Pak. J. Bot., 55(6): 2057-2066, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-6(33)

IMPACT OF PLANT GROWTH PROMOTING RHIZOBACTERIA BIOFERTILZERS ON BIOCHEMICAL ATTRIBUTES, ANTIOXIDANT ACTIVITIES, NUTRITIONAL VALUES AND PRODUCTIVITY OF MAIZE

AMJID KHAN[1,2], RASHID ABBAS KHAN[3], MUHAMMAD ALI[4] AND ZABTA KHAN SHINWARI[1*]

[1]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan*
[2]*Department of Botany, University of Mianwali, Mianwali 42200, Pakistan*
[3]*Department of Botany, University of Education, Lahore, Pakistan*
[4]*Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320, Pakistan*
**Corresponding author's email: shinwari2008@gmail.com*

Abstract

The quest for enhancing agricultural yields due to increased pressure on food production has inevitably led to the indiscriminate use of chemical fertilizers and other agrochemicals. The potential role of plant growth promoting rhizobacteria (PGPR) as a biofertilizer evolved as appropriate substitute to neutralize adverse environmental impacts wielded by manmade agrochemicals. The recent study was conducted to elucidate the role of plant growth promoting rhizobacteria as biofertilizer. Maize seeds were treated with PGPR (*Azotobacter chroococum & Planomicrobium chinense*) and in combination to observe the effects on physiology, hormonal activity, antioxidant enzymes, nutritional composition, and productivity. The study revealed that application of PGPRs and biofertilizer significantly (p<0.05) improved the physio-biochemical attributes including root length (222%), shoot length (85.1%), proline (%), phenolics (71%), flavonoids (85%) and protein (94%) as compared to control. The hormonal activity and plant-defense related antioxidant enzymes activities also improved leading to improve the yield, the observed increased in grain yield (81%) with 100 grain weight 25.37%. The application of PGPR resulted in increased soil fertility, maximum increase in soil organic matter (26.74%), total nitrogen (33.10%), available phosphorus (99.17%) and available potassium (48.55%). Similarly maximum increase in nitrogen was 54.4%, phosphorus 54.5%, potassium 34.72%, magnesium 78%, was observed. The present study clearly signifies that the use of biofertilizers and bioinoculants for sustainable yield production of maize is environment friendly and can used as alternative of the chemical fertilizers.

Key words: Antioxidant, *A. chroococum*, Biofertilizer, Biomass, Maize, *Zea mays,* Nutrients, Organic contents, *P. chinense,* Soil fertility.

Introduction

Zea mays L. commonly known as maize or corn belonging to family *Poaceae* (*Gramineae*) is food crop (Gul *et al*., 2017; Russo *et al*., 2019; Vocciante *et al*., 2022). Maize has been used worldwide for food purposes and being used in genetic research for different traits (Iqbal *et al*., 2014; Kong *et al*., 2020). It is a cereal crop with value worldwide. It is 3rd most important cereal crop after wheat and rice (Iqbal *et al*., 2015; Amjad *et al*., 2020). Maize has a significant nutritional value having starch (72%), protein (10.4%), fats (40.5%) mineral and oil (Imran, 2015; Arshiya *et al*., 2022). It also contains enough vitamins and minerals. The starch from maize plays a significant role in the maize processing industry (Ma *et al*., 2020). Phytohormones commonly known as plant hormones are basically trace endogenous compounds which play a significant role in growth and developmental process of plants, it can be stated that the PGPR plays an important role in hormone stimulation or increasing their potency (Liu *et al*., 2019; Sedri *et al*., 2022).

Plant growth promoting rhizobacteria also known as PGPR are basically a rhizosphere bacterium which enhances the plant growth by different mechanism like phosphate solubilizations, nitrogen fixation, siderophore production, rhizosphere engineering, phytohormones productions, antifungal activity, volatile organic productions, promoting beneficial plant microbe's symbiosis etc. (Bhattacharyya & Jha, 2012). These PGPRs can protect the plant from different plant pathogens (Gangopadhyay & Ghosh, (2019) PGPRs are classified into extracellular and intracellular PGPRs (ePGPR and iPGPR). The ePGPR are found on the rhizoplane or spaces between root cortex while iPGPRs are found inside the nodular structures of root cells (Mokabel *et al*., 2022; Mokoginta *et al*., 2022). PGPR plays a significant role in the sustainability of different crops as PGPR maintains plant health by nitrogen fixation and many other mechanisms. These microbes also provide resistance to plants as it enhances the activity of antioxidant enzymes and many other non-enzymatic antioxidants (Kumar *et al*., 2020). PGPR functions as bio-stimulant, biofertilizer and bioprotectants (Maryani *et al*., 2019). Cherif *et al*., (2018) have been reported that PGPR and endophytes enhances the nutrient uptake, reduce chemical fertilizer and chemical secretions which enhance the crop productivity, even under stressed environments (Choudhary & Varma, 2016; Afzal *et al*., 2017; Afzal *et al*., 2019; Javed *et al*., 2020; Sedri *et al*., 2022). Maize production can be increased by biofertilizer and chemical fertilizers but biofertilizer can avoid the harmful effects of chemical fertilizers (Ahmed *et al*., 2020). Many chemical changes are associated with the bacteria in which some strains of PGPRs directly regulate plant physiology by promoting synthesis of plant hormones, increased plant growth, nutrient and yields (Ram *et al*., 2013; Noman *et al*., 2018; Saboor *et al*., 2021; Ji *et al*., 2020).

Biofertilizers are basically microbial inoculants and can be defined as microorganisms having efficient strains for nitrogen fixations, solubilizing of phosphate and

phytohormone production (Mokabel *et al*., 2022; Mokoginta *et al*., 2022). Unlike other fertilizers they do not serve as food for a specific plant. However, these are basically organic substances with microbial cultures and can be used for plants, seeds, and soil for colonizing of rhizosphere to increase the nutrients for plant growth. Many studies reveal the direct and indirect benefits for growth of different agricultural crops (Filho *et al*., 2020). Since 1980 to onwards, PGPRs have been popular in India and China because they are referred as yield increasing bacteria followed by biofertilizers in India. Before the commercial market for PGPR as biofertilizers can be focused, significant work and effort needs to be done. Researchers have identified a number of possible PGPR; however, they have not been successfully commercialized. The preliminary research indicates that the PGPRs have potential beneficial effects on improving the productivity; however, further studies are required for better understanding of the interactions between microbes and plants, before upscaling at the commercial level. In the present study, we applied the PGPR strains for assessing their overall effects on the productivity of maize, which is considered an important cash crop.

Material and Methods

Seeds collection: The seeds of Maize (variety AGAITI 85) were collected from National Agricultural Research Centre (NARC), Islamabad, Pakistan.

PGPR isolation: *Planomicrobium chinense* (accession no. MF616408) already isolated from wheat rhizosphere (Khan *et al*; 2017) grown in rainfed areas and *Azotobacter chroococum* (MK567895) isolated from paddy soil (Fazal & Bano, 2010) were used. The Department of Biosciences at University Wah, Pakistan, provided the isolates.

Biofertilizer preparation: Biofertilizer of *P. chinense* and *A. chroococum* was prepared in Lab of National Agriculture Research Centre, Islamabad, Pakistan using 500 g autoclaved of sugarcane husk and broth culture of *P. chinense* (40 ml) and *A. chroococum* (40 ml) injected under sterile condition and kept for two weeks after uniform mixing of broths and carrier material. After two weeks the seeds were coated with biofertilizer using 0.6% sucrose solution for uniform coating. Experiment was performed in the green house at National Agriculture Research Centre, Islamabad under temperature range of 25-27°C.

Seeds treatment: Seeds were soaked in broth culture of *A. chroocoum*, *P. chinense* and combination of *P. chinense + A. chroococum.*

Experiments: The experiment was placed in a completely randomized design and a total of 20 pots were used, and seeds were treated with different dozes as mentioned in (Table 1).

Determination of phytohormone from plant leaves: Indole acetic acid (IAA), gibberellic acid (GA) and abscisic acid content of fresh leaves were determined following the method of Kettner & Dorffling, (1995). The phytohormone content of leaves was determined after two weeks of seed sowing. Fresh leaves (2 g) were crushed in methanol at 4°C with butylated hydroxytoluene (BHT) as an antioxidant. After 72 h extractions, centrifugation at 3000 rpm for 15 min at 4°C obtained the supernatant. The supernatant was dried using Rotary Film Evaporator (RFE) and obtained extract pH were adjusted to 2.5-3.0 using 1N HCl and portioned with 1/3 ethyl acetate and dried in RFE. The obtained hormones dissolved in 1ml methanol was run in HPLC column equipped with UV detector and C-18 column keeping the column of temperature 35°C. Commercially graded IAA, GA, and ABA (Sigma Chemical Company USA) were used to identify and analyze phytohormones. Methanol eluted IAA and GA at 280 nm and 254 nm. ABA was eluted using a linear gradient of methanol (30-70%) at 0.8 ml min-1 at 254 nm. The retention time of ABA was determined by using authentic standards (Hansen & Doerffling, 1999).

Table 1. Details of seeds treatments.

S. No.		Treatments
1.	*C*	Untreated control
2.	*T1*	Seeds inoculated with *A. chroococum*
3.	*T2*	Seeds inoculated with *P. chinense*
4.	*T3*	Seeds inoculated with combination of *A. chroococum + P. chinense*
5.	*T4*	Seeds coated with biofertilizer of *A. chroococum* and *P. chinense*

Chlorophyll (a & b) determination: Chlorophyll concentration was estimated following Arnon, (1949). Fresh maize leaves (0.1 g) were crushed in 3 mL of 80% acetone and centrifuged at 1,000 rpm for 5 min at 4°C. Acetone added 7 mL to the supernatant. Optical density was recorded at 663 nm and 645 nm using PerkinElmer Lambda 25 spectrophotometer. Chlorophyll a, b and Carotenoid was calculated by using the following formula, where w = fresh weight, v = volume of filter solution, D = Dilution factor.

Chlorophyll a = 12.3D663 – 0.86D663*d* x 100 x w × v
Chlorophyll b = 12.3D663 – 0.86D645d x 1000 x w × v

Antioxidant enzymes: The method of Beauchamp and Fridovich, (1971) with slight modification was followed to determine the Superoxide dismutase (SOD) activity, Peroxidase (POD) activity (Vetter *et al*., 1958) and method reported by Kumar and colleagues was used for estimation of Catalase activity (Kumar *et al*., 2010).

Biochemical analysis:

Proline contents: The method of Bates & co-workers (1973); Parvin *et al*., (2015) were followed to determine the proline contents. Fresh leaves were grinded in 5 ml of 3 % sulphosalycylic acid and mixture was blended at 3000 rpm for 15mints. Ninhydrin reagent and glacial acetic acid were mixed with 2 ml supernatant and incubated at 100°C for 1 h. Later 4 ml toluene were added. After exhaustive blending brick red shading showed up, at that point the toluene layer was isolated. The absorbance was taken at 520 nm.

Phenolics content: The methodology of Singleton & Jones, (1999) were pursued to assess the phenolic substance. Plant sample (1 ml) was mixed with 9 ml distilled water pursued by the addition of 1 ml FolinCiocalteu reagent and after that 10 ml sodium-carbonate (7%) was added to the blend and later incubate the mixture at room temperature for 90 min and absorbance was taken at 765 nm.

Flavonoids content: The methodology of Zhishen *et al*., (1999) were pursued to determine the flavonoids substance. The homogeneous mixture was prepared in 80% methanol; were centrifugated for 10 min at 3000 rpm. later the supernatant (2 ml) was mixed with $AlCl_3$ reagent 1 ml and H_2O (400 µl). After intensive blending the optical density were noted at 430 nm against blank.

Total soluble protein: Leaves protein content were measured by using the protocol of Lowery *et al*., (1951), and final calculation of proteins contents was done using this formula:

Protein (mg / g) = OD × K value × Dilution Factor/ sample weight
K value = 19.6

Determination of the Nutritional Status and Yield of Maize Plants: The measurement of the phosphorus (P) content by flame emission and colorimetry was used to evaluate the nutritional status of maize seedlings. (Ruget *et al*., 1996), nitrogen using the Kjeldahl method as described by Valdes *et al*., (2013) and potassium by the atomic absorption Spectrophotometer (Olsen & Sommers, 1982; Amogou *et al*., 2019). The evaluation of calcium and magnesium by following standard procedure defined by George *et al*., (2002) and following formula was used for determination of average grain yield defined by Valdes *et al*., (2013).

$$R = P \times 10.000 \div SI \times 10.000 \times 14\% \div H$$

Soil sampling and analysis: Soil samples were collected from each treatment pot. Soil analysis for soil organic matter, phosphorous, nitrogen, potassium and available potassium & available phosphorous were determined by Lu *et al*., (2000); Li *et al*., (2007).

Results and Discussion

Shoot and root length and weight: Fig 1(a) elaborates that all the treated plants showed an increase in shoot length of maize plants. The maximum increase is shown by biofertilizer. The PGPR *A. chroococum* showed 85.1% increase in shoot length as compared to control. A consortium of *A. chroococum* and *P. chinense* showed more synergism for shoot length in the form of carrier based Inocula as compared to liquid broth. *P. chinense* showed 52% increase in shoot length as compared to control. The increase in shoot length could be due to gibberellic acid production by PGPR. *Pseudomonas nitroreducens* have been reported to increase shoot length in wheat cultivars (Lee *et al*., 2019). Hindersah *et al*., (2019) have been reported that *Aztobacter* sp., has been reported to produce gibberellic acid as a secondary metabolite and effect the plant growth in saline soil. Golkar *et al*., (2019) demonstrated that *Aztobacter* sp., produced gibberellic acid in oil seed crops in cereals and increase the shoot length and shoot biomass.

Fig 1(b) depicted that all the treated plants showed an increase in shoot weight of plants. The maximum increase in shoot weight was observed in plants treated with biofertilizer of *A. chroococum* and *P. chinense*. PGPR *P. chinense* showed 84% increase in shoot weight of plants as compared to control. *A. chroococum* treated plants showed a 41% increase in shoot weight of plants as compared to control. *P. chinense* and *A. chroococum* showed synergistic effect with each other and increased shot weight at par in the form of biofertilizers as well as liquid Inocula. Htwe *et al*., (2019) have been reported that Biofertilizers based on PGPR *Bradyrhizobium japonicum* and *Streptomyces griseoflavus* increase the biomass in legume crops. The overall biomass of *vagina radiata* plants can be increased by *P. chinense* (Das *et al*., 2014; Mehnaz *et al*., 2017). The increase in the shoot weight of plants can be attributed to IAA production by PGPR. *P. chinense* can produce IAA and increase the biomass of *Helianthus annus* (Khan *et al*., 2018).

Fig 1(c) showed that all the treatments showed an increase in root length of plants. The maximum increase in root length was observed in plants treated with combination of *A. chroococum* + *P. chinense i.e*., 91% as compared to control. *A. chroococum* showed 61% increase in root length as compared to control. The effect of *P. chinense* and biofertilizer is parallel to each other. Noteworthy, the increase in root length is due to IAA producing ability of PGPR. *P. chinense* produced more IAA content that is attributed to more root length. Biofertilizer based on PGPR *Bradyrhizobium japonicum* and *Streptomyces griseoflavus* are reported to increase the root length of legume crops (Htwe *et al*., 2019; Secilia & Bagyaraj, 1992). The increase in root length has been attributed to IAA producing ability of PGPR. *P. chinense* can stimulate shoot elongation and root branching in *Helianthus annus* plants (Khan *et al*., 2018).

Fig 1(d) expected that the maximum root biomass was observed in consortium of *A. chroococum* and *P. chinense i.e.*, 93%. *P. chinense* showed 82% and *A. chroococum* showed 61% increase in root weight, as compared to control. The results suggest that *P. chinense* and *A. chroococum* showed more synergistic effect for root weight in the form of liquid inocula as compared to biofertilizer. Previous study showed that *Azospirillum* sp., along with *Pseudomonas* sp., as a biological fertilizer increase growth of maize roots, *P. chinense* has ability to produce exopolysaccharides (Khan *et al*., (2019; Naserzadeh *et al*., 2019).

Phenolics, flavonoids, proline, and protein content of leaves: Phenolics, flavonoids, proline and protein content of leaves increased in all treatments as compared to control when plants inoculated with *A. chroococum* and *P. chinense* increased by 48-42%, 13-26%, 33-39% and 20-28% respectively (Fig. 1e, f, g, h). A consortium of *A. chroococum* and *P. chinense* increased phenolics, flavonoids, proline, and protein content of leaves by

51%, 70%, 90% and 75% respectively. Maximum increase in phenolics, flavonoids, proline and protein contents were recorded in plants by 71%, 85%, 98% and 94% respectively when biofertilizer applied. PGPR *A. chroococum* and *P. chinense* produced the phenolics content in plants and showed similar effect when used alone or as bioinoculant or biofertilizer. Noteworthy high phenolics production makes PGPR used as biocontrol agent against pathogens. Velmouruugane *et al*., (2017) reported that *A. chroococum* when inoculated with chickpea, increase the phenolics content. *P. chinense* and consortia of *P. chinense* + *A. chrocoocum* increase the proline content, but the effect of both PGPR was not so much synergistic for proline production. Proline is osmoregulant and protects plants from osmotic shock, both PGPR can be used to induce tolerance in plants under stress conditions. *Pseudomonas putida* have been reported to increase the proline content in maize plants under sodic soil condition (Khan *et al*., 2022; Joshi *et al*., 2020; Kotzot *et al*., 2000; Nosheen *et al*., 2016). PGPR *A. chroococum* and *P. chinense* in the form of carrier based inocula can increase the flavonoids content and show synergistic effect, but effect was more pronounced in the form of biofertilizer as compared to bioinoculant. High flavonoids production by both PGPR predict their role in inducing biotic and abiotic stress tolerance in plants. *Bradyrhizobium japonicum* and *Bcillus subtilis* can increase flavonoids content in soybean (Marinkovic *et al*., 2018; Ashraf *et al*., 2019). Carrier based inocula of PGPR have more synergistic effect for protein production as compared to liquid inocula. Protein is secondary metabolite and can increase overall biomass of plants (Latef *et al*., 2020).

Antioxidant enzyme assay: PAL, POD, SOD and CAT activities of leaves increased in all the treatment when plants inoculated with *A. chroococum* and *P. chinense* alone with carriers which were 5.83-9.16, 2.17-1.9, 5.52-7.48 and 0.61-0.88 mg/g, respectively as compared to control (Fig. 2a, b, c, d). Co-inoculation of both the PGPR maximum increased the PAL and CAT activities of leaves which were 12.5 and 1.32 mg/g, respectively. SOD and POD activities were much higher when biofertilizers applied by 11.27 and 3.22 mg/g, respectively. Faize *et al*., (2011) showed that ROS detoxification by PGPR improves antioxidant enzyme activity. Reactive oxygen species (ROS) when their role as a second messenger is the major contributor (Yan *et al*., 2007; Sharma *et al*., 2012) showed that SOD, CAT, POD, and ascorbate peroxidase are highly active against ROS.

IAA, GA, and ABA contents: Fig. 2(e) showed that inoculation with PGPR and biofertilizer increases the IAA content of leaves. Biofertilizer-inoculated plants exhibited the maximum increases in IAA concentration of *A. chroococum* + *P. chinense i.e*., 26.33 µg/g. PGPR *A. chroococum* and *P. chinense* increase the IAA content of plants 9.48 and 19 µg/g, respectively. Noteworthy combination of *A. chroocum* and *P. chinense* showed effect at part in the form of biofertilizer as compared to liquid inocula. *Pseudomonas plecoglossicida* can increase IAA in maize plant, increase root and shoot biomass (Zerrouk *et al*., 2019). *Planomicrobium chinense* produced IAA in wheat plants and can increase plant biomass (Khan *et al*., 2019; Khan & Bano, 2019).

Fig. 2(e) showed that the maximum GA content was observed in plants treated with combination of *A. chroococum* + *P. chinense i.e*., 23.35 µg/g. The plants treated *A. chroococum* and *P. chinense* showed 18.3 and 12.3 µg/g increase in GA content of leaves respectively vs the control. Noteworthy, the combination of *A. chroococum* and *P. chinense* showed the GA production at par and showed pronounced effect for GA production. *Aztobacter chroonocum* isolated from saline soil can produced gibberellins in plants (Hindersah *et al*., 2019). Afzal & Asad, (2019) have been reported that biofertilizers containing *Rhizobium, Aztobacter, Pseudomonas* sp., has ability to produced phytohormones including IAA, GA and ABA is growth inhibiting hormone produced under stress conditions (Takahashi & Shinozaki, 2019).

Fig. 2(e) showed that all the treatments showed reduced production of ABA as compared to control but biofertilizer of *A. chroococum* and *P. chinense* was inhibitorier to ABA production as compared to control. The combination of *A. chroococum* and *P. chinense* showed 0.71 µg/g decrease in ABA production vs the control. *P. chinense* and biofertilizer showed parallel response for ABA production. Ul-Hye *et al*., (2019) have been reported that *Bacillus amyloliquefaciens* and *Agrobacterium fabrum* improved wheat production in drought stress and inhibit ABA production. *P. chinense* have been previously reported to reduce ABA production in wheat under rainfed condition (Khan & Bano, 2019).

Effect of PGPR and biofertilizer on the amount of organic matter and nutrients in the soil of the maize rhizosphere (Values are the mean of three replicates with standard error). LSD (least significant difference) at $p \leq 0.05$ indicates that values with various letters after them are statistically different. SOM is for soil organic matter, and TN, TP, TK, AP, and AK stand for total nitrogen, total phosphorus, and total potassium.

Organic matter and nutrient contents: Table 2 shows the organic matter and nutrient levels of soil samples from different treatment groups. Compared with the control treatment maximum increase in SOM (26.74%), TK (33.10%), AP (99.17%) and AK (48.55%) respectively using biofertilizer showing that biofertilizer made soil more fertile and that both the PGPR and biofertilizer had a significant impact on soil fertility, however the combination of both the PGPR *A. chroococum* and *P. chinense* maximum increased in content of TN (43.24%) and TP (44.23%) respectively. PGPR strains in the rhizosphere may stimulate soil nutrient uptake. PGPR and biofertilizer enhanced maize rhizosphere soil SOM, TN, TP, AK, and AP. These findings were consistent with those of previous investigations in maize plant (Wang *et al*., (2021). Lazcano *et al*., (2013) have shown that PGPR and biofertilizer improve rhizosphere soil organic matter and nutrients.

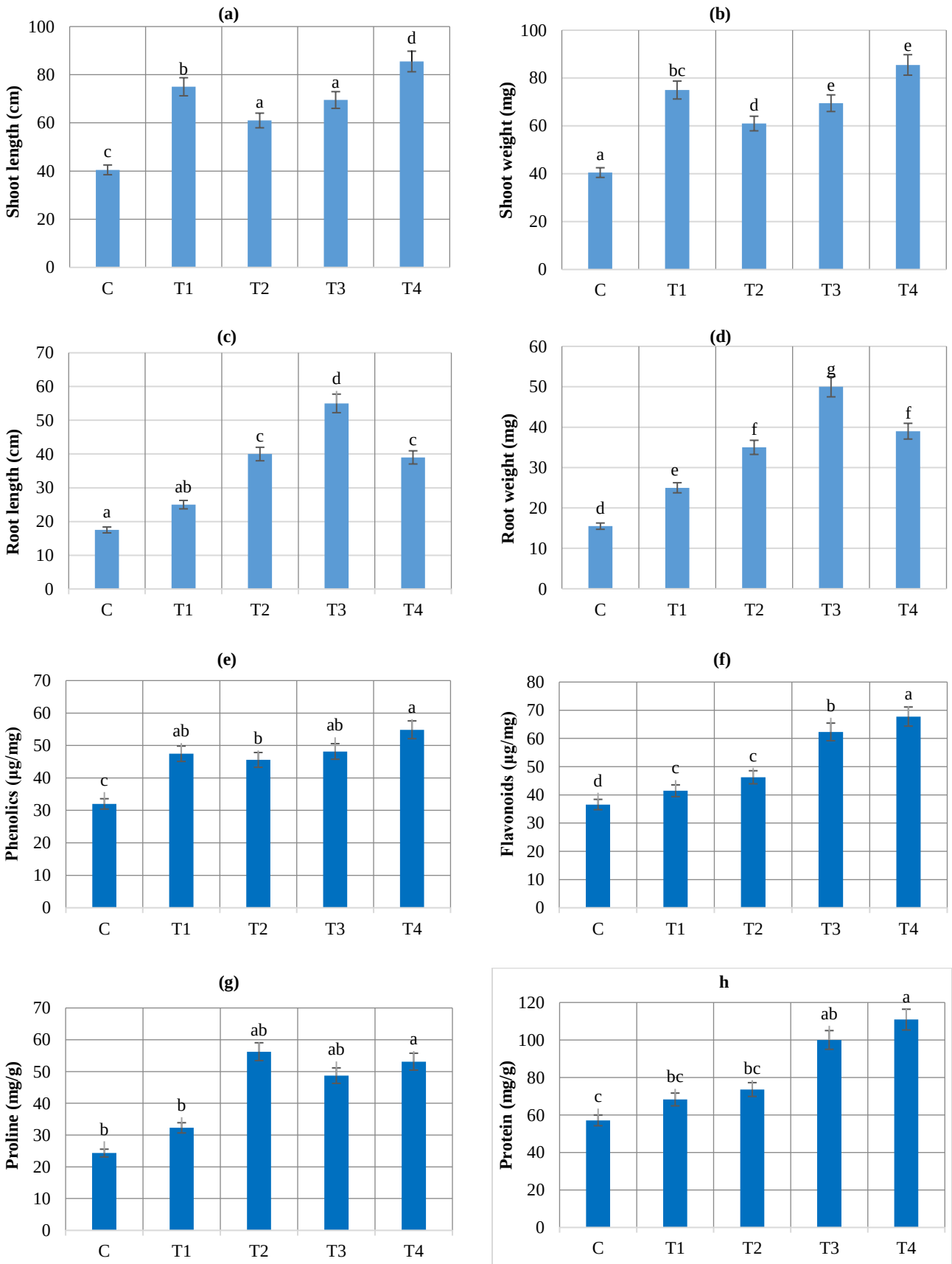

Fig. 1. Effects of carrier and Inocula based PGPR on shoot and root length, weight, phenolics, flavonoids, proline, and protein contents of maize. Values are the meaning of three replicates. Values followed by different letters are significantly different according to, $p \leq 0.05$, Tukey's honest significant difference. *C*= untreated control, *T1* = seeds inoculated with *Azotobacter chroococum*, *T2* = seeds inoculated with *Planomicrobium chinense*, *T3* = seeds inoculated with combination *Azotobacter chroococum* and *Planomicrobium chinense*, *T4* = seeds coated with biofertilizer of *Azotobacter chroococum* and *Planomicrobium chinense.*

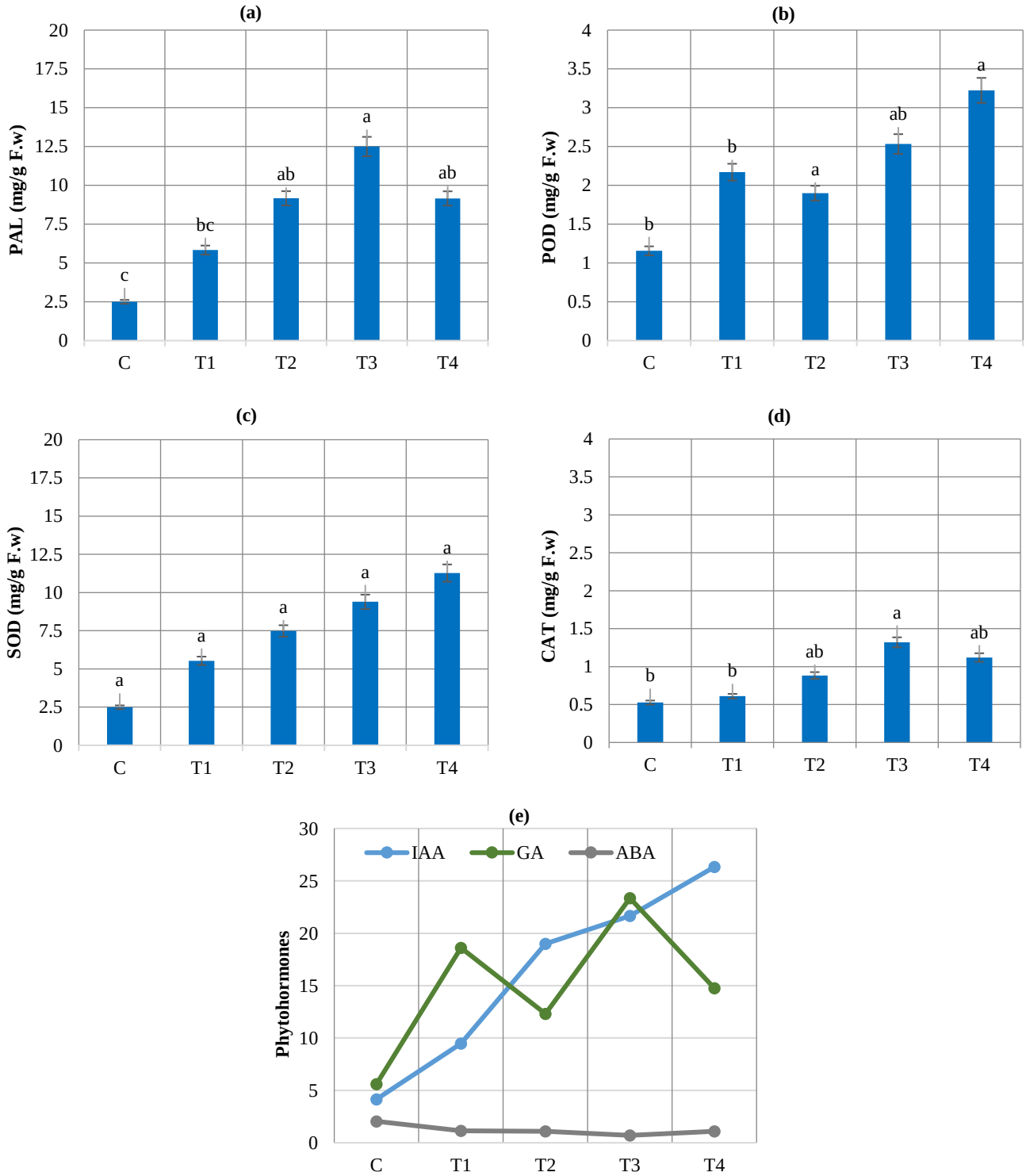

Fig. 2. Effects of carrier and Inocula based PGPR on PAL, POD, SOD and CAT activity of leaves, IAA, GA, and ABA content of maize. Values are the meaning of three replicates. Values followed by different letters are significantly different according to, $p \leq 0.05$, Tukey's honest significant.

Table 2. Organic matter and nutrient contents.

Treatments	SOM (g.kg ^{1})	TN (g.kg^{-1})	TP(g.kg^{-1})	TK (g.kg^{-1})	AP (mg.kg^{-1})	AK (mg.kg^{-1})
C	31.26c ± 0.72	0.37c ± 0.015	0.52c ± 0.92	14.89c ± 0.28	39.81e ± 0.92	115.33d ± 0.88
T1	28.82d ± 0.57	0.35c ± 0.92	0.44d ± 0.02	15.24bc ± 0.42	43.53d ± 0.55	136.33c ± 0.88
T2	32.59c ± 0.31	0.46b ± 8.81	0.61b ± 8.81	15.49bc ± 0.58	54.90c ± 1.24	140b ± 0.57
T3	35.72b ± 0.69	0.53a ± 1.24	0.75a ± 0.01	16.64b ± 0.66	60.74b ± 1.27	104.33e ± 1.76
T4	39.62a ± 0.73	0.50a ± 5.77	0.65b ± 0.01	19.82a ± 0.52	79.29a ± 0.86	171.33a ± 0.88
LSD (0.05)	2.08	0.03	0.05	1.62	3.49	1.94

Table 3. Nutritional status and yield.

Treatments	N %	P %	K %	Ca %	Mg %	Grain yield	1000 Grain weight (g)
C	1.25e ± 0.27	0.09b ± 2.96	0.72d ± 0.01	0.13d ± 0.08	0.13c ± 0.10	1.28d ± 0.01	133.22e ± 1.00
T1	1.51cd ± 0.24	0.37b ± 0.24	0.76cd ± 0.03	0.14c ± 0.01	0.17c ± 0.24	2.45bc ± 0.27	139.82d ± 1.41
T2	1.66c ± 0.01	1.18a ± 0.01	0.81c ± 0.01	0.15c ± 1.52	0.20c ± 0.08	2.07c ± 0.08	146.95c ± 0.57
T3	1.70b ± 0.08	1.19a ± 0.08	0.88b ± 1.45	0.18a ± 1.52	0.90b ± 0.05	2.80b ± 0.28	154.28b ± 0.86
T4	1.93a ± 0.01	1.39a ± 0.24	0.97a ± 0.01	0.15b ± 1.45	1.21a ± 0.10	4.19a ± 0.06	167.03a ± 1.65
LSD (0.05)	0.03	0.37	0.05	4.57	0.14	0.67	3.87

The effect of PGPR and biofertilizer on the nutritional status and yield maize plants values are mean of 3 replicates ± Standard error. Values followed by different letters are significantly different according to LSD (least significant difference) at $p \leq 0.05$.

Nutritional status and yield: In our study, data on effect of PGPR and biofertilizer on nutrient absorption and yield (Table 3) show that there is significant difference between the different treatments. Compared with the control treatment maximum increase in N (54.4%), P (54.5%), K (34.72%), Mg (78%), grain yield (81%) and 1000 grain weight (25.37%) respectively with biofertilizer suggesting that biofertilizers made soil more fertile and that the PGPR had a significant impact on soil fertility, however the combination of both the PGPR *A. chroococum* and *P. chinense* increased the nutrient contents of maize and maximum increase in Ca (38.46%) as compared to control. Maize and rice plant growth, nutrient content, and grain production were reported by different researchers (Akhtar *et al*., 2018; Rice *et al*., 2000; Farshchi *et al*., 2021; Cao *et al*., 2022). Rizwan *et al*., (2008) have reported that marked increase in yield components of cereals.

Conclusions

The treatments of biofertilizer and combination of *A. chroococum* and *P. chinense* showed improvement in growth parameters but more synergism for shoot length in the form of carrier based inocula as compared to liquid broth. The increase in shoot length is due to gibberellic acid production by PGPR while the treatments of plants with biofertilizer showed more promotion to antioxidant enzymes (SOD, POD, PAL) proline, phenolics and flavonoids contents and combination of *A. chroococum* and *P. chinense* showed more promotion to protein contents. There was a much contrast noted in the IAA, GA, and ABA contents of maize. The maximum IAA was noted in the plants treated with biofertilizer, while maximum GA contents were found in the combination of *A. chroococum* and *P. chinense*. The use of biofertilizer enhanced the yield productivity by increasing soil fertility. Hence both the treatments showed better promotion to maize growth, and this is an environment friendly technique that can be used in future.

Acknowledgements

We are grateful to the Dean, Faculty of Science, University of Wah, and Wah Cantt, Pakistan and Chairman, Department of Botany, University of Mianwali, Mianwali 42200, Pakistan for financial assistance.

References

Afzal, A. and S.A. Asad. 2019. Microbial Applications for Sustainable Agriculture. In: *Innovations in Sustainable Agriculture* Springer, Cham. pp. 43-77.

Afzal, I., I. Iqrar, Z. K. Shinwari and A. Yasmin. 2017. Plant growth-promoting potential of endophytic bacteria isolated from roots of wild *Dodonaea viscosa* L. *Plant Growth Regulation*, 81: 399-408.

Afzal, I., Z.K. Shinwari, S. Sikandar and S. Shahzad. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiological Research*, 221: 36-49.

Ahmed, A., T. Sultan, G. Qadir, O. Afzal, M. Ahmed, S.S. Shah, M. Asif, S. Ali and M.Z. Mehmood. 2020. Impact Assessment of Plant Growth Promoting Rhizobacteria on Growth and Nutrient Uptake of Maize (*Zea mays*). *Pak. J. Agri. Sci.*, 33(2): 234-246.

Akhtar, N., M. Naveed, M. Khalid, N. Ahmad, M. Rizwan and S. Siddique. 2018. Effect of bacterial consortia on growth and yield of maize grown in *Fusarium* infested soil. *Soil & Environ.*, 37(1): 35-44.

Ali, B., X. Wang, M.H. Saleem, A. Hafeez, M.S. Afridi, S. Khan and S. Ali. 2022. PGPR-mediated salt tolerance in maize by modulating plant physiology, antioxidant defense, compatible solutes accumulation and bio-surfactant producing genes. *Plants*, 11(3): 345.

Amjad, M., H. Raza, B. Murtaza, G. Abbas, M. Imran, M. Shahid and M.M. Iqbal. 2020. Nickel toxicity induced changes in nutrient dynamics and antioxidant profiling in two maize (*Zea mays* L.) Hybrids. *Plants*, 9(1): 1-15.

Amogou, O., G. Dagbénonbakin, N.A. Agbodjato, P.A. Noumavo, K.V. Salako, M.Y. Adoko and L. Baba-Moussa. 2019. Applying rhizobacteria on maize cultivation in Northern Benin: Effect on growth and yield. *Agric. Sci.*, 10(06): 763-782.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24(1): 1-15.

Ashraf, A., A. Bano and S.A. Ali. 2019. Characterisation of plant growth-promoting rhizobacteria from rhizosphere soil of heat-stressed and unstressed wheat and their use as bio-inoculant. *Plant Biol.*, 21(4): 762-769.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant & Soil*, 39(1): 205-207.

Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, 44(1): 276-287.

Bhattacharyya, P.N. and D.K. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.*, 28(4): 1327-1350.

Cao, M., J. Dong, J. Wang, Y. Cai, T. Ma, X. Zhou and R. Qin. 2022. Identification of a major stable QTL for spikelet number in wheat (*Triticum aestivum* L.) and its genetic effects analysis on yield-related traits. *Euphytica*, 218(7): 1-15.

Cherif, H., M. Neifar, H. Chouchane, A. Soussi, C. Hamdi, A. Guesmi and A. Cherif. 2018. Extremophile diversity and biotechnological potential from desert environments and saline systems of southern Tunisia. In: *Extremophiles*. CRC Press. pp. 33-64.

Choudhary, D.K. and A. Varma. (Eds.). 2016. *Microbial-mediated induced systemic resistance in plants*. New York, NY, USA: Springer. pp. 147-162.

Choudhury, D., S. Tarafdar and S. Dutta. 2022. Plant growth promoting rhizobacteria (PGPR) and their eco-friendly strategies for plant growth regulation: A review. *Plant Sci. Today*, 9(3): 524-537.

Das, R. and B.N. Tiwary. 2014. Production of indole acetic acid by a novel bacterial strain of Planomicrobium chinense isolated from diesel oil contaminated site and its impact on the growth of *Vigna radiata*. *Eur. J. Soil Sci.*, 62: 92-100.

Dos Santos, W.D., L.F. Maria de Lourdes, A. Finger, A.C. Teixeira and O. Ferrarese-Filho. 2004. Lignification and related enzymes in Glycine max root growth-inhibition by ferulic acid. *J. Chem. Ecol.*, 30(6): 1203-1212.

Farshchi, H.K., M. Azizi, M. Teymouri, A.R. Nikpoor and M.R. Jaafari. 2021. Synthesis and characterization of nanoliposome containing Fe2+ element: A superior nano-fertilizer for ferrous iron delivery to sweet basil. *Sci. Hort.*, 283: 110110.

Fazal, H. and B. Asghari. 2010. Effect of diazotrophs (Rhizobium and Azatebactor) on growth of maize (*Zea mays* L.) and accumulation of lead (PB) in different plant parts. *Pak. J. Bot.*, 42(6): 4363-4370.

Gangopadhyay, D. and A. Ghosh. 2019. Impact of plant growth promoting rhizobacteria in sustainable agriculture: An important natural resource for crop improvement. *Int. J. Plant and Env.*, 5(03): 210-214.

George, T.S., P.J. Gregory, M. Wood, D. Read and R.J. Buresh. 2002. Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. *Soil Biol. Biochem.*, 34(10): 1487-1494.

Golkar, P., S. Tabatabaei and N. Mosavat. 2019. A comparative study on the effect of gram negative and positive bacterial strains on germination and seedling growth of cereals and oil seed crops. *Biol. J. Microorg.*, 8(32): 139-151.

Gul, H., S. Kinza, Z. K. Shinwari and M. Hamayun. 2017. Effect of selenium on the biochemistry of Zea mays under salt stress. *Pak. J. Bot*., 49(SI): 25-32.

Hindersah, R., P. Suryatmana, M.R. Setiawati, B.N. Fitriatin, A. Nurbaity and T. Simarmata. 2019. Salinity resistance of azotobacter isolated from saline soil in West Java. In: *Plant Growth Promoting Rhizobacteria (PGPR): Prospects for Sustainable Agriculture* (pp. 323-334). Springer, Singapore.

Htwe, A.Z., S.M. Moh, K.M. Soe, K. Moe and T. Yamakawa. 2019. Effects of biofertilizer produced from *Bradyrhizobium* and *Streptomyces griseoflavus* on plant growth, nodulation, nitrogen fixation, nutrient uptake, and seed yield of mung bean, cowpea, and soybean. *Agronomy*, 9(2): 1-12.

Imran. 2015. Effect of germination on proximate composition of two maize cultivars. *J. Biol. Agri. Healthcare*, 53(8): 929-943.

Iqbal, J., Z.K. Shinwari and M.A. Rabbani. 2015. Maize (*Zea mays* L.) germplasm agro-morphological characterization based on descriptive, cluster and principal component analysis. *Pak. J. Bot*., 47(SI): 255-264.

Iqbal, J., Z.K. Shinwari, M.A. Rabbani and S.A. Khan. 2014. Genetic variability assessment of maize (*Zea mays* L.) germplasm based on total seed storage proteins banding pattern using SDS-PAGE. *Eur. Acad. Res*., 2(2): 2144-2160.

Javed, A., A.H. Shah, A. Hussain, Z.K. Shinwari, S.A. Khan, W. Khan and S.A. Jan. 2020. Potential of endophytic fungus *Aspergillus terreus* as potent plant growth promoter. *Pak. J. Bot*., 52(3): 1083-1086.

Ji, C., X. Wang, H. Tian, L. Hao, C. Wang, Y. Zhou and X. Liu. 2020. Effects of Bacillus methylotrophicus M4-1 on physiological and biochemical traits of wheat under salinity stress. *J. Appl. Microbiol.*, 5: 123-128.

Joshi, B., A. Chaudhary, H. Singh and P.A. Kumar. 2020. Prospective evaluation of individual and consortia plant growth promoting rhizobacteria for drought stress amelioration in rice (*Oryza sativa* L.). *Plant and Soil*, 457(1): 225-240.

Kettner, J. and K. Dörffling. 1995. Biosynthesis and metabolism of abscisic acid in tomato leaves infected with Botrytis cinerea. *Planta*, 196(4): 627-634.

Khan, A., S. Asad, A. Bano, R.A. Khan and T.A. Qadri. 2022. Evaluation of plant growth promoting rhizobacteria (PGPR) for the control of charcoal rot of mung bean. *Pak. J. Bot.*, 54(1): 285-289.

Khan, N. and A. Bano. 2016. Modulation of phytoremediation and plant growth by the treatment with PGPR, Ag nanoparticle and untreated municipal wastewater. *Int. J. Phytoremed.*, 18(12): 1258-1269.

Khan, N. and A. Bano. 2019. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. *PLoS One*, 14(9): e0222302.

Khan, N., A. Bano and M.A. Babar. 2019. The stimulatory effects of plant growth promoting rhizobacteria and plant growth regulators on wheat physiology grown in sandy soil. *Arch. Microbiol.*, 201(6): 769-785.

Khan, N., A. Bano and M.D. Babar. 2017. The root growth of wheat plants, the water conservation and fertility status of sandy soils influenced by plant growth promoting rhizobacteria. *Symbiosis*, 72(3): 195-205.

Khan, N., P. Zandi, S. Ali, A. Mehmood, M. Adnan Shahid and J. Yang. 2018. Impact of salicylic acid and PGPR on the drought tolerance and phytoremediation potential of *Helianthus annus*. *Front. Microbiol.*, 9: 2507.

Khan, R.A., A. Khan and T.A. Qadri. 2019. Influence of seed priming with $FeSO_4$ on germination, growth and biochemical aspects of mung bean (*Vigna radiata* L.) grown under NaCl stress. *J. Biosci. Appl. Res*., 5(4): 519-532.

Kong, D. X. Fu, X. Jia, W. Wang, Y. Li, J. Li and C. Ju. 2020. Identification of quantitative trait loci controlling ethylene production in germinating seeds in maize (*Zea mays* L.). *Sci. Rep.*, 10(1): 1-9.

Kotzot, D. M.J. Martinez, G. Bagci, S. Basaran, A. Baumer, F. Binkert and A. Schinzel. 2000. Parental origin and mechanisms of formation of cytogenetically recognisable de novo direct and inverted duplications. *J. Med. Genet.*, 37(4): 281-286.

Kumar, A. R. Kumar, M. Kumari and S. Goldar. 2020. Enhancement of plant growth by using PGPR for a sustainable agriculture: A review. *Int. J. Curr. Microbiol. Appl. Sci.*, 9: 152-165.

Kumar, P.P. S. Kumaravel and C. Lalitha. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *Afr. J. Biochem. Res.*, 4(7): 191-195.

Lazcano, C., M. Gómez-Brandón, P. Revilla and J. Domínguez. 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. *Biol. Fertil. Soils*, 49(6): 723-733.

Lee, S.G., H. Lee, J. Lee, B.C. Lee, H. Lee, C. Choi and N. Chung. 2019. Effect of plant growth promoting bacteria on early growth of wheat cultivars. *J. Appl. Biol. Chem.*, 62(3): 247-250.

Leme Filho, J.F., W.E. Thomason, G.K. Evanylo, X. Zhang, M.S. Strickland, B.K. Chim and A.A. Diatta. 2020. The synergistic effects of humic substances and biofertilizers on plant development and microbial activity: A review. *Int. J. Plant. Soil Sci*., 32(7): 56-75.

Li, Y.S., L.H. Wu, L.M. Zhao, X.H. Lu, Q.L. Fan and F.S. Zhang. 2007. Influence of continuous plastic film mulching on yield, water use efficiency and soil properties of rice fields under non-flooding condition. *Soil Tillage Res.*, 93(2): 370-378.

Lie, J.C., J. Shi, X.L. Zhao, G. Wang, H.F. Yu and Y.J. Ren. 1994. Separation and determination of three kinds of plant hormone by high performance liquid chromatography. *Fenxi–Huaxue*., 22: 801-4.

Liu, Y., X. Fang, G. Chen, Y. Ye, J. Xu, G. Ouyang and F. Zhu. 2019. Recent development in sample preparation techniques for plant hormone analysis, *TrAC Trends Anal. Chem.*, 113: 224-233.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193: 265-275.

Lu, R.K. 2000. Methods of soil and agro-chemical analysis. *China Agricultural Science and Technology Press, Beijing*, 127-332.

Ma, S.X., Y. Liu, J. Liu, J. Zhang and R. Liu. 2020. Changes in starch structures and in vitro digestion characteristics during maize (*Zea mays* L.) germination. *Food Sci. Nutr*., 8(3): 1700-1708.

Martínez-Viveros, O., M.A. Jorquera, D.E. Crowley, G.M.L.M. Gajardo and M.L. Mora. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.*, 10(3): 293-319.

Maryani, Y., W.S. Dewi and A. Yunus. 2019. Isolation and screening of calcareous and non-calcareous soil rhizobacteria producing osmoprotactant and indol acetic acid in Gunung Kidul, Yogyakarta, Indonesia. *Bulg. J. Agric. Sci. Ral. Sci.*, 25: 36-41.

Mehnaz, S. (Ed.). 2017. *Rhizotrophs: Plant growth promotion to bioremediation* (Vol. 2). Springer.

Mohammadi, K. and Y. Sohrabi. 2012. Bacterial biofertilizers for sustainable crop production: A review. *J. Agric. Biol. Sci.*, 7(5): 307-316.

Mokabel, S., Z. Olama, S. Ali and R. El-Dakak. 2022. The role of plant growth promoting *Rhizosphere microbiome* as *Alternative biofertilizer* in boosting *Solanum melongena* L. adaptation to salinity stress. *Plants*, 11(5): 1-22.

Mokoginta, R.F., S. Tumbelaka and R. Nangoi. 2022. The Effect of PGPR (plant growth promoting rhizobacteria) bio fertilization on the growth and production of lettage (*Lactuca sativa* L.). *J. Agroekoteknologi Terapan*, 3(1): 43-51.

More, N. A. Verma, R.N. Bharagava, A.S. Kharat, R. Gautam and D. Navaratna. 2022. Sustainable development in agriculture by revitalization of PGPR. In: *Bioremediation* (pp. 127-142). CRC Press.

Naserzadeh, Y. A.M. Nafchi, N. Mahmoudi, D.K. Nejad and A.S. Gadzhikurbanov. 2019. Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on the quality and components of corn forage in Iran. *J. Agron. Ani. Indust.*, 14(3): 209-224.

Numan, M., S. Bashir, Y. Khan, R. Mumtaz, Z.K. Shinwari, A.L. Khan, A. Khan and A.H. Ahmed. 2018. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiological Research*, 209: 21-32.

Nosheen, A., A. Bano, H. Yasmin, R. Keyani, R. Habib, S.T. Shah and R. Naz. 2016. Protein quantity and quality of safflower seed improved by NP fertilizer and Rhizobacteria (*Azospirillum* and *Azotobacter* spp.). *Front. Plant Sci.*, 7: 104.

Olsen, S.R. 1982. Phosphorus. *Methods Soil Anal.*, 2: 403-430.

Page, A.L., R.H. Miller and D.R. Keeney. 1982. Methods of soil analysis. Part 2. Chemical and microbiological properties. Agronomy, No. 9. *Soil Science Society of America, Madison, WI*, 1159.

Parvin, S., T. Javadi and N. Ghaderi. 2015. Proline, protein, RWC and MSI contents affected by paclobutrazol and water deficit treatments in strawberry cv. Paros. *Cercetări Agronomice în Moldova*, 161: 107-114.

Ram, R.L., C. Maji and B.B. Bindroo. 2013. Role of PGPR in different crops-an overview. *Ind. J. Seric.*, 52(1):1-13.

Riaz, U., G. Murtaza, W. Anum, T. Samreen, M. Sarfraz and MZ. Nazir. 2021. Plant growth-promoting rhizobacteria (PGPR) as biofertilizers and biopesticides. In: *Microbiota and Biofertilizers* (pp. 181-196). Springer, Cham.

Rice, W.A., N.Z. Lupwayi, P.E. Olsen, D. Schlechte and S.C. Gleddie. 2000. Field evaluation of dual inoculation of alfalfa with *Sinorhizobium meliloti* and *Penicillium bilaii*. *Can. J. Plant Sci.*, 80: 303-308.

Rizwan, A., M. Arshad, A. Khalid and A. Zahir. 2008. Effectiveness of organic bio-fertilizer supplemented with chemical fertilizer for improving soil water retention. Aggregate stability, growth, and nutrient uptake of maize. *J. Sustain. Agri.*, 34: 57-77.

Ruget, F., R. Bonhomme and M. Chartier. 1996. Estimation simple de la surface foliaire de plantes de maïs en croissance. *Agronomie*, 16(9): 553-562.

Russo, M.L., A.C. Scorsetti, M.F. Vianna, M. Cabello, N. Ferreri and S. Pelizza. 2019. Endophytic effects of *Beauveria bassiana* on corn (*Zea mays*) and its herbivore, Rachiplusia nu (lepidoptera: Noctuidae). *Insects*, 10(4): 1-9.

Saboor, A., M.A. Ali, S. Hussain, H.A. El Enshasy, S. Hussain, N. Ahmed and R. Datta. 2021. Zinc nutrition and arbuscular mycorrhizal symbiosis effects on maize (*Zea mays* L.) growth and productivity. *Saudi J. Biol. Sci.*, 28(11): 6339-6351.

Sahib, M.R. Z.H. Pervaiz, M.A. Williams, M. Saleem and S. DeBolt. 2020. Rhizobacterial species richness improves sorghum growth and soil nutrient synergism in a nutrient-poor greenhouse soil. *Sci. Rep.*, 10(1): 1-13.

Secilia, J. and D.J. Bagyaraj. 1992. Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice (*Oryza sativa* L.). *Biol. Fertility Soils*, 13(2): 108-111.

Sedri, M.H., G. Niedbała, E. Roohi, M. Niazian, P. Szulc, H.A. Rahmani and V. Fieziasl. 2022. Comparative analysis of plant growth-promoting rhizobacteria (PGPR) and chemical fertilizers on quantitative and qualitative characteristics of rainfed wheat. *Agronomy*, 12(7): 1-14.

Sertsu, S. and T. Bekele. 2000. Procedures for soil and plant analysis. National Soil Research Center. *Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia.* 110p.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*., 299: 152-178.

Suzuki, L.S., V. Herrig, M.L.L. Ferrarese, J.D. Rodrigues and O. Ferrarese-Filho. 2003. Simultaneous effects of ferulic and vanillic acids on peroxidase and phenylalanine ammonia-lyase in soybean (*Glycine max*) roots. *Phyton*, 43(1): 179-185.

Takahashi, F. and K. Shinozaki. 2019. Long-distance signaling in plant stress response. *Curr. Opin. Plant Biol.*, 47: 106-111.

Valdés, E.M.F., E.C. González, M.M. Serrano, H.R. Labrada, E.M. Báez, F.G. Hernández and F.A. Hernández. 2013. Experiencias obtenidas en el desarrollo participativo de híbridos lineales simples de maíz (*Zea mays*, L.) en condiciones de bajos insumos agrícolas. *Culti. Trop.*, 34(2): 61-69.

Vetter, J.L., M.P. Steinberg and A.I. Nelson. 1958. Enzyme assay, quantitative determination of peroxidase in sweet corn. *J. Agric. Food Chem.*, 6(1): 39-41.

Vocciante, M., M. Grifoni, D. Fusini, G. Petruzzelli and E. Franchi. 2022. The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant's environmental stresses. *Appl. Sci.*, 12(3): 1-16.

Wang, J., L. Liu, X. Gao, J. Hao and M. Wang. 2021. Elucidating the effect of biofertilizers on bacterial diversity in maize rhizosphere soil. *Plos One*, 16(4): e0249834.

Zafar-ul-Hye, M.S. Danish, M. Abbas, M. Ahmad and T.M. Munir. 2019. ACC deaminase producing PGPR *Bacillus amyloliquefaciens* and *Agrobacterium fabrum* along with biochar improve wheat productivity under drought stress. *Agronomy*, 9(7): 343.

Zerrouk, I.Z., B. Rahmoune, L. Khelifi, K. Mounir, F. Baluska and J. Ludwig-Müller. 2019. Algerian Sahara PGPR confers maize root tolerance to salt and aluminum toxicity via ACC deaminase and IAA. *Acta Physiol. Plant*, 41(6): 1-10

Zhishen, J., T. Mengcheng and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, 64(4): 555-559.

Zhong, W., T. Gu, W. Wang, B. Zhang, X. Lin, Q. Huang and W. Shen. 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant & Soil*, 326(1): 511-522.

(Received for publication 12 August 2022)