Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers

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Abstract

The work reported here evaluates whether bacteria populating arid and salty environments can confer resistance in tomato and pepper plants to water stress. Plant growth-promoting bacteria that have ACC deaminase activity were isolated from soil samples taken from the Arava region of southern Israel. One of these strains, Achromobacter piechaudii ARV8 [Mayak et al., Plant growth-promoting bacteria that confer resistance in tomato and pepper plants to salt stress, submitted for publication.] significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress. In addition, the bacterium reduced the production of ethylene by tomato seedlings, following water stress. During water deprivation the bacterium did not influence the reduction in relative water content; however, it significantly improved the recovery of plants when watering was resumed. Inoculation of tomato plants with the bacterium resulted in continued plant growth during both the water stress and after watering was resumed. Based on the results of the experiments reported herein, the use of plant growth-promoting bacteria such as A. piechaudii ARV8 may provide a means of facilitating plant growth in arid environments.

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1. Introduction

Plant growth-promoting bacteria are free-living soil bacteria that can either directly or indirectly facilitate the growth of plants [2,3]. Indirect stimulation of plant growth includes a variety of mechanisms by which the bacteria prevent phytopathogens from inhibiting plant growth and development [4,5]. Direct stimulation may include providing plants with—fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, soluble phosphate, or the enzyme ACC deaminase that can lower plant ethylene levels [6–8]. The suggested role of ACC deaminase is to ensure that the concentration of ethylene does not become elevated to the point that growth (especially of roots) is retarded [3,6,7]. Thus, mutant bacteria that lack ACC deaminase activity are no longer able to lower plant ethylene levels and thereby promote root elongation [9–11].

In addition to facilitating the growth of plant roots, plant growth-promoting bacteria can, protect plants from the deleterious effects of some environmental stresses [12,13] including heavy metals [14], flooding [15], salt [1] and phytopathogens [16].

Relatively few mechanisms have been unequivocally demonstrated to explain the increased resistance to environmental stresses including water stress of plants treated with plant growth-promoting bacteria. The mechanisms that have been suggested include reduction of stress ethylene production via the action of ACC deaminase [12] and increased expression of the ERD15 gene, which is responsive to drought stress [13,17].

In the present study, we evaluated the effect of the bacterium Achromobacter piechaudii ARV8 on the performance of tomato and pepper plants under drought stress conditions. The bacterium was recently isolated from soil samples collected in dry riverbeds in the Arava region in the southern part of Israel where rainfall is scarce. This bacterial strain was selected to contain ACC deaminase [2] and thus should be able to lower ethylene production in its host plants, and
2. Materials and methods

2.1. Bacterial strain and growth

The bacterium *Achromobacter piechaudii* ARV8 was isolated as described by Glick et al. [9] from a rhizosphere soil sample from a *Lycium shawii* plant growing in a dry riverbed in the Arava region of southern Israel, reported previously [1]. Bacterial suspensions for the treatment of plants were prepared as described previously [18]. Essentially, a single bacterial colony from solid DF medium containing ACC as a sole source of nitrogen was used to inoculate YT medium and then incubated for 24 h with vigorous shaking (i.e. approximately 250 rpm) to ensure proper aeration. Following growth, the bacterial cells were pelleted by centrifugation at 5000 × g for 10 min and then re-suspended in distilled water. This step was performed twice before the bacterial concentration was adjusted to 1.0 absorbance unit at 500 nm.

2.2. Plant material and growth conditions

Tomato (*Lycopersicum esculentum* Mill cv. F144) and pepper (*Capsicum annuum* L. cv. Maor) seedlings were started from seeds that were sown in plastic trays in wet vermiculite. After 1 week, uniform sized seedlings (shoot height approximately 3 cm) were selected and planted in vermiculite, one per 7 cm diameter plastic pot. During the 2nd week the seedlings were fertilized once with 40 ml of either 1/10 or 1/5 Murashige and Skoog (MS) medium [19] as indicated. Three days after fertilization some of the seedlings were treated with 40 ml of bacterial suspension (*A*_*_G* = 1.0), while others were watered with de-ionized water. Two weeks after the seedlings were transplanted, watering ceased and only resumed after 7 or 12 days as indicated. The seedlings were maintained in a growth chamber at a day/night temperature of 25/20 °C with 25 μmol photons m⁻² s⁻¹ or 75 μmol photons m⁻² s⁻¹ of light supplied for 12 h during the daytime.

2.3. Monitoring plant growth

Fresh (FW) and dry (DW) weights of tomato plants were measured 5 weeks after germination. Similar experiments were also conducted with pepper plants except that harvesting the plants and measurements were done 7 weeks after germination. In other experiments, FW and DW of tomato plants were also measured up to five times (0, 6, 12, 17 and 23 days) starting at 3 weeks after germination and continued thereafter at times indicated.

2.4. Ethylene production by seedlings in response to stress

Thirty seeds were placed in each 25 ml Erlenmeyer flask on sterile filter paper before 2 ml of water were added. Seeds were imbibed in water or a bacterial suspension (*A*_*_G* = 1.0) for 2 h before being placed in the flasks. After 8 days, when the cotyledons were expanded, the excess water was removed. The flasks were closed for 2 h with a rubber septum and the ethylene in the headspace was sampled and measured by gas chromatography (Shimadzu model GC-17A).

At the end of the ethylene accumulation period the stoppers were removed and the flasks were maintained in a growth chamber as described above except that the light irradiance was 10 μmol photons m⁻² s⁻¹. Whereas water was not added to the stressed seedlings for the rest of the experiment, sufficient water was added to each flask of the appropriate controls to be continuously visible on partially tilting the flask.

2.5. Water status

At times 0, 6, 12, 17 and 23 days, starting at 3 weeks after germination the relative water content (RWC) in tomato plants was determined. The fully turgid weight (FTW), defined as the weight of the shoot after the plant was held in 100% humidity conditions in the dark at 4 °C for 48 h, of each plant was recorded. The relative water content (RWC) was calculated, where:

\[
\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{FTW} - \text{DW}}
\]

2.6. Statistical analysis

Data were analyzed by analysis of variance (ANOVA), and pair wise comparisons were done using a student's *t*-test. All hypotheses were tested at the 95% confidence level.

3. Results

The bacterium *A. piechaudii* ARV8 was isolated from a soil sample from a desolate region in the southern part of Israel, where the annual rainfall is below 50 mm and the land has not been cultivated for many hundreds of years. The isolated bacterium was used to inoculate 10-day-old tomato seedlings and the dry and fresh weights of seedlings treated with *A. piechaudii* ARV8 were compared with those obtained from seedlings treated with the well-established plant growth promoting bacterium *Pseudomonas putida* GR12-2 [20,21]. Plants not treated with any bacterium served as an additional control. In this experiment, both *P. putida* GR12-2
Table 1
The effect of ACC deaminase-containing plant growth-promoting bacteria on the growth of tomato seedlings cv. F-144

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fresh weight (mg)</th>
<th>Dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bacterium added</td>
<td>155 ± 7.20 c</td>
<td>15.6 ± 0.65 c</td>
</tr>
<tr>
<td><em>P. putida</em> GR12-2</td>
<td>412 ± 22.4 b</td>
<td>41.0 ± 2.12 b</td>
</tr>
<tr>
<td><em>A. piechaudii</em> ARV8</td>
<td>574 ± 58.8 a</td>
<td>59.7 ± 5.43 a</td>
</tr>
</tbody>
</table>

During the 2nd week tomato seedlings grown in small pots were inoculated with a bacterial suspension. By the end of the 4th week watering was stopped for 2 weeks, and then watering was resumed. After 5 weeks fresh and dry weights were determined. Values are means of 10 replicates ± S.E. Superscripted letters indicate values within the same column that are either statistically significantly different (when the letters are different) or not (when the letters are the same).

and *A. piechaudii* ARV8 promoted plant growth to a significant extent compared to the non-bacterized control, with *A. piechaudii* ARV8 promoting growth significantly more than *Strain p. putida* GR12-2 (Table 1).

Table 2
The effect of ACC deaminase-containing plant growth-promoting bacterium *A. piechaudii* ARV8 on the growth of pepper seedlings cv. Maor, exposed to transient water stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FW (mg)</th>
<th>DW (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bacterium added</td>
<td>360 ± 50 b</td>
<td>30 ± 0.40 b</td>
</tr>
<tr>
<td><em>A. piechaudii</em> ARV8</td>
<td>960 ± 60 a</td>
<td>120 ± 2.60 a</td>
</tr>
</tbody>
</table>

Pepper seedlings were managed essentially as outlined in Table 1, except for few changes. By the end of 7 weeks after seed germination the fresh and dry weights were determined. Values are the means of 6 replicates ± S.E. Values with the same superscripts within columns indicate no significant difference with *P* ≥ 0.05.

To test whether the bacterium has the same effect on other plants, it was used to treat pepper seedlings subjected to the same regime as the tomato seedlings, and at the end of 7 weeks their fresh and dry weights were recorded. The data presented in Table 2 indicates that *A. piechaudii*
ARV8 significantly promoted the growth of pepper plants as manifested in the higher fresh and dry weights attained by the bacterially treated pepper plants. The ratio of bacterial treated/bacterial non-treated was higher in tomato plants compared with pepper plants. It should be realized however that the comparison is of limited value only (experimental protocol varied slightly). At present its value is suggestive and it needs to be improved.

In a more detailed study of the response of seedlings to a reduction in water availability, the fresh weight was monitored at 0, 6, 12, 17 and 23 days following the onset of the stress; at 17 and 23 days, the watering had been resumed. Although the seedlings were not supplied with water for 6 days their fresh weight increased (Fig. 1). This probably reflects the fact that during this period the plants were able to secure water from the growth medium. The observed increase in RWC supports this suggestion (Fig. 2). The increase in fresh weight was similar in control and stressed seedlings. On the other hand, no change in fresh weight in the stressed seedlings was observed during the next 6 days, during which water was not supplied.

After 12 days of drought stress watering was resumed but the measurements demonstrate that after five additional days (day 17 after the initiation of the stress) the fresh weight did not increase in the previously stressed seedlings. After 6 additional days (that is 23 days after the initiation of the stress) a small increase in the fresh and dry weight was measured in the previously stressed seedlings. In contrast, a continuous and significant increase in the fresh and dry weights of the stressed seedlings that were treated with the bacterial strain A. piechaudii ARV8 was observed. This increase in weight also occurred during the resumption of the water supply. At the end of the experiment (indicated as day 23 in Figs. 1 and 2) the fresh weight of the bacterially treated seedlings was about twice that of control plants. Not surprisingly, the changes in the dry weights of seedlings paralleled the changes in seedling fresh weight (Fig. 1B). The fresh weight attained by the bacterially treated, but not stressed seedlings was the highest compared with the other treatments.

During water deprivation the RWC in the plants declined in stressed plants, both treated and not treated with bacteria. The lowest value was measured at day 12 of the stress (Fig. 2). This reflects an increasing water stress [23]. When watering was resumed, the RWC increased, attaining higher values in the bacterially treated plants. In the presence of the bacterium, and independent of whether or not the plants were stressed, by 23 days after the onset of the stress the RWC was significantly greater in the presence than in the absence of the bacterium. Moreover, while the bacterium did not help the seedlings to maintain their RWC during periods of drought, the presence of the bacterium greatly facilitated the recovery of the seedlings upon re-watering.

### 3.1. Effect on ethylene production

Nine days after seedlings were exposed to water stress ethylene production began to increase. Thereafter, the rise in the rate of ethylene production continued to a value of 28 nl h⁻¹ at 13 days (Fig. 3). By comparison, control (hydrated) seedlings reached a rate of production of 1.5 nl h⁻¹.

By inoculating seedlings with A. piechaudii ARV8, and then exposing them to water stress, the rise in the rate of ethylene production occurred later and the rise in the rate of ethylene production was smaller than in stressed seedlings. At 13 days the rate of ethylene production has reached a lower value of 6.1 nl h⁻¹ (Fig. 3).

![Fig. 2. The effect of the ACC deaminase-containing plant promoting bacterium A. piechaudii ARV8 on the relative water content (RWC) of tomato plants cv. F-144 exposed to transient water stress. The bacterial suspension was applied once, 1 week after the plants were transplanted. Watering was stopped 21 days after germination (indicated as 0 on the time scale) and resumed 33 after germination (indicated as 12 on the time scale). Values are the means of 6 replicates ± S.E.](image-url)
stress period (between 6 and 12 days) (Fig. 1), when gain in fresh weight except for the second part of the tent than in control hydrated plants. A similar change was in a continued increase in dry weight to a greater ex-

biomass were stimulated in bacterially treated plants, result-

Apparently, the underlying processes causing a gain in plant was resumed (Fig. 1).

growth, and this inability to grow continued when watering period of reduced water availability resulting in cessation of growth processes were disturbed during the pe-

fresh weight was stopped. In the absence of bacterial treat-

duced (reflected in lowered RWC values), especially during that were exposed to water stress continued to accumulate

5. 4. Discussion

It has been suggested that bacteria containing ACC deam-

inase activity should all act to reduce the level of stress ethylene and thus confer resistance to various stresses [3]. Indeed this suggestion is supported by the results of the present experiments (Tables 1 and 2) and also those that were reported previously demonstrating increased resis-
tance to salt stress [1], flooding stress [15], heavy metal stress [14] and pathogen stress [16].

We have hypothesized that those bacteria populating sites where water is limited and repeated dry periods occur fre-

quently, are likely to be able to better promote plant growth compared with plant growth promoting bacteria isolated from sites where water sources are abundant. The results of our study support this hypothesis. The seedlings treated with A. piechaudii ARV8, a bacterial strain isolated from an arid site, were significantly larger than the seedlings treated with a bacterium, P. putida GR12-2, that was originally isolated from the rhizosphere of grasses in the High Canadian Arctic [20] where water is abundant.

In the present experiments, the bacterially treated plants that were exposed to water stress continued to accumulate plant biomass (Fig. 1), although water availability was re-

duced (reflected in lowered RWC values), especially during the 6 days between 6 and 12 days of the stress period (Fig. 2). Apparently, the underlying processes causing a gain in plant biomass were stimulated in bacterially treated plants, result-

ing in a continued increase in dry weight to a greater ex-
tent than in control hydrated plants. A similar change was observed in fresh weight except for the second part of the stress period (between 6 and 12 days) (Fig. 1), when gain in fresh weight was stopped. In the absence of bacterial treat-

ment, the growth processes were disturbed during the pe-

riod of reduced water availability resulting in cessation of growth, and this inability to grow continued when watering was resumed (Fig. 1).

The bacterium is envisioned as affecting the production of ethylene, which in turn influences the factors described above. This suggestion is supported by earlier observations that ethylene reduces the fluidity of membranes [24,25], influences phospholipid turnover in membranes [26], increases leakage of solutes from plant cells [27,28] and suppresses elongation of roots [22,29,30]. In addition stress is known to stimulate ethylene production [25,31,32]. Thus, under stress conditions, ACC deaminase-containing bacteria that restrain the production of ethylene (Fig. 3) may be effective in allevi-

ating a portion of the stress effect. However, other elusive factors evolved in bacteria populating ecological sites, where water is scarce and dry periods occur frequently, may also interact with plants to increase resistance to water stress.

The precise mechanisms notwithstanding, the use of plant growth promoting bacteria that decrease the damage to plants that occurs under drought conditions is a poten-

tially important adjuvant to agricultural practice in locales where drought is endemic.

References


