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INDUCED RESISTANCE TO STEM RUST DISEASE OF WHEAT BY ANTIOXIDANT COMPOUNDS APPLICATION

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ABSTRACT

Stem rust of wheat caused by *Puccinia graminis* f.sp *tritici* is a widespread disease in Egypt attacking wheat plants. The effect of spraying four antioxidant compounds *i.e.*, salicylic acid (SA), oxalic acid (OA), ascorbic acid (AA) and citric acid (CA) were studied to evaluate their induction of resistant against *P. graminis tritici* on wheat plants during 2011- 2012 growing season at seedling and adult stages. The obtained results proved that all the tested compounds were effective in inducing resistance to stem rust disease. Oxalic acid followed by salicylic acid was the most effective at 500 ppm when applied 24 hr., before inoculating the plants. Spraying the tested inducers 72 hr., before inoculation proved to be effective in changing the infection type from susceptible to resistant response as well as reducing pustule size particularly with oxalic acid and salicylic acid at 250 and 500 ppm. However, ascorbic acid and citric acid were inferior in this respect. Also, at adult stage, oxalic acid showed the highest efficacy, followed by salicylic acid, while citric acid and ascorbic acid showed the lowest efficacy values. The values of 1000 grain weight were in the same line with disease severity. Scanning electron microscope (SEM) of Sids- 6 cv. wheat seedlings sprayed with SA, OA, AA and CA 24 hr., before inoculation showed that urdiospores of the fungus were surface shrinkage and deformation of germ tubes.

Key words: Wheat, steam rust, induced resistance, antioxidant.

INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most important seed crops to the humans all over the world. Rust diseases have been a major concern and problem for breeders, farmers and commercial seed companies (Marsalis and Goldberg, 2006). However, wheat rusts have been reported as devastating, having the ability to destroy entire susceptible wheat cvs. resulting in large economical losses (Singh *et al.*, 2005 and Kuraparthy *et al.*, 2007).

Induced resistance is the phenomenon by which the plant can utilize the own defense mechanism to increase the level of resistance without alteration plant genome (Kuc, 1982). There are two types of induced resistance, *i.e.* local acquired resistance (LAR) which can be obtained on the pretreated part of the plant, and systemic acquired resistance (SAR) which developed in the tissues distant from the site of prior including treatment (Kessmann *et al.*, 1994 and Deverall, 1995).

The use of alternative control methods of diseases can effectively replace chemical fungicides (White, 1979 and Ward *et al.*, 1991). Induced resistance by chemicals is a promising approach to prevent diseases caused by *P. graminis* f. sp. *tritici* on leaves of wheat seedling inoculated with urediospore. SA at 5 mg/ml completely inhibited the development number and size of pustules on both upper and lower leaf surfaces. Disease severity represented by the total rusted leaf area was also decreased after the application of SA in both protective and therapeutic treatments (Abdelal, 1981).

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The objective of the present work is studying the effect of certain antioxidant compounds in inducing resistance against stem rust disease of wheat.

MATERIALS AND METHODS

The present work was carried out during 2011-2012 growing season at seedling and adult stages. Four synthetic compounds, *i.e.* salicylic acid (SA), oxalic acid (OA), ascorbic acid (AA) and citric acid (CA) were used in this study to evaluate their capabilities to induce resistance against *P. graminis* f.sp. *tritici* in wheat plants. These materials were used with three concentrations 250, 500 and 1000 ppm.

These materials were sprayed at three intervals, *i.e.* 24, 48 and 72 hours before inoculation. The highly susceptible wheat cultivar Sids-6 was used in this study.

Effect of the Tested Antioxidants at Seedling Stage

One hundred and eight of 7 cm diameter pots were used in this study. The pots were divided into 4 groups (27 pots for each treatment, concentration, and spray time). Five seeds of Sids-6, the highly susceptible wheat cv. were grown in each pot under greenhouse conditions. The pot groups were treated at 24, 48 and 72 hr., before inoculation at 7 days seedlings old. The all trials were arranged in a complete randomized block design with three replicates. All plants were inoculated according to (Tervet and Cassel, 1951) using the TTKTF pathotype of *P. graminis* .f.sp *.tritici*. Three pots were inoculated with the same stem rust pathotype were served as a control treatment. The following parameters were calculated for each treatment.

Infection type- five infection types were used according to (Stakman *et al.* 1962), *i.e.*, 0, 0; 1 and 2 (Resistant infection type), 3 and 4 (Susceptible infection type) as shown in Table 1

Number of pustules/cm^2(No.p./cm^2)- number of pustules per unit leaf area cm^2 (2.0 x 0.5 cm) on the upper side of the leaves were counted as described by (Parlevliet and Kuiper, 1977).

Pustule size (PS)-Pustule size was measured using the light microscope at 10x power magnification. Leaves were sampled 20 days after inoculation and pustules were fixed in boiled mixture of lactophenol and ethanol solution (1:2, v/v) for 3 minutes. Length (L) and width (W) of 10 randomly chosen pustules per one leaf were measured following formula suggested by (Broers, 1989) as follows:

$$\text{Pustule size} = 1/4 \times \pi \, L \times W \quad (\pi = 3.14)$$

Where; (L) was the length while (W) was the width of each pustule.

Scanning Electron Microscope (SEM) Study

SEM was used to describe the nature and mode of *P. graminis*.f.sp.*tritici* urediospores development on the treated plants with inducers. Samples of infected and treated leaves were taken at 10 days old (plants were sprayed with inducers at 500 ppm for salicylic and oxalic acid, 1000ppm for ascorbic and citric acid at 24 hr., before inoculation), fixed in 2.5% glutaraldehyde for 24 hours at 4°C then fixed in 1% osmium tetroxide (OSO_4) for one hour at room temperature. The samples were dehydrated with acetone critical point dried, and finally sputter coated with gold prior to the examination and photographed in a Jeol Scanning Electron Microscope (JSM-T330A) (Harley and Fergusen, 1990).

Effect of the Tested Antioxidants at Adult Stage

Field experiment was carried out at experimental farm of Sakha Agric. Res. St. during 2011-2012 growing season using the highly susceptible cv. (Sids-6). The experiment was carried out using randomized complete block design (RCBD) with three replicates. Each plot (2x1.5 m) contained 5 rows with 30 cm between rows. Each row was sown by 5 g wheat grains. Artificial inoculation was carried out using mixture of *P. graminis*.f.sp.*tritici* races at the rate of (1:20, w: w) using baby cyclone according to the method of (Tervet and Cassel, 1951).

Salicylic acid (500 ppm), oxalic acid (500 ppm), ascorbic acid (1000ppm) and citric acid (1000 ppm) were sprayed separately, 72 hr., before inoculation and 10 days latter. Rust severity (RS%), and 1000 grain weight (g), were assessed.

Statistical Analysis Procedure

All data were subjected to statistical analysis according to the procedures "ANOVA" reported by (Snedecor and Cochran, 1980). Treatments means were compared by the Least Significant Difference test (L.S.D) at 5% probability level (Duncan, 1955).

Table 1. The infection types of wheat stem rust reactions adopted by Stakman *et al.* (1962) at seedling stage

Infection Type			Symptoms
0			No uredia or other macroscopic sign of infection.
0;	Low	R*	No uredia, but hypersensitive necrotic or chlorotic flecks of varying size present.
1			Small uredia often surrounded by necrosis.
2			Small to medium uredia often surrounded by chlorosis or necrosis.
3	High	S**	Medium-sized uredia that may be associated with chlorosis or rarely necrosis.
4			Large uredia without chlorosis or necrosis.

*R: Resistant **S: Susceptible

RESULTS AND DISCUSSION

Greenhouse and Field Experiment

Data presented in Table 2 indicate the effect of the tested inducers which were sprayed 24 hours before inoculation. Significant differences were found among the used inducers as well as the concentrations used. In general, 500 ppm concentration was the most effective in all the applied inducers. Oxalic acid was the most effective inducer, especially at 500 ppm in reducing the mean of infection type (3), No., of pustules/cm^2 (1.6) and pustule size (0.0817) mm^2.

Salicylic acid came in the second rank in this respect releasing infection type (3), No., of pustules / cm^2 (1.8) and pustule size (0.0948) mm^2. The least effective one was ascorbic acid at all used concentrations comparing with control treatment.

Data in Table 3 show the effect of the tested inducers when applied 48 hours before inoculation. On contrast of spraying the inducers before 24 hours, the efficacy of salicylic acid was increased in reducing the previous parameters when sprayed 48 hours before inoculation. At 500 ppm concentration, salicylic acid occupied the first rank in reducing the pustules size (0.0786 mm^2) followed by oxalic acid (0.0948 mm^2). However, citric acid and ascorbic acid were the least effective in this respect.

Table 4 shows that spraying the tested inducer resistance chemicals 72 hours before inoculation proved to be effective in changing the infection type from susceptible to resistance response as well as decreased pustules size, especially with oxalic acid and salicylic acid at 1000 and 500 ppm. It could be noticed that all the tested inducers showed decreasing No., of pustules/cm^2 as well as pustule size when sprayed 72 hr., compared with 48 hr., before inoculation.

Data in Table 5 reveal that these inducers were mildly in their effects on stem rust development at adult stage. Data show that disease severity ranged between 23.33 to 43.33% comparing to the control treatment 51.11%. The highest efficacy was detected with oxalic acid (54.35%), followed by salicylic acid (50.00%). Citric acid came in the third rank (34.78%). While, ascorbic acid showed the lowest efficacy value (15.22%).

The values of 1000 grain weight were in the same line with disease severity. Oxalic acid gave the highest value (30.77g) releasing 39.23% increase comparing with the control treatment (22.10 g). Salicylic acid gave (30.65 g) releasing 38.68% increase. However, both of citric acid and ascorbic acid were the least effective (27.43 and 25.32 g, respectively) releasing 24.11 and 14.57% increase, respectively.

These results are in agreement with the finding of (Rauscher *et al.*, 1999) who demonstrated that treatment of broad bean (*Vicia faba*) leaves with salicylic acid (SA) or 2,6-dichloro isonicotinic acid (DcINA) induced resistance against the rust fungus *Uromyces fabae* resulting in inhibition of infection with hyphal and haustorial mother cell of the pathogen and reducing rust pustules density. Also, these

Table 2. Effect of spraying the synthetic inducer resistance chemicals, applied 24 hours before inoculation with urediospore of *P. graminis tritici*, on stem rust expressed as mean of infection type, No., of pustules/cm^2 and pustule size (mm^2) using the susceptible wheat cultivar Sids-6 under greenhouse conditions

Inducer chemicals	Concentration (ppm)	Infection type	No. of pustules /cm^2	Pustule size mm^2
Salicylic acid	**250**	4 b	2.9 ab	0. 1603 e
	500	3 a	1.8 a	0.0948 ab
	1000	4 b	2.8 ab	0.1183 c
Oxalic acid	**250**	4 b	4.6 c	0.1491 de
	500	3 a	1.6 a	0.0817 a
	1000	4 b	2.6 ab	0.1177 c
Ascorbic acid	**250**	4 b	8.0 e	0.1609 e
	500	4 b	6.3 d	0.1285 cd
	1000	4 b	4.6 c	0.1122 bc
Citric acid	**250**	4 b	4.2 bc	0.1686 e
	500	4 b	4.0 bc	0.1258 c
	1000	4 b	2.3 a	0.1111 bc
Control	—	4 b	23.99 f	0.1691 e

Means followed by a common letters are not significantly different at the 0.05% probability level.

Table 3. Effect of spraying the synthetic inducer resistance chemicals applied 48 hours before inoculation with urediospore of *P. graminis tritici*, on stem rust expressed as mean of infection type, No., of pustules / cm^2 and pustule size (mm^2) using the susceptible wheat cultivar Sids-6 under greenhouse conditions

Inducer chemicals	Concentration ppm.	Infection type	No. of pustules \ cm^2	Pustule size mm^2
Salicylic acid	**250**	4 b	11.83 c	0.1177 cd
	500	3 a	5.65 a	0.0786 a
	1000	4 b	5.83 a	0.1151 bc
Oxalic acid	**250**	4 b	9.66 b	0.1477 e
	500	3 a	5.33 a	0.0948 ab
	1000	3 a	8.99 b	0.1204 cd
Ascorbic acid	**250**	4 b	16.83 e	0.1399 de
	500	4 b	13.99 d	0.1230 cd
	1000	3 a	12.85 cd	0.1216 cd
Citric acid	**250**	4 b	18.33 f	0.1604 ef
	500	4 b	16.99 ef	0.1258 cd
	1000	3 a	12.49 c	0.1121 bc
Control	—	4 b	23.99 g	0.1691 f

Means followed by a common letters are not significantly different at the0.05% probability level.

Table 4. Effect of spraying the synthetic inducer resistance chemicals applied 72 hours before inoculation with urediospore of *P. graminis tritici* on stem rust expressed as mean of Infection type, No. of pustules / cm^2 and pustule size (mm^2) using the susceptible wheat cultivar Sids-6 under greenhouse conditions

Inducer chemicals	Concentration ppm.	Infection type	No. of pustules /cm^2	Pustule size mm^2
Salicylic acid	**250**	3 c	7.30 de	0.0935 ad
	500	1 a	5.2 bc	0.0742 ab
	1000	2 b	7.2 de	0.0863 abc
Oxalic acid	**250**	3 c	6.06 cd	0.0935 ad
	500	1 a	3.6 a	0.0627 a
	1000	1 a	4.00 ab	0.0922 ad
Ascorbic acid	**250**	4 d	8.00 e	0.1209 d
	500	3 c	7.90 e	0.1020 cd
	1000	3 c	7.60 e	0.1020 bcd
Citric acid	**250**	3 c	6.90 de	0.0986 ab
	500	3 c	5.4 c	0.0948 ad
	1000	3 c	5.2 c	0.0871 abc
Control	____	4 d	23.99 f	0.1691 e

Means followed by a common letters are not significantly different at the 0.05% probability level.

Table 5. Effect of spraying the synthetic inducers applied 72 hours before inoculation with urediospores of *P.graminis tritici* on development stem rust of wheat Sids-6 cultivar at adult stage, field experiment

Inducers	Disease severity,%	Efficacy, %	1000 G.w.	Increase, %
Salicylic acid	25.55 a	50.00	30.65 d	38.68
Oxalic acid	23.33 a	54.35	30.77d	39.23
Ascorbic acid	43.33 c	15.22	25.32 b	14.57
Citric acid	33.33 b	34.78	27.43 c	24.11
Control	51.11 d	00.00	22.10 a	00.00

Means followed by a common letter are not significantly different at the 5% probability level.

results are in accordance with the finding of (Seif El-Eslam *et al.*, 2003 and Ibrahim, 2010). However, Mahmut and Andrew (2000) found that, oxalic acid is one of the strongest organic acid and its oxalate salts, oxalates are widely distributed within the cells and cell walls of plants. While, Galal *et al.* (1997) reported that salicylic acid plays a physiological role in resistance of sunflower rust disease through enhancing some biochemical related reaction such as peroxidase activity. Also, Wang *et al.* (2000) stated that salicylic acid provides protection against biotic and a biotic stress and induces (PR) proteins, which are novel soluble proteins referred as "pathogen-related (PR) proteins" were effectively induced in response to infection by various pathogens. The biological functions of these (PR) proteins have been studied and some of these proteins were identified as chitinases and β- 1, 3- glucanases in several plant species. Also, Sallam and Hussain (2012) found that, the effect of spraying three novel polymeric compounds (Homopolymer, Pure acrylate and Styrene Co-polymer) at three different concentrations (5,10and 15%) on the development of wheat leaf rust. Spraying polymeric compounds before rust inoculation, significantly reduced pustule size and number of pustules/ cm^2 and leaf area but not affected incubation period. However, infection type was significantly affected through some of infection type produced only flecks which were not developed to pustules.

Scanning Electron Microscope (SEM) Experiment

Scanning electron microscope (SEM) gave an overview on the effect of spraying wheat seedlings with SA, OA, AA and CA 24 hours before artificial inoculation with *P. graminis tritici* urediospores (Figs. 2, 3,4 and 5) on Sids-6 wheat cultivar. It could be concluded that application of the tested antioxidant compounds led to shrinkage of surface of the urediospore and deformed of its germ tube as well as stomata were closed.

These results are in agreement with the finding of Edwards and Bowling, (1986) who suggested that the distribution of appressoria over the coated leaf surface is associated with disruption of the mechanisms coupled with orientation of the germinating urediospores toward the stomata and formation of appressoria. Also, these results are in line with the finding of (El-Deeb, 1999 and El-Naggar, 2005) which observed that through the SEM examination of Giza- 160 wheat cv. sprayed with bion compound, closed stomata and deformed appressoria were observed. However, Sallam and Hussain (2012) stated that, scanning electron microscope (SEM) examination showed polymeric compounds cause disturbance on leaf surface morphological characters led to alter the topography of the leaf surface. Also, Homopolymer compound inhibit urediospores germination, suppressed germ tube and appressoria formation which led to reduce rust development.

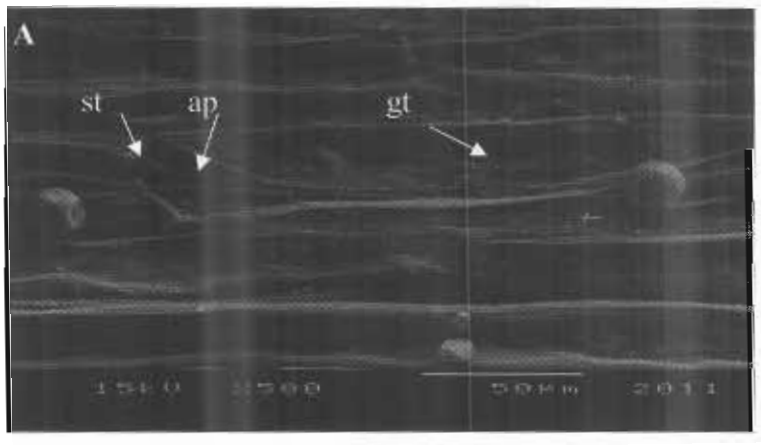

Fig. 1. Scanning electron microscope showing untreated leaf surface of Sids-6 cv. of wheat seedling as control plant. The ideal urediospores (us), germ tube (gt) and appersorium localized onto suitable site of opened stomat (st)

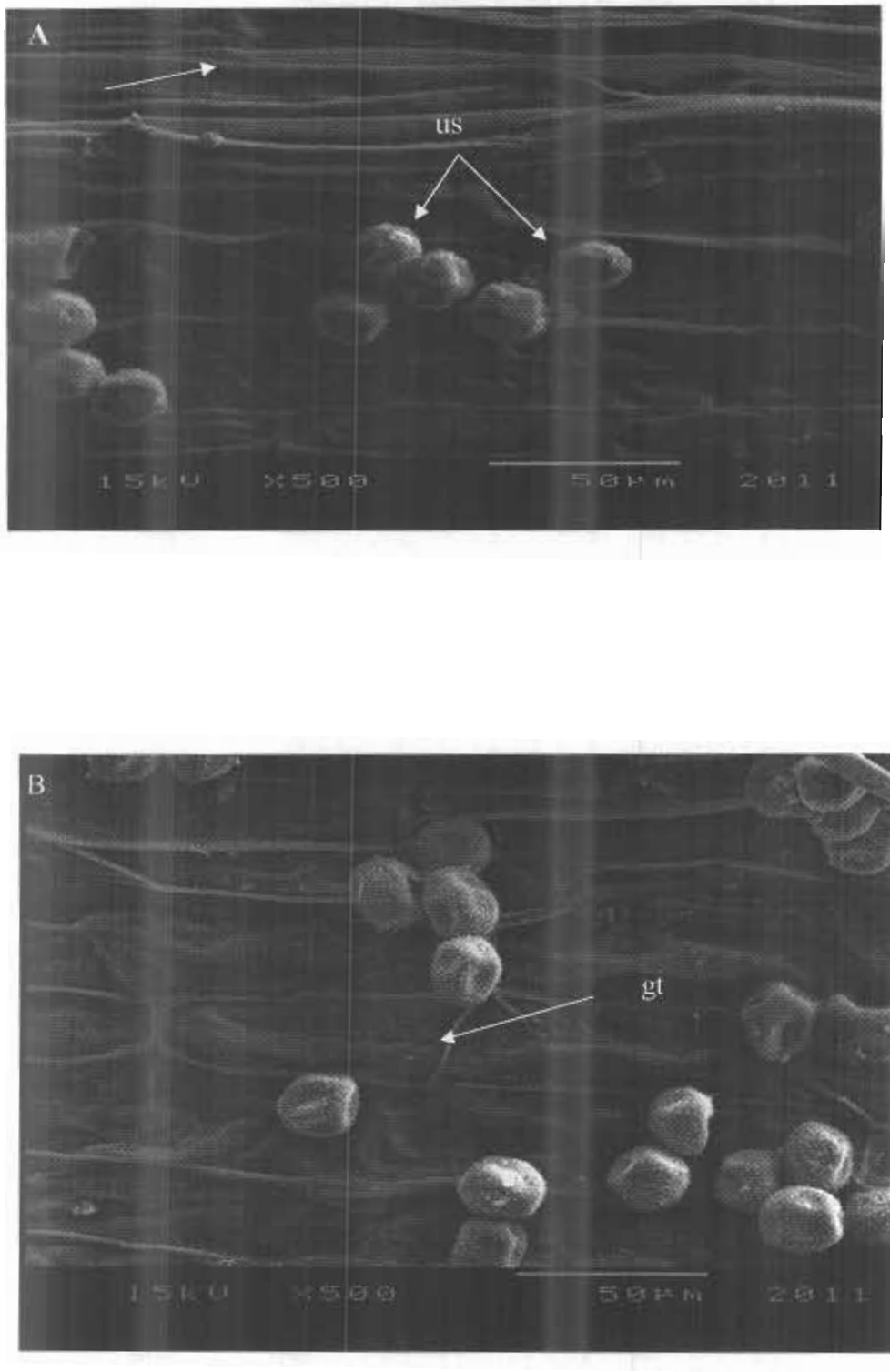

Fig. 2. Scanning electron micrograph showing leaf surface of Sids-6 cv. of wheat sprayed with salicylic acid 24 hr., before inoculation with urediospores of *P. graminis tritici* (A) Many of un-germinated urediospore are shown. (B) Show germinated urediospores without appresoria and shrinkage of their surfaces

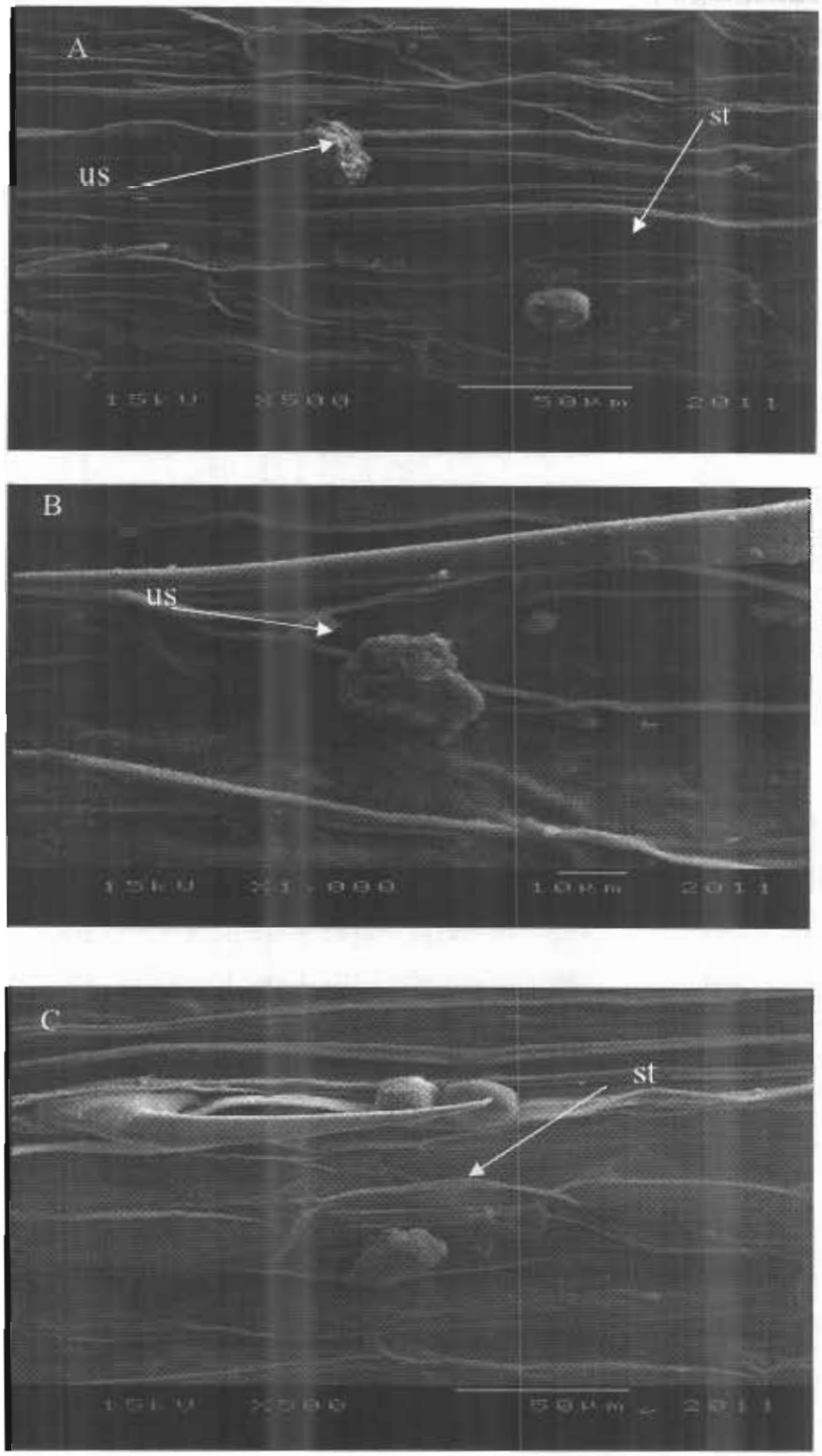

Fig. 3. Scanning electron micrograph showing leaf surface of Sids-6 cv. of wheat seedling sprayed with oxalic acid 24 hr., before inoculation with urediospores of *P.graminis tritici*. (A) Shrinkage of surface of urediospores. (B) Deformation of urediospore surface and germ tube as well (C) Un-germinated urediospore and closed stomata

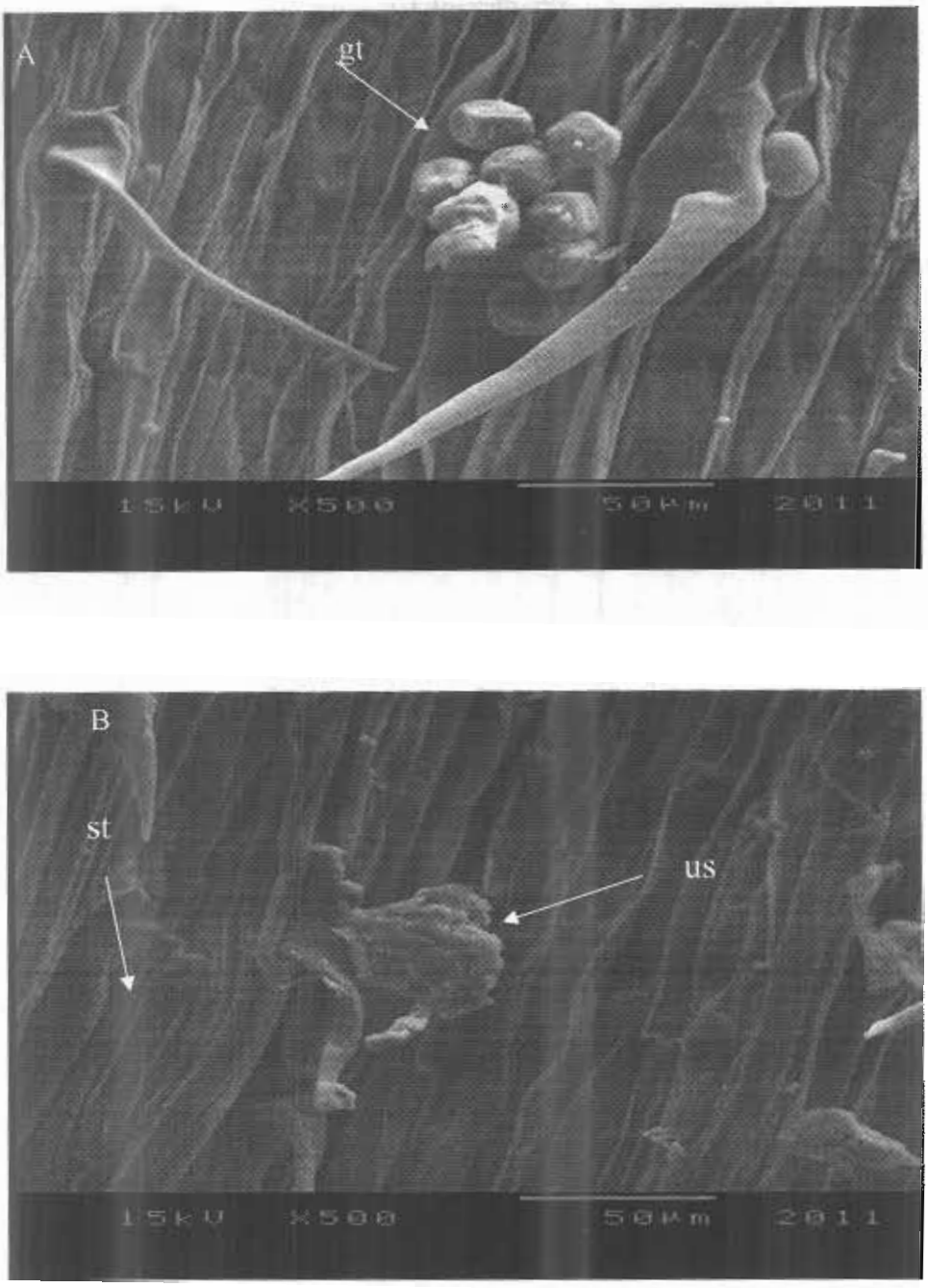

Fig. 4. Scanning electron micrograph showing leaf surface of Sids-6 cv. of wheat seedling sprayed with ascorbic acid 24 hr., before inoculation with urediospores of *P.graminis tritic*. (A) Deformation of germ tube. (B) Deformation of germ tube and urediospore surface in addition to stomata were closed

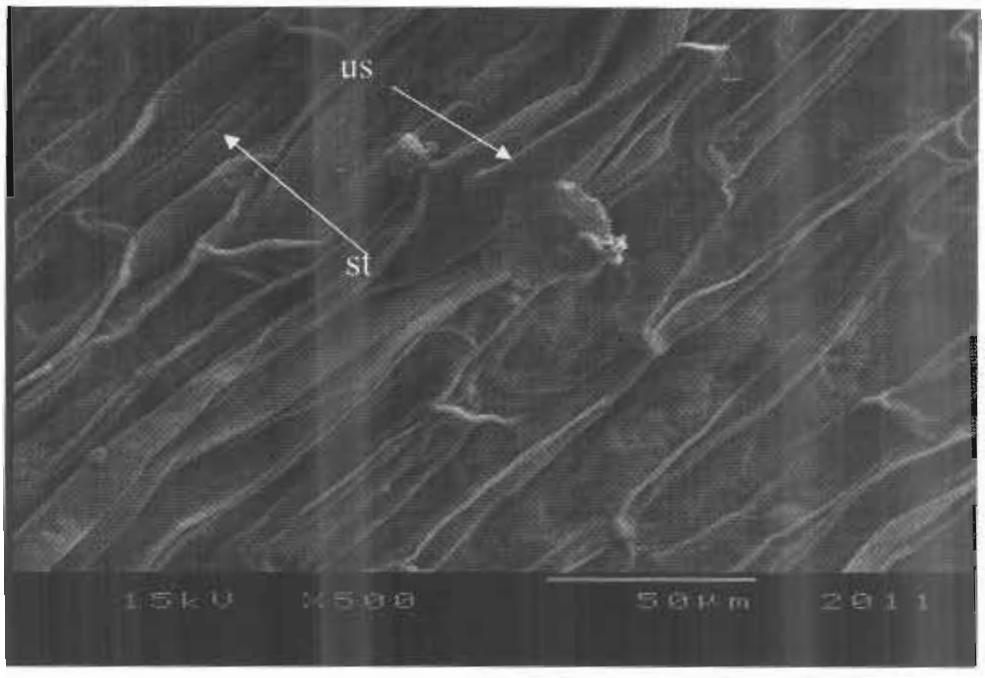

Fig. 5. Scanning electron micrograph showing deformation of germ tube on leaf surface of Sids-6 cv. of wheat seedling sprayed with citric acid 24 hr., before inoculation with urediospores of *P.graminis tritici*

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المقاومة المستحثة لمرض صدأ الساق في القمح باستخدام بعض المركبات المضادة للأكسدة

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يعتبر مرض صدأ الساق المتسبب عن فطر *Puccinia graminis* f.sp *tritici* من أهم الأمراض التي تصيب نباتات القمح في مصر، في هذا البحث تم استخدام أربعة مركبات لاستحثاث المقاومة فى الصنف سدس٦ القابل للإصابة بمرض صدأ الساق وهي حمض السالسيليك وحمض الأوكساليك وحمض الأسكوربيك وحمض الستريك وتم الرش بها بثلاث تركيزات فى مرحلة البادرة (٢٥٠ – ٥٠٠ – ١٠٠٠ جزء فى المليون) وذلك قبل العدوى ب ٢٤، ٤٨، ٧٢ ساعة. كما تم الرش بتركيز ٥٠٠ و ١٠٠٠ جزء فى المليون قبل العدوى بالحقل ب ٧٢ ساعة، وكانت هناك فروق معنوية بين المواد المستخدمة و بين التركيزات وكذلك مواعيد الرش، وثبت أن التركيز ٥٠٠ جزء فى المليون هو الأفضل من بين التركيزات المستخدمة. كان حمض الأوكساليك يليه حمض السالسيليك الأكثر كفاءة فى تقليل الإصابة بالمرض وكذلك عدد البثرات فى وحدة المساحة (سم٢) وحجم البثرة (مم٢) وكان حمض الستريك تلاه حمض الأسكوربيك هما الأقل كفاءة، أظهرت المواد المستخدمة كفاءة متوسطة فى مقاومة المرض فى مرحلة النبات البالغ مقارنة بغير المعاملة حيث كان حمض الأوكساليك الأكثر كفاءة تلاة حامض السالسيليك بنسبة ٥٤,٣٥ و٥٠,٠٠% على التوالى، بينما كان حمض الأسكوربيك تلاه حمض الستريك هما الأقل كفاءة بنسبة ١٥,٠٠ و ٣٤,٣٣%. كانت قيم وزن الألف حبة نتيجة لاستخدام هذه المواد مسايرا لشدة الإصابة إذ سجل حمض الأوكساليك أعلى القيم ٣٠,٣٧ جم بزيادة قدرها ٣٩,٣٣% مقارنة بالغير معاملة ٢١,١٠ جم، أوضح الفحص بالميكروسكوب الالكتروني لدراسة تأثير استخدام بعض المواد الكميائية لاستحثاث النبات لمقاومة مرض صدأ الساق في القمح أن استخدام هذه المواد يؤدي إلى إنكماش الجراثيم اليوريدية وكذلك تشوه في أنابيب إنبات تلك الجراثيم.

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