SYSTEMIC ACQUIRED RESISTANCE IN COTTON GENOTYPES AGAINST VASCULAR WILT DISEASE CAUSED BY *FUSARIUM OXYSPORUM* F.SP. *VASINFECTUM*

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Abstract

Systemic acquired resistance in cotton plants against infection with *Fusarium oxysporum* f.sp. *vasinfectum* (Atk) snyd. And Hank), (FOV) was expressed when cotton seeds of genotype Giza 80 X Australian genotype 19199 were soaked in solutions of various resistance elicitors (REs). All REs, Ascorbic acid (AA), benzoic acid (BA), benzothiadiazole (BTH), citric acid (CA), cinnamic acid (CiA), hydroquinone (HQ), salicylic acid (SA), sodium citrate (NaC), sodium metbisulfate (NaMS) and thiourea (THU) induced resistance that increased with increasing REs concentrations. BTH was the most efficient elicitor in inducing resistance in cotton plants. Efficiency of BTH was highly affected by the concentration and by cotton genotype. The most susceptible cotton genotype Giza 80 X Australian genotype 19199 showed the highest response to the application of BTH at all concentrations. A significant positive correlation was observed between susceptibility of cotton genotypes to FOV infection and efficiency of BTH in inducing resistance. In other words, susceptibility of the genotype positively correlated with the magnitude of response to the elicitor. Thus, infection accounted for 73 and 64 % of the total variation in BTH efficiency when it was applied at 50 and 100 ppm, respectively.

Key words: *Gossypium barbadense*, Ascorbic acid, Benzothiadiazole, Citric acid, Cinnamic acid, Salicylic acid

INTRODUCTION

The Egyptian race (race3) of *Fusarium oxysporum* f.sp. *vasinfectum* (Atk) snyd. and Hans, the causal agent of Fusarium wilt, has long been known in the Nile valley, where it remains as one of the most damaging pathogens to *Gossypium barbadense* cultivars (Watkins, 1981). This race caused serious losses in the commercial Egyptian cottons (*G. barbadense*) in the late 1950's (Bakry *et al.*, 1958). Since then, an extensive cotton-breeding program has been initiated to develop cultivars resistant to the disease. In this program, genotypes which showed high level of Fusarium wilt-resistance are used for final selection. However, breeding materials may include some genotypes, which are susceptible to *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) with desirable agronomic or technological traits. Such genotypes

are usually excluded from breeding programs, however; desirable characters are incorporated through back crossing. These genotypes could be used commercially if their seeds are treated with anti-oxidants to enhance their resistance to FOV. These compounds are inexpensive and environmentally safe (Elad, 1992 and Galal and Abdou, 1996). They may also help extend the durability of resistance to FOV in cotton cultivars with genes for resistance to specific FOV races (Romero *et al.*, 1998). Currently, a cibenzolar-*S methyl*, a benzothiadiazole (BTH) is released in Europe as Bion (Syngenta Ltd., Basel, Switzerland), and in the United States as Actigard (Syngenta Crop Protection Inc., Greensboro, North Carolina). It complies with the definition of a systemic acquired resistance (SAR) inducers as it leads to the expression of the same molecular and biochemical markers as biological inducers (*e.g.*, pathogenesis related proteins) and does not have direct antimicrobial activity (Kessmann *et al.*, 1994). BTH has been reported to induce resistance to fungal infections in wheat (Görlach *et al.*, 1996 and Morris *et al.*, 1998), bean (Siegrist *et al.*, 1997) and sunflower (Ismail *et al.*, 2006).

The main objective of the present study was to determine whether Fusarium wilt-resistance in cotton could be enhanced by the application of antioxidants as seed treatment.

MATERIALS AND METHODS

Pathogen:

Mixture of 35 isolates of FOV collected from various cotton producing areas of Egypt was used for soil infestation throughout this study. The experiments were carried out in the cotton pathology lab. and greenhouse, Plant Pathol. Res. Inst., Agric. Res. Center, Giza. Such a mixture represents the quantitative differences in virulence of various fungal populations of race 3.

Effect of certain resistance elicitors on cotton seed germination:

Nine antioxidant compounds, *i.e.*, ascorbic acid ($C_6H_8O_6$), benzoic acid (C_6H_5COOH), citric acid ($C_6H_8O_7.H_2O$), cinnamic acid ($C_6H_5CH=CHCO_2H$), hydroquinone (HQ), sodium citrate ($C_6H_5Na_3O_7.2H_2o$), sodium metabisulphate ($Na_2S_2O_2$), thiourea ($CH4N_2S$), salicylic acid ($C_7H_6O_3$) and benzothiodiazole (benzo 1,2,3 thiadiazole-7-carbothionic acid-S-methyl ester) were used. Tested compounds were dissolved in deionized distilled water (DDW) at two concentrations 50 and 100ppm and DDW served as control. Seeds of the cotton genotype Giza 80 X Australian genotype 19199 were delinted using 1.0% sulfuric acid then washed thoroughly with DDW and blotted dry immediately before soaking in the test solution. Seeds were soaked in the test

solutions separately for 12 hrs before planting. Treated seeds were distributed onto sterilized filter papers saturated with sterilized distilled water in sterilized Petri plates at the rate of 10 seeds per plate and were incubated at 25°C for 10 days. Germination % was measured as described by Strandberg and White (1989). Three plates were used per treatment and the experiment was repeated twice.

Effect of elicitors on growth of *FOV*:

Each elicitor was prepared in 50 ml of autoclaved distilled water, then added to a nutrient agar medium before solidifying to obtain the proposed concentrations (50 and 100 ppm), shaked gently, then poured into sterilized Petri dishes. Petri dishes were individually inoculated with one disk (5-mm diam.) of 10-day-old cultures of FOV and incubated at 27°C for 14 days. Three replicates were used for each treatment and the linear growth (mm) was recorded. For mycelial dry weight, concentrations of elicitor compounds (*i.e.* 50 and 100 ppm) were prepared in 50 ml of autoclaved nutrient broth liquid medium. Flasks were individually inoculated with one disk (5-mm diam) of 10-day-old cultures of FOV and incubated at 27°C for 14 days. Three flasks were used for each treatment. The mycelium was separated on filter paper Whatman No.1, dried at 70°C for 24 h., then the dry weight was recorded.

Efficiency of antioxidants in inducing resistance to Fusarium wilt:

Antioxidant compounds were dissolved in distilled water individually to obtain solutions of 50 and 100 ppm for seed soaking treatment.

The inoculum used in the present study was a mixture of equal weights of each of 35 isolates of FOV, according to the protocol of inoculation followed for screening Fusarium wilt resistance in the Cotton Pathology Dept., Plant Pathol. Res. Inst., ARC, Giza. Autoclaved clay loam soil was infested with the mixture of isolates at the rate of 10g/kg of soil. Substrate for growth of each isolate was prepared in 500 ml glass bottles, each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of bottle were autoclaved for 30 minutes. Isolate inoculum, taken from one week old culture on PDA was aseptically introduced into the bottle and allowed to colonize sorghum grains for 3 weeks. Infested soil was dispensed in 10 cm diameter clay pots and these were planted with 10 seeds per pot, with five replications (pots) for each genotype. Pots were distributed on greenhouse benches in a randomized complete block design of 5 replicates. The greenhouse was equiped with a heating system assuring that the minimum temperature in the greenhouse was maintained at 28 °C, however, due to the lack of a cooling system, the maximum temperature fluctuated from 30 to 35C depending on the prevailing temperature during the day.

Healthy seeds of cotton genotype Giza 80 X Australian genotype 19199 were delinted and surface disinfected as mentioned before, then washed thoroughly three times with sterile distilled water and soaked in the tested solutions for 12 h. Control seeds were soaked in distilled water.

Efficiency of BTH in inducing systemic resistance in different cotton genotypes:

Seeds of eight cotton genotypes susceptible to Fusarium wilt were treated with BTH at the concentrations of 50 and 100 ppm. These seeds were delinted as mentioned before then soaked in BTH solutions for 12 h before planting in soils infested with FOV. Disease incidence was recorded 45 days after planting.

Statistical analysis

Analysis of variance (ANOVA) of the data was performed with MSTAT-C statistical package (A Microcomputer program for the Design, Management and Analysis of Agronomic Research Experiments, Michiagan State Unvi., USA). Least significant difference (LSD) and standard error for means (SEM) were used to compare treatment means.

RESULTS AND DISCUSSION

Effect of elicitors on cotton seed germination:

Data summarized in Table (1) show that the tested chemicals (resistance elicitors) had no inhibitory effects on seed germination of the Fusarium wilt susceptible cotton genotype Giza 80 X Australian genotype 19199. Similar results were reported as to the effect of elicitors on sunflower seed germination (Ismail *et al.*, 2006).

***In vitro* effect of elicitors on growth of FOV:**

It is well established that the chemicals shown in Table (2) are resistance inducers. Therefore, it is assumed that they do not have direct antifungal activity (Kessmann *et. al.*, 1994). However, some of these chemicals showed a slight antifungal activity as they very slightly reduced mycelium dry weight and linear growth. They included benzothiadiazole, cinnamic acid and thiourea. Therefore, we can not completely rule out the possibility that these chemicals reduce infection with FOV, at a very low magnitude, low magnitude inhibitory effects on fungal growth. Data in Table (3) indicated that effect of elicitors on dry weight was not related to their effect on linear growth as the linear correlation coefficient are nonsignificant.

Table 1. Effect of some elicitors on seed germination of cotton genotype Giza 80 X Australian genotype 19199.

Chemicals	Conc. (ppm)	% Germination a
Ascorbic acid (AA)	50	85 ± 6 b
	100	80 ± 5
Benzoic acid (BA)	50	84 ± 6
	100	82 ± 4
Benzothiadiazole (BTH)	50	83 ± 4
	100	82 ± 4
Citric acid (CA)	50	83 ± 6
	100	85 ± 5
Cinnamic acid (CiA)	50	85 ± 4
	100	80 ± 3
Hydroquinone b(HQ)	50	85 ± 4
	100	80 ± 3
Salicylic acid (SA)	50	84 ± 2
	100	82 ± 5
Sodium citrate (NaC)	50	85 ± 4
	100	86 ± 4
Sodium metabisulfate (NaMS)	50	83 ± 3
	100	82 ± 2
Thiourea (THU)	50	81 ± 4
	100	84 ± 3
Control	0.0	86 ± 3

[a] Data are means of 2 experiments (each treatment included 3 replicates, each replicate (plate) contained 10 seeds.

[b] ± Standard error of means.

Table 2. *In vitro* effect of some elicitors on mycelial dry weight and linear growth of FOV.

Chemicals	Conc. (ppm)	Mycelial dry weight (mg / 50 ml liquid medium)[a]	Linear growth (mm)
Ascorbic acid (AA)	50	250 ± 12 b	82 ± 2
	100	246 ± 8	80 ± 4
Benzoic acid (BA)	50	240 ± 10	82 ± 3
	100	240 ± 12	80 ± 4
Benzothiadiazole (BTH)	50	254 ± 8	78 ± 4
	100	250 ± 10	78 ± 5
Citric acid	50	246 ± 8	82 ± 3
	100	245 ± 6	80 ± 4
Cinnamic acid (CiA)	50	240 ± 12	78 ± 5
	100	238 ± 10	78 ± 4
Hydroquinone (HQ)	50	250 ± 6	78 ± 4
	100	250 ± 8	78 ± 6
Salicylic acid (SA)	50	254 ± 4	80 ± 4
	100	250 ± 5	78 ± 4
Sodium citrate (NaC)	50	262 ± 8	82 ± 2
	100	258 ± 4	83 ± 2
Sodium metabisulfate (NaMS)	50	264 ± 8	80 ± 4
	100	262 ± 8	80 ± 4
Thiourea (THU)	50	245 ± 12	78 ± 5
	100	240 ± 10	78 ± 5
Control	0.0	260 ± 8	85 ± 0.0

[a] Data are means of 2 experiments (each included 3replicates, (plates or flasks).

[b] ± Standard error of means.

Table 3. Relationship between mycelium dry weight and linear growth of FOV when elicitors are added to growth medium at the concentrations of 50 and 100 ppm.

Elicitors concentration (ppm)	Linear correlation coefficient [a]
50	0.129 (N.S.)
100	0.377 (N.S.)

[a] Linear correlation coefficients are nonsignificant (N.S.).

Induction of resistance in cotton plants by different elicitors:

Pathogenic variability within FOV is well documented in the literature (Bakry *et al.*, 1958). In the present study, the tested genotypes were screened against a mixture of 35 different isolates collected from almost all cotton-growing areas in Egypt. The use of such a large number of isolates assures that resistant genotypes under greenhouse conditions will maintain their resistance under field conditions at as many locations as possible. On the contrary, if the genotypes were screened against a limited number of isolates, they may not perform as expected under many field conditions probably due to the presence of isolates differing in virulence from those used in the greenhouse test. In general, resistance to Fusarium wilt was enhanced when seeds were soaked in elicitors solutions at the concentrations of 50 and 100 ppm (Table4).

The efficiency of all elicitors except NaMS in enhancing resistance, was higher when the concentration was increased from 50 to 100 ppm. Efficiency of NaMS was not affected by concentration. BTH showed the highest level of efficiency regardless of the concentration. Ascorbic acid and salicylic acid showed higher efficiency when their concentrations were increased to 100 ppm. Other elicitors showed variable levels of efficiencies within each concentration. These results are consistent with the previous reports, relevant to the role of BTH and SA in inducing resistance to plant pathogens (Galal and Abdou, 1996, Morris *et al.*, 1998, Siegrist *et al.*, 1997, Kohler *et al.*, 2002 and Ismail *et al.*, 2006).

Table 4. Effect of seed soaking in solutions of some elicitors on susceptibility of cotton genotype Giza 80 X Australian genotype 19199 to FOV.

Chemicals	Conc. (ppm)	Infection (%)	Efficiency [a] (%)
Ascorbic acid (AA)	50	57.85	34.56
	100	51.86	41.33
Benzoic acid (BA)	50	84.27	4.67
	100	78.29	11.44
Benzothiadiazole (BTH)	50	46.33	47.6
	100	42.08	52.4
Citric acid (CA)	50	82.78	6.36
	100	78.68	11.00
Cinnamic acid (CiA)	50	77.30	12.56
	100	70.81	19.90
Hydroquinone (HQ)	50	83.73	5.23
	100	75.30	14.82
Salicylic acid (SA)	50	64.83	26.67
	100	49.87	43.59
Sodium citrate (NaC)	50	80.28	9.17
	100	76.30	13.69
Sodium metabisulfate (NaMS)	50	82.78	6.36
	100	82.78	6.36
Thiourea (THU)	50	64.33	27.23
	100	56.55	35.70
Control	0.0	88.40	

[a] Efficiency in inducing resistance was calculated according to the following formula $\frac{IC - IT}{IC} X100$

where IC is the infection in the control and IT is the infection in the treatment.

Effect of seed soaking in BTH on susceptibility of cotton genotypes to FOV:

Different cotton genotypes were studied as to their response to treatment with BTH with respect to infection with FOV. Due to the significance of concentration X genotype interaction Table (5), an interaction least significant difference (LSD) was calculated to compare between concentrations within each genotype. These comparisons showed that when BTH was applied at the lower concentration of 50 ppm , it significantly reduced infection in only four genotypes (Giza 90 X Pima 562, Giza 83 X Pima 56 X Dendara, Giza 91 X Pima 562.

Table 5. Effect of seed soaking in solutions of benzothiadiazole (BTH) on susceptibility of different cotton genotypes to FOV.

Cotton genotypes	Conc. (ppm) of BTH	Infection (%)	Efficiency [a] (%)
Giza 86 X Pima 56	0.0	75.90	-
	50	58.90	22.4
	100	47.50	37.4
Giza 89 X Pima 56	0.0	58.33	0.0
	50	47.83	18.00
	100	41.73	28.37
Giza 83 X Pima 562	0.0	71.10	0.0
	50	51.80	27.14
	100	46.70	34.31
Giza 90 X Pima 562	0.0	77.13	0.0
	50	55.20	23.4
	100	53.13	31.11
Giza 83 X Pima 56 X Dendara	0.0	82.50	0.0
	50	55.50	32.72
	100	52.20	36.70
Giza 91 X Pima 562	0.0	84.90	0.0
	50	57.13	32.70
	100	50.73	40.20
Giza 80 X Australian genotype 19199	0.0	90.45	0.0
	50	51.27	43.32
	100	42.80	53.37
Australian genotype 19199	0.0	80.04	0.0
	50	60.09	25.92
	100	52.05	34.97

LSD at 0.05 for concentration (A) = 7.75, genotypes (B) = 10.17 and interaction A x B = 21.43.

[a] Efficiency in inducing resistance was calculated according to the following formula $\frac{IC - IT}{IC} X 100$

where IC is the infection in control and IT is the infection in treatment.

and Giza 80 X Australian genotype 19199); however, when the concentration was increased to 100 ppm, BTH significantly reduced infection in all the tested genotypes except Giza 89 X Pima 56. Therefore, BTH was effective in inducing resistance to FOV in more genotypes when it was applied at 100 ppm. Of the tested genotypes, Giza 89 X Pima 56 failed to respond to BTH regardless of the applied concentration, while Giza 80 X Australian genotype 19199 showed the highest response regardless of concentration. The other genotypes showed variable responses between these two extremes.

These results indicated that systemic acquired resistance (SAR) to FOV was highly affected by cotton genotype. Ismail *et al.* (2006) reported similar results on studying SAR of sunflower against *Sclerotium rolfsii.*

Since it is well established that SA is an endogenous signal for the activation of SAR (Durner *et al.*, 1997), there has been increasing characterization of synthetic chemicals that are able to mimic SA. In many plants, enhancing disease resistance is frequently accompanied by the activation of genes encoding pathogenesis-related proteins (PRPs) (van Loon and van Strien, 1999). Because some of these proteins display antimicrobial activity, their accumulation has been assumed to contribute to SAR. Regarding the mode of action of BTH, this elicitor does not cause the biosynthesis of SA but triggered the same set of SAR genes such as those triggered by SA (Görlach *et al.*, 1996 and Lawton *et al.*, 1996). A significant positive correlation was observed between susceptibility of cotton genotypes to FOV infection and response to BTH in inducing resistance (Fig.1). This correlation indicates that the higher the susceptibility of genotypes the greater the response to the application of BTH. Thus, infection accounted for 73 and 64 % of the total variation in BTH efficiency when it was applied at the concentration of 50 and 100 ppm, respectively. Different elicitors may behave differently in different disease complexes. It is also speculated that genes triggered by the elicitors and consequently the products of these genes may differ from one disease to another, depending on the factors involved in the pathogenesis process such factors may vary for being toxins, enzymes, biochemicals, ...etc.

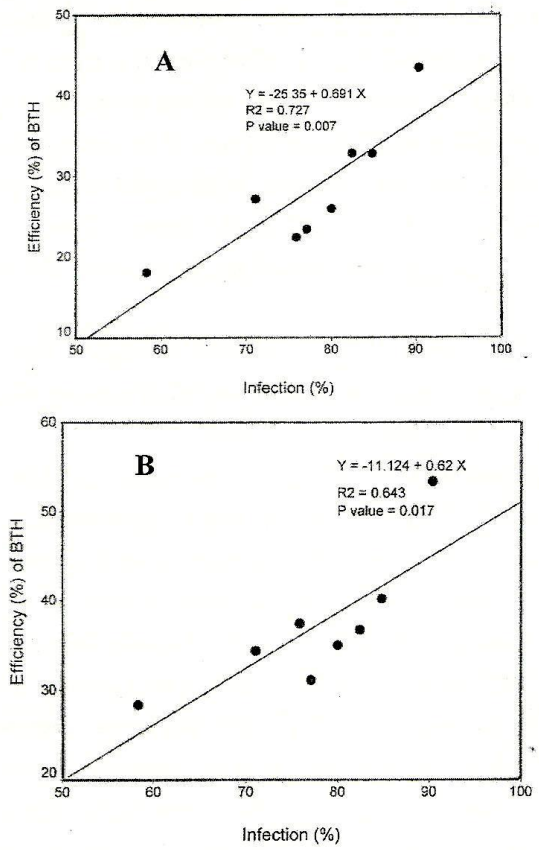

Fig. 1. Regression equations that describe the effect of susceptibility of cotton genotypes to FOV (infection) and efficiency of BTH in inducing resistance when it was applied at 50 (A) or 100 ppm (B).

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المقاومة الجهازية المكتسبة في نباتات القطن ضد مرض الذبول الوعائي الناجم عن الإصابة بالفطر *Fusarium oxysporum* f. sp. *vasinfectum*

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أمكن التعبير عن صفة المقاومة الجهازية المكتسبة في نباتات القطن ضد الإصابة بفطر الذبول الوعائي *Fusarium oxysporum* f. sp. *vasinfectum* و ذلك عند نقع بذور قطن التركيب الوراثي جيزة ٨٠ × التركيب الوراثي استرالي ١٩١٩٩ في محاليل مختلفة من محفزات المقاومة مثل حمض الأسكوربيك وحمض البنزويك والبنزوثيادايازول وحمض الستريك وحمض السينّاميك والهيدروكينون وحمض السالسيليك وسترات الصوديوم وميتابايسلفات الصوديوم والثيويوريا . كان المركب بنزوثيادايازول (منتج تجارى تحت اسم بيون في أوروبا وتحت اسم اكتيجارد فى أمريكا) هو الأكفأ في إكساب نباتات القطن صفة المقاومة ضد الذبول وقد اختلفت فعالية هذا المركب باختلاف كل من التركيز والتركيب الوراثي المختبر. أظهر التركيب الوراثي جيزة ٨٠ × التركيب الوراثي استرالي ١٩١٩٩ أعلى استجابة لهذا المركب بصرف النظر عن التركيز المستعمل والجدير بالذكر أن هذا التركيب الوراثي أظهر أيضاً أعلى درجات القابلية للإصابة بالمرض قبل المعاملة . لوحظ ارتباط موجب معنوي بين قابلية التراكيب الوراثية للإصابة بالمرض ودرجة الاستجابة لتأثير BTH في استحثاث مقاومة هذه التراكيب الوراثية ومن ثم فإن القابلية للإصابة فسرت ٧٣ و ٦٤ % من التباين الكلي في الكفاءة عند استعمال مركبBTH بتركيز ٥٠ و ١٠٠ جزء في المليون على التوالي.