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INDUCED SYSTEMIC RESISTANCE IN CHICKPEA AGAINST *ASCOCHYTA* BLIGHT BY SAFE CHEMICALS

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Abstract

Chickpea variety, C727 susceptible to *Ascochyta rabiei*, was grown in small plots of 20 sq feet area. Induction treatments were given by spraying aqueous solutions of 1.0 mM Salicylic acid, 50 mM K_2HPO_4 (analytical and commercial grade), 0.4 mM Bion and two types of neem leaves extract (50mg/ml, leaves boiled in methanol, methanol was evaporated then residue dissolved in water and in other extract neem leaves were boiled in water) at flowering stage of the plants in triplicates. Control plants were treated with water only. One week after induction treatments, all the plants were challenged with spore suspension of *A. rabiei* (10^6 spores/ml). Disease data was recorded after disease was fully developed on control plants. Plant tissues were collected at 0, 24, 48 and 72 hours after induction and challenge treatments for biochemical analyses of proteins and peroxidase enzyme activity as the part of defense mechanism. Blight disease was significantly reduced in chickpea variety C727 after spraying the plants with all the chemicals tested. Maximum reduction in the disease was obtained with Salicylic acid, followed by Bion. Slight increase in yield was observed in Bion, K_2HPO_4 (commercial) and neem extract treated plants as compared to control plants but the difference was non-significant. Plant tissues collected at different time intervals from induced and control plants after induction and challenged treatments were subjected to biochemical analyses of total proteins, SDS-PAGE, activity of peroxidase enzyme and Native PAGE for isozymes of peroxidase. A significant increase in total proteins and peroxidase activity was observed after induction treatments with all the chemicals tested. However, electrophoresis indicated that pattern of proteins and isoforms of peroxidase were similar in induced and control plants.

Introduction

Induced systemic resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signalling pathways as well as to its potential use in plant protection (Heil & Bostock, 2002). In ISR, elicitors enhance level of one or more translocatable signal chemicals, which in turn, results in coordinated induction of genes controlling diverse defense pathways (Kuc, 1997). The exogenous application of inducer chemicals lead to the induction of systemic resistance in crop plants (Edreva, 2004; Ahn, 2005; Saikia *et el.*, 2006).

Chickpea (*Cicer arietinum* L.) is an important pulse crop (70% of all pluses) of Pakistan, recognized as one of the major source of plant protein. The average production of chickpea in Pakistan is less than the world production. *Ascochyta* blight caused by *Ascochyta rabiei* (Pass) Lab is considered one of the major constrains to chickpea production. As the pathogen changes in the nature constantly (Jamil *et al*, 2000) and previously resistant cultivars will become susceptible after sometime, there is need to curtail infection by using the latent disease resistance mechanism in existing high yielding and high quality chickpea varieties.

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The present studies have been conducted to induce systemic resistance in chickpea against *Ascochyta* blight disease by using environmentally safe chemicals. The biochemical analysis of induced plants may provide information to understand the mechanism of induced resistance and help in future research of ISR.

Materials and Methods

Plant materials, induction and challenge treatments: Chickpea variety, C727 susceptible to *Ascochyta rabiei*, was grown in small plots of 20 sq feet area. Induction treatments were given by spraying aqueous solutions of 1.0 mM Salicylic acid, 50 mM K_2HPO_4 (analytical and commercial grade), 0.3 mM Bion and two types of neem leaves extract (50mg/ml, leaves were boiled in methanol, methanol was evaporated then residue was dissolved in water and in other extract, neem leaves were boiled in water) at flowering stage of the plants in triplicate. Control plants were treated with water only. One week after induction treatments, all the plants were challenged with spore suspension of *A. rabiei* (10^6 spores/ml). Half of the control plants left uninoculated (-ve control) and rest of the plants were inoculated with *A. rabiei* (+ve control)**.** Disease data was recorded after disease was fully developed on control plants. Plant tissues were collected at 0, 24, 48 and 72 hours after induction and challenge treatments for biochemical analyses of proteins and peroxidase enzyme activity as the part of defense mechanism. Plant tissues were stored at -80^{o}C until analyzed.

Disease assessment: Disease was recorded from 10 randomly selected plants from each treatment by counting total branches and diseased branches and reduction in blight disease was calculated on the basis of disease considered as 100% on control.

Protein analysis: Frozen plant tissues were ground in 0.1M Sodium phosphate buffer, pH 6.0 (1:4 ratio) in ice chilled pestle and mortar. Homogenized tissues were filtered through cheese cloth and centrifuged at 14000 rpm for 10 min., supernatant was freezed and used for protein/peroxidase estimation. Total proteins were determined quantitatively by standard Bradford method (Bradford, 1976) and qualitatively by gel electrophoresis on 10% polyacrylamide gels containing 0.1% SDS.

Peroxidase analysis: Peroxidase activity was assayed with guaicol, as hydrogen donor, as described by Hammerschmidt *et al.,* (1982). Native PAGE electrophoresis was conducted for detection of peroxidase isozymes. Isoforms were detected as described by Winterhalter & James (1983).

Results

Blight disease was significantly reduced in chickpea variety C727 after spraying plants with all the chemicals tested. Maximum reduction in blight disease, 63%, was obtained with Salicylic acid (SA), followed by Bion while similar reduction i.e. 44-48%, was observed with both types of K_2HPO_4 and Neem extracts (Fig. 1). Yield data was recorded at the end of the experiment. Slight increase in yield was observed in Bion, K_2HPO_4 (commercial) and neem extract treated plants as compared to control plants but the difference was non-significant. However, data of grain/5 plants indicated that Bion and SA treatments showed significant increase in number of grains per plants (Table 1).

Table 1. Effect of induction treatments on chickpea yield after challenge with *Ascochyta rabiei.*

Treatments	Yield (gm)	Grains/5 plants
Control	230	190^{d}
Bion	272	261^{b}
Salicylic acid	231	257^{bc}
K_2HPO_4 (analytical)	191	211^{d}
K_2HPO_4 (commercial)	271	214^{d}
Neem extract (organic)	260	214^{d}
Neem extract (water)	313	220^{cd}

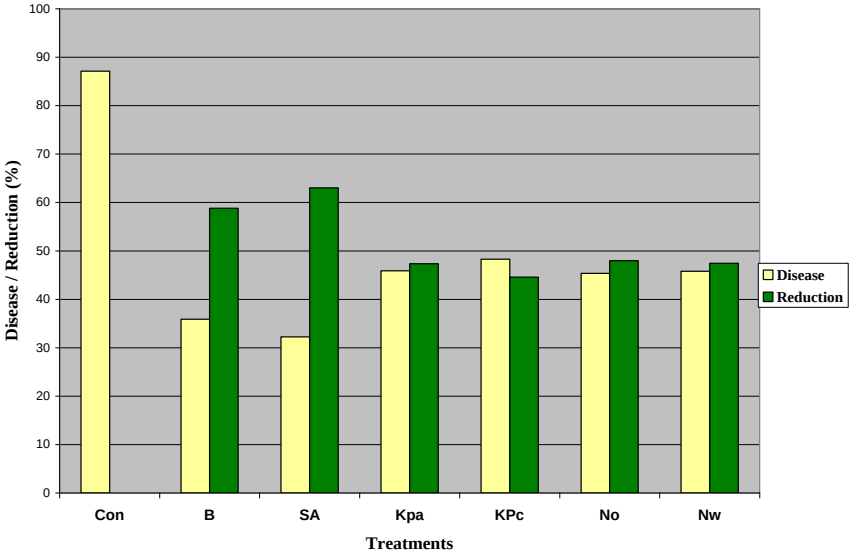
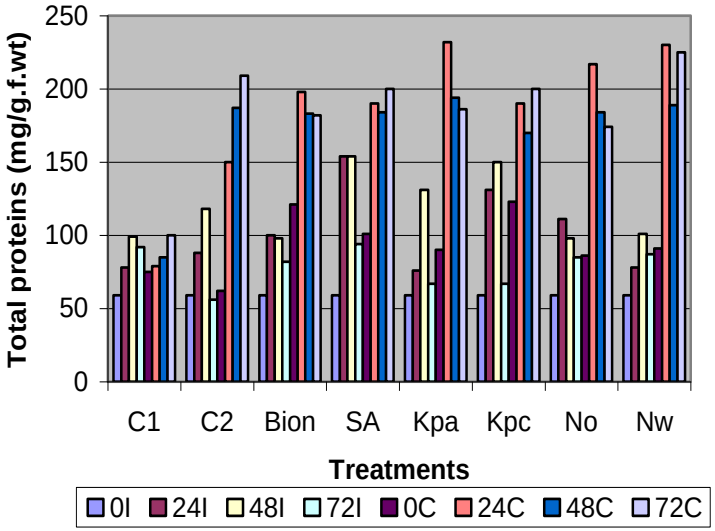


Fig. 1. Effect of induction treatments on blight disaese in chickpea after challenged with *A. rabiei* under field conditions.
Con: Control; B: Bion; SA: Salicylic acid; Kpa: K_2HPO_4 (analytical); Kpc: K_2HPO_4 (commercial); No: Neem extract (organic extraction); Nw: Neem extract (water extraction)

Significant increase in total proteins (mg/g.f.wt.) was observed 24 hrs after all the induction treatments (Fig. 2). Maximum amount of proteins were observed in plants treated with salicylic acid followed by K_2HPO_4 at 24 hrs after induction treatments. Rapid increase in protein contents were observed in all the treated plants 24 hrs after challenged treatments, but increase was higher in induced plants as compared to control ones. Proteins of different molecular weight from 10-200 kilodalton (KDa) were separated by SDS-PAGE electrophoresis from induced and control plants extracts (Fig. 3).

Peroxidase activity was increased in all the treated plants after 24 h of induction and maximum increase was observed with K_2HPO_4 followed by Salicylic acid and neem extract (Fig. 4). Native PAGE analysis showed that three isozymes of peroxidase were present in all the induced and control plants (Fig. 5). No difference in isozymes of induced or control plants were observed while peroxidase activity was significantly increased in chickpea plants after induction treatments.



Hours after induction treatments (I) Hours after challenge treatments (C)

Fig. 2. Total proteins in chickpea after induction with different chemicals and challenged with *A. rabiei*. C1: Control without induction and without challenged with *A. rabiei*; C2: Control without induction but challenged with *A. rabiei*; SA: Salicylic acid; Kpa: K_2HPO_4 (analytical); Kpc: K_2HPO_4 (commercial); No: Neem extract (organic extraction); Nw: Neem extract (water extraction)

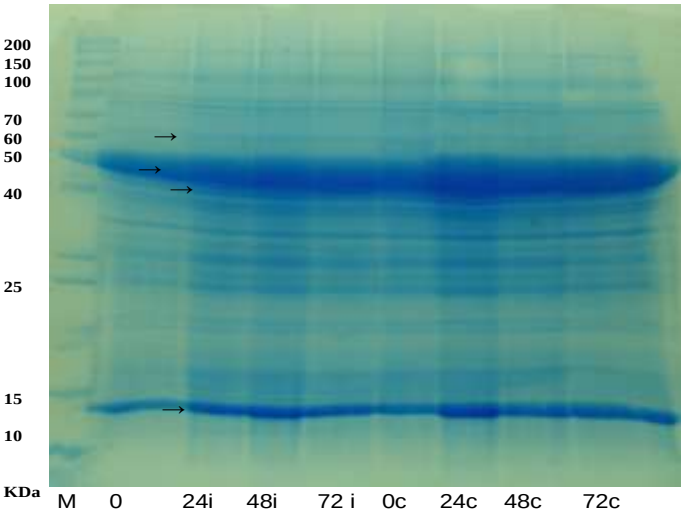


Fig. 3. SDS-PAGE of proteins from induced (with Bion) and challenged (with *A. rabiei*) chickpea plants. Lane: M marker; 0 before induction treatments; i after induction; c after challenged.

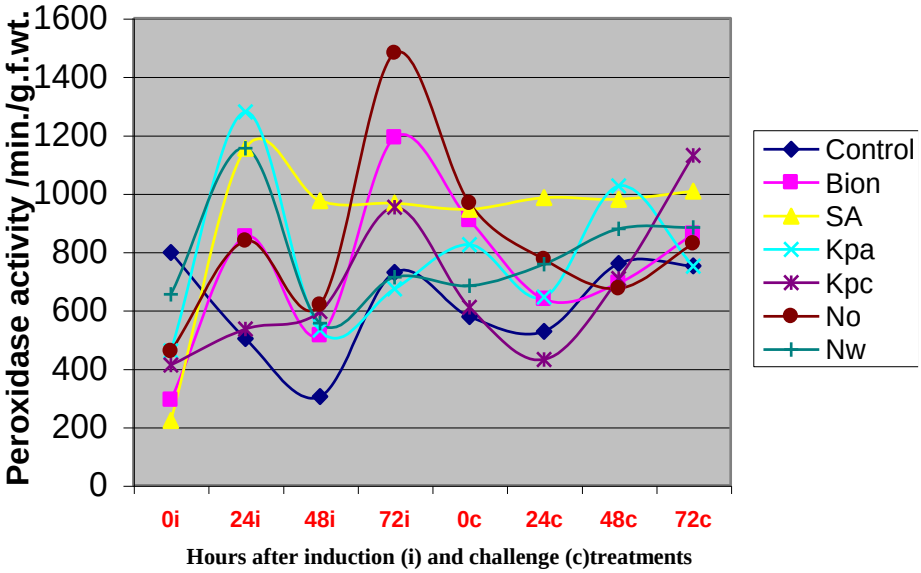


Fig. 4. Change in peroxidase activity of chickpea after induction with different chemicals and challenged with *A. rabiei* treatments.

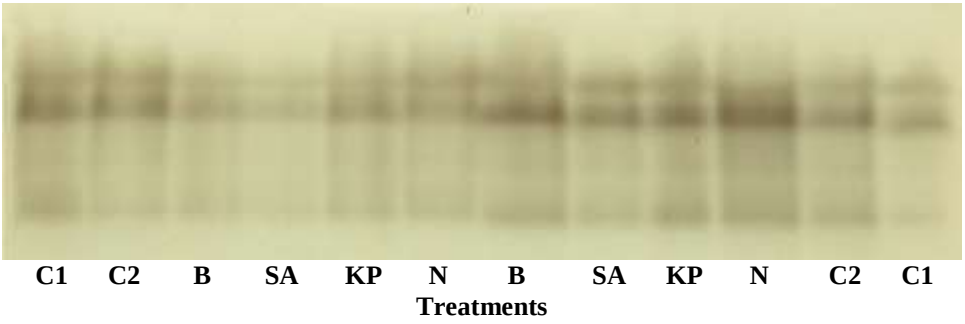


Fig. 5. Native PAGE gel showing isoforms of peroxidase enzyme in chickpea control and induced plants 24 hours after induction and challenged treatments.
C1: Control without induction and without challenged with *A. rabiei*; C2: Control without induction but challenged with *A. rabiei*; B: Bion; SA: Salicylic acid; Kp: K_2HPO_4 N: Neem extract. First 6 lines: extract from induced plants and rest of 6 lines: extract from induced and challenged plants

Discussion

Present study demonstrated that Bion Salicylic acid, K_2HPO_4 and neem extract induced systemic resistance against *Ascochyta* blight. Blight caused by *A. rabiei* consider as the most damaging disease of chickpea crop. These findings are in line with ISR studies conducted with different Plant-pathogen interactions. Earlier studies indicated that Bion, SA and Riboflavin can induce systemic resistance against another disease of chickpea, *Fusarium* wilt (Sarwar *et al*, 2003; Saikia *et al*., 2006). Similar results were observed in rice where systemic resistance against *Pyricularia grisea* was obtained by

foliar spray of K_2HPO_4, oxalic acid, $Na_2B_4O_7$ and vitamin B (Du *et al*., 2001; Ahn *et al*., 2005 **)**. Early and late blight of potatoes were significantly reduced by chemical inducers (Nadia *et al*., 2007). However, effect of induction treatments on chickpea yield was not comparable with the degree of reduction of disease this may be explained that vegetative growth was increased by induction of systemic resistance in chickpea by SA and Bion (Saikia *et al*., 2003; Sarwar *et al*., 2003).

Pathogenesis related proteins (PR-protein) were reported as one of the major defence mechanism involved in disease resistance of plants (Van Loon *et al*., 2006). Concentration of total protein and separated proteins increased in induced plants in present studies. These proteins may be pathogenesis related proteins (PR-protein) i. e., peroxidase, chitinases and β1,3 glucanases. These finding are similar with the earlier report by Saikia *et al*, (2005, 2006) that two chitinases (31, 62KDa) three β1,3 glucanases (23, 27 , 39 KDa) and peroxidase (42 KDa) were observed in induced chickpea plant against *Fusarium* wilt. The involvement of PR-protein in ISR could be related to their characteristic functions, such as some of them exert hydrolytic action (glucanase, chitinase), this suggesting a lytic effect on pathogen cell walls built-up of glucans and chitins (Gozzo, 2003; Van Loon *et al*., 2006).

These results were also similar with the earlier findings in chickpea that the peroxidase activity was significantly increased in chemically induced plants as compared to control ones (Chaudhry *et al*., 2001). Peroxidase is considered an important PR-protein (Van Loon *et al*., 1994). Plants express peroxidase activity during pathogen interaction (Sarwar *et al*., 2000; Saikia *et al*., 2006). In this study, we observed increased peroxidase activity in induced plants challenged with *A. rabiei* as compared to control ones.

Results of present findings suggest that all the chemicals tested systemically induced resistance in chickpea against *Ascochyta* blight and showed accumulation of proteins and peroxidase enzyme which are the part of defense mechanisms.

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