS treated plants (Wang, 2012) [12].

Transcriptional levels of rbcL gene was down regulated sharply till the fifth day post inoculation, but after the seventh day of inoculation it was strongly transcribed till the 13 days post inoculation. Whereas the transcriptional levels of rbcL were not detected after the ninth day of inoculation in the control plants. This shows that the transcriptional levels of rbcL and rbcS were higher in the BBS treatment than in the control, indicating the ability of BBS to maintain the transcriptional levels of rbcL and rbcS under drought stress (Wang, 2012) [12].

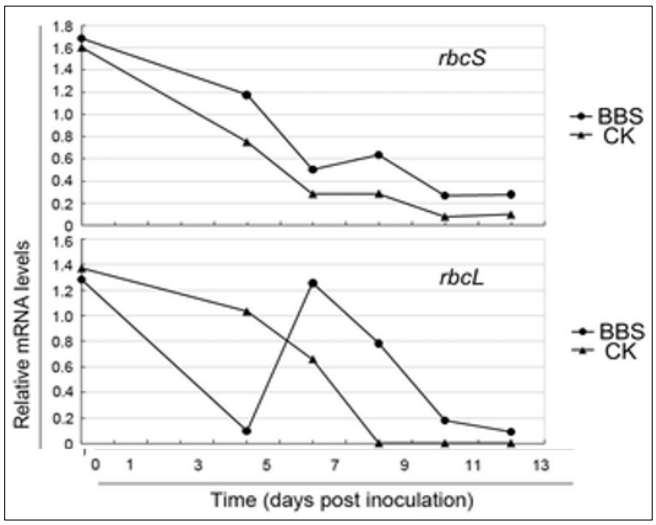

Fig 3: Transcriptional levels of rbcL and rbcS in BBS treated plants and in control plants under drought stress (Wang, 2012) [12].

Mainly drought stress increases reactive oxygen species (ROS) by the production of oxygen free radicals and inhibits plant growth. Reactive oxygen species harm the cell under osmotic stress by interacting with proteins, lipids, and DNA (Jha, 2014) [5].

The plant should develop an effective antioxidant system in order to decrease the toxic effect of drought. The anti-oxidative defense system is composed of enzymatic and non-enzymatic antioxidants, the plant possess this system in order to protect itself against overproduced ROS and oxidative damage (Jha, 2014) [5]. ROS increased levels due to drought stress leads to the oxidative stress. The antioxidant enzyme superoxide dismutase (SOD) activity in the leaf is studied to understand how BBS is able to induce tolerance to drought stress. Once applying BBS to the drought stressed plants and measuring the SOD activity, it was shown that the SOD activity had increased till the ninth day post inoculation, where it peaks. After the ninth day post inoculation, SOD activity fell down but remains higher than that in the control plants (Figure 4) (Wang, 2012) [12].

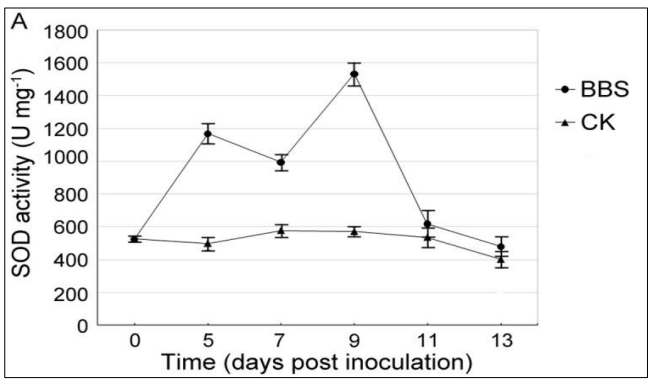

Fig 4: SOD activity in plants treated with BBS and in control plants under drought stress (Wang, 2012) [12]

1.2. ACC deaminase produced by PGPR conferred IST to plants

PGPR could affects plant growth in a direct or indirect way, or by both ways. PGPR direct mechanisms to effect plant growth are through providing plants with phytohormones, fixed nitrogen, soluble phosphate, or iron through bacterial siderophores, or ACC deaminases (Lim, 2013) [7].

ACC deaminase (1-aminocyclopropane-1-carboxylate) is secreted by many types of PGPR, it is able of inducing systematic tolerance to stress conditions like drought stress. When exposed to stress conditions the plant hormone ethylene reduces root and shoot growth by regulating homeostasis, but in the presence of ACC diaminase, ethylene precursor ACC is degraded and plant stress is released. In order to study the activity of the ACC deaminase, the amount of α-ketobutyrate released when ACC deaminase cleaves ACC is measured. PGPR strains that contain ACC deaminase are able to lower plants ethylene levels. Without the use of large amounts of agrochemicals and chemical fertilizers in drought affected regions, plants that produce auxin and ACC deaminase are able to reduce drought stress (Lim, 2013) [7].

The influence of PGPR strain on the plant growth under drought stress was studied through an experiment. In this experiment, pepper plants were grown under drought stress, the first sample was inoculated with PGPR (*B. licheniformis* K11), and the second sample was not inoculated with PGPR. A third sample was used as control where the pepper plants were grown under normal conditions and no drought stress was induced, they were treated with water normally (Lim, 2013) [7].

The comparison between the growth of pepper plants under drought stress with or without inoculation with PGPR (*B. lincheniformis* K11) and the growth of water treated plants shows that after drought stress the length of the root and shoot and the dry weight were decreased (Lim, 2013) [7].

Comparing the growth of the pepper plants under drought stress that were inoculated with PGPR (*B. lincheniformis* K11) to those that were not inoculated with PGPR showed that the inoculation with PGPR (*B. lincheniformis* K11) had increased the length of the root and the shoot under drought stress (Figure 5) (Lim, 2013) [7].

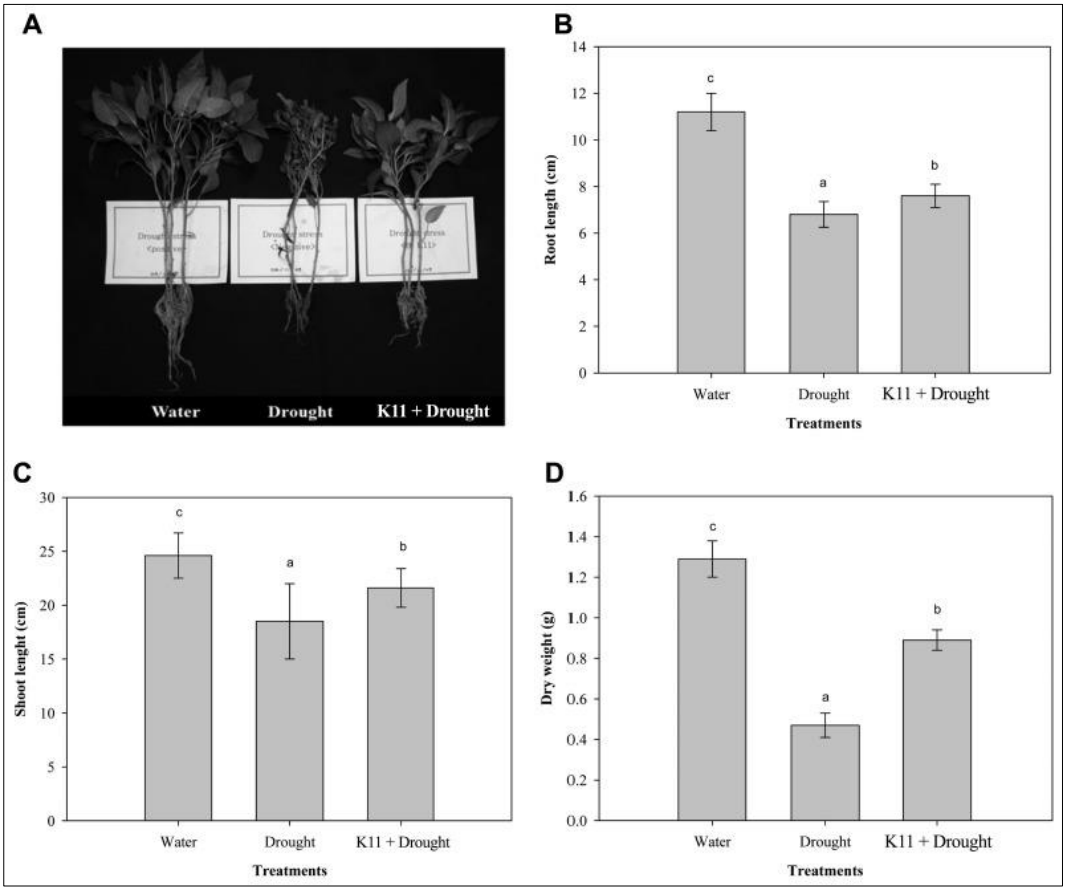

Fig 5: drought stress decreased the root and shoot length also decreased the dry weight of the pepper plants. Inoculating the pepper plants with PGPR (*B. lincheniformis* K11) increased the root and shoot length and increased the dry weight. (A) plant picture, (B) root length, (C) shoot length, (D) dry weight (Lim, 2013) [7].

1.3. PGPR increases photosynthetic performance

Plant Growth Promoting Rhizobacteria (PGPR) colonizes the rhizosphere of many plant species; the interaction of plant with these PGPR benefits the plant. One of the ways by which PGPR benefits the plant is by helping it to overcome and tolerate many stresses. Drought stress is the most serious stress that is affecting the agriculture these days. Serious water stress can lead to death due to irreversible cellular damages (Bresson, 2014) [3].

PGPR could induce systematic tolerance to drought stress by different ways; one of these ways is increasing photosynthetic

performance. Photosynthetic capacity under drought stress normally decrease mainly through stomatal closure and leaf senescence, incubating the plants by PGPR changes the photosynthetic capacity by changing the chlorophyll content, and changing photosynthetic PSII efficiency which leads to an increase in the photosynthetic outcome (Bresson, 2014) [3].

Chl-fluorescence is a powerful, rapid and minimally invasive indicator of photosynthetic performance by measuring the quantum yield of PSII (photosystem II).PGPR triggers improvement of plant photosynthetic performance by increasing the chlorophyll content (Bresson, 2014) [3].

Physiological processes in plant are greatly dependent on ABA (abscisic acid hormone). ABA plays an important role in late seed development stage and is essential for the response to environmental stresses (cold, drought, salt…) (Porcel, 2014) [9].

Under drought stress, PGPR induce an increase of ABA (abscisic acid) concentration in the leaf which leads to a decrease of transpiration rate. ABA has a critical rule in the response of plant to drought stress by controlling stomatal closure. The modifications in the ABA contents during drought stress by PGPR induce better survival of plants by improving their tolerance to dehydration. Thus an increase in the ABA content by PGPR leads to a decrease water loss under drought stress (Bresson, 2014) [3].

2. Improving plant growth and yield production by PGPR

Beneficial soil microorganisms induce the plant growth and increase yield production. The most beneficial soil microorganism is Plant Growth Promoting Rhizobacteria (PGPR). PGPR can exist in the rhizosphere, on the root surface or in the spaces between cells and in this case they are called extracellular bacteria. In the case that PGPR exist inside the cell they are called intracellular bacteria, mainly they are N_2 fixing bacteria (Porcel, 2014) [9].

PGPR affects the plant by direct or indirect mechanisms. Production of hormones by PGPR is an example of direct mechanism (Erturk, 2009) [4]. Plant hormones like auxin, gibberellins, abscisic acid (ABA) and cytokines induce growth and development of the plant (Porcel, 2014) [9].

2.1. Auxin production by PGPR

Many microorganisms that interact with plants are able to synthesize hormones like those produced by the plant itself to regulate growth, such as auxins, gibberellins and cytokines. Among these hormones auxin is the most studied hormone due to its important role in plant growth, it plays an essential role in the initial process of lateral and adventitious root formation and root elongation (Erturk, 2009) [4].

Synthesis of auxin in plants and in microbes needs a precursor, an amino acid, L-tryptophan (L-TRP). TRP is a naturally synthesized by the root exudates for rhizosphere microflora that may induce production of auxin in the rhizosphere (Khalid, 2003) [6].

Many PGPR strains are able to produce hormone IAA (indole-3-acetic acid). IAA is the most produced type of auxin in nature, and it is synthesized mainly depending on the presence of tryptophan (Erturk, 2009) [4].

Each strain of PGPR produces specific amounts of IAA. Strains grown in the presence of tryptophan produce larger amounts of IAA than the strains that were grown in the absence of tryptophan (Figure 6) (Erturk, 2009) [4].

Bacterial strains	IAA production (μg ml^{-1} (OD$_{600}$ unit)$^{-1}$)	
	Control	Tryptophan (25μg ml^{-1})
RC23	4.3±0.7b	20.4±1.6c
RC05	6.8±0.9ab	32.8±2.6a
OSU 142	6.3±0.8ab	22.4±2.1bc
RC03	5.9±0.6ab	27.3±2.7b
RC41	6.7±0.5ab	30.5±2.4ab
RC01	5.6±0.5ab	25.3±1.7bc
RC19	7.2±0.5a	33.6±2.6a

* Values in the same column with different lower-case letters in same clone are significantly different at $p<0.05$. Average ± standard error from three separate experiments. Data were means of three replicates IAA production in average 48, 72, and 168 h pure cultures.

Fig 6: The production of IAA by PGPR in the presence of various concentrations of tryptophan (Erturk, 2009) [4].

Great variation in the IAA production among the PGPR tested. In the absence of tryptophan amount of IAA produced varied between 4.3μg (*Bacillus RC23*) to 7.2μg (*Bacillus simplex RC19*). In the presence of tryptophan amount of IAA produced by PGPR increases. *Bacillus simplex* RC19 produces the highest amount of IAA, while *Bacillus* RC23 produces the lowest amount of IAA (Erturk, 2009) [4].

A correlation exists between auxin produced by rhizobacteria in vitro and growth of wheat seedlings especially root and shoot growth. This indicates that auxin production by PGPR causes the development of the root system, which increases the biomass production (Khalid, 2003) [6].

PGPR produces auxin themselves and effect the production of auxin in cuttings. Semi-hardwood stem cuttings of kiwifruit were treated with PGPR, higher numbers than water treated control stem cuttings were observed for the number of main roots, root length, root diameter, root dry weight, root quality and root percentage. Plants treated with IBA (indole-3-butyric acid), auxin family plant hormone, have the highest results (Figure 7) (Erturk, 2009) [4].

Treatments	The number of main roots per cutting	The highest root length (cm)	Average root diameter (mm)	Root dry weight per cutting (mg)	Root quality (1-5 scale)	Rooting (%)
Control	0.53f	0.70f	0.27e	2.56g	1.63e	12.50g
IBA 2000 ppm	4.89b	9.22ab	1.18b	19.63b	3.02b	57.50b
IBA 4000 ppm	5.18a	9.76a	1.30a	22.33a	3.33a	72.50a
RC23	2.67d	6.73cd	1.05c	13.89e	2.14d	32.50e
RC05	3.36c	6.17de	1.12bc	15.37d	2.82bc	40.00d
OSU142	2.47de	5.70de	0.92d	12.47f	2.40cd	25.00f
RC03	3.40c	6.40d	1.19b	17.18c	2.53c	47.50c
RC41	2.88cd	7.70c	1.03c	15.76d	1.70e	40.00d
RC01	2.47de	7.60cd	0.90d	14.86de	2.12d	32.50e
RC19	3.07d	8.63ab	1.13bc	17.29c	2.00de	47.50c
LSD	0.25	1.24	0.11	1.02	0.24	4.67

* Values in the same column with different lower-case letters are significantly different at p<0.05.

Fig 7: The effect of bacteria on rooting and root growth of semi hard-wood cuttings of kiwifruit (Erturk, 2009) [4].

The highest number of main roots per cutting, the greatest root length and diameter of stem cuttings, the best root dry weight, the highest root quality and the highest rooting percentage was obtained in plants treated with 4000 ppm IBA followed by 2000 ppm IBA. The lower results were for the control. Plants treated with PGPR strains have results that are lower than IBA treated plants but higher than control (Erturk, 2009) [4].

These results show that root induction occurs due to either auxin production by PGPR or due to auxin production by the cuttings themselves after the plant is incubated with PGPR (Erturk, 2009) [4].

Evaluating the effect of inoculation by PGPR *Azopirillum brasilense* on growth of spring wheat, they observed better germination in inoculated plants, an increase in the dry weight and early development and flowering of the root system and the upper plant parts (Khalid, 2003) [6].

To get high crop yield and quality, concentrated amounts of chemical fertilizers and pesticides are used, but this method is expensive and affects human health and the environment (Xu, 2014). These problems give more importance for the usage of PGPR as bio-fertilizers (Erturk, 2009) [4]. The best way to select effective PGPR for bio-fertilizer development biotechnology is the screening of rhizobacteria for invitro auxin production and growth promotion under axenic conditions (Khalid, 2003) [6].

2.2. PGPR source of organic nitrogen

Nitrogen is essential for the plants to make its proteins, nucleic acids and other biological molecules. Plants absorb organic nitrogen from the soil. To get higher yield of crops, inorganic N fertilizers are greatly used. But these inorganic N fertilizers are considered as environmental polluters because only 30-50% of it is absorbed by the plant and the remaining amount is lost by run-off leaching or volatilization (White, 2015) [13].

Alternative source of nitrogen that is considered friendly to the environment is through oxidative N scavenging (ONS) process. ONS process provides proteases that degrade oxidatively denatured proteins during the day. While denatured protein degradation and absorption occur at night.

These proteins that are degraded through ONS, by direct oxidative degradation, are mainly PGPR proteins that are present around the root. Roots secrete reactive oxygen that is responsible for denaturing the proteins. The denaturing of proteins by reactive oxygen leads to protein unfolding, which in turn increases the exposure of peptide bonds to proteases.

Plant and PGPR secrete proteases that degrade proteins into oligopeptides and peptides, which are smaller and may be absorbed by the root. These degraded proteins are considered as an important source of nitrogen to the plant (Figure 8) (White, 2015) [13].

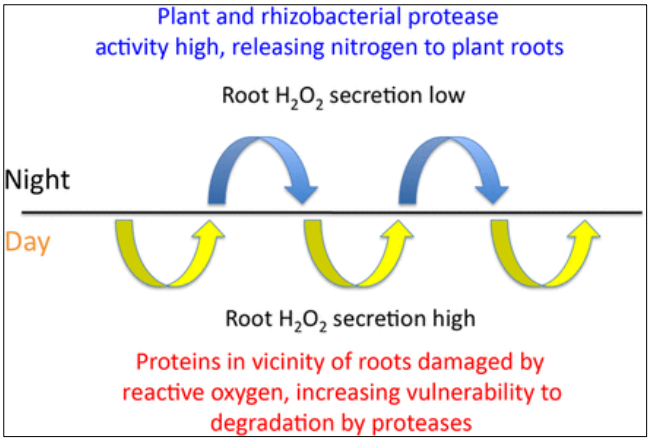

Fig 8: Proposed cyclic model of the oxidative nitrogen scavenging process in grasses. In daylight, roots secrete hydrogen peroxide in order to denature proteins (PGPR proteins) around roots. At night plant and PGPR proteases degrade oxidized proteins to form smaller peptides or oligopeptides that may be absorbed by roots (White, 2015) [13].

2.3. PGPR stimulates plant growth under rising atmospheric CO_2

Under nutrient limited environments, plant performance decreases, but incubating the plants with PGPR increases its performance. The future climate is characterized by an increase in CO_2, which may be a severe condition facing the plants. PGPR is able to help plants tolerate this condition and continue growing normally. PGPR could minimize the harmful effects of elevated CO_2 (Nie, 2014) [8].

The association of elevated CO_2 with PGPR, positively affects the plant by increasing the total plant biomass C. The highest plant productivity which is expressed by the total plant biomass C, was shown when the plant is treated with the elevated CO_2 and PGPR (Figure 9) (Nie, 2014) [8].

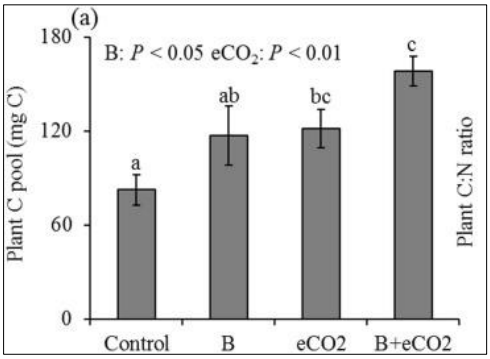

Fig 9: Plant C pool size per pot**.** Control: ambient CO_2 and without bacteria addition; B: ambient CO_2 and with bacteria addition; eCO_2: elevated CO_2 and without bacteria addition; B + eCO_2: elevated CO_2 and with bacteria addition (Nie, 2014) [8].

When combined, PGPR and elevated CO_2 increased the plant C:N ratio, indicating that plants can store more C per unit of N in tissue when CO_2 is high (Figure 10) (Nie, 2014) [8].

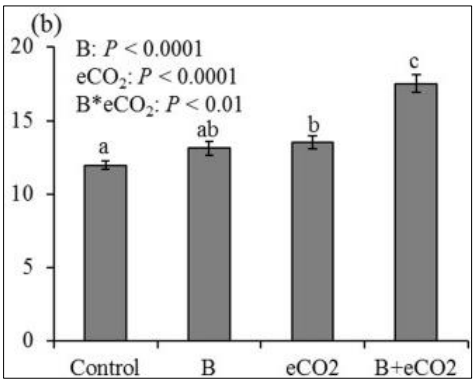

Fig 10: Biomass C: N ratio. Control: ambient CO_2 and without bacteria addition; B: ambient CO_2 and with bacteria addition; eCO_2: elevated CO_2 and without bacteria addition; B + eCO_2: elevated CO_2 and with bacteria addition (Nie, 2014) [8].

3. PGPR protects the plants from pathogens

PGPR benefits the plant through an antagonism against plant pathogens by producing siderophores, protease, cellulose, cyanide and antibiotics. Spore-forming PGPR have a lot of advantages over non-spore formers in product formulation and stable maintenance in soil (Xu, 2014) [14].

3.1. Seven strains of *Paenibacillus* protect tomato from FCRR disease

Fusarium crown and root rot (FCRR) is a disease that damages tomato production. It is caused by the fungus *Fusarium oxysporum f. sp. Radicis-lycopersici* (FORL). This disease leads to the loss of the crops in greenhouses or in open field.

Seven strains of bacteria were able to inhibit the mycelial growth of FORL in an in vitro dual culture. These strains were identified to be *Paenibacillus* spp (SC02-09, SC09-2, SG09-01, SG09-02, SR04-02, SR04-16 and SR07-23) according to their specific characteristics like producing spores. This study was done in order to estimate the effectiveness of the seven *Paenibacillus* strains in the reduction of FCRR disease under greenhouse conditions (Xu, 2014) [14].

These bacterial strains were cultured with FORL in order to screen them for antagonistic activity, after 14 days of culturing the inhibition zones were measured. In the dual culture plate containing SC09-21, the antagonistic activity against FORL was the highest (12.2mm inhibition zone) (Figure 11) (Xu, 2014) [14].

Paenibacillus strain	Sampling source	Inhibition zone (mm)[a]
SC02-09	Chinese cabbage field	8.9 ± 0.3 e
SC09-21	Chinese cabbage field	12.2 ± 0.3 a
SG09-01	Garlic field	10.4 ± 0.2 c
SG09-02	Garlic field	9.7 ± 0.2 d
SR04-02	Paddy field	11.6 ± 0.4 b
SR04-16	Paddy field	11.3 ± 0.4 b
SR07-23	Paddy field	9.9 ± 0.4 d

Fig 11: Antagonistic activity of *Paenibacillus* strains against FCRR by measuring the inhibition zone (Xu, 2014) [14].

SG09-01, SR04-02, and SR04-16 strains formed an inhibition zones >10mm, the remaining three strains produce inhibition zones between 8.9mm and 9.7 mm in the dual culture assays. The fungal mycelia covered the entire plate surface of the control plate and no inhibition zones were observed (Figure 12) (Xu, 2014) [14].

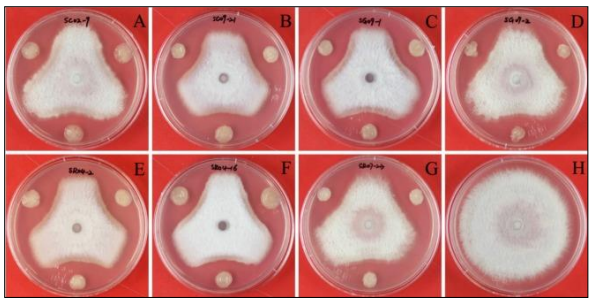

Fig 12: Dual culture assay for *in vitro* inhibition of mycelial growth of FORL by *Paenibacillus* strains. Inhibition zones were measured after incubation for 14 days. Results are shown for: A, SC02-09; B, SC09-21; C, SG09-01; D, SG09-02; E, SR04-02; F, SR04-16; G, SR07-23; H, Control (Xu, 2014) [14].

The results showed that all these seven strains of *Paenibacillus* were able to reduce the effect of disease that was induced by experimental inoculation of the plant pathogen FORL into host plants, compared to the control. The disease severity of FCRR was reduced by >80% using the strain SC09-21, whereas it was reduced by >60% using the strains SC02-09 and SG09-02 (Figure 13) (Xu, 2014) [14].

Treatment	Disease severity (%)	Control value (%)
SC02-09	31.7 ± 13.7 abc[a]	68.3 ± 16.7 ab
SC09-21	18.3 ± 6.2 ab	81.7 ± 8.5 ab
SG09-01	51.1 ± 7.5 abc	48.9 ± 13.3 bc
SG09-02	32.2 ± 11.7 abc	67.8 ± 12.8 ab
SR04-02	58.3 ± 13.2 cd	41.7 ± 18.4 bc
SR04-16	78.3 ± 8.1 de	21.7 ± 12.2 c
SR07-23	46.1 ± 11.1 bc	53.9 ± 18.9 bc
Tapseed[b]	22.8 ± 6.9 abc	77.2 ± 10.9 ab
Metaconazole	2.8 ± 0.5 a	97.2 ± 4.8 a
Control	100.0 ± 0.0 e	

Fig 13: The suppressive effect of seven *Paenibacillus* strains against FCRR in tomato (Xu, 2014) [14].

3.2. Induced systemic resistance by PGPR against leaf spot pathogens

One of the mechanisms by which PGPR reduces plant diseases is through induced systematic resistance (ISR). ISR in the plants is induced by non-pathogenic microorganisms; it starts in the roots then extends to the shoots.

The ability of three PGPR strains (*Azospirillum brasilense Sp7, Chryseobacterium balustinum AUR9 and P. flourescens AUR6*) to induce systematic resistance by reducing disease severity in *Arabidopsis thaliana* against the leaf spot pathogen *Pseudomonas syringae*pv.*tomato* DC3000 was recorded. The three strains were able to reduce disease severity compared to the control plants that were untreated with PGPR (Figure 14) (Solano, 2008) [11].

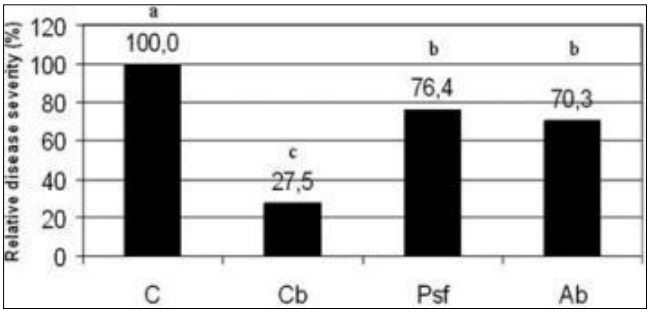

Fig 14: Relative disease activity in *Arabidopsis thaliana* plants incubated with different strains of PGPR: *Chryseobacterium balustinum AUR9* (Cb), *Azospirillum brasilense Sp7* (Ab), *P. flourescens AUR6* (Psf), and untreated controls. All these four were exposed to leaf spot pathogen *Pseudomonas syringae*pv.*tomato* DC3000 (Solano, 2008) [11].

Chryseobacterium balustinum AUR9 reduced disease severity by more than 72% compared with control plants, which is the highest value. The other two PGPR strains *Azospirillum brasilense Sp7* and *P. flourescens AUR6* decrease disease severity by 29.7% and 23.6% respectively, which are less percentages than that of *Chryseobacterium balustinum AUR9* (Solano, 2008) [11].

3.3. Bacillus sp. strain RMB7 exhibited antifungal activities

127 isolated bacterial strains were screened for their PGPR activities, among them the best PGPR and biocontrol activities were detected for the bacterium named RMB7. To test the biocontrol activity of RMB7, a broad-spectrum pathogen infecting wide-range of crops, *Pythium irregular*, was applied to Arugula plant.

Strain RMB7 shows wide spectrum antifungal activity in vitro by inhibiting more than 70% of mycelia growth of all the tested fungal pathogens. Each type of fungus has a specific growth inhibition percentage (Figure 15), example *Pythium irregular* (85%), *Aspergillusniger* (76%), *Fusarium oxysporum* (71%) and *Rhizoctoniasolani* (70%) (Ali, 2014) [2].

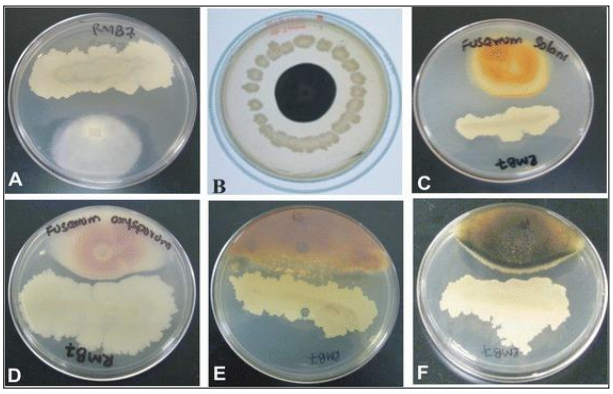

Fig 15: Antifungal dual plate assay of *Bacillus sp.* strain RMB7 with (A) *Pythium irregular* (B) *Aspergillusniger* (C) *Fusariumsolani* (D) *Fusariumoxysporum* (E) *Rhizoctoniasolani* (F) *Alternaria alternate* (Ali, 2014) [2].

IV. PGPR can have a role in decreasing soil pollution

Solid wastes such as feathers, hairs, nails, skin and wool are keratinous wastes that are degraded with great difficulty in the environment. Among these keratinous wastes, feathers are the mostly produced around the world as a by product of poultry processing plants, where the production of feathers can reach millions of tons in one year (Anwar, 2014) [1].

90% of feathers composition is made up of keratin. Keratins are a major type of animal proteins that is stable in nature and unable to be degraded. The accumulation of such type of proteins may lead to environmental pollutions.

A type of proteolytic enzymes called keratinase is able to degrade keratin and it is produced by PGPR. Morphological changes in feathers treated with isolated PGPR and the control was detected (Figure 12A). The control shows smooth and homogenous morphology. While treated feathers shows wrinkled structure with cavities (Figure 12B). This difference in structure proposes that keratinase enzyme produced by PGPR is responsible for surface deterioration of feather (Anwar, 2014) [1].

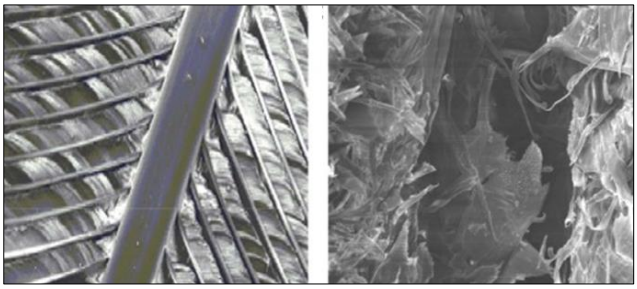

Fig 12: *In-vitro* determination of feather degrading activity. Control feather (A). Degraded feather treated with isolated PGPR (B) (Anwar, 2014) [1].

Conclusion

The usage of bio fertilizers and bio pesticides is gaining attention in the agriculture sector world-wide due to the food safety issues and increasing environmental concerns. These synthetic bio fertilizers and pesticides are pollutants to the environment, also they are expensive.

PGPR enrich the soil environment with micro- and macro-nutrients, protects the plants from pathogens and helps the plant tolerates environmental stresses. Depending upon their function, they may serve as partial replacements for chemical fertilizer or pesticides as an eco-friendly and cost-effective alternative as compared to their synthetic counterparts. In the future, PGPR can be the key players in the sustainable agriculture.

References

1. Anwar M, Siddique M, Verma A, Rao Y, Nailwal T, Ansari M. *et al*. Multitrait plant growth promoting (PGP) rhizobacterial isolates from *Brassica Juncea* rhizosphere Keratine degradation and growth promotion. Landes Bioscience. 2014; 7(1):1-9.
2. Ali S, Hameed S, Imran A, Iqbal M, Lazarovits G. Genetic, physiological and biochemical characterization of Bacillus sp. Strain RMB7 exhibiting plant growth promoting and broadspectrum antifungal activities. *Microbial Cell Factories*. 2014; 13(144): 1-15.
3. Bresson J, Vasseur F, Dauzt M, Labadie M, Fabrice V, Touraine B. *et al*. Interact to Survive: *Pyllobacterium brassicacearum* Improves Arabidopsis Tolerance to Severe Water Deficit and Growth Recovery. *PLOS ONE*. 2014; 9(9):1-12.
4. Erturk Y, Ercisli S, Haznedar A, Cakmakci R. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidiadeliciosa*) stem cuttings. *Biological Research*. 2010; (43):91-98.
5. Jha Y, Subramanian R. PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. *PhysiolMolBiol Plants*. 2014; 20(2):201-207.
6. Khalid A, Arshad M, Zahir Z. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology. 2004; 96:473-480.
7. Lim J, Kim S. Induction of Drought Stress Resistance by Multi-functional PGPR *Bacillus licheniformis* K11 in Pepper. *The Korean Society of Plant Pathology*. 2013; 29(2):201-208.
8. Nie M, Bell C, Wallenstein M, Pendall E. Increased plant productivity and decreased microbial respiratory C loss by plant growth-promoting rhizobacteria under elevated CO_2. *Scientific Reports*. 2014; 5(9212):1-6.
9. Porcel R, Zamarreno A, Garcia-Mina J, Aroca R. Involvement of plant endogenous ABA in Bacillus megaterium PGPR activity in tomato plants. BioMed Central Plant Biology. 2014; 14(36):1-12.
10. Shavit R, Lalzar M, Burdman S, Morin S. Inoculation of tomato plants with rhizobacteria enhances the performance of the phloem-feeding insect Bemisiatabaci. Frontiers in Plant Science. 2013; 306(4):1-12
11. Solano B, Maicas J, Iglesia M, Domenech J, Manero F. Systemic Disease Protection Elicited by Plant Growth Promoting Rhizobacteria Strains: Relationship Between Metabolic Responses, Systemic Disease Protection, and Biotic Elicitors. The American Phtopathological Society. 2008; 4(98):451-457.
12. Wang C, Yang W, Wang C, Gu C, Niu D, Liu H. *et al*. Induction of Drought Tolerance in Cucumber Plants by a Consortium of Three Plant Growth-Promoting Rhizobacterium Strains. *PLOS ONE*. 2012; 12(7):1-10.
13. White J, Chen Q, Torres M, Mattera R, Irizarry I, Tadych M, Bergen M. *et al*. Collaboration between grass seedlings and rhizobacteria to scavenge organic nitrogen in soils. *AoB Plants*. 2015; 7(93):1-11.
14. Xu S, Kim B. Biopcontrol of Fusarium Crown and Root Rot and Promotion of Growth of Tomato by *Paenibacillus* Strains Isolated from Soil. The Korean Society of Mycobiology. 2014; 42(2):158-166.