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**Research Article**

# Evaluation of diacetylphloroglucinol producing pseudomonads for their biocontrol potential against *Ralstonia* wilt in brinjal

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**ABSTRACT**: Diacetylphloroglucinol (DAPG) producing isolates of pseudomonads were screened against bacterial wilt in brinjal caused by *Ralstonia solanacearum* under greenhouse conditions. Thirty *Pseudomonas* isolates were obtained from the culture collection of NBAII, Bangalore that include seven isolates of *Pseudomonas putida* (CK8C, CK24E, Pf4K, RPF9, RPF13, OTN5E2, GR1ARS1), one *P. mosselli* (CK24C) isolate, three isolates of *P. fluorescens* (GR3ARS3, Pf-DWD, CHAO), three isolates of *P. plecoglossicida* (BA11D1, BA16-2, BA3-D1) and sixteen isolates of *P. aeruginosa* (CK13C, CK19E, AFP3, AFP4, AFP6, RFP7, OTN8, AFP9, AFP8, PDB8, PDB1, AFP7, AFP5, AFP13, ND4-IARIB, RPF8). Bacterial wilt susceptible brinjal variety MEBH-9 was used in the screening. The brinjal seedlings were dipped in *Pseudomonas* suspension for five minutes before transplantation and plants were observed for wilt symptoms like stunted growth, drooping of leaves, loss of rigidity and eventually death of the plant. Four weeks later, the plants were uprooted and length and weight of both roots and shoots were recorded. The highest percentage of wilt disease reduction was observed in seedlings treated with *P. plecoglossicida* BA11D1 (95.8%), *P. putida* CK24E (62.5%) and *P. plecoglossicida* BA3D1 (41.7%). Average shoot length, root length and shoot weight, root weight were comparatively lesser in most of the pseudomonads treated plants than the control plants.

**KEY WORDS**: *Ralstonia solanacearum,* bacterial wilt, plant growth promotion, pseudomonads, diacetylphloroglucinol.

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## INTRODUCTION

*Ralstonia solanacearum* (syn. *Pseudomonas solanacearum*) is a destructive soil-borne plant pathogen that causes wilt disease in more than 200 plant species belonging to over 50 families of higher plants including several crops like brinjal, potato, tomato, banana, ginger and chilli and causes serious economic damage (Smith, 1896; Hayward, 1991; Yabuchhi *et al*., 1996). Using beneficial bacteria for the biocontrol of bacterial wilt of tomato has been found effective when compared to other control measures that include plant breeding, sanitation, bactericides and other chemical methods. Lemessa and Zeller (2007) screened 118 rhizobacteria *in vitro* and selected six isolates having good inhibitory effect. They showed that in greenhouse tomato seedlings treated with the antagonists having disease control ability (fluorescent pseudomonad APF1 and *Bacillus subtilis* B2G) significantly reduced disease incidence and increased dry weight of tomato plants. The seedlings dipped in pseudomonads suspension before transplanting showed biocontrol ability against wilt in *Eucalyptus urophylla* (Ran *et al*., 2005). In this study, greenhouse experiment was carried out to study the biocontrol ability of thirty DAPG producing pseudomonad isolates by seedling dip method against bacterial wilt in brinjal.

## MATERIALS AND METHODS

### *Bacterial isolates and plant material*

*Ralstonia solanacearum* G3 virulent strain was used for the experiments. Pathogenicity of the isolate was tested on brinjal seedlings and was re-isolated from the affected plant to fulfill Koch's postulates. Thirty pseudomonad isolates were collected from NBAII culture collection, Bengaluru which include seven isolates of *Pseudomonas putida* (CK8C, CK24E, Pf4K, RPF9, RPF13, OTN5E2, GR1ARS1), one *P. mosselli* (CK24C) isolate, three isolates of *P. fluorescens* (GR3ARS3, Pf-DWD, CHAO), three isolates of *P. plecoglossicida* (BA11D1, BA16-2, BA3-D1) and sixteen isolates of

*P. aeruginosa* (CK13C, CK19E, AFP3, AFP4, AFP6, RFP7, OTN8, AFP9, AFP8, PDB8, PDB1, AFP7, AFP5, AFP13, ND4-IARIB, RPF8). Seedlings of susceptible brinjal variety (MEBH9) for were used for greenhouse experiments.

*Green house experiments*

The bacterial wilt pathogen *R. solanacearum* G3 isolate was grown in CPG broth and all *Pseudomonas* isolates were grown in nutrient broth. The broth was incubated overnight in shaker at 28°C and then cultures were centrifuged at 5000 x g for 10 min. at room temperature and the pellet was resuspended in sterile water. This procedure was repeated twice to remove traces of the medium and finally the suspension was adjusted to the concentration of $10^8$CFUs per ml. Pathogen inoculation was done by drenching five ml of bacterial suspension directly to cocopeat. Seedling dip method was used for pseudomonads treatment (root portion of the plants was completely immersed in bacterial suspension for five minutes). Three replicates, each with eight plants were maintained for each bioagent isolate. Plants were transplanted after dipping in pseudomonads suspension and observed for bacterial wilt disease symptoms such as stunted growth, wilting and/or epinasty upto four weeks. Thirty days after the treatment, wilt disease incidence was recorded.

*Growth promotion assessment*

At the end of the experiment (when all plants in the pathogenic control died due to wilt), plants were uprooted separately and plant growth parameters were recorded. Healthy plants were counted and their length and weights also recorded to find out any significance difference in growth promotion between the pseudomonads compared with the untreated control.

*Statistical analysis*

IRRISTAT software was used for statistical analysis. Measurements of length and weight of root and shoot were used for ANOVA in order to find the best treatment among the treatments with different *Pseudomonas* isolates.

**RESULT AND DISCUSSION**

*Biocontrol ability*

The thirty pseudomonad isolates tested under greenhouse showed varying degree of wilt disease reduction in brinjal. All the *Pseudomonas* isolates showed a considerable reduction in percentage of wilt disease that varied from 8.3% to 95.8%. Among the thirty isolates, the highest percentage of wilt disease reduction was observed in seedlings treated with *P. plecoglossicida* BA11D1 (95.8%) followed by *Pseudomonas putida* CK8C (91.7%) and *P. auruginosa* RPF7 (79.2%) (Table 1). In greenhouse experiments conducted by Guo *et al*. (2004) three strains of plant growth-promoting rhizobacteria (PGPR), *Serratia* sp. J2, fluorescent pseudomonad J3 and *Bacillus* sp. BB11, were found effective in the biological control of bacterial wilt of tomato caused by *R. solanacearum*. In our study, among 30 isolates screened, isolates of *P. plecoglossicida* and *P. putida* strains have shown the highest percentage of disease reduction.

*Plant growth promotion by bacterial antagonists*

Among thirty pseudomonads, shoot length were maximum in plants treated with Pf4K (132.1 cm) followed by CHAO (129.6), RFP9 (127.5 cm) and CK19E (121.9 cm) compared to 112.5 cm recorded in untreated plants (Table 1). When pathogen was also co-inoculated with the bioagents, the maximum root length was recorded in plants treated with CK24E (108.8 cm) followed by CK13C (106.7 cm), Pf4K (100.8 cm) and OTN5E2 (100.2 cm) compared to 79.0 cm in pathogen alone treated plants. The root weight did not change significantly when plants were treated with pseudomonads while there was variation in the shoot weight. Maximum shoot weight was observed in plants treated with BA11D1 (16.0 g) and CK8C (13.0 g) when pathogen was co inoculated. The shoot weight was ranging between 5.0 g and 10.3 g in plants treated with other isolates of pseudomonads and pathogen was co-inoculated on these plants. The shoot weight in pathogen alone inoculated plants was 0.6g. Without pathogen inoculation, plants treated with CK19E and Pf4K recorded maximum shoot weight (27.7 and 23.0 g respectively).

Earlier a few reports on the effect of bioagents on the bacterial wilt pathogen have been reported. When potato tubers were treated with the selected antagonistic isolates prior to planting in pathogen-infested soil, there was significant suppression in the incidence of bacterial wilt and increase in its survival rate of plants by 59.83% (Kuarabachew *et al.*, 2007). The bioagents *P. fluorescens*, *P. putida* and *B. subtilis* were effective in controlling brown rot disease of potato when used separately and *P. putida* was the most efficient (Mahmoud, 2007). Ramesh *et al.* (2009) observed that in greenhouse experiments, in the plants treated with *Pseudomonas* isolates (EB9, EB67), *Enterobacter* isolates (EB44, EB89) and *Bacillus* isolates (EC4, EC13) showed reduced incidence of bacterial wilt by more than 70% in brinjal compared to control treatment.

**Table 1. Incidence of wilt in various treatments**

| Treatment | % wilt incidence |
|---|---|
| AFP13 | 66.0[1ij] |
| AFP3 | 53.2[fgh] |
| AFP4 | 41.8[def] |
| AFP5 | 49.8[efg] |
| AFP6 | 29.5[bc] |
| AFP7 | 58.0[ghi] |
| AFP8 | 41.9[def] |
| AFP9 | 62.4[hij] |
| BA11D1 | 4.8[a] |
| BA16-2 | 82.[7l] |
| BA3D1 | 58.7[ghi] |
| CHAO | 58.7[ghi] |
| CK13C | 61.5[hij] |
| CK19E | 29.3[bc] |
| CK24C | 38.1[cde] |
| CK24E | 38.1[cde] |
| CK8C | 7.5[a] |
| GR1ARS1 | 61.4[hij] |
| GR3ARS3 | 32.3[cd] |
| ND4-IARIB | 70.3[jk] |
| OTN5E2 | 79.5[kl] |
| OTN8 | 53.2[fgh] |
| PDB1 | 53.2[fgh] |
| PDB8 | 53.2[fgh] |
| Pf4K | 53.2[fgh] |
| Pf-DWD | 70.3[jk] |
| RFP13 | 86.4[l] |
| RFP7 | 82.7[l] |
| RFP8 | 20.8[b] |
| RFP9 | 90.7[l]m |
| Control | 100.0[m] |
| CD ($P$ = 0.01) | 11.3 |

Most of the selected antagonists produced an antibiotic DAPG, which inhibited *R. solanacearum in vitro*. *Bacillus subtilis* and Bacteriophage (PT21) were highly effective in reducing the bacterial wilt incidence in tomato by 68.69 and 68.24%, respectively (Kumar *et al.*, 2009). Further, the antagonists reduced pathogen population by 1000– and 100-folds in soil and rhizosphere, respectively.

A negative correlation between the population of *P. fluorescens* and per cent bacterial wilt incidence was observed by Bora and Deka (2007) when tomato plants were treated with *P. fluorescens* and there was increase in yield corresponding to the reduction in the disease incidence. Vanitha *et al.* (2009) studied the effects of tomato seed treatments with *P. fluorescens* in the control of bacterial wilt under greenhouse conditions and the results revealed that the treatments protected plants against soil-borne infections of the bacterial wilt organism. Seed treatment with antagonistic *P. fluorescens* strain significantly improved the quality of seed germination and seedling vigour. The disease incidence was significantly reduced in plants raised from *P. fluorescens* treated seeds followed by challenge inoculation with *R. solanacearum*. The present study also confirms the possibility of using bacterial antagonists for the management of bacterial wilt in solanaceous crops like tomato, chilli and brinjal. The bacterial antagonists along with phages and other cultural practices can be employed to minimize the yield loss due to bacterial wilt disease.

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**Table 2: Plant growth promotion in plants treated with fluorescent pseudomonads with or without the co-inoculation of pathogen**

| Treatment | Shoot length | | Root length | | Shoot weight | | Root weight | |
|---|---|---|---|---|---|---|---|---|
| | FP | FP+RS | FP | FP+RS | FP | FP+RS | FP | FP+RS |
| AFP13 | 107.7 de | 99.2 h | 40.6 bc | 46.7 ghi | 9.3 ab | 7.3 bc | 1.0 | 0.8 |
| AFP3 | 99.2 b | 69.4 ab | 69.6 ik | 55.8 ijkl | 13 .0 abc | 5.7 b | 2.0 | 1.3 |
| AFP4 | 100.2 bc | 72.9 bc | 58.3 fghi | 65.0 klm | 11.7 abc | 7.0 bc | 1.7 | 1.3 |
| AFP5 | 100.6 bc | 97.9 h | 37.5 ab | 37.5 defg | 6.0 a | 6.0 b | 0.8 | 0.6 |
| AFP6 | 102.1 bc | 81.3 e | 78.1 l | 60.6 jkl | 15.3 bcde | 8.7 bc | 2.0 | 2.0 |
| AFP7 | 122.1 h | 99.6 h | 51.7 ef | 32.1abcde | 9.3 ab | 5.0 b | 3.7 | 0.6 |
| AFP8 | 107.5 def | 95.8 h | 42.5 bcd | 22.1 a | 11.3 abc | 6.0 b | 0.8 | 1.7 |
| AFP9 | 100.0 bc | 99.2 h | 32.1 a | 37.9 defg | 9.3 ab | 5.3 b | 1.3 | 0.8 |
| BA11D1 | 107.4 de | 98.5 h | 75.0 kl | 83.3 n | 16.7 bcdef | 16.0 e | 3.0 | 1.7 |
| BA16-2 | 121.5 gh | 86.5 fg | 37.5 ab | 39.4 defg | 10.7 abc | 7.3 bc | 1.3 | 0.9 |
| BA3D1 | 106.9 de | 99.6 h | 46.0 cde | 42.5 defg | 10.3 ab | 6.0 b | 1.7 | 0.6 |
| CHAO | 129.6 i | 98.8 h | 40.0 bc | 39.4 defg | 14.7 bcde | 5.3 b | 1.7 | 0.8 |
| CK13C | 109.6 e | 106.7 i | 50.0 de | 25.8 abc | 17.0 bcdef | 7.3 bc | 1.7 | 0.6 |
| CK19E | 121.9 i | 79.8 e | 75.0 kl | 65.4 lm | 27.7 g | 7.7 bc | 2.7 | 1.7 |
| CK24C | 93.1 a | 83.8 ef | 61.2 ghij | 72.9 mn | 8.7 ab | 7.3 bc | 1.3 | 1.3 |
| CK24E | 105.0 d | 108.8 i | 52.5 efg | 39.2 defg | 14.7 bcde | 7.0 bc | 1.3 | 1.2 |
| CK8C | 112.1 e | 97.9 h | 76.9 kl | 73.9 mn | 15.0 bcde | 13.0 de | 2.7 | 2 |
| GR1ARS1 | 116.5 f | 98.1 h | 44.8 bcde | 46.0 ghi | 15.3 bcde | 10.3 cd | 1.0 | 0.7 |
| GR3ARS3 | 110.2 e | 70.8 ab | 64.8 ij | 60.0 ijkl | 19.0 cdef | 5.7 b | 2.7 | 1 |
| ND4-IARIB | 113.1 ef | 97.9 h | 42.5 bcd | 35.4 bcdef | 10.7 abc | 6.3 bc | 2.3 | 0.9 |
| OTN5E2 | 111.0 e | 100.2 h | 37.7 ab | 49.8 hij | 11.3 abc | 7.7 bc | 1.3 | 0.6 |
| OTN8 | 111.0 e | 65.2 a | 64.4 ij | 56.5 ijkl | 16.7 bcdef | 5.0 b | 2.7 | 1.3 |
| PDB1 | 102.1 bc | 99.2 h | 42.1 bc | 30.0 abcd | 10.7 abc | 6.0 b | 2.9 | 0.7 |
| PDB8 | 112.1 e | 95.0 h | 41.3 bc | 24.2 ab | 12.7 abc | 5.3 b | 1.3 | 0.8 |
| Pf4K | 132.1 i | 100.8 hi | 52.5 efg | 28.3 abc | 23.0 ef | 5.7 b | 4.3 | 0.6 |
| Pf-DWD | 118.0 g | 88.8 fg | 55.6 fgh | 30.0 abcd | 16.0 bcdef | 4.3 ab | 1.7 | 0.5 |
| RFP13 | 103.1 bc | 96.9 h | 40.2 bc | 38.5 defg | 9.3 ab | 6.3 bc | 1.3 | 0.8 |
| RFP7 | 108.8 def | 76.7 bcd | 74.2 kl | 55.0 hijk | 15.6 bcde | 7.0 bc | 1.7 | 1.3 |
| RFP8 | 110.6 e | 98.1 h | 40.4 bc | 44.0 gh | 9.3 ab | 6.0 b | 2.7 | 1.0 |
| RFP9 | 127.5 i | 89.4 g | 45.0 bcde | 37.1cdefg | 10.3 ab | 7.0 bc | 1.0 | 0.7 |
| Control | 111.2 e | 79.0 e | 62.1 hij | 30.2 abcd | 10.2 ab | 0.6 a | 2.3 | 0.2 |
| Cd = @ P=0.01 | 3.52 | 5.93 | 7.8 | 11.2 | 8.3 | 4.2 | ns | ns |

Values shown here are mean of 3 replicates. Length in mm and weight in g. P-*Pseudomonas*, R-*Ralstonia solanacearum*.

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