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# PR-proteins Accumulation in Tomato Plant Due to Application of Resistance Inducing Chemicals During Period of Induced Resistance Against *Alternaria* Leaf Blight

S.A. Raut and S.G. Borkar
Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth Rahuri, 413722, India

## ABSTRACT

**Background:** Resistance Inducing Chemicals (RIC) viz., Salicylic Acid (SA), $-aminobutyric acid (BABA), chitosan (CHT) and 2, 6-dichloroisonicotinic acid (INA) are known to play an important role in induction of resistance by increasing the activity of PR-proteins. Therefore the study was done to test these RIC's for accumulation of PR-proteins in tomato plant to confer resistance against *Alternaria* blight disease. **Methods:** The activity of PR-proteins viz., chitinase and $ 1,3-glucanase in tomato leaves were measured in response to treatment of these RIC's at different concentrations as seed dip treatment for 8 h, seedling dip treatment for 2 h and seed + seedling treatment. The RIC treated plants were challenged inoculated with *Alternaria alternata*, a tomato leaf blight pathogen at 10 days interval from germination and observed for disease development. Chitinases and $ 1,3-glucanase activity was determined spectronically by quantifying the release of mg of N-acetylglucosamine and of glucose, respectively at 30 days of RIC treatment as the persistence of resistance induced by RIC was up to 50 days. **Results:** Maximum activity of chitinase and $ 1,3-glucanase was observed in seed + seedling treatment with BABA at 15 mM concentration and the increase in chitinase and $ 1,3-glucanase activity was 32.00 and 56.59% more, respectively over control and was followed by treatment with salicylic acid at 1.5 mM concentration. **Conclusion:** The application of RIC SA and BABA elicits the increased activity of PR-proteins viz., chitinase and $ 1,3-glucanase which inhibit the process of pathogenesis in susceptible tomato cultivars to exhibit the resistance.

**Key words:** Salicylic acid, $ 1,3-glucanase, tomato leaves, *Alternaria* blight, chitinase, $ 1,3-glucanase

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## INTRODUCTION

Pathogenesis related proteins viz., chitinase and $ 1,3-glucanase are known to induce resistance against the plant pathogens[1,2,3,4,5] in otherwise known as susceptible plant. The induction of PR-proteins in the susceptible plants are therefore important where the resistant varieties are not readily available.

Tomato is an important vegetable fruit crop which is susceptible to *Alternaria* leaf blight causing upto 80% losses[6] and the disease is prevalent in most of the tomato growing areas of the world[7]. As the resistance is not readily available in most of the cultivated tomato varieties against the disease, besides fungicidal disease management, an alternative option seem to be induced resistance through resistance inducing chemicals.

Resistance inducing chemicals particularly salicylic acid[8,9,10,11,12], $-aminobutyric acid[13,14,15,16] and chitosan[17,18,19] are reported to induced resistant in crop plants against variety of diseases. Therefore these chemicals were tested for accumulation of PR-proteins in tomato plant to confer resistance against *Alternaria* blight disease.

**Corresponding Author:** S.A. Raut, Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth Rahuri, 413722, India

## MATERIALS AND METHODS

**Materials:** Seeds of tomato var. Dhanashree, 0.1% $HgCl_2$ solution, distilled water, resistance inducing chemicals viz., salicylic acid, $-amino butyric acid, chitosan and 2, 6-dicholoroisonicotinic acid, mortar and pestle, centrifuge.

**Methods:** Tomato seeds of var. Dhanashree were surface sterilized with 0.1% $HgCl_2$ for 30 sec. followed by three subsequent washings with sterilized distilled water. The sterilized seeds were dipped in the solution of resistance inducing chemicals for 8 h. Seeds dipped in distilled water served as a control. After 8 h the treated seeds were sown in the pots containing sterilized soil for raising the seedlings. The raised seedlings were spray inoculated

with the suspension of the *Alternaria* pathogen at an interval of 10 days to note the development of leaf spot symptoms.

In another set tomato seedlings of var. Dhanashree were raised in glasshouse. The 30 days old seedlings were uprooted gently after adding sufficient quantity of water to loosen the soil. The roots of seedling were washed with distilled water and then dipped in Resistance Inducing Chemical (RIC) of required concentrations separately for 2 h for each treatment and transplanted in pots filled with sterilized soil. The experiment was laid out with three replication in CRD design. The seedlings dipped in sterile distilled water served as a control. The seedlings were then spray inoculated with the spray of spore suspension of *Alternaria* pathogen at 10 days interval to note the development of leaf spot symptoms.

In another set 8 h seed treatment followed by 2 h seedling treatment was given to the seeds of var. Dhanashree as described above. The seedlings were challenged inoculated with the spray of spore suspension of *Alternaria* pathogen at 10 days interval to note the development of symptoms.

The activity of PR-proteins viz., chitinase and $-1,3-glucanase were studied in tomato leaves raised from RIC treated seed (8 h seed dip method), seedling (2 h seedling root dip in RIC) and both RIC treated seed + seedling at 30 days of RIC treatment as the resistance induced persisted up to 50 days.

**Assay of $-1,3-glucanase activity:** The assay of $-1,3-glucanase from the fresh tomato leaves (0.25 g) was carried out as per the method described by[20]. Reducing sugar released in to the solution at the end of reaction was estimated by Nelson-Somogyi's method[21]. The protein content in the crude enzyme extract was estimated according to the method of[22]. The $-1,3-glucanase activity was expressed as mg of glucose released mg$^{-1}$ soluble protein min$^{-1}$.

**Assay of chitinase activity:** The chitinases activity from the fresh tomato leaves (0.25 g) was estimated as per the methods described by[23,24]. An aliquot of 0.5 mL was taken for the estimation of N-acetylglucosamine as per the method of Nelson-Somogyi's[21]. The protein content in the crude enzyme extract was estimated according to the method of [22]. The chitinase activity was expressed as mg of N-acetylglucosamine released mg$^{-1}$ soluble protein min$^{-1}$.

**Statistical analysis:** The statistical analysis was carried as given by[25].

## RESULTS AND DISCUSSION

The resistance inducing chemicals increased the PR protein activity in the treated leaves. The increase in $-1,3-glucanase activity in the leaves of tomato due to the seed treatment with RIC's was in the range of 0.006-0.046 mg and varied with different resistance inducing chemicals. The maximum increase i.e., 34.33% was observed with $-aminobutyric acid at 15.0 mM concentration. Whereas the least increase i.e., 4.48% was observed due to 2, 6-dichloroisonicotinic acid. The increase due to salicylic acid was in the range of 13.43-16.42% over control. Similarly treatment of tomato seedling (as root dip) with salicylic acid and $-aminobutyric acid also increased the $-1,3-glucanase activity in tomato leaves which were statistically significant over the control. Higher increase in $-1,3-glucanase activity was observed with $-aminobutyric acid at 15.0 mM concentration followed by 10.0 mM concentration. The maximum increase in $-1,3-glucanase activity g$^{-1}$ of sample was observed to be 0.034 mg. Similar results were obtained when seed + seedling treatment were done with resistance inducing chemicals. The maximum increase was observed for $-aminobutyric acid at 15.0 mM concentration which was 56.69% more over control. The increase in $-1,3-glucanase activity was statistically significant for seed, seedling and seed + seedling treatment with resistance inducing chemicals over the control (Table 1).

Table 1: $-1,3-glucanase activity in tomato leaves as influenced by different resistance inducing chemicals

| | | $-1,3-glucanase activity in tomato leaves (mg of glucose released/min/mg soluble protein) due to | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | Conc. (mM) | Seed treatment | Percent increase in $-1,3-glucanase activity over control | Seedling treatment | Percent increase in $-1,3-glucanase activity over control | Seed + seedling treatment | Percent increase in $-1,3-glucanase activity over control |
| Salicylic acid | 1.0 | 0.152 (0.018) | 13.43 | 0.156 (0.026) | 20.00 | 0.184 (0.057) | 44.88 |
| Salicylic acid | 1.5 | 0.156 (0.022) | 16.42 | 0.160 (0.030) | 23.08 | 0.190 (0.063) | 49.61 |
| $-amino butyric acid | 10.0 | 0.169 (0.035) | 26.12 | 0.163 (0.033) | 25.38 | 0.175 (0.048) | 37.80 |
| $-amino butyric acid | 15.0 | 0.180 (0.046) | 34.33 | 0.164 (0.034) | 26.15 | 0.199 (0.072) | 56.69 |
| Chitosan | 15.0 | 0.143 (0.009) | 6.72 | 0.145 (0.015) | 11.54 | 0.155 (0.028) | 22.05 |
| 2, 6-dichloroisonicotinic acid | 10.0 | 0.140 (0.006) | 4.48 | - | - | - | - |
| Control (non treated) | - | 0.134 | | 0.130 | | 0.127 | |
| *SE ± m | | 0.004 | | 0.005 | | 0.004 | |
| *CD (p = 0.01) | | 0.011 | | 0.015 | | 0.011 | |

*CD: Critical difference, SE: Standard error, Values in parenthesis indicates increase in $-1,3-glucanase activity (mg) over control

Table 2: Chitinase activity in tomato leaves as influenced by different resistance inducing chemicals

| Treatment | Conc. (mM) | Chitinase activity in tomato leaves (mg of N-acetyl glucosamine released/min/mg soluble protein) due to Seed treatment | Percent increase in chitinase activity over control | Seedling treatment | Percent increase in chitinase activity over control | Seed + seedling treatment | Percent increase in chitinase activity over control |
|---|---|---|---|---|---|---|---|
| Salicylic acid | 1.0 | 0.379 (0.063) | 19.94 | 0.380 (0.063) | 19.87 | 0.404 (0.079) | 24.31 |
| Salicylic acid | 1.5 | 0.388 (0.072) | 22.78 | 0.389 (0.072) | 22.71 | 0.423 (0.098) | 30.15 |
| $-amino butyric acid | 10.0 | 0.373 (0.057) | 18.04 | 0.373 (0.056) | 17.67 | 0.414 (0.089) | 27.38 |
| $-amino butyric acid | 15.0 | 0.377 (0.061) | 19.30 | 0.391 (0.074) | 23.34 | 0.429 (0.104) | 32.00 |
| Chitosan | 15.0 | 0.331 (0.015) | 4.75 | 0.340 (0.023) | 7.26 | 0.395 (0.070) | 21.54 |
| 2, 6-dichloroisonicotinic acid | 10.0 | 0.343 (0.027) | 8.54 | - | - | - | - |
| Control (non treated) | - | 0.316 | - | 0.317 | - | 0.325 | - |
| *SE ± m | | 0.004 | | 0.002 | | 0.002 | |
| *CD (p = 0.01) | | 0.011 | | 0.006 | | 0.006 | |

*CD: Critical difference, SE: Standard error. Values in parenthesis indicates increase in chitinase activity (mg) over control

The increase in chitinase activity in the leaves of tomato was in the range of 0.015-0.072 mg and varied with different resistance inducing chemicals. The maximum increase i.e., 19.30% was observed with salicylic acid at 1.5 mM concentration. The increase in chitinase activity due to $-aminobutyric acid at 10.0 and 15.0 mM concentration was 0.057 and 0.061 mg, respectively, whereas chitosan and 2, 6-dichloroisonicotinic acid increased chitinase activity by 0.015 and 0.027 mg, respectively. The increases in chitinase activity due to seed treatment with resistance inducing chemicals were statistically significant over control. Similarly seedling treatment (as root dip) with resistance inducing chemicals also increased the chitinase activity in tomato leaves which were statistically significant over the control. Higher increase in chitinase activity was observed with $-aminobutyric acid at 15.0 mM concentration followed by salicylic acid at 1.5 mM concentration. The maximum increase in chitinase activity per gram of sample was observed to be 0.074 mg. Similar results were obtained when seed + seedling treatment were done with resistance inducing chemicals. The maximum increase was observed for $-aminobutyric acid at 15.0 mM concentration which was 32.00% more over control. The increase in chitinase activity was statistically significant for seed, seedling and seed + seedling treatment with resistance inducing chemicals over the control (Table 2).

Tosun *et al.*[26] reported salicylic acid induced accumulation of PR-proteins i.e., chitinase and $-1, 3-glucanase in tomato plants which confers resistance against *Phytophthora infestans*. Gao *et al.*[27] also reported increase in glucanase activity in cotton plant with application of salicylic acid to confirm resistance against *Verticillium* wilt. Barilli *et al.*[28] reported the enhancement of PR-proteins such as $-1,3-glucanase and chitinase by application of benzothiadiazole and $-aminobutyric acid in pea plants that confer the resitance against pea rust pathogen. Iriti *et al.*[29] and El-Hadrami *et al.*[30] also reported the increase in PR-proteins with application of chitosan.

## CONCLUSION

The resistance inducing chemicals particularly salicylic acid and $-aminobutyric acid elicits the increased activity of pathogenesis related proteins (PR-proteins) $-1,3-glucanase and chitinase to induce resistance in susceptible tomato cultivars against *Alternaria* leaf blight disease.

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