INDUCTION OF RESISTANCE IN FABA BEAN AGAINST CHOCOLATE SPOT AND RUST USING ETHEPHON SEED TREATMENT

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Abstract

Ethephon treatment proved efficient in inducing resistance of faba bean plants against both chocolate spot (*Botrytis fabae* Sard.) and rust (*Uromyces viciae fabae* (Pers.) de Bary). Seed Soaking in 800 µg/ml showed a significant reduction in severity of both diseases and a noticeable increase in yield of seeds/plant. The same treatment caused a pronounced reduction in percentage of browning and spreading of chocolate spot beneath inoculum droplets placed on the abaxial surface of detached leaves. However, treatment with 1000 µg/ml led to lower levels of both diseases. Treatments with 600 and 800 µg/ml of ethephon showed an increase in phytoalexin accumulation in leaf tissues after 24 h of elicitation by each of *B. fabae* or *B. cinerea*.

INTRODUCTION

Fruit ripening and plant senescence are all subject to ethylene treatment (Abeles 1973). Stress factors, including pathogen, attack, increases the biosynthesis of ethylene (Lieberman 1979). It has been reported that ethylene enhances the synthesis and activity of different enzymes involved in defence mechanism (Daly *et al.* 1970, Sheen and Diashun 1976, Boller *et al.* 1983, Haruyoma *et al.* 1988, Aly and Afifi 1989). Ethylene production has been correlated with the induction of several proteins that presumably function in defence response against fungal and bacterial attack (Toppan *et al.* 1982). Hargreaves *et al.* (1977) found phytoalexins accu-

mulation in tissues bearing limited lesions caused by *Botrytis cinerea* or *B. fabae.* Wyerone acid was the predominant phytoalexin produced by leaves, particularly during the first three days after inoculation. Ethylene treatment may induce more phytoalexin accumulation. Induction of phytoalexin by ethylene was reported by Chaultze and Stahmann (1969). On the other hand, Paradies *et al.* (1980) reported that ethylene does not appear to function as elicitor recognition signal for the induction of phytoalexin. Induction of resistance in barley plants against powdery mildew was reported by Aly and Afifi (1989). In faba bean, Aly (1989) found ethephon treatments (800 and 1000 μg/ml) were effective against chocolate spot and Stemphylium blight in detached leaves and whole plant tests.

The present work was designed to test the effect of ethephon treatment in inducing resistance in faba bean grown under field conditions to some fungal diseases and its effect on yield. The effect of this treatment on phytoalexin accumulation in relation to induction of disease resistance was also investigated.

MATERIALS AND METHODS

The field experiment was carried out at Bahtim Agricultural Research Station during 1989-1990 growing season.

Seeds of faba bean (Giza 3 Cv.) were soaked, before sowing, in solutions of 600, 800 and 1000 μg/ml ethephon (2-chloroethylphosphonic acid) for 20 hours. Soaking in the same volume of tap water was carried out and served as control. In addition, a fungicidal treatment with Dithane M 45 (0.3 %) was used. Three applications were carried out at the middle of January, the 1st and mid February 1990.

Sowing was carried out in rows, each of 4 m length. Seeds were drilled in doubles, 30 cm apart. Four replicates were specified for each treatment, each consisted of 3 rows, and agricultural practices were performed as commonly practiced.

Fungal inoculation

Botrytis fabae was grown on salted faba bean extract agar medim. Spore suspension (10^5 conidia / ml.) was sprayed over the plants after sunset during the 3rd week of January. Rust was left for natural infection.

Disease assessment

Chocolate spot disease was assessed as percentage of leaf lamina affected. Average values for three leaves from the flowering nodes were calculated for each plant. Ten plants for each replicate were used. The assessment was carried out at the medium stage of growth (at mid March). As for rust disease, it was assessed at the beginning of April. The scale of Berneir *et al.* (1984). ranging from 1to 9 was followed for disease assessment.

Inoculation of detached leaves

Leaves without any signs of damage were detached from the middle of the flowering nodes. the abaxial surface of detached leaves were gently washed with distilled water and dried with tissue paper before inoculation. Leaves were placed on moistened filter paper in plastic boxes, then inoculated on their abaxial epidermis with 20 ul droplets, containing 200 conidia of *B. fabae* or 200 conidia of *B. cinerea.* Incubation was carried out at 18 $\pm$ 2^{o}C under fluorescent light. Data were recorded as browning of infection sites and spreading of lesions according to Mansfield and Deverall (1974).

Extraction and determination of wyerone acid by thin layer chromatography (TLC)

Leaf discs , 1cm, from inoculation sites and their peripheral tissues were cut 24 and 48 hours after inoculation. The leaf discs were homogenized using mortar and pestle with diethyl ether, the ether were tipped off and the debris centrifuged at 3000 rpm for 3min. The supernatent was collected and dried in a rotary evaporator at 35^{o}C . The residues were washed with 10ml absolute ethanol then evaporated till dryness. The latter residual was resuspended in absolute ethanol at the rate of 1 ml/g fresh weight of the sample for phytoalexin determination. The method of Letcher *et al.* (1970) was used for separation and determination of wyerone acid. The concentration of wyerone acid was calculated from the formula :

ug wyerone acid ml^{-1} = optical density at max (356 nm) X 9.04

Effect on growth , yield and yield component

Determination of the plant height (cm), number of branches, number of pods/ plant, number of seeds/pod, weight of 100 seeds (g) and seed yield /plants -for each replicate -were done on randomly chosen plants. Statistical analysis was car-

ried out using Tuker multiple range test according to Neler *et al.* (1985)

RESULTS AND DISCUSSION

Effect of ethephon treatment on chocolate spot and rust diseases as compared to Dithane M45:

Results in Table (1) show that ethephon treatments at 100 and 800 ug/ml significantly reduced chocolate spot severity. Treatments with 1000 and 800 ug/ml reduced disease severity by about 65% and 50% , respectively. Ethephon treatment (600 ug/ml) and Dithane M45 foliar application showed similar results and both reduced disease severity by about 30%

As for rust disease, Dithane M45 foliar application showed the highest significant reduction in disease severity. Meantime, all ethephon Treatments i.e. 600, 800 and 1000 ug/ml were less effective.

Table 1. Effect of ethephon seed soaking as compared to Dithane M45 foliar application on chocolate spot and rust diseases of faba bean.

Treatments	Disease sverity	
	Ch. spot1	Rust2
Ethephon 600 ug/ml	8.30 ab3	5.10 ab
Ethephon 800 ug/ml	6.20 b	5.35 ab
Ethephon 1000 ug/ml	4.28 b	4.80 ab
Dithane M45 (0.3 %)	8.80 ab	4.70 ab
Control	12.30 a	6.90 a

1 . Percentage of leaf lamina area affected.

2 . Rating scale ranged from (1-9) where 9 the highest susceptible .

3 . Figutes with the same letter are not significantly different.

Influence of ethephon treatments as compared to Dithane M45 foliar treatments on growth, yield and yield components of faba bean

Results in Table (2) indicated that ethephon treatment significantly reduced plant height. Treatments with 800 and 1000ug/ml showed higher reduction followed

by 600 ug/ml . Other agronomical characters i.e. numbr of branches, number of pods per plant, number of seeds per pod. weight of 100 seed and seed yield per plant showed no significant differences.

Table 2 . Influence of ethephon seed soaking and Dithane M45 foliar application treatments on growth, yield and yield components of faba bean.

Plant Treatments	height (cm)	Number of branches	Weight of pods/ plant	number of seeds/ pod	of 100 seed (g.)	yield plant (g.)
Ethephon 600 ug/ml	60.0ab	2.6a	18.7a	3.2a	61.9a	36.6a
Ethephon 800 ug/ml	57.9c	3.1a	19.0a	3.3a	63.4a	39.9a
Ethephon 1000 ug/ml	55.5c	3.0a	16.2a	3.2a	60.0a	31.3a
Dithane M45 (0.3 %0)	68.6ab	3.2a	19.6a	3.3a	59.8a	38.1a
Control	72.la	3.4a	18.1a	3.2a	57.8a	32.6a

Figures with same letter are not significantly different

Effect of ethephon treatments on percentage of browning and spreading of lesions of *Botrytis fabae* and *B. cinerea* on detached leaves

Results in Table (3) indicate that all ethephon treatments reduced both percentage of browning and spreading of lesions of *Botrytis fabae*. Treatments with 800 ug/ml showed pronounced effect, specially in reducing the percentage of spreading lesions 48h after inoculation.

Table 3 . Effect of ethephon treatments on percentage of browning and spreading of lesions of *Botrytis fabae* and *B. cinerea* on detached leaves of faba bea.

Ethephon treatment ppm	*B. fabae*				*B. cinerea*			
	24h		48h		24h		48h	
	Br.[3]	Spr.[4]	Br.	Spr.	Br.[3]	Spr.[4]	Br.	Spr.
0	72.2	57.0	96.6	87.2	17.1	0.0	23.4	15.3
600	57.0	27.4	82.0	47.6	8.0	0.0	20.9	0.0
800	47.2	28.7	77.0	28.0	11.0	0.0	20.4	0.0
1000	57.3	28.1	79.5	33.3	8.6	0.0	10.0	0.0

1. Inoculum droplets 20ul each containing 200 conidia.
2. Inoculum droplets 20ul each containing 200 conidia
3.Percentage of browning beneath the inoculum droplet.
4. Percentage of spreading lesions.

All ethephon treatment reduced percentage of lesions browning and restricted spreading of *B. cinerea* 24 and 48h after inoculation.

Effect of ethephon treatments on the elicitation of wyerone acid by *Botrytis fabae* and *B. cinerea* .

Ethephon seed treatments showed a slight effect on the accumulation of wyerone acid elicited by each of *B. fabae* or *B. cinerea*. Treatments with 600 and 800 ug/ml showed an increase in wyerone acid conentration 24h after inoculation by either of *B. fabae* or *B. cinerea*. Meanwhile, 48h after inoculation wyerone acid content showed no clear differences between tissues of treated or untreated plants (Table 4).

Similar trend of the results was shown in leaf tissues inoculated with *B. cinerea*. As for 1000 ug/ml ethephon treatment, general reduction in wyerone acid level was detected 24h and 48h after inoculation with *B. Cinerea* and 48h after inoculation with *B. fabae*.

Elthephon treatmen proved its efficiency in inducing resistance of faba bean against both chocolate spot and rust diseases. The higher and significant effect was found with chocolate spot disease, the most effective treatments was seed soaking in 800 ug/ml.

Table 4. Effect of ethephon treatment on the elicitation of wyerone acid[1] by *Botrytis fabae* and *B. cinerea.*

Ethephon treatment ug/ml	*B. fabae* [2]		*B. Cinerea*[3]	
	24 H	48 H	24 H	48 H
0	22.5	31.9	29.0	35.0
600	29.8	29.9	36.5	38.1
800	27.3	30.2	34.6	36.2
1000	24.0	28.2	24.2	28.7

1. ug/g fresh weight.
2. Inoculum droplets, 20ul each containing 200 conidia.
3. Inoculum droplets, 20ul each containing 200 conidia.

This concentration was significantly effective in controlling chocolate spot disease more than the recommended fungicidal treatment (Dithane M45) . The obtained results for chocolate spot were in complete accordance with those obtained by Aly (1989) . Our results concerning wyerone acid accumulation in response to *B. fabae* or *B. cinerea* indicated that 600 and 800 ug./ml ethephon treatment caused a slight increase, 24h after inoculation. Wyerone acid content showed negligible increase after 48 hours. These results indicate that wyerone acid did not play a principal role in restricting the infection of *B. fabae.* It could be attributed to the ability of *B. fabae* to metabolize wyerone acid into less toxic products (Mansfield and Deverall 1974). Other defence mechanism could be involved in reducing disease severity in the treated plants. It has been reported that ethylene stimulated the accumulation of hydroxyproline rich glycoprotein in the cell wall which is considered a common defence mechanism against pathogens in dicots (Toppan *et al.* 1982 and Roby *et al.* 1985). Treatment with ethylene or ethylene induced oxidative enzyme activit ies including peroxidase, PO and polyphenol oxidase PPO (Sheen and Diochun 1976, Horuyama *et al* 1988 and Aly and Afifi 1989). The increase in PO activity enhances lignification in response to infection which may restrict the penetration (Ride 1983) . On the other hand, activity of PPO and PO oxidize phenolic compound into their oxidized products. Deverall and Wood (1961) reported that oxidized phenolics inactivate pectic enzymes of *B. fabae*, such inhibition of cell wall degrading enzyme may affect the rate of coloniztion by the fungus and subsequetly tissue maceration. Recently, it was found that ethylene treatent increased chitinase activity (Roby *et al.* 1985) and gluconase activity (Mouch and stachelin 1989). Both enzymes have been implicated in defence reaction of plants against potential pathogens (Abeles *et al.* 1971 and Boller *et al.* 1983).

Generally , ethephon application minimized severity of chocolate spot and rust diseases of faba bean plants and to some extent increased seed yield.

REFERENCES

1 . Abeles, F.B. 1973. Ethylene in Plant Biology., Academic Press. New York. pp. 302.

2 . Abeles, F.B., R.P. Bosshart, L.E. Forrence, and W.E. Halbig. 1971. Preparation and purification of gluconase and chitinase from bean leaves . Plant Physiol., 47: 129-134.

3 . Aly, M.M. 1989. Induced resistance against plant disease using ethephon (2-

chloroethylphosphonic acid treatment. II : Chocolate spot and stemphylium blight of broad bean. VII. Conf. of Microbiol . Cairo, Nov. 1989. pp. 316 - 327.

4 . Aly M.M. and W.M. Afifi. 1989. Induced resistance against plant disease using ethephon (2-chloroethylphosphonic acid) treatment. I. Powdery mildew and net blotch of barley . VII. Conf. of Microbiol. Cairo , Nov. 1989, pp.. 299-315.

5 . Aly , M.M. S.A. Habib, and Soad M. Abdallah. 1988. Induced resitance in barely plants against powdery mildew disease using ethephon treatment, Proc. of 2nd Conf. of Agric. Develop., Ain Shams Univ. Vol. 3 pp. 47 - 56.

6 . Bernier, C.C., S.B. Hanounik, M.M. Hussein, and H.A. Mohamed. 1984. Field manual of common Faba bean Disease in the Nile Valley. Information Bull. No. 3, International Centre for Agricultural Research in the Dry Areas (ICARDA) p. 35.

7 . Boller, T.A. Gehri, F. Meuch, and U. Vogeli. 1983. Chitinase in bean leaves : Induction by ethylene , purificantion properties and possible function. Plants, 157: 22-31.

8 . Chaultz, E. and M.A. Stahmann. 1969. Induction of pisation by ethylene. Phytopathology, 59 : 750 - 755.

9 . Daly, J.M. P.M. Seevers and P. Ludden . 1970. Studies on wheat stem rust resistance correlated to the Sr6 locus. III - Ethylene and diseases resistance . Phytopathology, 60 : 1648 - 1652.

10 . Deverall, B.J. and R.K.S. Wood. 1961. Chocolate spot of bean (*Vician faba* L.) - interaction between phenolase of host and pectic enzyme of pathogen. Ann. Appl. Biol., 49 : 473 - 487.

11 . Hargreaves, J. A., J.W. Mansfield and S. Rossall. 1977. Changes in phytoalexin concentration in tissues of broad bean plant (*Vicia faba* L.) following inoculation with species of Botrytis, Physiological Plant Pathology, 11 : 227-242.

12 . Haruyama, T. S. Urushizaki, K. Matsumoto H.M. Kano, H. Haga and Y. Sekizawa. 1988. Dependence on ethylene in induction of phenylalanine ammonialyase , peroxidase and lipoxygenase in rice leaves induced with blast fungus. 5th International Congress of Plant Pathology , Keyoto, Japan. P. VI-2-88 (Abstr.).

13 . Letcher, R.M. D.A. Widdoswson, B.J. Deveral and J.W. Monsfied. 1970. Indentification and activity of wyerone acid as phytoalexin in Broad bean (*Vicia fabae*) after infection by *Botrytis*, Phytoschemistry, 9:249 - 252.

14 . Liberman. M. 1979. Biosynthesis and action of ethylene. Annu. Rev. Plant Physiol., 30:533 - 591.

15 . Mansfield, J. W. and B. J. Deverall 1974. The rate of fungal development and lesion formation in leaves of *Vicia faba* during infection by *Botrytis cinerea* and *B. fabae* . Ann. Appl. Biol., 76:77-89.

16 . Mouch, F. and L.A. Stachelin.1989. Functional implications of the subcellular localization of ethylene-induced chitinase and B 1-3 gluconase in bean leaves . The Plant Cell, 1: 447 - 457.

17 . Neler., J., W. Wasserman, and M.H. Kutner., 1985. Applied linear statistical models. Regression, analysis of variance and experimental design. 2 Ed . Richard D Lrwin Inc. Homewood, Illinois.

18 . Paradies, I. J.R., Kontze, E. Elsener and J. Paxton. 1980. Ethylene indicator but

not inducer of phytoalexin in soybean. Plant physiol., 66. 1105-1109.

19 . Ride, J.P. 1983. Cell walls and other structural barriers in defence . In Calloz J.A. (ed). Biochemical Plants Pathology. John Wiley and Sons. New York.

20 . Roby, D., A. Toppan and M.T. Esquerre-Tugaye 1985. Cell surfaces in Plant - Microoragnisms Interanction. V. Effect of fungal and of plant origin trigger on the synthesis of ethylene and of cell wall hydroxyproline - rich glycoprotein in plants. Plant Physiol., 77 : 700 - 704.

21 . Sheen, S.J. and S. Diachun. 1976. Relationship of polyphenoloxidase and peroxidase activity with virus symptoms in red clover. Plant Growth Regulator (Abstracts.), 4:1956.

22 . Toppan, A. D. Roby and MT. Esquerre Tugaye .1982. Cell Surface in Plant Microorganism Interanction . III: in vivo effect of ethylene on hydroxyproline rich glycoprotein accoumulation in cell wall of diseased plants. Plant Physiol., 70:82-86.

إحداث مقاومة ضد مرض التبقع البني والصدأ فى الفول البلدى بمعاملة البذور بالأثيوفون

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معاملة الفول البلدي بالأثيفون أحدثت مقاومة ضد مرض التبقع البني والصدأ. أظهرت النتائج الحقلية والمعملية ان نقع بذور الفول في تركيز ٨٠٠، ١٠٠٠ ميكروجرام / مليلتر منه سبب نقصا معنويا في شدة الإصابة بمرض التبقع البني والصدأ ، وكذلك فإن تركيز ٨٠٠ ميكروجرام / مليلتر اظهر أيضا نقصا ملحوظا في نسبة التلون وانتشار مرض التبقع البني عند استخدام طريقة الاوراق المنفصلة.

وجد أن المعاملة بتركيزات ٨٠٠، ٦٠٠ ميكروجرام /مليلتر احدثت زيادة في تراكم الفيتواليكسين (حمض الويرون) في انسجة الأوراق بعد ٢٤ ساعة من الحقن بفطري بتريتس فابي وبتريتس سنريا. ومن ناحية اخري فان معاملة بذور الفول بتركيز ١٠٠٠ ميكروجرام / مليلتر اظهرت نقضا محدودا في شدة الاصابة بمرض التبقع البني والصدأ.