Plant Archives Vol. 20, Supplement 1, 2020 pp. 3389-3394 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID DEAMINASE PRODUCING FLUORESCENT PSEUDOMONAS ON THE GROWTH OF EGGPLANT UNDER DROUGHT STRESS

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Abstract

The aim of this research is to evaluate the effect on inoculated eggplants with two different Pseudomonas strains which contain the enzyme ACCD under water deficit stress conditions. Both the strains *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21 have showed The ACC deaminase gene (acdS) in the PCR amplification of that gene. In the present study two pre-isolated strains of fluorescent Pseudomonads were evaluated for ACC deaminase ctivity, where *Pseudomonas putida* SAB10 had the highest ACC deaminase activity. One of the main problems is drought, it is major challenges affecting worldwide agricultural production and it is predicted to get worse in future. Our results showed that bacteria have the enzyme ACCD could improve growth of plants compared with their identical. Irrigation every 6 days (S1) had caused significant decrease of plant growth parameters but inoculation significantly recovered the negative effects of stress. The work in this research confirmed the role of PGPR containing the ACCD enzyme in enhancing plant growth under water deficit conditions.

Key words : Pseudomonas; PCR; *Solanum melongena* L.; water deficit; proline.

Introduction

Ethylene hormone (C_2H_4) is synthesized from its precursor 1-aminocyclopropane- 1-carboxylic acid in plant tissues that has inhibitory effects in different physiological and developmental processes of plants (Bleecker and Kende, 2000). Numerous authors have proposed that ethylene can control response of plants to biotic and abiotic stress (Ali *et al.*, 2012). Under appropriate conditions, plants produce necessary required of ethylene, which have useful implications on growing and developing plants but the production of ethylene is often significantly increased by biotic and abiotic stresses which accumulate in the root, which is one of the ways that drought stress damages some plants. This reduces root growth, and causes loss of plant yields (Ali *et al.*, 2012).

Beneficial fluorescent pseudomonads colonize roots very well and produce different metabolites and enzymes which protect the plants from various types of biotic and abiotic stress (Vivekananthan *et al.*, 2004). It would be interesting to determine whether these beneficial bacterial strains also have the enzyme ACC deaminase, specially in different species of the genus Pseudomonas. This type of enzyme activity has been detected in P.brassicacearum (Belimov *et al.*, 2007), P. entomophila (Kamala-Kannan *et al.*, 2010), P. fluorescens (Saravanakumar and Samiyappan, 2007), P. grimontii (Shagol *et al.*, 2014), P. maltophilia (Li *et al.*, 2012), P. marginalis, P. putida (Ahmad *et al.*, 2013), and P. thivervalensis (Zhang *et al.*, 2011). Additionally, these bacteria can produce phytohormones and secondary metabolites that are capable of improving plant growth (Bergsma-Vlami *et al.*, 2005).

Degradation of ACC by enzyme of ACC deaminase would decrease ethylene in the plant and diminish the damage to plants (Glick, 2014). ACCD producing bacteria associated with plants can play a role as a sink for plant. In the presence of ACC deaminase containing bacteria in the rhizosphere, it will be possible to work on reducing ethylene levels in host plants. Previous studies of horticultures, nuts and wheat have shown that the use of

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bacteria ACC deaminase could improve plant growth and plants are capable of withstanding the negative effects of different environmental stresses (Li *et al.,* 2012).

Drought stress is one of main limitations of crop production over large areas of Earth (Wu *et al.,* 2013).Under drought stress conditions, Plants respond to the stress of water through a variety of morphological and physiological changes. The one of the main responses of plants is the increasing in ethylene production. Proline play key roles in speed up water absorption under soil-water deficit (Gomes *et al.,* 2010). Studies showed that plants have been inoculated with beneficial microorganisms contain ACC deaminase increase plant drought tolerance (Glick, 2014).

This research work was aimed at evaluating the activity of the enzyme ACCD in beneficial plant growth promoting bacteria of strains of Pseudomonas and detect the existence of *acdS* gene in both strains were used in this study. In addition to above objective, we assess the impact of two different strains of Pseudomonas containing ACCD on eggplants under water deficit stress conditions.

Materials and Methods

Bacterial Isolates

The two pre-isolated strains of ACC deaminase containing pseudomonas were used in this study. The strains were *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21, they were selected on the basis of previous research results in the laboratory and growth room experiments (Fathalla *et al.,* 2015).

Amplification of acdS gene

The primers AccF 5'-ATG AAT CTG AAT CGT TTT GAA C-3' and 5'-TCA GCC GTT GCG GAA CAG-3' (Duan *et al.,* 2009) were used for PCR amplification of (*acd*S) gene. PCR cycle consisted of the thermal profile for amplification was 2-min initial denaturation at 94ºC, followed by 35 cycles of 1-min denaturation at 92ºC, annealing at 58ºC, and 1 min of elongation at 72ºC for 1 min and the final extension at 72ºC for 5 min. The results were visualized in agarose gel electrophoresis which was stained with ethidium bromide stained.

Quantification of ACC deaminase activity

Determination of ACC deaminase activity by measurement of the amount of α-ketobutyrate content produced by bacterial strains *in vitro* conditions according to Penrose and Glick, 2003. Nutrient broth (NB) were inoculated with old bacterial cultures and incubated in a shaker incubator at 150 rpm for 28ºC for 48hours. Culture broths were centrifugation at 1500rpm for 5min. The pellets were incubated over night with 5 ml DF minimal medium adjusted with final ACC concentration of 3.0 mM. The bacterial cultures were centrifuged at 8000 g for 10 min at 4°C and the pellets with 0.1 M Tris HCl, pH (8.5) and washed more than three times.

By adding 30 µl toluene, the bacterial cells were labilized and homogenized for 30 s at maximum s peed. The toluenized cell suspension was observed at 540 nm to determine ACC deaminase activity. α-ketobutyrate standard solutions were prepared from1 to 0.1 µmol concentrations in 200 µl in test tubes and mixed with 300 µl of 2, 4-dinitrophenylhydrazine then vortexed, tubes were kept at 30 °C for 30 min. After this period, 2 ml of 2N Na OH was added to each tube. Finally, the amount of standard solutions absorbance was read at 540 nm, and standard curves were drawn accordingly.

Plant experiment

The inoculum was prepared by growing the bacterial strains in nutrient broth (NB) till they reach to log phase. Eggplant (*Solanum melongena* L seeds were sterized by a 2 percent solution of sodium hypochlorite for 6 minutes then washed with 3 times by deionized sterilized water. The sterilized seeds were treated with a prepared bacterial suspension of *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21 for 3h. During the same period of time the control seeds were submerged in deionized sterilized water. After 3h incubation period seeds were sown into trays were filled with wet peat in 10 replications for each treatment. All plants were watered once every 2 days through the first 10 days of the experiment and fertilized with Hoagland solution to get nutrients every week. Ten days after planting, seedlings were transplanted in 15 cm diameter pots using 3 kg of autoclaved sandy soil which mixed well with bacterial culture (10^8 CFU ml^{-1}), The physical characters of soil are presented in Table1. In the control plants, bacterial suspension was replaced by 0.85% saline solution. After the transplanting, the plants were watered once every 3 days (S0) in the normal condition and once every 6 days (S1) in the drought conditions. The experiment ended after 40 days. Plants have been harvested, and growth parameters were reported (plant height, fresh, dry weight of plants).

Total proline content

Free Proline content in leaf tissue was measured by spectrophotometric analysis at 520nm, the method was suggested by Bates *et al.,* (1973) using acid ninhydrin reagent.

SDS-PAGE electrophoresis

Polyacrylamid gel electrophoresis (PAGE) using sodium dodecyl sulphate was conducted according to the method of (Laemmli, 1970) in order to find out the genetic differences between genotypes under studying.

Statistical analysis

According to steel and Torrie (1981) collected data were statistically analyzed using the correct variance analysis. The assigned experiment date was absolutely randomized with three replicates, in one way. The experiment data was analyzed using computer program software CoStat version 6.311. Least significant difference (LSD) at 5% level was used separately to evaluate the response of each character.

Results and Discussion

Screening of *acdS* gene by polymerase chain reaction (PCR)

ACC deaminase (acdS) gene in strain *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21was amplified with PCR. Acds gene amplification was observed approximately (1 kb) in both strains (Fig. 1).

Quantification of ACC deaminase activity

In this work, 2 representative pseudomonads strains were selected to quantify ACC deaminase activity. Optical density (540 nm) of *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21 showed the activity of ACC deaminase 275 and 498 nmol ketobutyrate/mg protein /h respectively.

Table 1: Physical properties of the soil.

Properties	
Particle size distribution (%)	
Sand	94.72
Silt	1.49
Clay	0.81
Textural class	Sand
Field Capacity %	16.6
pH	7.60

Pot experiment

The presented study illustrates how the isolated endophytic ACC deaminase containing *pseudomonas* effect of on growth of *Solanum melongena* L. under water deficit which was evaluated in a pot experiment. Comparatively more plant length and weight were acquired by the inoculated plants with bacteria than identical without bacteria (Fig. 2). Inoculated unstressed control plants indicated maximum plant length and weight as compared to uninoculated plants under different water levels (Fig. 2). Inoculation with strains *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21 every 6 days significantly increased the plant height up to 37.3.7% and 18.1% respectively when compared to the plant height of uninoculated identical. Maximum increase in Plant height was detected when plants were inoculated with strain *Pseudomonas putida* SAB10 at unstressed condition. Dry weight of shoots and roots increased at all treatments compared to uninoculated identical (Fig. 2). Strains *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21 increased the shoot dry weight up to 39. 4% and 20.2% respectively a at the treatment S1 compared to uninoculated identical and the same trend in root dry weight.

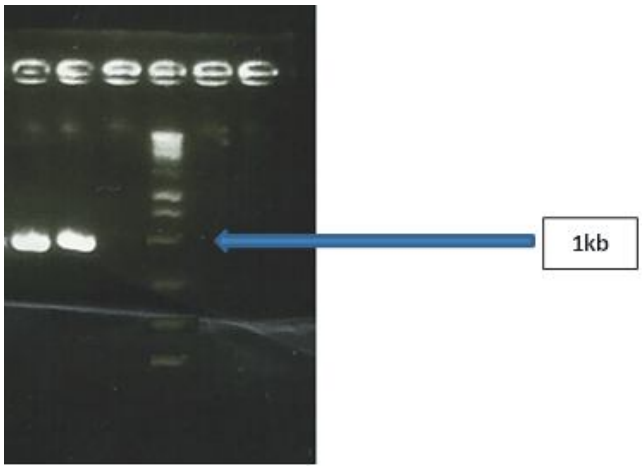

Fig. 1: The two fluorescent pseudomonad strains showed the amplification of acdS gene fragment in Agarose gel photograph.

Total proline content

The results showed that the proline concentration in plant leaves was found to be higher in uninoculated plants at the drought treatment (S1) as compared to their corresponding inoculated ones (Fig. 2). The treatments with inoculum *Pseudomonas putida* SAB10 and *Pseudomonas palleroniana* SAW21 under S1 conditions caused decreasing in proline by rate 34.5 % and 20.8% respectively comparing with their corresponding uninoculated ones.

SDS-Protein electrophoresis

The SDS- electrophoresis patterns (Fig. 3 and Table 2) showed that the number of polymorphic and monomorphic bands among the treatments under investigation is 3 and 2 respectively. Different treatments induced changes in protein pattern. There were two new bands having molecular weights 185.234 and 161.427 kda that appeared only by the effect of *Pseudomonas putida* SAB10 as a compared to control under drought

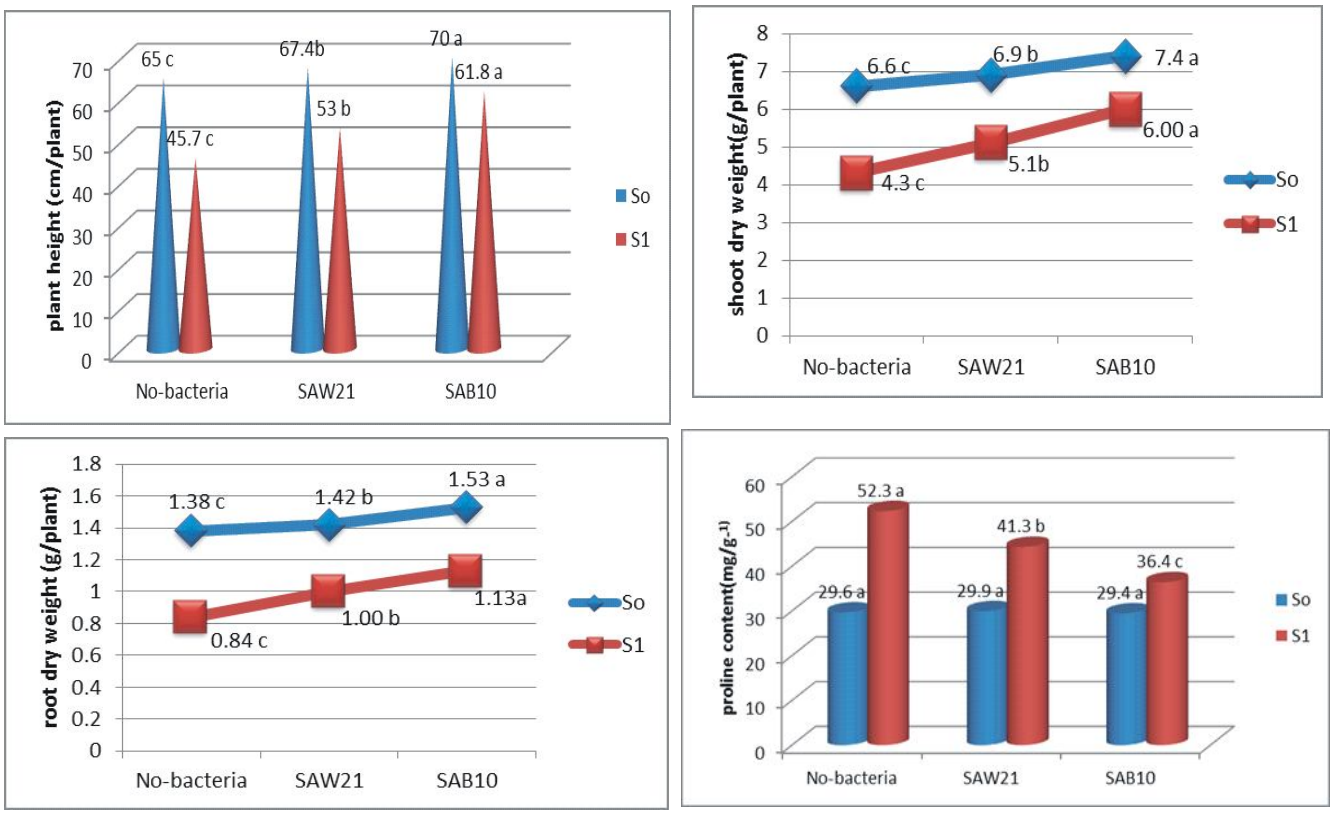

Fig. 2: Effect of the two selected ACC deaminase containing *pseudomonas* on plant height, shoot dry weight, root dry weight and proline accumulation mg/g *Solanum melongena* L. under unstressed condition (S0) and stressed condition.

Table 2: Protein banding patterns in Eggplant genotypes under normal and drought stress conditions.

MW	Without stress			With stress			Frequency	Polymorphism
	Control	SAB10	SAW21	Control	SAB10	SAW21		
185.234	0	0	0	0	1	0	0.167	Unique
176.527	0	1	1	1	0	0	0.5	Polymorphic
161.427	0	0	0	0	1	0	0.167	Unique
159.77	1	0	0	0	0	0	0.167	Unique
140.679	1	0	0	0	0	0	0.167	Unique
109.069	1	1	1	1	1	1	1	Monomorphic
93.109	0	0	1	1	0	0	0.333	Polymorphic
63.126	1	0	0	0	0	0	0.167	Unique
55.203	0	0	1	0	0	0	0.167	Unique
54.636	1	0	0	0	0	0	0.167	Unique
36.161	1	0	0	0	0	0	0.167	Unique
8.858	1	1	1	1	1	1	1	Monomorphic
8.045	1	0	1	0	0	0	0.333	Polymorphic

treatments. As a result of treatment with *Pseudomonas putida* SAB10, Two bands disappeared with molecular weights 176.527 and 93.109 kda. The same two bands missed with the treatment with *Pseudomonas palleroniana* SAW21 under drought treatments as a compared to control. The two new bands with molecular weights 185.234 and 161.427 kda which appeared only As a result of treatment with *Pseudomonas putida* SAB10 under stress condition in The SDS- electrophoresis patterns. Maybe these bands related with the activation aminocyclopropane-1-carboxylate (ACC) deaminase or proline accumulation.

Discussion

Previous studies have confirmed the promotion of plant growth and drought resistance by

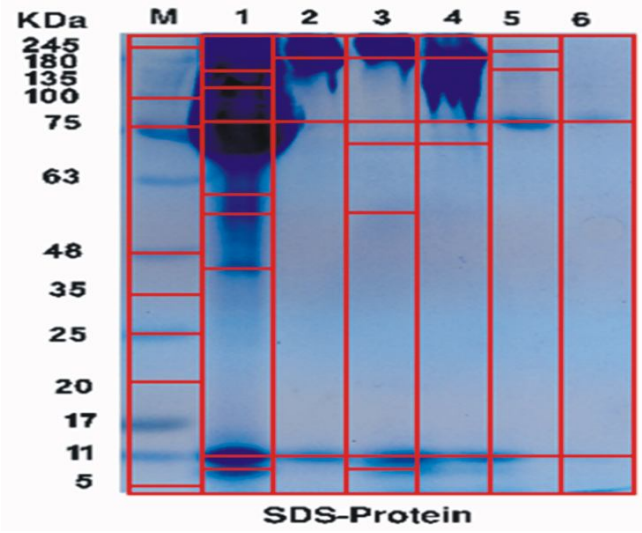

Fig. 3: SDS-PAGE patterns of canola Eggplant protein under non stress (S0) and drought (S1) conditions.

beneficial bacteria containing the ACC deaminase gene (Penrose *et al.*, 2001; Ahmad *et al.*, 2013). The present study has shown the role of ACC deaminase containing bacteria in enhancing the Eggplant growth and biomass of under water deficiency conditions. Our results are similar with many previous researchers, who have recorded that a number of bacteria isolated from different environments contain ACC deaminase enzyme, and these bacteria are capable of improving the growth of various crops (Bangash *et al.*, 2013; Glick, 2014).

The accumulation of proline is one of the most commonly reported changes caused by salt and water stress, and other stresses in plants (Verbruggen and Hermans, 2008).

The two new bands with molecular weights 185.234 and 161.427 kda which appeared only As a result of treatment with Pseudomonas putida SAB10 under stress condition in The SDS- electrophoresis patterns. Maybe these bands related with the activation of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase or proline accumulation. Results were coordinated with (Gong *et. al.*, 2005), who reported that, electrophoretic protein bands and isoenzymes polymorphism used for to define and measure the correlation between altered specific genes expression and environmental changes. These variations in gene expression would be involved in adaptation and could be used as stress molecular markers. One dimensional polyacrylamide gel electrophoresis of proteins has been widely used for strain- and species level identification and classification to screen the variation among population and select desirable genotypes.

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