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Endophytic Colonization of Rice Root with *Azospirillum irakense* and Its Effect on Some Growth and Biochemical Parameters

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Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world which occupies a large area of the world's arable land and constitutes the food of more than half of the world's people. (Filgueiras et al., 2020).

Several plant hormones participate in the modification of the cell wall; the most important one is auxin (IAA) and gibberellin (GA3). The interaction between IAA and GA3 shows that these hormones increase the activity of cell wall lytic enzymes, which cause its destruction by breaking some essential links in the polysaccharides structure (White et al., 2018).

Azospirillums are a group of heterotrophic and anaerobic bacteria that are often seen around the roots of *Geramineae* plants (Cosgrove, 2016). This bacterium plays an important role in the production of plant hormones such as auxin, gibberellin, cytokinin, biological nitrogen fixation, increasing absorption of water and nutrients, increasing the solubility of nutrient elements, especially phosphorus, and biological control of plant pathogens (EKhawas & Adachi, 2010). In this research, for the first time, the effect of inoculation of *Azospirillum iraknse* on some growth and biochemical parameters in rice seedlings has been studied. Also, the process of bacterial entry and their colonization was investigated. The results of the present research can identify a new species that is compatible with the rice plant, as well as formulating a suitable combination of bacteria and hormone suspension, which can be used as a biological fertilizer to improve the growth and yield of agricultural cultivars.

Material and Methods

Plant treatment and bacterial inoculation: Azospirillum irakense has been obtained from the "Golestan Agricultural and Natural Resources Research Center, Soil and Water Research Department (Gorgan-Iran). The bacteria were incubated in liquid NFB medium at 30 °C for 48 h until reaching to a concentration of 106 colony forming unite (CFU).

The seeds of rice (*Oryza sativa* cv. hashemi) were obtained from the National Rice Research Institute (NRII, Amol-Iran). The seeds were surface sterilized through sequential washing with NaOCl (3 %, 5 min), EtOH (75 %, 30 s), and double distilled water (3 times), and were allowed to germinate for 1 week. The seedlings were grown hydroponically (Yoshida et al, 1976) in a culture room with a photoperiod of 18 h day (33°C) and 6 h night (22°C), relative humidity of 71 %. The roots of 21-days-old seedlings were treated in Yoshida medium with 5 mL of bacterial suspension solution containing with or without different concentrations (0, 100 and 200 ppm) of IAA and /or GA3. The bacterial suspension solutions were added (1/10, V/V) in Yoshida medium.

In order to observe bacterial colonization, the roots were stained with 0.05 % aqueous solution of Aniline blue. The root samples were squashed and examined under light microscope, equipped with a digital camera (BH2, Olympus, Japan).

Measurement of dry and wet weight and relative growth rate of samples: To determine the fresh weight, the samples were weighed with a scale and for dry weight; the samples were dried in an oven at 37°C for 3 days and weighed again. The relative growth rate of the seedlings was calculated based on the amount of total dry weight per day according to Eq. (1):

$RGR= (\ln W_2- \ln W_1)/ (T_2- T_1)$ (1)

Quantitation of hydrogen peroxide, soluble sugar and protein, phosphorus rates: For measuring the content of H_2O_2, the root samples were extracted with trichloroacetic acid followed by centrifugation (12,000 ×g, 20 min). The reaction of the supernatant with KI was monitored at 390 nm (Sergiev et al., 1997). The amount of soluble sugar and protein were measured according to the phenol sulfuric acid (Kochert, 1978) and Bradford (Bradford, 1976) methods, respectively. The amount of phosphorus was determined by ammonium-vanadate solution according to Afa's standard method (Afa, 1985).

Result and Discussion

The results obtained from microscopic studies showed that the entry of bacteria is accompanied by the destruction of the cell wall and finally it settled down in the root parenchymal layer as single colonies. It seems that seedlings bacterial inoculation may induce oxidative stress and increase oxygen free radicals such as hydrogen peroxide in plants. Finally, the oxygen free radicals can destroy cell wall (White et al., 2018). Also, the formation of colonies inside the cell indicates the ability of bacteria to grow and be placed in conditions that are adapted to their place of residence in terms of nutrition, temperature and time. In other words, the successful entry of bacteria into the cell and the creation of a favorable symbiotic relationship with the host plant are confirmed by the formation of colonies (Carole et al., 2013).

Bacterial inoculation in combination with hormonal treatment had a positive and significant effect on the growth parameters of rice (*Oryza sativa* cv.hashemi) seedlings. It seems that the bacteria, by affecting the growth of the root and increasing its surface to absorb water and nutrients, has a positive effect on the growth of aerial parts and ultimately increased its performance (Filgueiras et al., 2020). Since *Azospirillum* is native to rice plants and have been isolated from them, they have shown great ability to stimulate growth. So far, several reports have been published concerning the effects of inoculation of *Azospirillum* on the growth of different plants (Compant et al., 2019).

Soluble sugars play an important role in plant structure and metabolism. The soluble sugars accumulation is a fast response to the stress conditions (Kannan & Ponmurugan, 2010). Also, the increase of soluble sugars helps the plant to keep its carbohydrate reserve at the optimal level to maintain the basic metabolism. The signaling pathways of soluble sugars during biological stress can accelerate the necessary responses to alleviate stress by affecting the metabolic pathways and defense systems of the plant. In this way, the cell can distinguish the symbiotic bacteria from the pathogen and allows entry of them into the cell (Guixiang et al., 2019).

The present results showed that the amount of soluble proteins was increased in the seedlings inoculated with *Azospirillum irakenese* in combinations with IAA and GA3. The increase in the amount of soluble proteins, probably indicates the changes in the activity of some enzymes involved in the process of wall degradation, which allow the bacteria to enter into root cell (White et al., 2018).

The bacteria around the roots play a role in converting the insoluble forms of phosphorus into soluble forms and affect the further availability of phosphorus for plant roots. In the present study, the increase in the amount of phosphorus in inoculated plants was consistent with the results of other published reports, which show that different species of the *Azospirillum* genus have the ability to dissolve phosphate element (Bashan & de-Bashan, 2010).

Conclusions

The current results showed that the successful colonization of *Azospirillum irakense* in the rice root seedlings caused change in the root morphology and improvement of growth and biochemical parameters. Also, hormone treatment has a synergistic effect on bacterial function. Therefore, the use of appropriate concentration of hormones can improve the methods of inoculation of bacteria with plants. The application of *Azospirillum irakense* as growth-promoting bacteria in combinations with IAA and GA3 treatment is a powerful strategy to improve rice the growth, which will reduce the use of chemical fertilizers.

Keywords: Azospirillum, Auxin, Destruction of cell wall, Gibberellin, Growth indicators, Rice.

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Declaration of conflict of interest

The authors declare that they have no conflicts of interest.

Statement on ethics

This material is the authors' own original work, which has not been previously published elsewhere.