THE EFFECT OF TWO BACILLUS ISOLATES ON ROOT ELONGATION OF WHEAT SEEDLINGS

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Beneficial rhizobacteria called as Plant Growth Promoting Rhizobacteria (PGPRs) are commonly used as inoculants for improving the growth and yield of agricultural crops. PGPRs colonize rhizosphere and roots of plants, and assist plants by direct and indirect mechanisms including modification of root system. The present study was focused on determining the effect of two rhizobacterial isolates (belonging to Bacillus genera namely; Gu2 and 127b) on root elongation of wheat seedlings under controlled conditions. Experiment was conducted according to randomized plot design. The wheat seeds coated with the isolates Gu2 and 127b were sown in pots and kept in controlled growth room for 4 weeks. To evaluate the effect of isolate, efficiency percentages of isolate were calculated based on means of linear measurements of roots and statistical analysis were performed by One Way ANOVA. Additionally, the seeds were germinated in petri dishes containing filter paper just to observe the changes on root hair visually. There was a slight increase in the efficiencies obtained from Gu2 isolate (1.87%) whereas 127b decreased primary root at the rate of 2.39%. There were no statistical significant differences between isolate treated and control plants. The results exhibited that there was no considerable impact of rhizobacteria on root elongation; however, there was observable increase in the hairs of wheat root both isolates treated in vitro.

Keywords: Rhizobacteria, growth promotion, wheat, root, growth.

INTRODUCTION

Wheat is grown on 20% of the cultivated land area of the world and is a main food resource for 40% of the world’s population (Braun et al., 2010). In 2019, the forecast of wheat production is at nearly 771 million tonnes, 5.6 percent high than previous year’s (FAO, 2019). In 2050, the world’s population is expected to reach 9 billion, thus it is estimated that cereal production needs to increase by 50% by 2030 (Alexandratos and Bruinsma, 2012). The wheat production is suffering substantial losses of biotic and abiotic stress factors (Elad and Pertot, 2014; Kan et al., 2017).

PGPRs (Plant Growth Promoting Rhizobacteria) are a group of beneficial bacteria living in the rhizosphere, the phyllosphere, or in the plant tissues as entophytes (Ahemad and Kibret, 2014; Miliutė et al., 2015). PGPRs can promote plant growth against stresses by direct and indirect mechanisms or a combination of both (Ertürk et al., 2010; Siddikee et al., 2010; Kusek and Činar, 2012; Imriz et al., 2014; Unlu and Aysan, 2016; Telek et al., 2019). Indirect mechanisms comprise the suppression of pathogens through the production of antibiotics and extracellular hydrolytic enzymes and the action of siderophores, Inducing Systemic Resistance (ISR), exo-polysaccharides production. Direct mechanisms involve making the natural nutrition source ready to use for plants including fixation of atmospheric nitrogen, solubilisation of phosphorus, potassium and iron; production of siderophores; producing phytohormones like auxins, cytokinins and gibberellins, (Ahemad and Kibret, 2014) or by the activity of 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, an enzyme which can hinder the “plant stress ethylene” that is typically arose by a number of environmental stresses such as flooding, extreme temperature, the presence of organic and inorganic toxicants, phytopathogens, drought or high salt concentrations (Cheng et al., 2007; Glick, 2014; Gamalero and Glick, 2015).

Figure 1. The effect of phytostimulating of PGPR on RSA. The figure is adapted from Vacheron et al. (2013).

PGPR can modulate root development and growth through the production of phytohormones, secondary metabolites and enzymes. The most commonly observed effects are a reduction of the growth rate of primary root, and an increase of the number and length of lateral roots and root hairs. PGPR
also influence plant nutrition by nitrogen fixation, solubilization of phosphorus, or siderophore production, and modify root physiology by changing gene transcription and metabolite biosynthesis in plant cells.

Beneficial rhizospheric bacteria associated with plant roots can improve root development and growth by producing of phytohormones or enzymatic activities. The effects of PGPR on the roots and the other part of plants are awaiting to be understood by exhaustive studies. An approach is to inoculate roots with a PGPR in vitro and monitor the resulting effects on plant. PGPR may reduce the growth rate of the primary root (Dobbelaere et al., 1999), increase the number and/or length of lateral roots (Combes-Meynet et al., 2011; Chamam et al., 2012), and stimulate root hair elongation in vitro (Dobbelaere et al., 1999; Contesto et al., 2008). Thus, the uptake of minerals, water, and the growth of the whole plant can be increased. Some of these effects, including increased root and shoot biomass, are also reported for PGPR-inoculated plants (El Zemrany et al., 2006; Minorsky, 2008; Veresoglou and Menexes, 2010; Walker et al., 2012). Root system architecture (RSA) is formation of root system topology, primary and lateral roots, and the number and length of different types of roots. PGPR modify RSA and the structure of root tissues particularly through the synthesis of various hormone and enzyme including ethylene and Acc-deaminase (Vacheron et al., 2013). Modifications of root cell wall structure are caused by mainly from PGPR-triggered changes in plant gene expression (Fig. 1). An isolate of Bacillus subtilis (GB03) promotes Arabidopsis growth by producing VOCs that were shown to modulate the a number of genes expressions with known functions associated with cell wall structure (Ryu et al., 2003; Zhang et al., 2007). Ku et al. (2018) demonstrated the colonization and promotion of different to soybean (Glycine max L. Merr.), wheat (Triticum aestivum L.) and Chinese cabbage (Brassica rapa L.) under different conditions by a GFP-tagged Bacillus cereus strain YL6. Strain YL6 similarly promoted the growth of these plants by dissolving inorganic and organic phosphorus and producing IAA and GA in soil. In the present study, it was aimed to assess the effect of two isolates belongs to Bacillus genera on root elongation of wheat seedlings.

MATERIALS AND METHODS

The experiment was carried out in a growth room adjusted to 24±2°C and 80% relative humidity by using the sterile pots, in 5 cm of diameter and 25 cm in depth, containing sterile soil mix (1:1 mix of gardening peat-sand).

Rhizobacterial isolates: Two isolates Gu2 and 127b, previously identified as belonging Bacillus genera based on MALDI Biotyper classification, were included in the experiments. The isolates were selected from Rhizobacterial Culture Collection (Gul IMRIZ, Dicle University) based on their Acc-deaminase activity and bio-control potency. The data belonging to the isolates is given in Table 1. The features ACC-deaminase activity and bio-control potency of both isolates had been determined in previous study (Imriz et al., unpublished data).

Table 1. The names, locations, species and origin plant of the rhizobacterial isolates.

| Isolate | Species       | Location | Plant origin of isolates |
|---------|---------------|----------|--------------------------|
| Gu2     | Bacillus sp.  | Çumra    | Wheat                    |
| 127b    | Bacillus sp.  | Ilgın    | Wheat                    |

Seed inoculations and experimental design: The rhizobacterial isolates gave highest ACC-deaminase activity and bioagent potency in previous study were included in vivo pot trials to evaluate their efficiencies on the growth wheat seedlings under controlled conditions. Durum wheat cultivar ‘Kızıltan-91’ seeds were sterilized by dipped in 1% NaOCl for 5 minute and followed by rinsing twice with sterile distilled water (SDW) and dried. The seeds were inoculated with pure culture of rhizobacterial isolates grown for 48 hours by dipping the seeds in the bacterial suspensions at a density of 10° cells mL⁻¹ and shaken for 60 minutes at 80 rpm and then let to dry. For control, the seeds were dipped only in SDW for the same period. The treated seeds were sown in pots containing sterilized soil mix and kept in the growth room whose conditions mentioned above. The inoculated seeds were sown into pots with 3 replications for each application (each contained 4 plants) according to the randomized plot design. At the same time, 30 treated seeds for each application were placed on petri dishes with moistened blotter and germinated at temperature and photoperiod regime of 24±2°C (light/dark, 12h/12h) for two weeks just to observe visual change on root hair. The whole process was carried out under sterile conditions up to pot trial stage.

Impact evaluation of isolates on root growth: Four weeks old plants were removed from the pots and washed up with tap water carefully not to damage the root part of the plants. The root length of each plant was measured and recorded in cm. Isolate efficiency was calculated by the formula as below:

\[
\text{Isolate efficiency} (\%) = \frac{X - Y}{X} \times 100,
\]

Where, \(X\) is average root lengths in control, \(Y\) – average root lengths of the treated parcels.

Statistical analysis of data collected: Data were analysed using statistical package for social sciences (SPSS). The means of the data obtained from the effect of rhizobacterial isolates on root length were analysed using One Way Anova Test. The means were separated and compared with standard error the Tukey HSD test (p<0.05).

RESULTS AND DISCUSSION
The data obtained on the means of root lengths and percentage of each treatment is shown in Table 2. There was no statistical difference on the basis of root length between control and rhizobacterial treated plants (F=3.206; df=2.33; \( P=0.053 \)). However, in terms of efficiency percentage for each application, Gu2 provided a very slight difference of 1.87% on the root elongation in comparison with SDW treatment, similar to the finding of Telek et al. (2019) that PGPR isolates did not have statistically significant effects on root length while there was a slight effect on root elongation in red chilli (Capsicum annum L.) plants.

### Table 2. The effect of isolates on the wheat seedling root elongation.

| Isolates | Root length (cm) | Efficiency (%) |
|----------|------------------|----------------|
| Gu2      | 15.25 ± 0.454    | 1.87           |
| 127b     | 12.58 ± 1.271    | -2.39          |
| Control (SDW) | 14.97 ± 0.653 | -              |

*In the columns, values having the same letter are not statistically different \( P<0.05 \) (Tukey HSD test).

Furthermore, isolate 127b did slightly minus effect (-2.39%) on the primary root development of the wheat plants (Fig. 2). Dobbelære et al. (1999) reported a finding that PGPRs may reduce the growth rate of the primary root which is in accordance with our results.

Figure 2. Means of root lengths and efficiency percentages of applications.

In comparison with the control plants (SDW treated), the isolate Gu2 made a slight increase (1.87%) whereas 127b decreased the primary roots at the rate of 2.39%.

In the observational part of the study, it was noticed a great visual difference with an increase in root hair of the germinated wheat seeds in petri dishes treated with rhizobacterial isolates in comparison with those untreated seeds. Even though it is not based on numerical data, it's ineluctable to emphasize about obtained visual observation as the increase of root hair positively affects the plants such as aiding water and nutrient uptake. Likewise, a number of studies on PGPR impact associated plant roots were documented increasing in the number and/or length of lateral roots whereas primary root growth is decreased (Combes-Meynet et al., 2011; Chamam et al., 2012; Telek et al., 2019) and stimulating root hair elongation (Dobbelære et al., 1999; Contesto et al., 2008) which has significant impact on the uptake of minerals and water by plant as shown in Figure 1 (Vacheron et al., 2013).

**Conclusion:** There was no significant impact based on root lengths between control and rhizobacterial treated plants. The efficiencies of the isolates, even though, on root elongation of wheat seedlings are not significant, a noticeable impact in root hair increase both isolates is engrossing. This effect to be considered may have an importance in terms of mineral and water intake and therefore the growth of the whole plant. There is no doubt that further studies in controlled and natural conditions should be conducted to reveal root and shoot biomass effect of Gu2 and 127b rhizobacterial isolates on wheat varieties.

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