

INDUCTION OF SYSTEMIC RESISTANCE IN CHICKPEA AGAINST *FUSARIUM* WILT BY SEED TREATMENT WITH SALICYLIC ACID AND BION

NIGHAT SARWAR, M. HAYAT ZAHID CH., IKRAMUL HAQ,
AND F. F. JAMIL

*Nuclear Institute for Agriculture and Biology,
P. O. Box 128, Jhang Road, Faisalabad, Pakistan*

Abstract

Seeds of chickpea variety AUG424, susceptible to *Fusarium oxysporum ciceri* (FOC), were surface sterilized with sodium hypochlorite, thoroughly rinsed with sterilized water and immersed in two concentrations of Salicylic acid (1.0 & 1.5 mM) and Bion (0.3 & 0.4 mM). Seeds treated with 2% Benlate were used as standard. Control represented, the seeds soaked in distilled water. Control and treated seeds were sown in two sets of pots containing sterilized soil (experiment 1) and soil inoculated with FOC (experiment 2). Chemically treated and control seeds were grown under controlled environment. Two week old seedlings grown in sterilized soil were up rooted, roots were cut at 1cm from tip and immersed in spore suspension (10^6 micro conidia/ml) of FOC for three hours and then the seedlings were transplanted into new pots containing sterilized soil. Plants of both sets were observed daily for up to 40 days to record wilt disease by counting the total and wilted plants in each pot. At the end of the experiment, surviving plants were cut at collar region for observing the fungus growth inside the vascular tissues. Fresh and dry weight of the shoots and roots were recorded. Wilt disease was significantly reduced with all the treatments in both experiments. On the basis of disease rating done after root cutting, wilt incidence was significantly less in chemically treated plants as compared to control ones. Fresh and dry weights of shoot and root were higher in treated plants as compared to control ones especially in plants grown from Bion treated seeds.

Introduction

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *ciceri* (FOC), is a widespread disease that occurs in most chickpea growing areas. In Pakistan it has reduced the share chickpea on irrigated land from 50% to 10% (Hanif *et al.*, 1999). FOC is a soil-borne pathogen that infects plants through roots at all stages of plant growth. Various strategies for controlling FOC have been introduced over the years e.g., soil cultural practices, fungicide treatments etc., but serious losses still occur, largely because the effectiveness of these approaches is variable and often short lived (Jarvis, 1988). In addition, chickpea cultivars with strong resistance to FOC are not commercially available in Pakistan. Recent advances in our understanding of the mechanism underlying the expression of plant defence gene upon microbial infection (Dixon & Lamb, 1990) have led to the conclusion that artificial manipulation of the natural plant defence system could provide a biologically/environmentally safe and commercially valuable alternative to the existing pathogen control methods (Sequeira, 1990; Dong & Cohen, 2002). Several lines of evidence have shown that all plants, resistant and susceptible, respond to pathogen attack by induction of an array of defence reactions designed to affect pathogen growth and viability (Lamb *et al.*, 1989). However, in cases where plant- pathogen interactions result in disease establishment, successful host colonization by the parasite is likely due to

delayed plant defence expression, rather than the absence or inactivation of defence mechanisms (Dixon & Lamb, 1990). Thus, the speed and extent of the plant response to microbial attack appears to be the key determinants in the outcome of a given interaction, and it is reasonable to assume that a faster response to a pathogen may enhance the resistance in a previously susceptible plant. Enhanced disease resistance in plants was found to occur in response to biotic (pathogens or non pathogens) and abiotic inducing agents such as Salicylic acid, K_2HPO_4 , oxalic acid, chitosan etc. (Gaffney *et al.*, 1993; Kuc', 1993; Benhamou *et al.*, 1994; Bashir *et al.*, 1997). The present paper reports that seed treatment with Salicylic acid and Bion induces systemic resistance in chickpea seedlings against Fusarium wilt.

Material and Methods

Fungus culture and growth conditions

Highly virulent strain of *Fusarium oxysprum* f.sp. *ciceri* (FOC) was isolated from infected chickpea plant by using Komada's medium (Komada, 1975) and confirmation of *Fusarium* was made on carnation leaf agar medium (Fisher *et al.*, 1982). After confirmation it was transferred on potato dextrose agar (PDA) medium and stored in the refrigerator till further use. FOC was grown on different media in plates, which were incubated in cooled incubator (Gallen kamp) at $25 \pm 2^\circ C$ in dark for one week. For the preparation of spore suspension of FOC to inoculate chickpea seedlings, FOC culture was inoculated on PDA slants, incubated for one week, micro conidia were harvested by adding 5 ml of sterilized water in each tube and surface of medium was scraped with the help of sterilized spatula. Spore suspension from each tube was passed through two layers of muslin cloth to remove mycelial mass. Spores were counted with haemocytometer and suspension was adjusted to 10^6 spores/ml by adding sterilized distilled water.

Seed treatment with chemicals and fungal inoculation

Seeds of chickpea variety AUG424, highly susceptible to FOC, were surface sterilized with sodium hypochlorite, thoroughly rinsed with sterilized water and soaked into different concentrations of Salicylic acid (1.0 & 1.5 mM) and Bion (0.3 & 0.4mM) for 24 hours. Seeds immersed for 24 hours in the suspension of 2% wet able powder of Benlate were used as standard. For control plants, seeds were soaked in sterilized water. Control and chemical treated seeds were sown in two sets of pots, one containing sterilized soil only (experiment 1) and 2nd containing sterilized soil inoculated with FOC (experiment 2). Chemically treated and control seeds were sown in three plastic pots (5 inch dia.) and each pot contained five plants. Plants were grown in growth room under controlled environment (temp. $22 \pm 2^\circ C$, 12 hours photo period with 24,000-26,000 Lux fluorescent and incandescent lights). Two week old seedlings, grown in sterilized soil, were up rooted, washed under tap water, and dipped in spore suspension (10^6 spores/ml) of FOC for three hours for inoculation. After inoculation the seedlings were transplanted into new pots containing sterilized soil. Plants of the both sets were observed daily, up to 40 days, to record wilt disease by counting the total wilted plants in each pots. At the end of the experiment surviving plants were uprooted, washed in tap water to remove soil particle and kept immersed in tap water till observation. Each plant was cut at collar region for observing the fungus growth inside the vascular tissue according to 0-5 rating scale (Haq & Jamil, 1995). Fresh and dry weight of the shoots and roots were recorded.

Table 1. Effect of induction treatments on fresh and dry weights (gm) of shoots and roots of chickpea plants after inoculation with *Fusarium oxysprum* F. sp. *ciceri*.

Treatments	Experiment 1				Experiment 2			
	Shoots		Roots		Shoots		Roots	
	F	D	F	D	F	D	F	D
Control	1.967	0.467	1.400	0.200	0.567	0.100	0.533	0.027
B1	3.533	0.900	3.533	0.500	2.667	0.500	1.267	0.167
B2	3.033	0.700	3.200	0.500	2.767	0.667	1.200	0.167
Sa1	2.767	0.633	1.733	0.367	2.067	0.600	1.067	0.167
Sa2	2.600	0.600	0.800	0.100	2.633	0.500	1.067	0.167
Ben	3.700	0.900	1.800	0.300	2.845	0.640	1.723	0.358

B1= Bion (0.3 mM), B2= Bion (0.4mM), S1= Salicylic acid (1.0 mM),

S2= Salicylic acid (1.5mM), Ben= Benlate (2%)

F= Fresh weight; D= Dry weight

Results

Wilt disease was significantly reduced with all the treatments except with lower concentration of Bion (Fig. 1). Only 7% plants wilted when seeds were treated with higher concentrations of Salicylic acid, Bion and Benlate. On the basis of disease rating done after root cutting, wilt incidence was significantly less in chemical treated plants as compared to control ones (Fig. 2). Fresh and dry weights of shoot and root were higher in treated plants as compared to control ones especially in plants grown from Bion treated seeds (Table 1).

Plants from control seeds sown in FOC inoculated soil showed 80% wilting at the end of the experiment, while plants grown from seeds treated with higher concentrations of Salicylic acid and Bion had only 27% wilting (Fig. 3). Plants grown from seeds treated with lower concentrations of Salicylic acid and Bion showed 53 and 30% wilting, respectively whereas in case of Benlate 25% the plants wilted. On the basis of disease rating done after root cutting, disease was significantly less in plants grown from seeds treated with Bion, higher concentration of Salicylic acid and Benlate. No significance difference was observed between control and plants grown from lower concentration of Salicylic acid (Fig. 4). Fresh and dry weights of shoots and roots were significantly higher in treated plants (Table 1).

Discussion

In the past two decade, elicitor-mediated induced resistance has become one of the most challenging research areas in plant pathology (Ryan & Farmer, 1991) Extensive studies, initially using model systems of reduced complexity such as elicitor-treated cell-suspension cultures (Scheel *et al.*, 1986), have provided a conceptual basis for designing new strategies to enhance plant resistance to microbial attack (Hadwiger *et al.*, 1988; Jarvis, 1989).

Results of the present studies demonstrated that seed treatments induced systemic resistance in susceptible chickpea variety. These findings were in line with earlier studies on tomato plants, where susceptible plants developed a systemic induced resistance to *Fusarium oxysprum* f.sp. *radicis-lycopersici* infection in response to chitosan application given by seed treatment (Benhamou *et al.*, 1994).

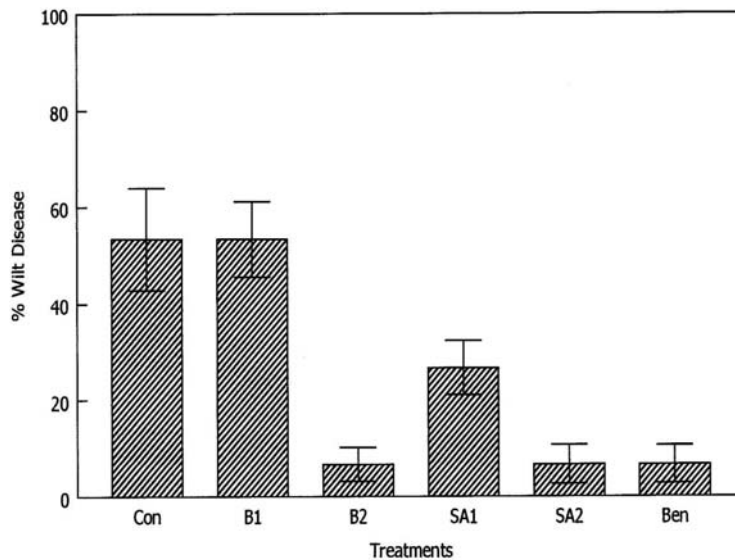


Fig. 1. Incidence of wilt disease in chickpea after seed treatment with two concentrations of Bion, Salicylic acid and Benlate (standard fungicide).

Con = Control , B1 = Bion (0.3mM), B2 = Bion (0.4mM), SA1 = Salicylic acid (1.0mM), SA2 = Salicylic acid (1.5mM), Ben = Benlate (2%)

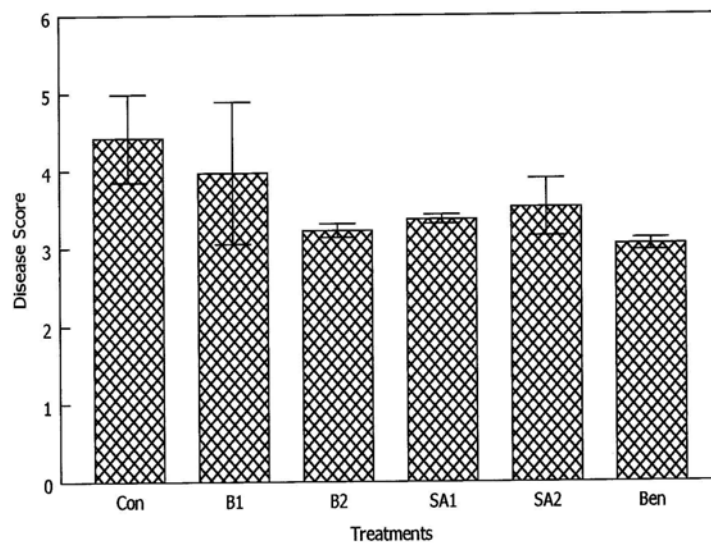


Fig. 2. Incidence of wilt disease in root's vascular tissues of chickpea after seed treatment with two concentrations of Bion, Salicylic acid and Benlate (standard fungicide).

Con = Control , B1 = Bion (0.3mM), B2 = Bion (0.4mM), SA1 = Salicylic acid (1.0mM), SA2 = Salicylic acid (1.5mM), Ben = Benlate (2%)

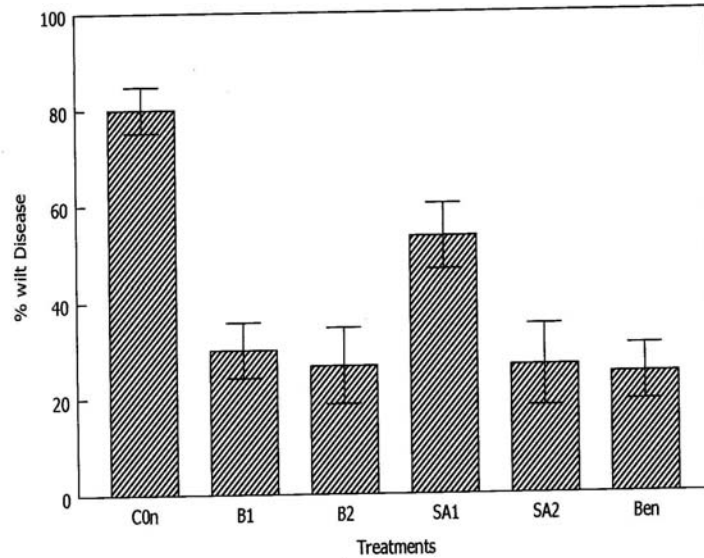


Fig. 3. Incidence of wilt disease in chickpea after seed treatment with two concentrations of Bion, Salicylic acid and Benlate (standard fungicide).

Con = Control , B1 = Bion (0.3mM), B2 = Bion (0.4mM), SA1 = Salicylic acid (1.0mM), SA2 = Salicylic acid (1.5mM), Ben = Benlate (2%)

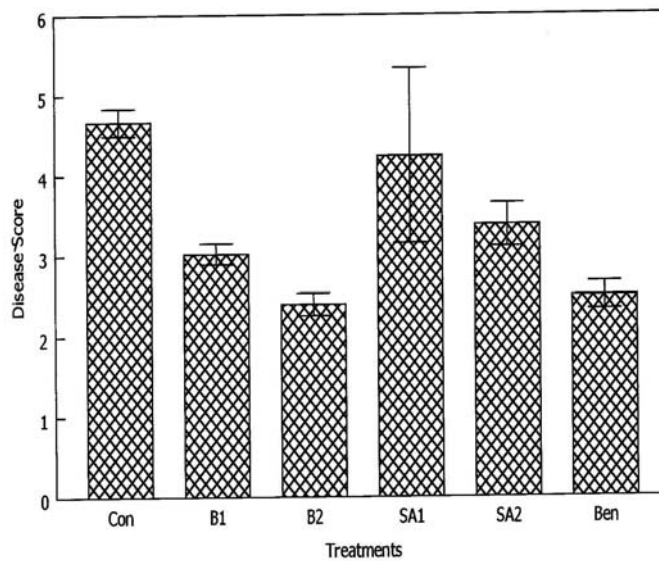


Fig. 4. Incidence of wilt disease in root's vascular tissues of chickpea after seed treatment with two concentrations of Bion, Salicylic acid and Benlate (standard fungicide).

Con = Control , B1 = Bion (0.3mM), B2 = Bion (0.4mM), SA1 = Salicylic acid (1.0mM), SA2 = Salicylic acid (1.5mM), Ben = Benlate (2%)

These results are in agreement with the earlier studies that Benothiazole (BTH) was the best among many elicitor tested on tomato plant against several bacterial and fungal pathogens (Inbar *et al.*, 1998); BTH and 3-aminobutyric acid (INA) were able to induce resistance in cotton against *Verticillium* wilt and *Alternaria* leaf spot diseases (Colson-Hanks *et al.*, 2000). Treatments that reduced the incidence of root infection also increased fresh and dry weight of shoots and roots. These observations are similar with the report of Ali *et al.*, (2000) where Bion reduced root infection of *Pinus radiata*, *Banksia integrifolia* and *Isopogon cuneatus* caused by *Phytophthora cinnamomi* and increased plant height in all the species and dry weight in *I. cuneatus*. The results presented here show that exogenously applied Salicylic acid and Bion provided protection to chickpea plant similar to that of Benlate, which is a standard fungicide. This protection may be due to induction of a set of plant defence reactions that culminate in the creation of a toxic environment adversely affecting the pathogen and causing fungal growth inhibition. These observations are in consistence with the proposed role of Salicylic acid and Bion as active signaling molecules and shed more light on the potential of induced resistance as a valuable alternative means of disease control.

References

- Ali, Z., I. Smith and D.I. Guest. 2000. Combinations of Potassium Phosphonate and Bion (acibenzolar-S-methyl) reduce root infection and dieback of *Pinus radiata*, *Banksia integrifolia* and *Isopogon cuneatus* caused by *Phytophthora cinnamomi*. *Australas. Plant Pathol.*, 29: 59-63.
- Bashir, N., M.I. Hashmi and F.F. Jamil. 1997. Induction of systemic acquired resistance by Oxalic acid in chickpea (*Cicer arietinum*) against *Ascochyta rabiei*. *Pak. J. Phytopathol.*, 9: 18-20.
- Benhamou, N., P.J. Lafontaine and M. Nicole. 1994. Induction of systemic resistance to *Fusarium* crown and root rot in tomato plants by seed treatment with chitosan. *Phytopathol.*, 84: 1432-1444.
- Colson-Hanks, E.S., S.J., Allen and B.J. Deverall. 2000. Effect of 2,6-dichloroisonicotinic acid or benzothiadiazole on *Alternaria* leaf spot, bacterial blight and *Verticillium* wilt in cotton under field conditions. *Australas. Plant Pathol.*, 29: 170-177.
- Dixon, R.A. and C.J. Lamb. 1990. Molecular communication in interaction between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 41: 339-367.
- Dong, H. and Y. Cohen. 2002. Induced resistance in cotton seedlings against *Fusarium* wilt by dried biomass of *Penicillium chrysogenum* and its water extract. *Phytoparastica*, 30: 1-11
- Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*, 72: 151-153.
- Gaffney, T., L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Ward, H. Kessmann and J. Ryals. 1993. Requirement of salicylic acid for the induction of systemic induced resistance. *Science* (Paris), 261: 754-756.
- Hadwiger, L.A., C. Chiang, S. Victory and D. Horovitz. 1988. The molecular biology of chitosan in plant-pathogen interactions and its application to agriculture. In: *Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications*. (Eds.): G. Skjak, B.T. anthonsen and P. Sandford. Elsevier Applied Sciences, Amsterdam. PP 119-138.
- Hanif, M., F.F. Jamil and I. Haq. 1999. Effect of various sowing depths on wilt incidence of chickpea in a wilt-sick field in Pakistan. *Intl. Chickpea and Pigeonpea News Letter*, 6: 11.
- Haq, I. and F.F. Jamil. 1995. Comparison of vascular discoloration and growth of *Fusarium oxysporum* in various segments of chickpea grown in wilt sick plot in Faisalabad, Pakistan. *Intl. Chickpea and Pigeonpea News Letter*, 2: 30-32.
- Inbar, M., H. Doostdar, K.M. Sonoda, G.L. Leibe and R.T. Mayer. 1998. Elicitors of plant defensive systems reduced insect densities and disease incidents. *Journal of Chemical Ecology*, 24: 135-149.

- Jarvis, W.R. 1988. *Fusarium* crown and root rot of tomatoes. *Phytoprotection*, 69: 49-64.
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Protection Research*, 8: 114-125.
- Lamb, C.J., M.A. Lawton, M. Dron and R.A. Dixon. 1989. Signals and transduction mechanisms for activation of plant defense against microbial attack. *Cell*, 56: 215-224.
- Ryan, C.A. and E.F. Farmer. 1991. Oligosaccharide signals in plants: A current assessment. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 42: 651-674.
- Sequeira, L. 1990. Induced resistance: Physiology and biochemistry. In: *Alternatives for Suppression Agricultural Pests and Diseases*. (Ed.): Alan R. Liss. New York. pp. 663-678.
- Scheel, D., K.D. Hauff, W. Jahnen and K. Hahlbrock. 1986. Stimulation of phytoalexin formation in fungus-infected plants and elicitor-treated cell culture of parsley. In: *Recognition in Microb-Plant Symbiotic and Pathogen Interactions*. (Ed.): B. Lugtenberg. Springer-Verlag, Berlin. pp. 325-331.

(Received for publication 29 January 2005)