JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 40, No. 1 2000

PROTECTION OF PLANTS AGAINST VIRUSES BY BENZOTHIADIAZOLE

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Abstract. Inoculation of tobacco cv. Xanthi nc or bean plants with the mixtures of benzothiadiazole (Bion) and tobacco mosaic tobamovirus (TMV) or alfalfa mosaic virus (AlMV), respectively did not show any inhibition of the number and size of the local lesions. Protective treatment of plants with Bion caused a significant decrease in disease incidence. In the case of tobacco cv. Xanthi nc and TMV or bean plants and AlMV that protective effect increased day by day and 6-7 days after treatment the production of local lesions was inhibited almost completely. Bean plants treated with Bion demonstrated resistance ranging between 60-90% also in nontreated parts. Bean and tomato plants pretreated with 0.01% Bion were effectively (in 60-70%) protected against systemic infection by tomato black ring nepovirus (TBRV).

Key words: tobacco mosaic tobamovirus, alfalfa mosaic virus, tomato black ring nepovirus, tobacco, bean, tomato, benzothiadiazole, Bion, protection, induction of resistance

I. INTRODUCTION

Plants possess various defense mechanisms against pathogens including constitutive and inducible ones. Recent finding that benzo-(1,2,3)-thiadiazo-7-carbothioic acid S-methyl ester (benzothiadiazole, BTH), the plant defense activator induces systemic acquired resistance (SAR) has attracted a great deal of interest of this form of resistance for plant disease controlling. Benzothiadiazoles proved very effective in wheat, tomato and *Arabidopsis thaliana* (Dommes et al. 1998; Görlach et al. 1996; Maćkowiak and Pospieszny 1999). We are exploring the possibility of application of benzothiadiazole for plant protection against viral diseases.

II. MATERIALS AND METHODS

Most of experiments were performed using mosaic tobamovirus (TMV) and alfalfa mosaic virus (AlMV), both causing the local necrotic lesions in tobacco cv. Xanthi nc. and bean plants, respectively. We have studied an effect of Bion, a commercial form of benzothiadiazole on the systemic viral infection in Tobacco cv. Samsun and TMV or bean, tomato plants and tomato black ring nepovirus (TBRV) as a model systems. Plants were cultivated in the standard soil, under greenhouse conditions at 20-25°C. Young plants were treated protectively with a range concentration of Bion (Novartis) and at various intervals between treatment and virus inoculation. The compound in granulated form was suspended in water and sprayed onto plants until run-off. Induction of SAR was monitored by challenge of treated plants with viruses. Plants were infected mechanically, using carborundum as an abrasive. The presence of viruses in systemic infection of plants was checked by an ELISA test.

III. RESULTS AND DISCUSSION

Experiments were performed in conditions increasing the possibility of the infection: i) a high concentration of the virus in inoculum, ii) plants were infected using carborundum which increases the possibility of the infection, iii) in the case of viruses causing systemic infections two leaves were inoculated, iv) plants were treated with Bion once only.

For the purpose of the determination of the direct effect of Bion on infectivity of viruses and first steps of viral infection, plants of tobacco cv. Xanthi nc or bean were inoculated with respective mixture of TMV or AlMV and 0.01% Bion. In both cases no inhibition in the number and diameters of necrotic lesions was observed (Tab. 1).

Table 1

Direct effect of Bion on viral infectivity

Treatment[1)]	Average number of lesions per half leaf	Inhibition of lesions production
Control, TMV in water	85.5	
TMV in 0.01% Bion	88.0	0
Control, AlMV in water	105.5	
AlMV in 0.01% Bion	109.5	0

[1)] Viruses were kept for 30 min. in water (control) or 0.01% Bion before inoculations
0 no inhibition.

On the other hand a protective treatment of plants caused suppressing symptoms evoked by viruses. Using various models of plant – virus it was clearly shown that the most effective concentration of Bion was 0.01%. At this concentration induction of SAR was high and did not cause phytotoxic side effects. When concentration of benzothiadiazole was higher induction of SAR increased but plants showed symptoms of damages, probably due to chemical shock. Lower concentrations of the preparation were often much less effective. It was shown, applying two models i.e. tobacco cv. Xanthi nc – TMV and bean – AlMV that protective effect increased day by day and 7 days after treatment reduction of the production of necrotic lesions caused by both viruses reached 100%. It was also a long lasting effect (Tabs. 2, 3).

Table 2

Effect of Bion on the production of local lesions by Alfalfa mosaic virus (AlMV) on bean plants

Treatment	Interval between treatment and virus inoculation (days)	Average number of lesions	Inhibition of lesions production (%)
Control		115.8	
0.01% Bion	2	69.8	39.7
Control		68.1	
0.01% Bion	3	27.4	59.8
Control		69.6	
0.01% Bion	4	17.8	74.4
Control		66.6	
0.01% Bion	5	5.2	92.8
Control		126.1	
0.01% Bion	7	4.4	96.5

Table 3

Effect of Bion on the production of local lesions by tobacco mosaic virus (TMV) on tobacco cv. Xanthi nc

Treatment	Interval between treatment and virus inoculation (days)	Average number of lesions	Inhibition of lesions production (%)
Control		93.0	
0.01% Bion	3	51.2	45
Control		62.7	
0.01% Bion	7	1.1	98.2
Control		83.5	
0.01% Bion	10	1.2	98.6
Control		59.9	
0.01% Bion	13	1.1	98.8

In the case of tobacco and TMV three days after treatment with Bion SAR was manifested by the 75% reduction of the number of local lesions as well as their diameters. Bean – AlMV model showed SAR not only on first leaves but also second ones where reduction of the number of local necrosis ranged between 60-90%.

Serological test ELISA indicated that ca. 85% of tobacco plants cv. Samsun infected with TMV in the concentration of 1 mg/ml after treatment with 0.01% Bion were set free from virus.

In the case of TBRV that cause the systemic infection of bean and tomato, the lowest concentration, causing 100% infection in control was applied. In this conditions ca. 60 and 70% of bean and tomato plants respectively, treated with Bion were virus – free (Tab. 4). Symptoms of disease were less intensive also in infected plants. When concentration of the virus was that low that only 50% of the control infected 100% of Bion treated plants were virus – free.

Table 4

Effect of Bion on the systemic infection of tomato and bean plants by tomato black ring nepovirus (TBRV)

Plant	Treatment[1)]	Number of plants infected/inoculated	Percentage of healthy plants
Bean	Control	18/18	0.0
	0.01% Bion	6/18	66.7
Tomato	Control	14/16	6.6
	0.01% Bion	6/16	61.5

[1)] Plants were treated once with 0.01% Bion 7 days before TBRV inoculation.

Table 5

Temperature effect vs. Bion on the local lesions production caused by alfalfa mosaic virus (AlMV)

Temperature after treatment	Average number of lesions	Inhibition of lesions production (%)
Control at 24-28°C	62.7	
0.01% Bion at 24-28°C	1.1	98.2
Control, 17h at 40°C followed by 24-28°C	85.7	
0.01% Bion, 17h at 40°C[1)] followed by 24-28°C	2.2	98.5

[1)] Bean plants were kept for 17h at 40°C followed by treatment by Bion.

At the summer the temperature in greenhouse is over 30°C. We also checked an effect of the temperature on the level of SAR. Plants kept at 40°C after Bion treatment did not show inhibition of SAR development (Tab. 5).

IV. CONCLUSIONS

These results show that plants tested are responsive to benzothiadiazole in developing of systemic acquired resistance to various mechanically transmitted viruses.

Application of Bion for protection of plants against viral diseases needs definition of its influence on viruses transmitted by vectors, especially aphids, the most important ones.

V. LITERATURE

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STRESZCZENIE

Badano możliwość zastosowania benzothiadiazolu (Bion) do ograniczania infekcji wirusowych przenoszonych na drodze mechanicznej. Bion dodany do inokulum przed inokulacją nie inhibitował ani liczby, ani średnicy plam nekrotycznych powodowanych przez wirus mozaiki tytoniu (TMV) i wirus mozaiki lucerny (AlMV) odpowiednio na tytoniu odmiany Xanthi nc oraz fasoli. Potwierdza to fakt , że Bion bezpośrednio nie oddziałuje na patogeny, w tym na wirusy. Prewencyjne zastosowanie Bionu powodowało znaczne ograniczenie infekcji wirusowych zarówno lokalnych jak i systemicznych. W przypadku tytoniu odmiany Xanthi nc i fasoli odporność indukowana przez Bion, przejawiająca się inhibicją tworzenia plam przez wirus, narastała z dnia na dzień, aby po 6-7 dniach osiągnąć maksymalny poziom. Efekt ten utrzymywał się przez dłuższy czas (co najmniej kilkanaście dni). Bion indukuje odporność, nie tylko w traktowanych częściach rośliny, ale także poza nimi, systemicznie, która na fasoli wyrażała się 60-90% redukcją liczby plam nekrotycznych. Około 60% roślin fasoli i 70% pomidora traktowanych Bionem było wolnych od systemicznej infekcji wirusem czarnej pierścieniowej plamistości pomidora (TBRV). Powyższe wyniki świadczą o tym, że odporność wzbudzona przez Bion w roślinie jest skierowana także przeciwko wirusom. Dla praktycznego zastosowania bardzo istotne jest określenie w jakim stopniu aktywność Bionu przenosi się na infekcje roślin wywołane przez wirusy, które są przenoszone przez wektory, głównie mszyce będące podstawowym sposobem rozprzestrzeniania się wirusów w naturze.