STUDY THE EFFECT OF BACTERIAL 1-AMINOCYCLOPROPANE-1-CARBOXYLATE DEAMINASE (ACC deaminase) ON RESISTANCE TO SALT STRESS IN TOMATO PLANT

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Abstract: 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) produced by rhizobacteria could be remove the ethylene precursor and stimulate plant growth. Aim of the work was investigation on effect of rhizosphere bacteria *Pseudomonas mendocina* containing plasmid carrying gene encoding ACC deaminase on resistance of tomato plant to salinity. Amplification of acds gene in selected *Pseudomonas* was performed; the gene was cloned in *Escherichia coli* and was cloned subsequently in *P. mendocina*. Enzyme activity was determined in cloned *Escherichia coli* and cloned *P. mendocina* for confirmation of gene expression. Effect of bacterial ACC deaminase on resistance of tomato plants to NaCl was studied in Pot and Greenhouse. In pot experiment, tomato plant treated by cloned *P. mendocina* was compared with plants treated by *P. mendocina* (without plasmid) and control group. Salinity were established by adding 172 and 207 mM of NaCl to irrigated water. Greenhouse experiments were conducted in similar groups of bacteria in 207 mM of NaCl. Results obtained from pot experiment revealed that plants treated by cloned *P. mendocina* in 172 mM of NaCl was showed increasing content of growth than ones treated by *P. mendocina* and control as 11%, 18.4% growth for the shoot, 16.6%, 3.7% for roots and 9.6%, 27.5% for wet weight after five weeks, respectively. In 207 mM of NaCl, the results were as 14.9 %, 9.7% for shoot, 94.3%, 15.7% for roots and 96.4%, 50.6% for wet weight, respectively. In greenhouse experiment, results in same parameter in 207 mM of NaCl were revealed as 63.7%, 7 times for shoot, 2.8, 14 times for roots and 66.1%, 154 times for wet weight, respectively. We concluded that recombinant *P. mendocina* producing ACC deaminase by reduction of ethylene content of tomato plant in high salt concentrations could result in improvement of plant resistance to salinity.

Keywords: *Pseudomonas mendocina*, ACC deaminase, ethylene, salt stress.

INTRODUCTION

Between 30% - 40% of the world irrigated agricultural lands are prone to salinity [2, 29]. Salt stress affects the plant metabolism and, as a result, growth is reduced. Excess salt in the soil solution may affect plant growth either through osmotic inhibition of water uptake by roots. In the other hand, specific ion effects may cause direct toxicity, the insolubility or competitive absorption of ions may affect the plant's nutritional balance [10, 29]. Salinity increase the uptake of Na^+ or decrease the uptake of Ca^{2+} and K^+ [21]. The bacteria that stimulate plant growth are free living soil rhizosphere microorganisms. They live in the rhizosphere and are in close relationship with plant roots [3]. One of the main mechanisms used by bacteria to stimulate plant growth is the reduction of plant hormone ethylene [24]. Ethylene is an important signaling molecule involved in many processes occurring in plants, including germination, flower development, fruit ripening and responses to many environmental factors [26, 29].

Sharply increased production of ethylene occurs during stress and tissue damage. Excess amount of ethylene inhibits root elongation, seedlings, stops the growth of leaves in plants [2, 8].

One of the major challenges of modern biotechnology is the creation of highly productive strains of microorganisms capable of synthesis of biologically active compounds. Plant growth promoting rhizobacteria (PGPR) can facilitate plant growth indirectly by reducing plant pathogens, or by phosphorous solubilization, nitrogen fixation, iron chelating, phytohormone production (e.g. auxin or giberellin), and/or enzymatic lowering of plant ethylene levels [4, 11]. It was proved that PGPR-group, can promote plant growth by synthesize an enzyme that can regulate the level of ethylene in plants. This enzyme, 1-aminocyclopropane-1-carboxylate - deaminase, hydrolyses the 1-aminocyclopropane-1-carboxylate - the immediate precursor for the biosynthesis of ethylene in plants [8, 20], and plays an important role in the interaction of plants and microorganisms [5, 15]. Particularly promising in this way are bacteria of the genus Pseudomonas [1, 12]. For many members of the genus *Pseudomonas* has been shown ACC deaminase activity [3, 18, 22, 27], and in some cases, the ability to synthesize this enzyme is a key factor stimulating the growth of plants by bacteria of the genus [7, 9, 13, 16].

In the present study, selected biological treatments were evaluated to increase tomato plants resistance and growth under saline conditions.

MATERIALS AND METHODS

Screening and ACC deaminase activity assay

Screening of cultured collection of 18 isolates of *Pseudomonas* that were isolated from rhizosphere of plants grown was performed by PCR for the presence of acdS-gene encoding the synthesis of ACC deaminase *in vitro*. *Pseudomonas* strains capable synthesize of ACC deaminase were selected. ACC deaminase activity of the selected isolates was

determined by measuring the production of Alpha ketobutirate as described by M. Honma and T. Shimomura method [14] [unpublished data].

Molecular Assay and Growth Promotion Assay

DNA extraction, acdS-gene amplification and cloning in heterologous host cells - *Escherichia coli* were performed. Expression of the ACC deaminase activity of the gene in this bacterium was studied. Then acds-gene was cloned in cells of rhizosphere *Pseudomonas* producers of biologically active substances. Furthermore, expression of the gene in cloned bacterium assaied.

Effect of bacterial ACC deaminase on resistance of tomato plant to salinity was studied. All experiments were performed in.

Pot experiments

Sterile tomato seeds (variety "Tomato Excellent 176") were sown in Petri dishes between two sterile moist papers. After 4 days was cultured subsequently in moist soil in 3 cm deep of a big and flat pot (25 cm in diameters and 9 cm height). After a week, sized buds were selected and five buds were cultured per any plastic pots (8 and 5 cm top and bottom diameters, respectively, and 10-cm height, with holes in the bottom in two groups). Salinity treatments were established by adding 172 and 207 mM of NaCl to water. The composition of soil was (%): total N, 0.15; P_2O_5, 0.1; K_2O, 0.3; organic material content 50%, humidity 60%.

Every group were divided in three sections: the first part was treated by 10 ml 10^7 cell/ml cloned *Pseudomonas mendocina* (containing plasmid carrying acds gene), the second part by 10 ml 10^7 cell/ml solution containing *P. mendocina* and threes part without any treatments as control.

Plants were irrigated manually to saturation as needed with 172 or 207 mM saline solutions to maintain the level of salinity. Experiments were conducted in controlled conditions. Plants were maintained at a temperature of 25/20 °C (day/night) with 13 h photoperiod during the day time.

After five weeks the results for wet weight, shoot and root lengths were measured for test and control groups.

Greenhouse experiments:

Sterile tomato seeds were sown in sterile Petri dishes on wet filter paper. Through 4 days of germinated seeds were sown in moist soil.

After 1 week of growth of seedlings of equal size were selected and transplanted into individual plastic bag (37 cm in diameters and 50 cm height) by three repeats. In each bag 10 kg of sieved soil have been added. Soil preparation was done as following: The soil taken from Campus farmland. Compound soil was collected from depth of 0-30 cm, was air dried, sieved and mixed uniformly.

All plastic bags were divided in four sections. First section considered as control (first control - without bacteria). The second three bags (second section) were treated by 500 ml bacterial suspension of *P. mendocina*, third section by 500 ml of cloned *P. mendocina* and last section by 500 ml of distilled water (second control without bacteria).

Then, three first sections irrigated with a solution of 207 mM NaCl to maintain the level of salinity. The results were studied after 9 weeks.

Experiments were conducted in controlled greenhouse conditions. All bags plants were maintained randomized on benches in the greenhouse at a temperature of 28/24 °C (day/night) with 13 h photoperiod during the day time.

RESULTS

1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) gene purified from rhizobacteria, amplified and cloned in *E. coli* and *Pseudomonas mendocina* from rhizobacteria. Results of enzyme activity assay revealed properly expression of gene in both bacteria. *P. mendocina* was used for experiments because of high level of enzyme production and activity [unpublished data].

Results of experiments in Pot

The results were obtained within 5 weeks. In the salt concentration of 172 mM NaCl, tomato plant treated with a suspension of cloned *Pseudomonas mendocina* (containing plasmid) in comparison with tomato plants treated by *P. mendocina* had 11% greater length of stem, 16.6% greater length of root and 9.6% more biomass.

In the other hand, treatment of tomato by cloned *P. mendocina* had 18.4% greater length of stem, 3.7% greater root length and 27.5% more biomass in comparison by control (plants without *P. mendocina*) (Table 1).

In concentration of 207 mm, treated plants by cloned P. *mendocina* have 14.9% greater length of stem, 94.3% more root length and 50.6% more biomass than tomato treated by *P. mendocina*. Furthermore, plants treated with a suspension of cloned *P. mendocina*, in comparison with plants without bacterial treatment, had 9.7% greater length of stem, 96.4% more biomass and 15.7% greater length of the root (Table 2).

Table 1. Tomatoes treated by saline solution of 172 mM NaCl in pot.

Parameters	Cloned *P. mendocina*	*P. mendocina*	Control (Without *P. mendocina)*
Shoot length, Cm	18.6 ± 1.25	16.75 ± 1.3	15.75 ± 1.25
Root length, Cm	15.3 ± 1.2	13.125 ± 1.1	14.75 ± 1.15
Biomass, gr	4.106 ± 0.22	3.745 ± 0.17	3.22 ± 0.15

The greatest plant weights were obtained with cloned *P. mendocina* at 207 mM of NaCl. The largest differences were observed in the mass of plants. This indicates that plants treated with cloned *P. mendocina*, had not only a greater increase in stem and root, but also the best development in comparison with other treatments.

Table 2. Tomatoes treated with saline solution with a concentration of 207 mM of NaCl.

Parameters	Cloned *P. mendocina* (pACD)	*P. mendocina*	Control (Without *P. mendocina)*
Shoot length, Cm	18.1±1.15	15.75±1.35	16.5±1.2
Root length, Cm	13.6±1.2	7.0±0.9	11.75±1.1
Biomass, gr	4.292±0.18	2.85±0.12	2.185±0.11

Results of Greenhouse experiments

According to the results, the plants treated by bacterial suspension of cloned *P. mendocina*, had almost 2.8 times in length of the root, 63.7% greater length of stem, 66.1% more biomass than plants treated by *P. mendocina*.

Furthermore, treatment of plants by suspension of cloned *P. mendocina*, showed an increase as 7 times in length of the stem, 14 times in length of the root 154 times the biomass than plants in saline water without bacterial treatment (Table 3).

It was proved that cloned *P. mendocina* could significantly promote growth of tomato plant. Concentration of 207 mmol / L NaCl, causes a reduction in the length of the stem and roots of tomato, and their biomass. In this case, cloned *P. mendocina*, decreased the degree of suppression of growth, resulting in growth enhancement (Table 3).

Table 3. Ability of the bacteria cloned *P. mendocina* to improve the stability of tomato plants to salt stress in salt concentration of 207 mm in the greenhouse.

Parameters	Saline solution with a concentration of 207 mM of NaCl			Plants irrigated by distilled water (without bacteria)
	Without *P. mendosina*	*P. mendosina*	Cloned *P. mendosina*	
Shoot length, Cm	5.0±1.7	22.3±3.3	36.5±5.8	57.8±8.3
Root length, Cm	2.0±0.8	9.9±2.7	28.0±3.9	34.4±5.1
Biomass, gr	0.13±0.03	12.1±3.4	20.1±4.5	60.7±9.0

Plants treated by a suspension of cloned *P. mendocina* in concentration of 207 mM NaCl had not only a greater length of stem and root, but also had more development in comparison with other treatments. This suggests that these bacteria may reduce some of the negative effects of stress caused by salt stress.

DISCUSSION

The present study demonstrated that salinity adversely affected tomato plant growth and biological treatment increased its growth in comparison to the control that was exposed to stress.

The study proved the ability of recombinant *P. mendocina* in enhancement of the stability of tomato plants to salt stress. Recombinant *P. mendocina* is capable of reducing higher levels of C_2H_4 in plants through the activity of enzyme ACC-deaminase that hydrolyzes ACC into α-ketobutyrate and ammonia, instead of ethylene.

The biosynthesis of ethylene in plant roots is significantly affected by the concentration of salts [28]. Ethylene evolution would be increase at high salt concentration and decrease at low and medium salt concentration [23]. Low levels of ethylene (as low as 10 μg L^{-1}) enhance root initiation and growth, while higher levels of ethylene (as high as 25 μg L^{-1}) lead to inhibition of root elongation [17, 19].

Reduction in endogenous levels of ethylene in plants results in the formation of better root system. This study demonstrates the screening of rhizobacteria containing ACC deaminase to promote lentil growth under axenic conditions.

Pot experiments proved the ability of recombinant *P. mendocina* in increasing stability of tomato plants to salt stress. The largest differences observed in plant biomass treated by cloned *P. mendocina* in comparison with plants without bacterial treatment. This indicated that plants treated by cloned *P. mendosina*, had not only a greater increase in stem and root, but also the best development in comparison with control. Overall, resistance to salinity was shown in 207 mM of NaCl greater than 172 mM of NaCl by cloned bacteria.

Results showed that inoculation was even more effective in the presence of higher salinity level (207 mM) under potted and greenhouse conditions.

It was previously indicated that the activity of 1-aminocyclopropane-1-carboxylate deaminase enzyme was the key factor in the ability of plant growth promoting rhizobacteria to stimulate the elongation of plant roots [6, 25].

In the present work, resistance analysis was shown that growth of tomato plants in concentration 207 mM of NaCl was greater than growth in 172 mM of NaCl. In comparison results of experiments by 207 and 172, it was assumed that in high concentration of salt, an increase in the amount of ethylene and subsequently raising level of produced ACC deaminase greater than low concentration of salt was happened.

We concluded that treatment by recombinant *P. mendocina* in excess amount of salinity, could be better demonstrate his growth enhancing effect in comparison with treatment by *P. mendocina* and control. This

advantage would proved in greenhouse experiment that showed, in cultivation of tomato plants in salt concentration of 207 mM NaCl, treated by a suspension of cloned *P.mendocina*, the degree of growth suppression was much lower in comparison with plants without bacterial treatment or treated with a suspension of *P. mendocina*.

In conclusion, the study showed that bacteria capable to increased production of 1-aminocyclopropane-1-carboxylate deaminase could enhance the resistance of tomato plants to salt stress. This suggests that these bacteria may reduce some of the negative effects of stress caused by salt stress.

Acknowledgements: We sincerely thank our colleagues at the Laboratory of Plant Biotechnology, Department of Genetics and Biotechnology; Faculty of Biology, Belarusian State University, Minsk, Belarus, for their assistance and kind help during data collection.

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Received: 4 June 2011
Accepted: 20 July 2011
Published Online: 22 August 2011
Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433