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# Induction Of Bacterial Blight (Xanthomonas Oryzae Pv. Oryzae) Resistance In Rice By Using Systemic Acquired Resistance (Sar) And Induced Resistance (Ir) On Selected Genotype.

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**ABSTRACT**

*Bacterial blight of rice caused by Xanthomonas oryzae pv. oryzae is a common and destructive disease of rice (Oryza sativa). The disease can cause yield losses of 20 to 60 per cent (Adhikari et al., 1994; Exconde et al., 1971 and Ou, 1985). It is the most extensively studied disease with particular emphasis on resistant cultivars, their nature of resistance, explicitly of gene pyramiding for resistance, incorporation of wild source of resistance etc. Systemic acquired resistance (SAR) is the phenomenon whereby a plant's own defense mechanisms are induced by prior treatment with either a biological or chemical agent. The use of SAR as part of a disease management strategy in relation to received little scientific investigation despite offering some potential for pathogen control. Whereas, Induced resistance (IR) is a physiological state of enhanced defensive capacity elicited by specific environmental stimuli, whereby the plant's innate defens are potentiated against subsequent biotic challenges.*

*The efficacy of salicylic acid, oxalloacetic acid (Chemical agent) and biological agents in enhancing systemic acquired resistance and induced resistance against bacterial blight development was studied in pyramid variety IRBB-60 possessing 4 resistance genes (i.e. Xa 4 + xa 5 + xa 13 + Xa 21) and IRBB-59 (possessing three resistance genes i.e. Xa 5 + xa 13 + Xa 21) respectively. Salicylic acid (100 ppm.), Salicylic acid (150 ppm), Salicylic acid (200 ppm), Oxalloacetic acid (100 ppm), Oxalloacetic acid (150 ppm), Oxalloacetic acid (200 ppm) and Control (only Water) were taken as treatments for SAR and Pseudomonas fluorescenes (2g/ liters); Pseudomonas fluorescenes (3g/ liters); Bacillus subtilis (2g/ liters); Bacillus subtilis (3g/ liters); Trichoderma harzianum (T4) (2g/ liters); Trichoderma viride (T12) (2g/ liters) and Control (only Water) for IR, respectively.*

*Chemical agents and biological agents both could able to induce resistance in rice plants which was reflected in the forms of suppressed bacterial leaf blight severity / incidence as well as higher yield and yield components. Amongst chemical agents and their concentrations, Salicylic acid at 200ppm concentration could induce more resistance and thus effectively suppress disease severity as well as enhancing the yield and yield components i.e. bundle weight, grain weight etc. followed by oxalloacetic acid. Induced resistance was investigated taking into the consideration of microbial antagonism of Pseudomonas fluorescence and Bacillus subtilis as possible methods for control of bacterial blight of rice. Amongst biological agents, Pseudomonas fluorescence was found more effective in suppressing the disease severity as well as increasing yield and yield components than Bacillus subtilis. Present study therefore revealed that bacterial leaf blight severity can be suppressed up to certain level by inducing systemic acquired resistance and induced resistance using chemical and biological agents. Dempsley and Klessing (1995) also reported that salicylic acid induced resistance by signal mechanism inducing the biochemical activity.*

**KEYWORDS :**

## 1. Introduction

Bacterial blight of rice caused by Xanthomonas oryzae pv. oryzae is one of the most extensively studied disease with particular emphasis on resistant cultivars, their nature of resistance, explicity of gene pyramiding for resistance, incorporation of wild source of resistance etc. The gene expression also depends up on largely on the climatic conditions of that region, also on the pathogen virulence spectrum and to some extent the management practices adopted. Most virulent isolates are reported to be prevalent in this region (Thrimurty et al., 1993 and AICRIP reports, 2002-2007). Systemic acquired resistance (SAR) is the phenomenon whereby a plant's own defense mechanisms are induced by prior treatment with either a biological or chemical agent. The use of SAR as part of a disease management strategy in relation to received little scientific investigation despite offering some potential for pathogen control. The importance of some chemicals in imparting acquired resistance prior to the infection at the site of activity in host pathogen interaction system. Stimulants / catalase, being an antioxidant enzyme, play a major role in combating the toxic effect of reactive oxygen species (ROS) in plant cells. Induced resistance (IR) is a physiological state of enhanced defensive capacity elicited by specific environmental stimuli, whereby the plant's innate defens are potentiated against subsequent biotic challenges. Effect of different stimulants and bio-agents in imparting resistance against Xanthomonas oryzae pv. oryzae were studied.

## 2. Materials and Methods

### 2.1 Systemic acquired resistance (SAR) induced by chemicals

The efficacy of salicylic acid and oxalloacetic acid in enhancing the systemic acquired resistance against bacterial blight development was studied in pyramid variety IRBB-60 during 2007-08 and 2008-09.

Three replications for each treatment were maintained following Randomized Complete Block Design. Basal fertilizers were incorporated @ 60 kg N and 50 kg P $ha^{-1}$. Two top dressings @ 30 kg N $ha^{-1}$ were given at tillering and panicle initiation stage of the crop. Salicylic acid (100 ppm.), Salicylic acid (150 ppm), Salicylic acid (200 ppm), Oxalloacetic acid (100 ppm), Oxalloacetic acid (150 ppm), Oxalloacetic acid (200 ppm) and Control (only Water) were taken as treatments.

When plants were at maximum tillering stage, first the treatments were sprayed with stimulants. After 72 hours of treatments application first four leaves from the top were inoculated by clip inoculation in each plant and thus selected ten plants for each treatment. Top four leaves from the tagged plants were assessed for disease development. Observations were recorded from 7 day onwards at an interval of 7 days for a period of 21 days i.e., three observations. Similarly, disease severity was calculated using the formula-

$$\text{Per cent Disease severity (\% DS)} = \frac{\text{Total lesion length}}{\text{Total length of leaf}} \times 100$$

Yield (kg plot$^{-1}$) were estimated (bundle weight and grain weight) after harvesting. Per cent reduction over control were calculated using the following formula:

$$\text{I or D} = \frac{T - C}{C} \times 100$$

Where,

I or D = % Increase or Decrease over control
C = % Disease Severity in control
T = % Disease Severity in treatment

### 2.2 Role of bio-agents in inducing resistance/ reducing disease

The experiment was conducted to know the bio-agents in inducing resistance on bacterial blight of rice during *Kharif* season in two years i.e. 2007-08 and 2008-09. Seedlings were grown in wet seed beds and transplanted 25 days after sowing (DAS) of IRBB-59 (*Xa 5 + xa 13 + Xa 21*) into main field. Individual plot size was 1 m x 1 m. Plants were spaced 20 cm between row and 15 cm between plants (within rows). Three replications for each treatment were maintained following Randomized Complete Block Design. Basal fertilizers were incorporated @ 60 kg N and 50 kg P ha$^{-1}$. Two top dressings @ 30 kg N ha$^{-1}$ were given at tillering and panicle initiation stage of the crop. The treatments *viz., Pseudomonas fluorescenes* (2g/ liters); *Pseudomonas fluorescenes* (3g/ liters)**;** *Bacillus subtilis* (2g/ liters)**;** *Bacillus subtilis* (3g/ liters); *Trichoderma harzianum* ($T_4$) (2g/ liters); *Trichoderma viride* ($T_{12}$) (2g/ liters) and Control (only Water).

The treatments were applied 72 hours before inoculation by thoroughly mixing the specific solution of bio-agents (approximately 108 cfu ml-1) and sprayed on the foliage of the plants with the help of atomizer, till surface of the entire leaf covered with minute droplets of the solution that appeared. The rice plants were also sprayed with sterilized water to serve as control. After 72 hours of treatment application first four leaves from the top were inoculated by clip inoculation in each plant and selected ten plants at random for each treatment to record the observations. Four leaves from the tagged plants were assessed for disease development (by measuring the lesion length and total leaf length). Observations were recorded at every seven days interval after inoculation up to a period of 21 days i.e. three observations. Similarly, disease severity and per cent increase or decreases over control were calculated using the formula given in 1.1. Yield (kg plot-1) were also estimated (bundle weight and grain weight) after harvesting.

## 3. Results and Discussions

### 3.1 Systemic acquired resistance (SAR) induced by chemicals

Among the two years, salicylic acid treatment (at all three concentrations) reduced the disease severity significantly over the oxalloacetic acid treatments and control. Among the oxalloacetic acid treated (at all the concentrations) and control, the former showed significant reduction in per cent disease severity (Table 1).

Salicylic acid at 200ppm concentration could induce more resistance as compared to the other concentration of the same chemical also.

In seven days, fourteen days and twenty one days after inoculation, spraying of salicylic acid 200ppm ($T_3$) (0.64%, 1.34% and 5.67% respectively) showed the mean minimum per cent disease severity followed by salicylic acid 150ppm ($T_2$) (1.22%, 2.10% and 8.50% respectively) and salicylic acid 100ppm ($T_1$) (1.83%, 2.76% and 11.53% respectively) in comparison with control (7.91%, 32.13% and 48.81% respectively).

Mean maximum per cent reduction in disease severity on the final observations was observed in salicylic acid 200ppm ($T_3$) treated plants i.e. (88.38 %) over untreated control.

Dempsely and Klessing (1995) reported that salicylic acid induced resistance by signal mechanism inducing the biochemical activity. Further rice seedlings were reported to contain highest level of this chemical among the plants also supports the view that plant inbuilt resistance mechanism adopted several other workers (Iwata *et al*., 1980; Malamy *et al*., 1992; Silverman *et al*., 1995; Xiao *et al*., 1996; Durner *et al*., 1997; Liu and Wang, 2000; Rohilla *et al*., 2001; Song *et al*., 2001 and Babu *et al*., 2003) reported the involvement of salicylic acid in resistance development against *Xanthomonas oryzae* pv. *oryzae* pathogen. The present results are in confirmation of the above findings. Similar findings on the role of salicylic acid in the host pathogen interactions were also reported to impart the resistance (Wang *et al*., 2000; Katrin *et al*., 2001; Zhang *et al*., 2001; Nakashita *et al*., 2002; Ratnam *et al*., 2004; Umesha and Girish, 2005; Salam, 2007; Miersch, 2007; Mahmood *et al*., 2006 and 2007; Meirong *et al*., 2008 and Ding *et al*., 2008). Applications of low concentrations of jasmonic acid (JA) to plants induce proteinase inhibitors, proline-rich cell wall protein, and a range of enzymes involved in plant defense reactions (Sticher *et al*. 1997).

### 3.2 Effect of chemical stimulants on yield

The bundle weight (kg/plot) and grain weight (kg/plot) were recorded and presented during both the years of experimentation and their mean of two years data (Table 1.1).

The over all mean bundle weight was 1.85 kg/plot, with the range of 1.49 kg/plot ($T_7$) to 2.09 kg/plot ($T_3$). On the basis of two years mean data, spraying of salicylic acid 200ppm ($T_3$) recorded significantly higher bundle weight (2.09 kg/plot) over control (1.49 kg/plot) and oxalloacetic acid treated plants.

The salicylic acid at 200ppm recorded significantly higher bundle weight over the other concentrations of the same chemicals also. The yield (i.e. grain/plot) were non-significant.

Role of bio-agents in inducing resistance/ reducing disease

The effect of bio-agents in imparting resistance in the selected genotype for the study i.e. IRBB-59 (*Xa 5 + xa 13 + Xa 21*) was studied. The bio-agent treatments reduced the per cent disease severity in all the dates of observations over control treatment (water only). The mean data of the two years clearly indicated that $T_2$ (*Pseudomonas fluorescence*) sprayed @ 3g/lit. superiorly reduced the disease severity over control and at par with $T_3$ (*Bacillus subtilis* @ 3g/lit.).

**Fourteen days after inoculation:**

All the treatments significantly reduced the disease severity over untreated control. The $T_2$ (*Pseudomonas fluorescence* @ 3g/lit.) superiorly performed in reducing the disease severity over all the other treatments. This was followed by $T_3$ (*Bacillus subtilis* @ 2g/lit.); $T_1$ (*Pseudomonas fluorescence* @ 2g/lit.); $T_4$ (*Bacillus subtilis* @ 3g/lit.); $T_5$ (*Trichoderma harzianum* @ 2g/lit.) and $T_6$ (*Trichoderma viride* @ 2g/lit.).

**Twenty one days after inoculation:**

The *Pseudomonas fluorescence* treated plants ($T_2$@ 3g/lit. and $T_1$@ 2g/lit.) significantly reduced the disease severity over control and other treatments. They were followed by $T_4$ (*Bacillus subtilis* @ 3g/lit.); $T_3$ (*Bacillus subtilis* @ 2g/lit.); $T_6$ (*Trichoderma viride* @ 2g/lit.) and $T_5$ (*Trichoderma harzianum* @ 2g/lit.) treatments.

Maximum mean per cent reduction in disease severity on the final observations was observed in *Pseudomonas fluorescence* treated plants ($T_2$ @ 3g/lit. and $T_1$ @ 2g/lit.) i.e. (87.95 %) and (83.77 %) over untreated control respectively.

The importance of *Pseudomonas fluorescence*, *Bacillus* species, *Erwinia herbicola* had been reported as potential bio-agents against *Xanthomonas oryzae* pv. *oryzae* (Hsieh and Buddenhagen, 1974; Randhawa and Schoad, 1985; Anuratha and Gnanmanickam, 1987; Sivamani *et al*., 1987; Santhi *et al*., 1987; Saikia and Chowdhary, 1993; Gnanamanickam, 1999 and Vidhyasekaran *et al*., 2000). This confirms the present findings also in addition to the above in the present studies the efficacy of *Trichoderma* species were also effective in reducing the disease severity. Several other workers also emphasized the importance of bio- control agents like *Azospirillum brasilense*, *Bacillus polymixa*, *Azotobacter*, *Enterobacter cloacae, Serratia marcescens* and *Alcaligenes paradoxus* in disease management. (Pandey and Iswaran, 1982; Yang *et al*., 1999; Kemple and Sequeira, 1983; Suneja

*et al*., 1994; Kim and Misaghi, 1996; Anuratha and Gnanamanickam, 1987; Gnanamanickam *et al*., 1999; Someya *et al*., 2002; Rangrajan *et al*., 2003 and Mirik *et al*., 2008).

### 3.4 Effect of bio-agents on yield

The bundle weight (kg/plot) and grain weight (kg/plot) were recorded and presented during the mean of two years data.

The over all average bundle weight was for the two years mean 1.81 kg/plot, with the range from 1.46 kg/plot ($T_7$) to 2.32 kg/plot ($T_2$). On the basis of two years mean data, spraying of *Pseudomonas fluorescence* 3g/lit. ($T_2$) significantly increased the bundle weight (2.32 kg/plot respectively) over control (1.46 kg/plot). However, it was at par with *Pseudomonas fluorescence* 2g/lit. ($T_1$) (2.04 kg/plot) and *Bacillus subtilis* 3g/lit. ($T_3$) (1.90 kg/plot).

During both the years of study the grain weight among the treatments were non-significant. However, there was an increase in grain weight over the control in all the treatments and $T_2$ recorded highest weight followed by $T_1$, $T_4$, $T_3$, $T_5$ and $T_6$ respectively.

### Conclusions

Recent demonstrations of the effectiveness of SAR and ISR in field situations presents interesting opportunities for the control of *Xanthomonas oryzae* pv. *oryzae* of rice. Experiments with rice plants have shown that SAR and ISR can lead to long-lasting, broad spectrum disease control and can be used preventively to bolster general plant health. Treated plants with salicylic acid and other chemicals increased phenolics and accumulate phytoalexins, pathogenesis-related proteins faster than non-treated plants. Ample evidence suggests that SAR is based on multiple natural defense mechanisms, and this makes it less likely that a pathogen can readily develop resistance to this control measure. Bacterial siderophores inhibit plant pathogens through competition for iron, antibiotics suppress competing microorganisms, and chitinases and glucanases lyse microbial cells. The availability of this long-lasting, broad-spectrum and potentially stable solution to disease control may have a positive impact on bacterial blight management.

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**Table 1: Effect of chemicals in imparting resistance on genotype IRBB-60**

| Trt. | Treatments | Rate (ppm.) | Disease severity (%) | | | Percent reduction over control |
|---|---|---|---|---|---|---|
| | | | 7 DAI[a] | 14 DAI[b] | 21 DAI[b] | |
| $T_1$ | Salicylic acid | 100 | 1.83 | 2.76 | 11.53 | 76.38 |
| | | | (1.35) | (9.57) | (19.85 | |
| $T_2$ | Salicylic acid | 150 | 1.22 | 2.1 | 8.5 | 82.59 |
| | | | (1.1) | (8.43) | (16.96) | |
| $T_3$ | Salicylic acid | 200 | 0.64 | 1.34 | 5.67 | 88.38 |
| | | | (0.8) | (6.66) | (13.77) | |
| $T_4$ | Oxalloacetic acid | 100 | 3.92 | 11.86 | 28.12 | 42.39 |
| | | | (1.98) | (10.09) | (32.02) | |
| $T_5$ | Oxalloacetic acid | 150 | 4.11 | 14.91 | 33.97 | 30.4 |
| | | | (2.03) | (22.72) | (38.68) | |
| $T_6$ | Oxalloacetic acid | 200 | 1.16 | 5.47 | 19.43 | 60.19 |
| | | | (1.08) | (13.53) | (26.16) | |
| $T_7$ | Control | Water | 7.91 | 32.13 | 48.81 | 0 |
| | | | (2.81) | (33.57) | (44.32) | |
| **General Mean** | | | 2.97 | 9.29 | 22.28 | 54.33 |
| (**1.8**) (**14.94**) | | | (**27.39**) | | | |
| SEm ± | | | 0.05 | 0.3 | 0.5 | |
| CD (P = 0.05) | | | 0.16 | 0.92 | 1.56 | |

[a] Bracket values represents square root transformed values
[b] Bracket values represents Arc sine transformed values
DAI: Days after inoculation

**Table 1.1: Effect of chemicals on yield (kg/plot) parameters of IRBB-60**

| Trt. | Treatments | Rate (ppm) | Yield (kg / plot) | |
|---|---|---|---|---|
| | | | Bundle weight | Grain weight |
| $T_1$ | Salicylic acid | 100 | 1.85 | 0.97 |
| $T_2$ | Salicylic acid | 150 | 1.99 | 1 |
| $T_3$ | Salicylic acid | 200 | 2.09 | 1.05 |
| $T_4$ | Oxalloacetic acid | 100 | 1.69 | 0.72 |
| $T_5$ | Oxalloacetic acid | 150 | 1.76 | 0.63 |
| $T_6$ | Oxalloacetic acid | 200 | 2.11 | 1.05 |
| $T_7$ | Control | Water | 1.49 | 0.52 |
| General Mean | | | 1.85 | 0.83 |
| SEm± | | | 0.02 | 0.02 |
| CD (P = 0.05) | | | 0.05 | NS |

**Table 2: Effect of bio-agents inducing resistance on genotype IRBB-59**

| Trt. | Treatments | Rate/ litre (grams) | Disease severity (%) | | | Percentage reduction over control |
|---|---|---|---|---|---|---|
| | | | 7DAI[a] | 14 DAI[b] | 21DAI[b] | |
| $T_1$ | Pseudomonas fluorescenes | 2 | 1.79 | 5.32 | 7.93 | 83.77 |
| | | | (1.52) | (13.33) | (16.26) | |
| $T_2$ | Pseudomonas fluorescenes | 3 | 1.4 | 3.23 | 5.89 | 87.95 |
| | | | (1.38) | (10.2) | (13.96) | |
| $T_3$ | Bacillus subtilis | 2 | 1.5 | 4.5 | 18.52 | 62.1 |
| | | | (1.4) | (12.24) | (25.49) | |
| $T_4$ | Bacillus subtilis | 3 | 1.72 | 5.72 | 18.46 | 62.22 |
| | | | (1.48) | (13.83) | (25.37) | |
| $T_5$ | *Trichoderma harzianum* ($T_4$) | 2 | 2.95 | 9.51 | 32.03 | 34.45 |
| | | | (1.85) | (17.96) | (34.34) | |
| $T_6$ | *Trichoderma viride* ($T_{12}$) | 2 | 2.16 | 9.83 | 31.13 | 36.29 |
| | | | (1.63) | (18.26) | (33.91) | |
| $T_7$ | Control | Water | 6.45 | 31.88 | 48.86 | 0 |
| | | | (1.3) | (34.37) | (44.35) | |
| **General Mean** (**1.52**) (**17.17**) | | | 2.57 | 9.99 | 23.26 | 52.39 |
| | | | (**27.68**) | | | |
| SEm ± | | | 0.08 | 0.38 | 0.48 | |
| CD (P = 0.05) | | | 0.25 | 1.15 | 1.49 | |

a Bracket values represents square root transformed values,
b Bracket values represents arc sine transformed values,
DAI: Days after inoculation

**Table 2.1: Effect of bio-agents on yield (kg/plot) parameters of IRBB-59**

| Trt. | Treatments | Rate/liter (grams) | Yield (kg/plot) | |
|---|---|---|---|---|
| | | | Bundle weight | Grain weight |
| $T_1$ | Pseudomonas fluorescenes | 2 | 2.04 | 0.97 |
| $T_2$ | Pseudomonas fluorescenes | 3 | 2.32 | 1.16 |
| $T_3$ | Bacillus subtilis | 2 | 1.79 | 0.69 |
| $T_4$ | Bacillus subtilis | 3 | 1.9 | 0.78 |
| $T_5$ | *Trichoderma harzianum*($T_4$) | 2 | 1.66 | 0.47 |
| $T_6$ | *Trichoderma viride* ($T_{12}$) | 2 | 1.54 | 0.39 |
| $T_7$ | Control | Water | 1.46 | 0.33 |
| General Mean | | | 1.81 | 0.68 |
| SEm± | | | 0.02 | 0.02 |
| CD (P = 0.05) | | | 0.05 | NS |

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