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Research Article

Aminocyclopropane 1-carboxylic acid deaminase producing bacteria inoculation for improving the maize seed germination and seedling growth

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ABSTRACT

Maize (Zea mays L.) is the most important and profitable crop in the world. Growth and productivity of maize has been declined recently due to improper nutrient management and unfavorable soil conditions which are contributing in food insecurity. That situation exerts a tremendous pressure on agricultural scientists for substantial increase in food production where plant growth promoting rhizobacteria (PGPR) played their part for enhance the growth of maize via different mechanisms and crop-bacteria specificity. A study was conducted to compare the effect of ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) producing bacteria two b strains (Bacillus sp. MN54 and Burkholderia phytofirmans PsJN) on germination of inoculated and un-inoculated three genotypes of maize (Arfat, Sunehri and Sultan). The germination was recorded after the 9 days of sowing and seedlings will be harvested after 21 days of sowing. The following parameters will be recorded seed germination rate, seedling growth, length of root, length of shoot, root and shoot ratio, relative growth ratio and plant vigor. In addition, models will be presented to explain the phenotypic response of maize varieties to ACC deaminase producing bacteria inoculation. The genotype named Arfat and its combined treatment with bacterial strains Bacillus sp. MN54 and Burkholderia phytofirmans PsJN performed very well in all parameters. In case of Genotype Sunehri the bacterial strain named Burkholderia phytofirmans PsJN performed high in in all parameters. The third genotype named Sultan was inoculated with bacterial strain Bacillus sp. MN54 performed very strongly in all parameters.

Keywords: *ACC Deaminase, ANOVA, Biplot, Correlation*

1. INTRODUCTION

The king of cereals, maize (Zea mays L.) is a member of family Poaceae and mainly chief source of diet around the world and specially in Pakistan. Maize utilized for multi purposes such as staple food, animal feed and raw material for agro based enterprises. In Pakistan it ranked third among cereals after wheat and rice. In GDP the contribution of maize is 0.5% and in value addition in agriculture is 2.6%. During the year of 2018, cultivated land under maize crop was 1251 thousand hectares and the yield was 5902 million tonnes (Economic Survey of Pakistan, 2018-2019). When farmers started to grow maize instead of cotton and sugarcane, the production and availability of improved variety of seed increased, along with better economic returns. The human population is growing at a higher pace in Pakistan and hindering the food security. Among cereal maize gain importance and known as king of crops. Maize crop is reported to provide higher yields per acre than other crops in the world. Crop germination percentage reduced due to extreme environmental conditions and major

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diseases. These are consisting of temperature, drought, radiation, light, insect attack, soil pH, salts in the soil and various soil pathogens.

Due to the abiotic stresses plant growth and overall crop yield decreasing and leading to food insecurity (Masood *et al*.2011). For the development of the society use of modern methods in the crops for better crop production and enhancement of overall yield. In some developing countries modern equipment and some modern strategies are using to overcome the deleterious effect of in the crops due to environmental changes. Agronomists are using the plant growth enhancers by the foliar application, seed priming (Naumovo *et al*., 2013). The cost of production of any crop decrease by using plant growth enhancers and have more positive effects as compared to chemical fertilizers (Bar-ness *et al*., 2011). Plant hormones regulates the overall plant growth and development. Among major plant growth hormones auxin is very important which increase the main root formation. It is fact that more strong crop root system leads to more uptake of nutrients and vise virsa (Pierik *et al.*,2005). In the other types of growth hormones, Cytokinines and Gibberellines that play a vital role in the stimulation of shoot development. In agriculture the beneficial bacteria in the forms of inoculum are used to enhance the crop productivity. Bacterial inoculum are applied in different method, either directly mixed with soil or treated seed before sowing. The ultimate aim of the inoculum is to improve crop production by increasing the nutrient uptake efficiency (saleem *et al*., 2007).

During growth and developmental stage, the foliar application of the plant growth regulators enhances the nutrient absorption to the leaf. Germination of seed and efficacy of seedling establishment are determined by development and growth of plant (Hadas, 2006). At the stage of seedlings stress factor can decrease crop production and due to extreme conditions results in nonuniform seedlings development (Zeid *et al*., 2001). Stress severity is directly affected rate of seed germination, seedling growth and poor imbibition of maize crop. (Glick *et al*., 2007). Germination index of maize is improved by plant growth promoter bacteria inoculation. The small amount of ethylene production in the plants play important role to improve the plant growth, while the moderate and high levels of hormones can disturb the root growth process(Abeles *et al.*,2002). A large number of beneficial bacteria has been found naturally that contain the ACC deaminase enzyme, which helps to decrease the ethylene level in a new established seedling and stressed plant. The beneficial bacterial strain thereby high concentration of enzyme so that it cannot be reach a harmful level to reduced root growth (Jacobson *et al.*,2009).

The beneficial microorganisms such as rhizobacteria, are characterized (i) In the form of colonize on the surface of root (ii) increase survival rate, multiply and can fight with other harmful microbiota, to show their protection activities (Nautiyal *et al,* 2000). Bacterial species are grown between and around the tissues of plant which regulate the plant growth by the complex mechanisms are collectively known as PGPR (Vessey 2003). In the non occcrance of potentially pathogen ic microorganisms, plant growth promoting bacteria can increase the plant growth.

On the basis of functional activities PGPR categories as (i) Biofertilizers (Improves nutrient requirement to plants), (ii) Phytostimulators (promotion of plant growth by the use of harmons), (iii) Rhizoremediators (decomposing of organic compounds) (iv) Biopesticides (controlling diseases by producing natural metabolites) (Antoun *et al*., 2005). When the improvement of the entire community of microbes in the

uper layer of soil, niches through the development of different types of substances then the promotion of plant development occurs (Zhuang *et al*., 2007). PGPR directly encourage plant growth either by encouraging the acquisition of resources (some essential nutrients and minerals) or by changing plant growth hormone levels, or indirectly by alternating the reducing effects of different pathogens on the growth of plant and the production of antimicrobial agents. PGPB regulates the number of bacteria that act as pathogen by microbial antagonism, which is accomplished by engaging with pathogens for nutrients, by producing antibiotics and by creating anti-fungal metabolites (Bar-ness *et al*., 2011). Apart from antagonism, other interactions between bacteria and plants can trigger the mechanisms in which the plant can better protect itself against pathogenic bacteria, fungi and viruses (Wang *et al*., 2005). This is known as Systemic Resistance Induced (ISR), and was first discovered by Van Peer in 1991. The inducing rhizobacteria causes a root reaction that produces a signal that travels throughout the plant, resulting in the activation of defensive mechanisms such as plant cell wall reinforcement, the development of anti-microbial phytoalexins, and pathogen-related protein synthesis (Marschner *et al*., 2004). Bacterial components which can activate ISR include lipopolysaccharides (LPS), salicylic acid, flagella and sideophores. Beneficial microorganisms such as bacteria containing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which regulate the plant growth by decreasing the plant ethylene level (Wang *et al.,* 2001). The occurrence of ACC deaminase is very common in the micro-organisms of soil. Range of bacterial isolates have been recognized for the presence of ACC genes including bacterial isolates consisting of *Azospirillum, Rhizobium, Agrobacterium, Achromobacter, Burkholderia, Ralstonia, Pseudomonas and Enterobacter* (Blaha *et al*., 2006). In different locations throughout the world, range of *Pseudomonas* (62 out of 88) strains has been reported to contain ACC deaminase activity.

Under stress condition, higher plants synthesis maximum concentration of growth hormones. Then higher production of auxin in crop plants support them in the stress condition by stimulating the elongation in plant cell and root growth of plant for nutrients uptake. After this process auxins produce the SAM (S-Adenosyl methionine) amino acid, which is precursor for the ACC in plants. With increase in the production of special hormones that cause the high production of SAM. When ACC produced in plants and their cataliste produced on the uper surface of plant root by the soil microorganisms. ACC oxidase produced in plants convert ACC into ethylene which stop the plant growth by inhabiting the growth and root initiation. Whereas ACC deaminase produced in the rhizosphere convert ACC into α-ketobutyrate and ammonium, which act as essential nutrient for the seedling growth (Penrose *et al.,* 2001). The production of ACC is higher in plant and lower in the rhizosphere of plant where ACC come out of plant in rhizosphere due to concentration gradient. The beneficial microbial (PGPR) strains give specific responses only for specific plants in specific soil conditions therefore it can be said that plant, strains and soil are specific for the expression of better plant-microbe interaction.

2. MATERIALS AND METHODS

A research trial was conducted in the glasshouse of the Institute of Plant Breeding and Biotechnology, MNS University of Agriculture Multan.

2.1. Collection of maize hybrids

Three maize hybrids were collected from CIMMYT, Pakistan and Maize and Millets Research Institute (MMRI)

Yousafwala. These maize hybrids are Arfat, Sunehri and Sultan.

2.2. Collection of bacteria

Two bacterial strains (*Bacillus* sp. MN54 and *Burkholderia phytofirmans* PsJN) were collected from Soil Microbiology and Biochemistry Lab., Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. The collected strains were saved in glycerol (*Eppendorf Tube) and stored at -80 ºC freezer*.

2.3. Inoculum Preparation

For the fresh inoculum preparation Petri plates, nutrient agar media and conical flasks were autoclave at 15 psi, 121°C for 20 minutes. The media was poured to Petri plates in laminar flow hood and cooled. Media containing Petri plates were streaked by bacteria in laminar flow hood and incubated at 28±1°C for 24 h. The freshly grown culture of bacteria on agar plates were used for broth culture preparation. A loop full of culture are inoculated in autoclaved nutrient broth and incubated in a shaking incubator at 100 rpm and 28±1°C for 72 h (Feldmann *et al*., 2009).

2.4. Seed Disinfection

Seed was disinfected using 5% sodium hypochlorite solution for 1 min and then rinsed with sterilized DI water for 4 times (Moral *et al*., 2012).

2.5. Seed inoculation

Freshly prepare inoculum was centrifuged at 10,000 rpm, 4°C for the 5 minutes in a refrigerated centrifuge. Cell pellets obtained after centrifuge were used to prepare a uniform cell density [0.5 Optical Density (OD) at 600 nm to equate 10^8 cells ml^{-1}] using a spectrophotometer. The inoculum, 15% sugar solution, peat, and clay were mixed in ratio (2:1:6:1) for coating on maize seeds. The coated seeds were dried on a filter paper sheet in the laminar flow for 6-8 h (Lifshitz *et al*., 2015).

2.6. Treatment plan

T_0 = No PGPR inoculation

T_1 = *Bacillus* sp. MN54 inoculation

T_2 = *Burkholderia phytofirmans* PsJN inoculation

T_3 = *Bacillus* sp. MN54 and *Burkholderia phytofirmans* PsJN

The genotypes were sown according to factorial arrangement under complete randomized design (CRD) with three repeats. Seed germination of the maize hybrids with or without PGPR inoculation were recorded. Afterwards the seedlings were transplanted in sand jars and harvested after 15 days of transplanting. Ten seeds per hybrid were used in the experiment and total 360 seedlings were planted. There were three seedlings per jar.

2.7. Germination trial

Ten sterilized seeds of each maize genotype were inoculated with PGPR as per treatment keeping an uninoculated seed set as control on a moist filter paper in Petri plates. The treatments were replicated thrice. Inoculated and uninoculated seeds were incubated in germinator at ambient temperature 26±1ºC for 7 days. The moisture of the plates was maintained as per the requirement. The germination data was collected on daily basis.

2.8. Pot trial

About three maize cultivars were used for evaluation in response to PGPR seed inoculation. The pots containing autoclaved peat were sown with the inoculated seeds. After sowing, the pots were irrigated with ½ strength Hoagland solution for balanced nutrition after each 5 days interval. Whereas pots are irrigated with distilled and autoclaved water daily. The following treatment plan was followed and arranged as per completely randomized design (CRD) in factorial settings with three replications.

- Factor A:
  - Genotypes: 3
- Factor B:
  - Inoculation: 4
- Replication: 3

- Total treatments: 3*4 = 12
- Total treatment units: 12*3 = 36

The trial was harvested 21st day after germination and following parameters were recorded.

2.9. Observations

The following parameters were studied during that experiment.

- ✓ **Germination of seed (%)**
- ✓ **Root length (cm)**
- ✓ **Shoot length (cm)**
- ✓ **Fresh weight of root (g)**
- ✓ **Dry weight of root (g)**
- ✓ **Fresh weight of shoot (g)**
- ✓ **Dry weight of shoot (g)**
- ✓ **Root and Shoot ratio**

2.10. Statistical analysis

The data recorded was analyzed via analysis of variance and means were compared using least significance difference test at 5% probability (Steel et al., 1997).

3. Results and Discussion

The research on the selection of maize plant to PGPR specific interaction was conducted in the glasshouse of the Institute of Plant Breeding and Biotechnology, MNS University of Agriculture Multan. The results obtained were analyzed through analysis of variance, genetic variability and correlations modules. Analysis of variance (ANOVA) showed that all the traits give significant response to treatments and genotypes. Compared with genotypes and inoculation treatment factors resulted in higher variation. Variable results were obtained due to different genotypes, genotypes and treatment factors. In the case of root length, shoot length, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, seed germination, and root and shoot ratio, high variability due to treatments was observed. Analysis of variance was performed for various plant traits as set out in Appendix 1-8. For all characters under study there were statistically significant differences among genotypes. The results of the different parameters that were analyzed are listed as follows.

3.1. Root Length (cm)

Root length and surface area can be used to monitor the water and nutrient absorption variables. Separate the root from the top (cut at surface of soil). The root length for each plant is recorded separately for the evaluation of variance. Maize genotypes showed statistically significant variations for root length are describes in (Appendix. 1). In root lengths the genotypic means of twelve maize genotypes are given in Table 4.9. The results described that genotype 2014 had maximum value root length (22.667 cm) followed by 2016 (21.667 cm) while genotype 2012 showed minimum value for root length (20 cm).

3.2. Shoot Length (cm)

The new growth which grows upward from seed germination is a shoot where leaves will emerge. Separately weigh and record the shoot length form each plant to identify the mean difference. At first, they grew in all directions, then the shoots started to go toward light, while roots started to grow toward water. For shoot length statistically significant differences as shown in (Appendix. 2). The genotypic mean of twelve maize genotypes is given for the shoot length in Table 4.10. The results described that genotype 2016 had maximum value shoot length (18.33 cm) followed by 2012 (18.20 cm) while genotype 2014 showed minimum value for shoot length (16.33 cm).

3.3. Fresh root weight (g)

Root fresh weights varies widely (usually within the range of 4–10 percent, but up to 18 percent) and were significantly influenced (P percentage 0.05) by air temperature and relative humidity, air currents, different light intensities during substratum extraction, period of soil extraction (based on the size of the experiment and the form of substratum for plant growth), and the absorbent form of

paper used to wipe excess water from harvested roots. Specific technicians' measurements did not impact the fresh or dry weight values. Fresh weight recorded immediately following harvesting of a plant or part of a plant. For fresh root weight statistically significant differences as shown in (Appendix. 3). The genotypic mean of twelve maize genotypes is given for fresh root weight in Table 4.11. The results described that genotype 2012 had maximum value shoot length (27.883 g) followed by 2016 (23.900 g) while genotype 2016 under T_0 showed minimum value for fresh root weight (18.967 g).

3.4. Dry root weight (g)

Dry weight recorded after drying the plant or part of a plant at temperatures more than the ambient air temperature (e.g., around 65 - 100 degrees C), to drive off the water. Get more precise measurement of biomass without the large weight fluctuations caused by changes in water content, which can occur pretty quickly depending on the moisture status of a plant when you harvest it, or after some period in storage. Statistically significant differences for dry root weight as shown in (Apendix. 4). For dry root weight the means of maize genotypes are given in Table 4.12. The results described that genotype 2012 had maximum value dry root weight (7.4133 g) followed by 2016 (5.6233 g) while genotype 2014 showed minimum value for dry root weight (5.0133 g).

3.5. Fresh shoot weight (g)

Shooting fresh weights ranged widely (usually between 30 and 50 percent, but up to 60 percent) and were significantly influenced by air temperature and relative humidity, air currents, various light intensities during plant extraction from the substratum, Period of soil extraction (depending on experiment size and type of plant growth substratum) and type of absorbing paper used to blot excess water from collected shoots. Similar technician measurements did not affect the fresh or dry weight values. Fresh weight recorded immediately after the plant or part of a plant is harvested. Statistically significant differences for fresh shoot weight as shown in (Appendix. 5). For fresh shoot weight the means of maize genotypes are shows in Table 4.13. The results described that genotype 2016 had highest value fresh shoot weight (9.5667 g) followed by 2012 (9.1000 g) while genotype 2014 showed minimum for fresh shoot weight (6.3000 g).

3.6. Dry shoot weight (g)

Dry weight recorded after drying the plant or part of a plant at temperatures more than the ambient air temperature (e.g., around 65 - 100 degrees C), to drive off the water. Get more precise measurement of biomass without the large weight fluctuations caused by changes in water content, which can occur pretty quickly depending on the moisture status of a plant when you harvest it, or after some period in storage. Statistically significant differences for dry shoot weight as shown in (Appendix. 6). For dry shoot weight the genotypic means of twelve maize genotypes is presented in Table 4.14. The results described that genotype 2016 had maximum value dry shoot weight (3.2867 g) followed by 2012 (92.9467 g) while genotype 2014 showed minimum value for dry shoot weight (2.2300 g).

3.7. Germination of seed (%)

Germination is a critical process and it consists of stages that occurs like absorption of water, respiration, impact of Light, mobilization of reserves, development of the embryo axis. Temperature, moisture, air, and light conditions are involved in the seed germination process. Germination percentage provides an indicator of the viability of a seed population. Statistically significant differences for germination of seed as shown in (Apendix 7). For germination of seed the maize genotypes is presented in Table 4.15. The results described that genotype 2016 had maximum germination of seed (100%) followed by

2012 (100%) while genotype 2014 showed minimum value for germination of seed (100%).

3.8. Root and Shoot ratio

The plants with a higher ratio of roots can compete more effectively for soil nutrients whereas those with a higher ratio of shoots can compete more effectively and can collect more light energy. Statistically significant differences for root and shoot ratio as shown in (Appendix. 8). For root and shoot ratio the mean of maize genotypes is presented in Table 4.16. The results described that genotype 2014 had maximum value root and shoot ratio (22.667) followed by 2016 (21.667) while genotype 2012 showed minimum value for root and shoot ratio (20.00).

Biplot for the treatment shows the correlation between the active variables and active observations in fig. 4.2. In this graph for fresh shoot weight and germination, T1 is performed very well and T2 is worse for the fresh shoot weight and germination. T1T2 strongly correlate with dry weight of root and fresh root weight and T1 and T2 worse for dry weight of root and fresh root weight. The treatment T2 performed best for the root length and shoot length.

Biplot for the genotype shows that correlation between the active variables and active observations in fig4.3. In this graph for fresh shoot length and germination, genotype 2016 is performed very well. 2014 is worse for the fresh shoot length and germination. Genotype 2012 strongly corelate with dry weight of root and fresh root weight and genotype 2016 is worse for root dry weight and fresh root weight. For the root length and shoot length 2014 performed best.

4. DISCUSSION

The inoculation of plant growth promoting rhizobacteria to seeds may improve germination, seeding vigor and ultimately the establishment of crop plants for higher yield (Simons *et al*., 2010). The present study demonstrated the specific interaction of three genotypes of maize (M1, M2 and M3) with PGPR strains MS-54 and PsJN alone and in combination. The germination parameters as germination percentage, time to 50% germination and final germination significantly improved due to PGPR inoculation in all maize genotypes. However, the interaction between M2 and combination of MN-54 and PsJN proved most efficient among the treatments. The improvement in germination could be due to specific interaction between maize genotype and PGPR strain. Moreover, the plant growth promoting traits of PGPR such as IAA production, phosphate solubilization and higher colonization of seed and influence on the ethylene homeostasis through ACC deaminase would have contributed for the positive response (Ruzicka *et al*., 2007). The germination might have been improved due to biopriming impact (Visca *et al*., 2009).

Analysis of variance (ANOVA) showed that all the traits give significant response to treatments and genotypes. Compared with genotypes and inoculation treatment factors resulted in higher variation. In the case of fresh root weight, root length, dry root weight, shoot length, fresh shoot weight, seed germination, dry shoot weight and root and shoot ratio, variability due to treatments was greater. In Appendix 1-8 analysis of variance was performed for various plant traits as set out in Appendix 1-8. For all characters under study there were statistically high significant differences among genotypes.

Maize genotypes showed statistically significant variations for root length are describes in (Apendix. 1). Maize genotypes in root length are given in Table 4.9. The results prescribed that genotype 2014 had maximum value root length (22.667 cm) while 2016 (21.667 cm) and genotype 2012 showed minimum value for root length (20 cm). For shoot length statistically significant changes as shown in (Apendix. 2). In shoot

length the genotypic mean of twelve maize genotypes is given for the shoot length in Table 4.10. In results the genotype 2016 had maximum value shoot length (18.33 cm) followed by 2012 (18.20 cm) while genotype 2014 showed minimum value for shoot length (16.33 cm). For fresh root weight statistically significant variations as shown in (Appendix. 3). The genotypic mean of twelve maize genotypes is given for fresh root weight in Table 4.11. In results the genotype 2012 had maximum value shoot length (27.883 g) followed by 2016 (23.900 g) while genotype 2016 under T_0 showed minimum value for fresh root weight (18.967 g). Statistically significant variations for dry root weight as shown in (Apendix. 4). For dry root weight the maize genotypes are given in Table 4.12. In results the genotype 2012 had maximum value dry root weight (7.4133 g) followed by 2016 (5.6233 g) while genotype 2014 showed minimum value for dry root weight (5.0133 g). For fresh root weight the genotypic means of twelve maize genotypes are given in Table 4.13. In results the genotype 2016 had maximum value fresh shoot weight (9.5667 g) followed by 2012 (9.1000 g) while genotype 2014 showed minimum for fresh shoot weight (6.3000 g). Statistically significant variations for dry shoot weight as shown in (Apendix. 6). For dry shoot weight the maize genotypes are given in Table 4.14. In results the genotype 2016 had maximum value dry shoot weight (3.2867 g) followed by 2012 (92.9467 g) while genotype 2014 showed minimum value for dry shoot weight (2.2300 g). Statistically significant variations for germination of seed as shown in (Apendix. 7 (20.00).

The PCA of normal trial have only two eigenvector that have value above 01 and the 1st eigenvector represent 78% variability, whereas 2nd eigenvector represents 13.5% variability of the total (Table 4.10). The factor loading values are given in table 4.11. The 1st eigenvector represents 78.173% variability containing the major variability for the root length (rl), shoot length (sl), fresh root weight (frw), dry root weight (drw), fresh shoot weight (fsw), germination (g), dry root weight (drw). In 2nd PCA component the highest loading values were recorded for root and shoot ratio.

5. CONCLUSION

In addition, model is presented to explain the phenotypic response of maize varieties to ACC deaminase producing bacteria inoculation. The genotype named Arfat and its combined treatment with bacterial strains *Bacillus* sp. MN54 and *Burkholderia phytofirmans* PsJN performed very well in all parameters done 100% germination and high in root and shoot length, fresh weight of root and shoot and dry weight of root. In case of Genotype Sunehri the bacterial strain named *Burkholderia phytofirmans* PsJN performed high in in all parameters also done 100% germination and high in root and shoot length, fresh weight of root and shoot and dry weight of root. The third genotype named Sultan was inoculated with bacterial strain *Bacillus* sp. MN54 performed very strongly in all parameters and set high score in seed germination, high in root and shoot length, high in fresh weight of root and shoot and dry weight of root and shoot.

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