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Isolation and Molecular Identification of Plant Growth-Promoting Bacteria from Phyllosphere and Rhizosphere of *Triticum aestivum* L.

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Introduction

Phyllosphere and rhizosphere of plants are suitable habitats for bacteria and other microorganisms. Researches have shown that many agricultural fields are facing the problem of nitrogen deficiency. Nitrogen fertilizers, especially urea will cause environmental problems by creating NO, N_2O and NH_3 greenhouse gases (Vessey 2003, Khan et al 2020. 1998 Atlas & Bartha). In organic agriculture, biological fixation of nitrogen is of great interest (Din et al 2021, Orr et al 2011, Ren et al 2020, Saharan & Nehra 2011).

A significant number of these microorganisms have the ability to produce plant growth stimulating hormones (Ashrafuzzaman et al 2009, Knief et al 2012, Mwajita et al 2013). The ability of bacteria to produce auxin hormone in the rhizosphere depends on the availability of raw materials for the biosynthesis of this substance in the soil, as well as the ability to absorb the produced auxin by plant roots (Keswani et al 2020, Park et al 2021).

In agriculture, phosphate is added to the soil in the form of phosphate fertilizer. A significant number of bacteria living in the rhizosphere by secreting organic acids, inorganic acids and proton ions cause a decrease in soil pH, which can reduce the solubility of soil elements (Eramma et al 2021, Khan et al 2007, Kummar et al 2018).

The aim of this research was to isolate and identify molecular nitrogen fixing bacteria, phosphate solubilizer and indole acetic acid (auxin) producer. It can play an effective role in the prosperity of organic agriculture.

Material and Methods

Thirty bacterial samples were isolated from phyllosphere and rhizosphere of wheat. Nitrogen-free culture medium was used to identify nitrogen-fixing bacteria, and NBRIP culture medium (National Botanical Research Institute's phosphate growth medium) was used to evaluate the ability of solubilization of insoluble phosphate. The ability of the isolates to produce indole acetic acid was also investigated by the Salkowski method. The isolates that had the most ability to fix nitrogen, solubilize phosphate and produce auxin were selected and molecular methods were used to accurately identify their genus and species. For this purpose, first, using the DNA extraction kit (fermentase K0512), the genome of the bacteria was extracted and using the pair of universal primers F27 with the nucleotide sequence (5'AGAGTTTGATCMTGGCTCAG3') and R1492 with the nucleotide sequence (5'GGTTACCTTGTTACGACTT3') which partially from the 16S rDNA gene, polymerase chain reaction was performed. In this research, Chi square statistical test was used to examine and compare the obtained data regarding the number and performance of phyllosphere and rhizosphere isolates. Finally, the effect and yield of two superior isolates on the longitudinal growth of stem and root of lentil were investigated.

Result and Discussion

Out of 14 phyllospheric isolates and 16 rhizospheric isolates, 2 isolates that had the highest ability for the desired parameters were identified by sequencing a portion of 16SrDNA gene. Based on the obtained results, 5 phyllosphere isolates (36 %) and 7 rhizosphere isolates (44 %) were able to fix nitrogen. 4 phyllosphere isolates (29 %) and 4 rhizosphere isolates (25 %) were able to dissolve insoluble phosphate salt around them. Also, 4 phyllosphere isolates (29 %) and 7 rhizosphere isolates (44 %) had the ability to produce indoleacetic acid. Compared to the phyllosphere, the rhizosphere of plants is a more suitable ecological place in terms of the number and diversity of microorganisms, because the phyllosphere is constantly exposed to environmental stresses such as temperature, dryness, ultraviolet rays of the sun, and wind. But the rhizosphere area is almost immune from most of these stresses. In addition, rhizospheric bacteria have the advantage of using the organic secretions of plant roots (Knief et al 2012, Mwajita et al 2013). In the present research, it was found that more and more diverse bacteria can be isolated from the rhizosphere of wheat. Most of the bacteria isolated in this research, especially the bacteria of the plant rhizosphere, were rod type. Rod bacteria have a higher surface area to volume ratio compared to spherical bacteria, so they can better absorb water, salts and other nutritional needs in the soil environment (Atlas & Bartha 1998). Most isolates (71 percent of phyllosphere isolates and 63 percent of rhizosphere

isolates) were gram positive. Gram-positive bacteria have a thicker cell wall and are able to withstand higher osmotic pressures caused by the dryness of the environment. Seven percent of phyllosphere bacteria and 50 percent of rhizosphere bacteria were spores.

In the current research, it was determined that the phyllosphere and rhizosphere areas of wheat have nitrogen-fixing bacteria that are free and non-symbiotic. No statistically significant difference can be observed between these two areas ($P < 0.05$). Nitrogen-fixing bacteria are found in greater numbers in the rhizosphere (compared to the phyllosphere) (Ashrafuzzaman et al 2009, Knief et al 2012, Mwajita et al 2013). In the present research, regarding the results of phosphate solubilization, Chi-square statistical analysis was performed and the results showed that no significant difference can be observed between the number of phyllosphere bacteria and rhizosphere bacteria in terms of the amount of phosphate solubilization. ($P < 0.05$). Although the numerical results indicated the presence of more bacteria producing indole acetic acid in the rhizosphere region of the wheat plant, compared to the phyllosphere region of this plant, the chi-square statistical analysis did not show a significant difference between these two regions ($P < 0.05$). In the current research two isolates that had the most ability regarding the desired indicators were selected for molecular identification. One isolate with nitrogen fixation and phosphate solubilization ability was 98% similar to Bacillus cereus and the other isolate with indole acetic acid production capacity was 99 % similar to *Arthrobacter globiformis*. The effect of the presence of the examined bacteria on the longitudinal growth rate of the stem and root of the lentil plant was investigated. Both isolates significantly increased the longitudinal growth of lentil stems.

Conclusions

The phyllosophere and rhizosphere regions of the wheat can contain bacteria capable of nitrogen fixation, phosphate solubilization, and auxin production. Applying strains with the mentioned abilities can play an effective role in improving plant growth and reducing the use of chemical fertilizers. Nowadays, due to the increase in world population, reduction of fresh water resources and soil erosion, the need to use new methods to increase soil fertility and produce more crops is felt more than ever.

Keywords: Arthrobacter globiformis, Indole acetic acid, Bacillus cereus, Nitrogen fixation, Phosphate solubilization.

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Declaration of conflict of interest

The authors declare that they have no conflicts of interest.