Transgenic canola plants over-expressing bacterial catalase exhibit enhanced resistance to *Peronospora parasitica* and *Erysiphe polygoni*

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ABSTRACT

Transgenic canola (Brassica napus.L) plants expressing the bacterial catalase katE in the chloroplasts were obtained by the Agrobacterium-mediated transformation method. Resistance and susceptibility of the transgenic canola plants were evaluated against the airborne pathogenic fungi, Peronospora parasitica causing downy mildew and Erysiphe polygon causing powdery mildew under artificial infection in the greenhouse. The bioassays of the transgenic plants demonstrated that the growth of both fungi and the development of disease incidence were significantly inhibited in the leaves of the transgenic canola plants compared to controls. Chemical analyses of the transgenic plants revealed constitutive expression of the enzymes catalase, peroxidase and polyphenoloxidase as well as higher levels of free polyamines i. e. putrescine, spermidine and spermine compared with the control plants. Together, these data indicate an enhanced resistance of the transgenic canola plants expressing the bacterial catalase to the downy mildew and powdery mildew pathogens.

Key words: *Transgenic, canola, catalase, fungi, downy mildew and powdery mildew.*

INTRODUCTION

Canola (*Brassica napus. L*) is considered one of the most important oil crop worldwide. It ranks the third among the oil crops, following palm oil and soy oil and the fifth among economically important crops, following rice, wheat, maize and cotton. Powdery mildew caused by *Erysiphe polygoni*, is a major fungal disease of canola and can cause serious yield loss in wet seasons (Cardoso, *et al.*, 1996 and Gaetn and Madia, 2004). Downy mildew, caused by *Peronospora parasitica*, occurs sporadically and until recently rarely causes any yield loss (Lanoiselet *et al.*, 2003). However, in 1998, severe downy mildew was widespread in Western Australia on seedlings, and in some areas appeared to retard seedling growth and vigor severely. Cultivars resistant to these diseases have not been identified so far. Genetic engineering of canola using antifungal genes offers an opportunity to develop mildews resistance in canola.

Plants initiate various defense responses when attacked by microbes such as fungi and bacteria. Whereas some of these defense mechanisms are induced only after pathogen attack, others are preformed and constitute generalized physical and physiological barriers

that hinder pathogen infection. Similar to animal immune responses following pathogen infection, plant responses to microbes involve a network of signal transduction pathways leading to regulation of gene expression (Yang *et al.* 1997). The association of reactive oxygen species (ROS) in plant-microbe interactions has been clearly established in the last 10 years. During many plant-pathogen interactions ROS are produced by plant cells at a very high rate (oxidative burst) and are thought to activate plant defenses, including programmed cell death (Dangl *et al.*, 1996; Delledonne *et al.*, 2001; Hammond-Kosack and Jones, 1996) and systemic acquired resistance (SAR) (Sandermann, 2000). SAR is thought to protect the plant against the systemic spread of the invading pathogen and requires the expression of defense genes in healthy plant cells.

The objective of this study was to evaluate the potential function of the bacterial catalase overexpressed in the chloroplasts of canola plants to enhance the defense against the pathogens *P. parasitica* and *E. polygoni.*

MATERIALS AND METHODS

Bacterial strains and plasmid

The *Agrobacterium tumefaciens* strain LBA4404 was used for transformation. The construction of the transformation vector pBI101katE is illustrated in (Fig. 1-A). The transformation vector was generously provided by Dr. Shigeru Shigeoka, Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Japan.

Plant materials

The seeds of canola (*Brassica napus. L.* cv. Semu-249) used for transformation were kindly provided by the Field Crops Institute, Agricultural Research Center (ARC), Giza, Egypt.

Agrobcterium-mediated transformation

The transformation construct pBI101-KatE was introduced into canola hypocotyl segments (0.5 cm in length) excised from 6-day-old *in vitro* grown seedlings with *A. tumefaciensn* according to the optimized protocol for regeneration and transformation in canola reported by Moghaieb *et al.* (2006).

Growth of T_0 plants

Transgenic and wild-type plants were grown to maturity in the greenhouse. The T_0 plants were self-pollinated to produce T_1 progeny.

Genomic DNA isolation and PCR analysis

Genomic DNA from plant leaves (0.5 to 1 g FW) was prepared by the method of Dellporta *et al.* (1983). Specific primers for the *kat*E *and nptII* genes were designed on the basis of the published sequence of the genes (Ossowski *et al.*, 1991). For *kat*E, the sequences of forward and reverse primers were: 5-AAAAACTCACCGGACGTGAC-3' and 5-TAATTCGC CGGGTTAGTGTC-3', respectively, amplifying a fragment of 568 bp from position 923 to 1490 of the sequence. For *nptII*, the sequence of forward and reverse primers were: 5`-CGCAGGTTCTCCGCCGCTTGGGTGG- 3'and 5`-GCAGCCAGTCCC-TTCCCGCTTCAG-3', respectively, amplifying a fragment of 254 bp from position 24 to 277 of the sequence. The 20 µl amplification reaction mixture contained approximately 0.1 µg of plant genomic DNA, 200 µM of each dNTP, 2 mM $MgCl_2$, 1 µM of each primer and 1 unit of Red Hot Taq DNA polymerase (ABgene Housse, UK). The PCR reaction was conducted at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min in 30 cycles followed by 7 min at 72°C in controlled conditions.

Total RNA isolation and RT-PCR analysis

Total RNA was isolated from canola leaves (1.0 g FW) using the Rneasy Plant Mini Kit (QiIAGEN, USA) and following the procedure provided in the instruction manual.

The total RNA was converted into first strand cDNA using the commercially available kit (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas, EU) following the procedure provided in the instruction manual. The first strand cDNA was used for PCR reaction as a template. The reaction was conducted at the same conditions as mentioned above.

Plant growth conditions and pathogen treatment

Seeds of the transgenic canola were sown in 15 cm- diameter plastic pots (5 seeds per pot) containing peat, vermiculite and sand (1:1:1). Plants were grown for 30 days in the greenhouse under controlled conditions of temperature (22 to 24°C), 90 % relative humidity and 12 hr photoperiod.

Mildews and *In vitro* bioassay

Intensely colonized areas of canola leaves naturally infected with *P. parasitica* and *polygoni* were cut into leaf disks (50 mm^2). To inoculate canola plants, the procedure described by Okuno *et al.* (1991) and Reuveni *et al.* (1997) was used. Spores were harvested from infected disks using 10 ml of the solution containing 0.01 % tween 80 as surfactant. The spores were adjusted to 10^5 spores ml^{-1} with a hemacytometer and lightly atomized onto leaves. Five leave disks (1 cm^2) below the apex of transgenic and control plants were surface sterilized with 0.1% sodium hypo-chloride and rinsed three times in sterile water (all experiments involved five replicate disks). Two ml of spore suspension (10^5) were sprayed over the disks before placing them inside petri dishes (90 mm. in diameter) containing wetable filter paper. Then, the disks were incubated with *P parasitica* and *E. polygoni*, at 20±1°C and 25±1°C, respectively, in darkness. After four days disease incidence was determined under an Olympus microscope. Disease incidence of downy mildew was carried out according to Reuveni (1983) using color indices and infected area. The color index was as follows; 0, no symptoms; 1, change in color; 2, greenish; 3, yellowish; 4, yellow; 5, brown. The infected area index was as follows: 1= indicates symptoms on 25% or less of leaf area; 2= 26- 50; 3= 51-100% of leaf area. Multiplication of the color and infected area indexes for each leaf yields a value of disease severity. Assessment of powdery mildew severity was performed according to Reuveni *et al.* (1997). Powdery disease severity was rated on 0-5 scale: 1=1-25, 2=26-50, 3=51-75, 4=76-100, 5>100 colonies per leaf disk.

Greenhouse bioassay

Disease assessment

Inoculum suspension of *P parasitica* and *E. polygoni* was sprayed onto adaxial (upper) and abaxial (lower) leaf surfaces, respectively and exposed to 18 hr light for four days before assaying the disease severity.Disease severity of downy mildew and powdery mildew were carried out according to Reuveni (1983) and Reuveni *et al* (1997), respectively. Spores of each *P. parasitica* and *E. polygoni* / cm^2 were counted in the leaf infected areas, 10 days after artificial inoculation. Leaves were taken at the early morning and immersed in a jar containing 10 ml of distilled water. Spores were released from lesions using a brush, then they were counted using hemicytometer. The data was calculates as the means of five replicates each contained three leaves.

I. Scanning Electron Microscope (SEM)

Leaf lesions (1 cm^2) were rapidly frozen in the vapor phase above liquid nitrogen and placed in a glass vacuum container prior to lyophilization. The frozen dried leaf segments were then mounted on aluminum stubs and coated with gold using vacuum evaporation prior to SEM examination. Micrographs were taken on a Polaroid type positive film with UV-haze and 0.2 orange filters. Two pretreated samples were examined.

II.Chlorophyll-a, chlorophyll-b and carotenoid contents

Leaf samples (5 leaves) from each replicate were separately collected to determine the photosynthetic pigments, chlorophyll a and b as well as carotenoids according to the method described by Wetestein (1957) at wavelengths of 662, 640 and 440 nm, respectively.

III. Determination of free polyamine contents

Free polyamines were extracted and hydrolysed using the method described by Slocum and Galston (1985). Polyamines were extracted with 2 ml of 0.5*M* $HClO_4$ overnight at room temperature, derivatized with benzoyl chloride and quantitated with high performance liquid chromatography (HPLC) using standard chemicals (Sigma chemicals). Separation and quantification of derivatized polyamines were performed with a Shimadzn Lc-6A HPLC equipped with a UV detector. The analytical condition was as follows: 6×150 mm in column size; 45°C column temperature; 64% methanol mobile phase and detection on 254 nm.

IV. Peroxidase, polyphenoloxidase and catalase assays

The enzymes were extracted from canola leaves and the supernatant was prepared according to the method of Tuzun *et al.* (1989). Peroxidase activity was evaluated according to the method described by Allam and Hollis (1972) as one unit of peroxidase activity was expressed for the change in absorbance at 425 nm/min /g fresh weight. Polyphenol oxidase activity was quantitatively determined according to the methods described by Matta and Dimond (1963) as one unit of polyphenol oxidase was expressed for the change in absorbance at 420 nm/ 30 min /g fresh weight. Specific catalase activities were determined as previously described (Brown *et al.*, 1995). A specific catalase activity unit is defined as 1.0 μmol of H_2O_2 degraded / min / g of total protein^{-1}.

Statistical analysis

The collected data were statistically computed using the software SPSS for Windows (release7.5.1, Dec. 20, 1996, SPSS Inc.). AUDPC and yield data for all treatments were subjected to an Analysis of Variance in order to obtain the standard error of the difference (SED) which was used to assess the significance of differences.

RESULTS

Genetic transformation, growth and fertility and molecular characterization of the regenerated plants

Hypocotyl explants of the canola plants cv Semu-249 were infected with *A. tumefaciens* carrying the *katE* and *nptII* genes in the vector plasmid. Organogenic calli were selected on kanamycin-containing medium, and R_0 plants that regenerated independently from these calli were selected as putative transgenic plants. PCR analysis of these candidate plants revealed the presence of *katE* and *nptII* genes in some plants which were selected as transgenic plants and named T_0 (Fig. 1-B, C). All transgenic plants had normal morphology and growth habits compared with non-transgenic controls. After self-pollination of T_0 plants, overall, 55% of the transgenic plants were fertile and set viable seeds.

The RT-PCR analysis for the T1 plants showed the occurrence of the mRNA for the *katE* gene in all of the transgenic plants assayed (Fig. 1-D).

***In vitro* bioassay**

Transgenic canola plants were assayed for their resistance to the foliar plant pathogens included *P. parasitica* and *E. polygoni* (Fig. 2). There were prominent differences ($P<0.05$) between *P. parasitica* and *E. polygoni* disease incidence, symptoms

area in transgenic and wild type plants, whereas the level of resistance greatly varied among transgenic lines and the control plants. After 4 days of spraying with the pathogens, the leaves of the wild type plants showed typical downy mildew disease symptoms by an extensive disease incidence (15.2 %) and severity (33.3 %). At the same time, leaves of the transgenic plants were highly resistant to downy mildew with a lower disease incidence (1. 6%) and severity (0.53%). For the powdery mildew, the leaves of the transgenic plants revealed greatly lower disease incidence (4.45%) and severity (0.91%) compared to those of the wild type plants (60.6 % and 76.8%), respectively.

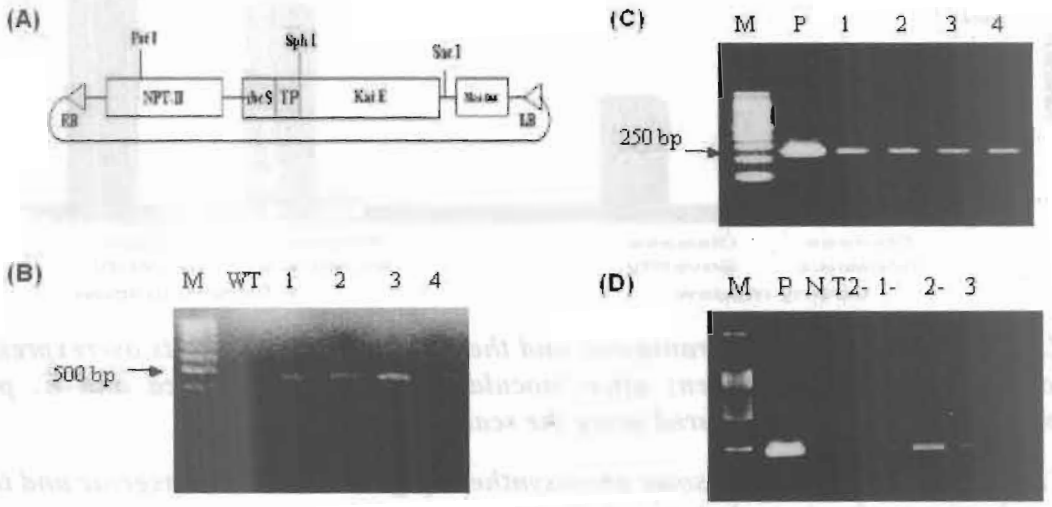

Fig. (1): Confirmation of transformation of canola plants with the bacterial catalase (katE) gene.A, the transformation vector pBI121-KatE. B, PCR assay of the T1 Plants of the transgenic canola plants using specific primers for catalase and for the kanamycin resistantance gene NPT II (C): D, RT-PCR analysis of some F_2 transgenic plants. M is DNA marker (100 base ladders), WT is wild-type plant N is negative control and P is the positive control.

Greenhouse bioassay

I. Disease assessment

Resistance of transgenic plants to mildew diseases was evaluated under artificial infection conditions with pathogens in the greenhouse (Fig. 3). Diseases rating were scored during eight days of inoculation based on symptoms graded from 0 to 5, as shown in Fig. (3). There were prominent differences ($P<0.05$) between diseases severity in the transgenic and the wild type plants, whereas the level of resistance varied greatly among transgenic line and the wild type plants. After 8 days, the inoculated wild type plants showed typical disease symptoms by an extensive of disease severity of either downy mildew (34.3%) or powdery mildew (71.5%). At the same time, the transgenic line consistently performed better than the controls and grew equally well as non-infected plants showing high resistance, where the maximum level of disease severity of downy mildew and powdery mildew reached 0.53 and 4.52 %, respectively. The same trend holds true for

pathogen sporulations observed 8 days after the infection (Fig.3). The wild type plants showed high number of spores of *P. parasitica* and *E. polygoni* (96.2 and 104.3 spore/ cm^2, respectively). Meanwhile, the leaves of the transgenic plants exhibited reduction in spore counts. The maximum reduction in the spore counts was observed in *P. Parasitica* (6.32 spore/ cm^2) followed by *E. polygoni* (10.5 spore/ cm^2) in the leaves of infected transgenic plants.

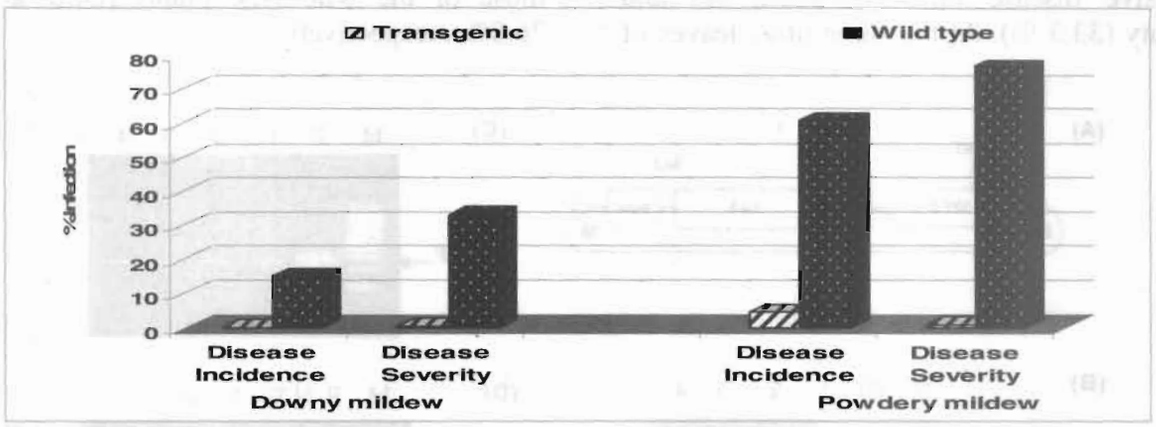

Fig. (2): Leaf disk bioassay of the transgenic and the wild type canola plants overexpressing the bacterial catalase (katE) gen) after inoculation with P. parasitica and E. polygoni. Disease incidence was measured using the scale from 0 to 5.

Table (1): Changes in the contents of some photosynthetic pigments in the transgenic and the wild type canola plants after inoculation with P. parasitica and E. polygoni.

Treatment	*Peronospora parasitica*			*Erysiphe polygoni*		
	Chlorophyll mg/g fresh weight		Caroteins mg/g fresh weight	Chlorophyll mg/g fresh weight		Caroteins mg/g fresh weight
	a	b		a	b	
Wild type	0.057	0.039	0.090	0.057	0.039	0.090
Inoculated Wild type	0.026	0.015	0.070	0.041	0.024	0.081
Transgenic	0.089	0.091	0.139	0.089	0.091	0.139
Inoculated transgenic	0.094	0.083	0.128	0.083	0.086	0.136
LSD	0.006	0.007	0.004	0.007	0.006	0.005

II. Scanning Electron Microscope (SEM)

Morphological and microscopical examinations, using SEM, revealed decreased growth level of the *P. parasitica* and *E. polygoni* fungi in the leaves of transgenic plants compared to the wild type plants (Fig.4). In the inoculated wild plants, both pathogens completely covered the leaf surface. In contrast, *Peronospora parasitica* was greatly inhibited on the transgenic plants, however, little sporulation of *E. polygoni* was observed.

III. Chlorophyll-a, chlorophyll-b and carotenoid contents

The data presented in Table (1) revealed that the contents of the photosynthetic pigments i.e. chlorophyll-a, chlorophyll-b and

carotenoides were markedly increased in transgenic plants to reach 0.089, 0.091 and 0.139 mg/g fresh weight, respectively, compared with those of wild type plants (0.057, 0.039 and 0.090, respectively). Severe decrease in the contents of the photosynthetic pigments was shown in the wild type plants inoculated with either *P. parasitica* or *E. polygoni* compared with the uninoculated wild type plants. Inoculation of the wild type plant with *P. parasitica* resulted in significant decrease of chlorophyll a, b and carotenoids to reach 0.026, 0.015 and 0.070 mg/g fresh weight, respectively. While, the contents of the same photosynthetic pigments decreased to reach 0.041, 0.024 and 0.081 mg/g fresh weight in the leaves of wild type plants infected with *E. polygoni*. Likewise, no significant differences in the concentrations of chlorophyll-a, chlorophyll-b and carotenoids were observed between non inoculated and inoculated transgenic plants with *P. parasitica* or *E. polygoni*.

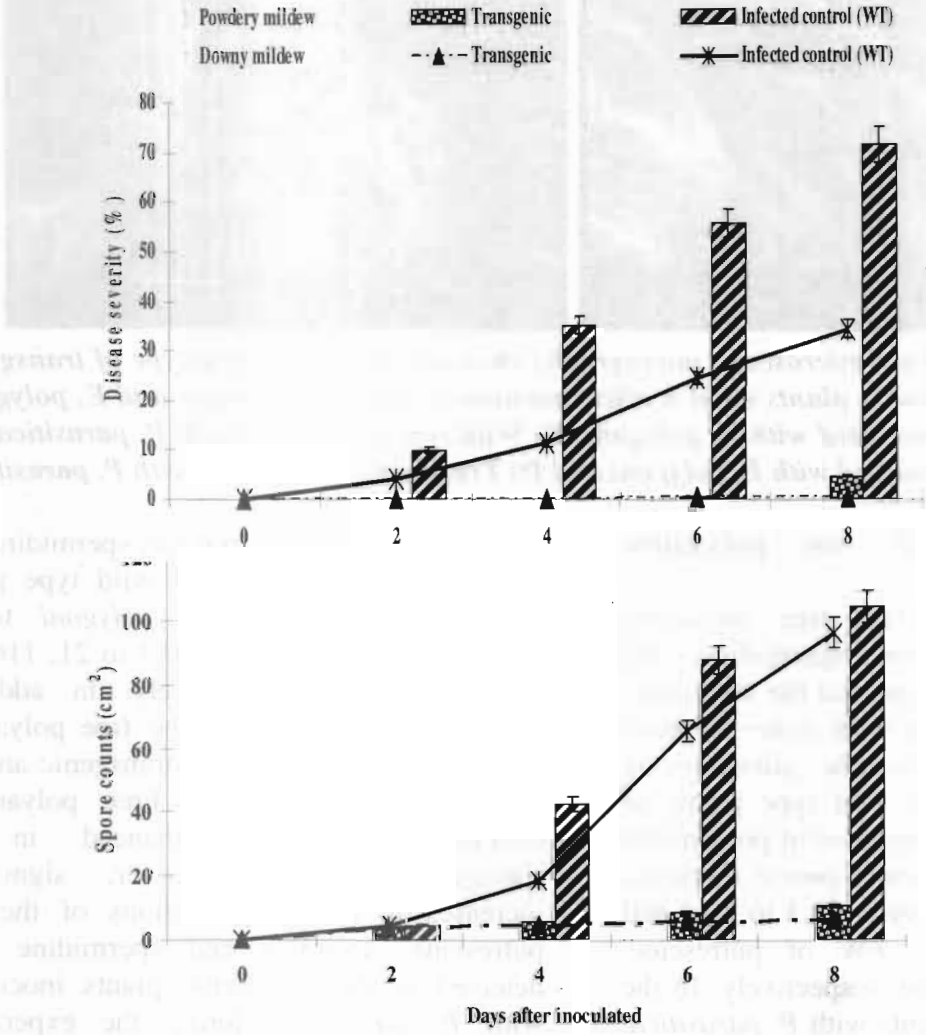

Fig. (3): Spore counts and disease severity on the leaves of transgenic and the wild type canola plants inoculated with P. parasitica and E. polygoni.

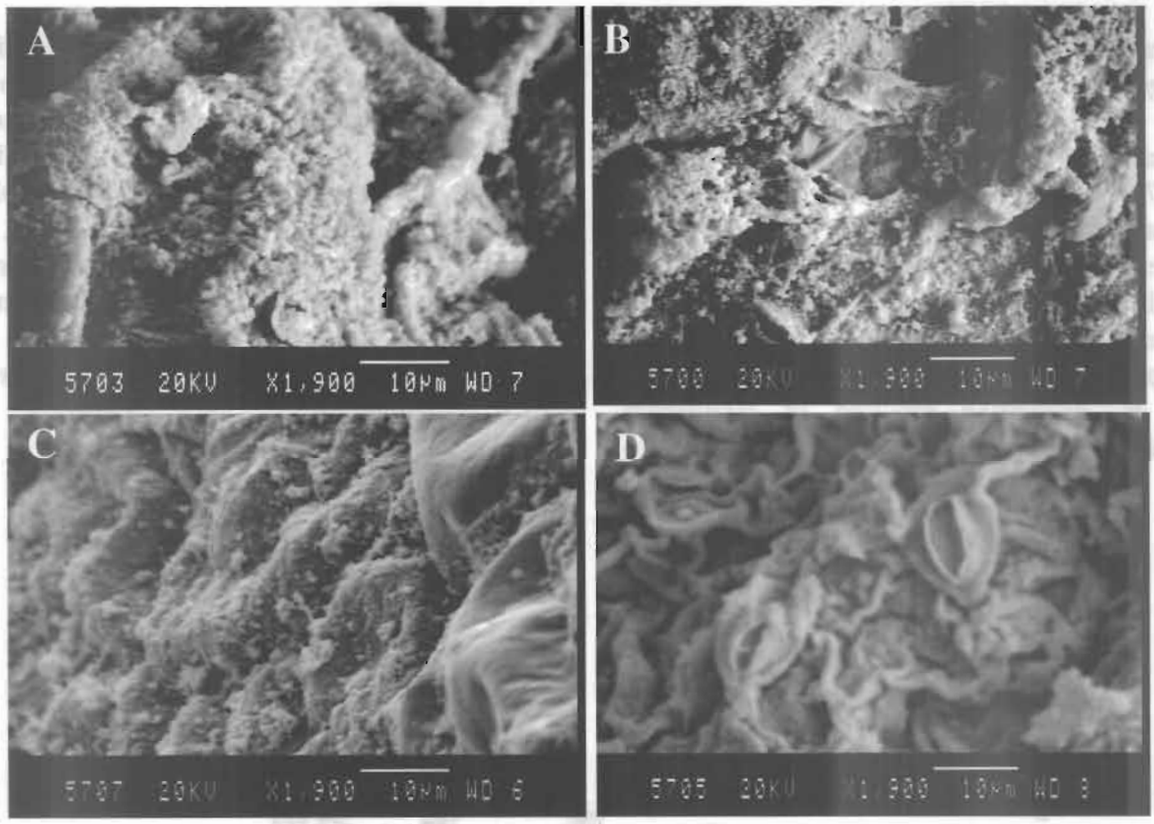

Fig.(4): Scanning electron microscope micrographs showing infection phenotype of transgenic and wild type canola plants eight h after inoculation with P. parasitica and E. polygoni. A) Wild type inoculated with E. polygoni; B) Wild type inoculated with P. parasitica; C) Transgenic inoculated with E. polygoni and D) Transgenic inoculated with P. parasitica.

IV. Determination of free polyamine contents

Concentrations of the free polyamine forms (*i. e.* putrescine, spermidine and spermine) in the wild type and the transgenic canola leaves inoculated with either *Peronospora parasitica* or *Erysiphe polygoni* are shown in Fig. (5). The wild type plants of canola contained moderate level of polyamines throughout the experimental period (8 days), ranged from 312.5 to 399.9, 52.3 to 59.3 and 124.3 to 129.3 nmol^{-1} FW of putrescine, spermidine and spermine, respectively. In the inoculated wild type plants with *P. parasitica,* the polyamines were reduced to ranging from 305 to 229, 50.3 to 31 and 116.3 to 68 nmol^{-1} FW, respectively. Meanwhile, concentrations of free polyamines, putrescine, spermidine and spermine, decreased in the wild type plants inoculated with *Erysiphe polygoni* to be ranging from 305 to 189, 50.3 to 21, 116.3 to 58 nmol^{-1} FW, respectively. In addition, significant differences in the free polyamine contents were found in the transgenic and the wild type canola plants. Free polyamines concentrations were enhanced in the transgenic plants. Moreover, significant increases in the concentrations of the free putrescine, spermine and spermidine were detected in the transgenic plants inoculated with *P. parasitica* during the experiment period (8 days) which ranged from 602.3 to 691.3, 93.5 to 132.5 and 301.5 to 385.5 nmol^{-1} FW, respectively. However, the concentrations

of the free polyamines, putrescine, spermidine and spermine, decreased in the transgenic plants inoculated with *E. polygoni to* be ranging from 602.3 to 672.3, 93.5 to 109.5 and 301.5 to 375.5 nmol^{-1} FW, respectively.

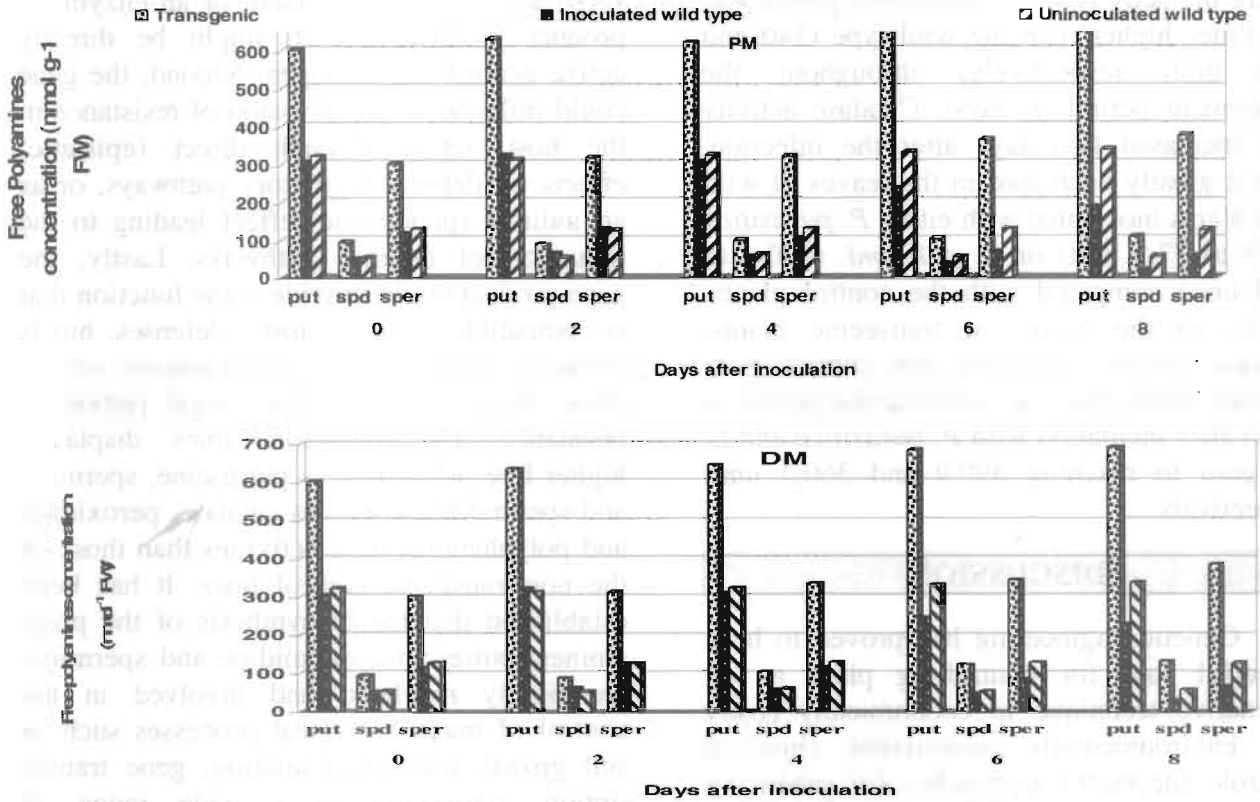

Fig. (5): Free polyamines, i.e., putrescine (put), spermidine (spd) and spermine (sper) contents expressed in the transgenic with the bacterial catalase (katE gene) and the wild type canola inoculated with P. parasitica (Dm) and E. polygon (Pm).

V. Peroxidase, polyphenoloxidase and catalase assay

The specific peroxidase and polyphenoloxidase as well as catalase activities of the wild type and transgenic canola plants were determined before and after inoculation with *P. parasitica* or *E. polygoni* (Fig. 6). The wild type of canola contained moderate activities of peroxidase and polyphenoloxidase ranged from 7.54 to 7.21 and 0.235 to 0.264 unit, respectively, throughout 8 days of the experimental period that. The results revealed that peroxidase and polyphenoloxidase activities were greatly decreased in the leaves of wild type canola plants inoculated with either *P. parasitica* (7.5 to 3.98 and 0.245 to 0.095 unit) or *E. polygoni*. (10.5 to 4.02 and 0.258 to 0.073 unit) compared with the control. Significant differences in the enzyme activities were found in the transgenic and wild type canola plants. The highest increase of the peroxidase and polyphenoloxidase activities was obtained in the transgenic plants inoculated with *P parasitica* (9.54 to 14.2 and 8.54 to14.6, respectively) and *E. polygoni* (0.415 to 0.887 and 0.403 to 0.734 unit) as

compared with the control plants .Significant differences in catalase activity were found in the wild type and the transgenic canola plants where the activity in the transgenic plants was 1.6 times higher than the wild type (140 and 100 unit, respectively) throughout the experiment period (8 days). Catalase activity was increased two days after the infection, then it greatly decreased in the leaves of wild type plants inoculated with either *P. parasitica* (11.5 to 77.5 unit) or *E. polygoni.* (100.4 to 56.0 unit) compared with the control plants. While, in the leaves of transgenic plants, catalase activity increased and continued to increase throughout the experimental period (8 days) after inculation with *P. parasitica* and *E. polygoni* to reaching 390.9 and 360.3 unit, respectively.

DISCUSSION

Genetic engineering has proved to be a powerful tool for controlling plant as an alternative technique to economically costly and environmentally undesirable chemical control. Successful approaches for enhancing disease resistance of plants include constitutively overproducing antifungal compounds (Lorito *et al.*, 1998) and expressing genes encoding peptides that are involved in the biosynthesis of antifungal compounds (Ali *et al.*, 2003, Saker *et al.*, 2008). The transgenic plants constitutively expreseds defense-related genes and displayed broad-spectrum disease resistance (Peever, *et al.*, 2000).

In the present study, the effect of constitutive expression of catalase in conferring resistance to powdery and downy mildew diseases in the transgenic canola was initially evaluated by direct infection of wild type and transgenic plants. While the control plants developed strong disease incidence and severity within 8 days, the transgenic plants appeared to be significantly more resistant to the infection. For a single, constitutively expressed foreign gene to confer disease resistance to a susceptible plant, there are three broad categories of possible mechanisms. First, the gene product itself, or an enzymatic product produced by it, might be directly active against the pathogen. Second, the gene could influence the expression of resistance in the host, either through direct (epistatic) effects on defense regulatory pathways, or as an indirect (pleiotropic) effect leading to the induction of defense pathways. Lastly, the gene product could provide some function that is compatible with the host's defenses, but is normally lacking in the incompatible interaction. When evaluated for fungal pathogens resistance, the transgenic lines displayed higher free polyamine as putrescine, spermine and spermidine as well as catalase, peroxidase and polyphenoloxidase activities than those of the non-transgenic control lines. It has been established that the biosynthesis of the polyamines: putrescine, spermidine and spermine, are tightly regulated and involved in the control of many biological processes such as cell growth and differentiation, gene transcription, translation in a wide range of organisms and in plant responses to stress (Walters, 2003). Von Ropenack and Schulze (1998) found that the polyamine conjugate, ρ-coumaroyl-hydroxyagmatine accumulated to significant levels in a resistant barley variety challenged with powdery mildew. Moreover, positive correlation between putrescine, spermine and spermidine concentrations with secondary metabolite production including free polyamines forms in the transgenic plants and the fungal pathogens resistance was shown. Polyamines conjugated to phenolic compounds, hydroxycinnamic acid amides (HCAAs), have been shown to accumulate in incompatible interactions between plants and a variety of pathogens, while changes in the diamine catabolic enzyme diamine oxidase suggest a role for this enzyme in the

production of hydrogen peroxidase during plant defense responses.

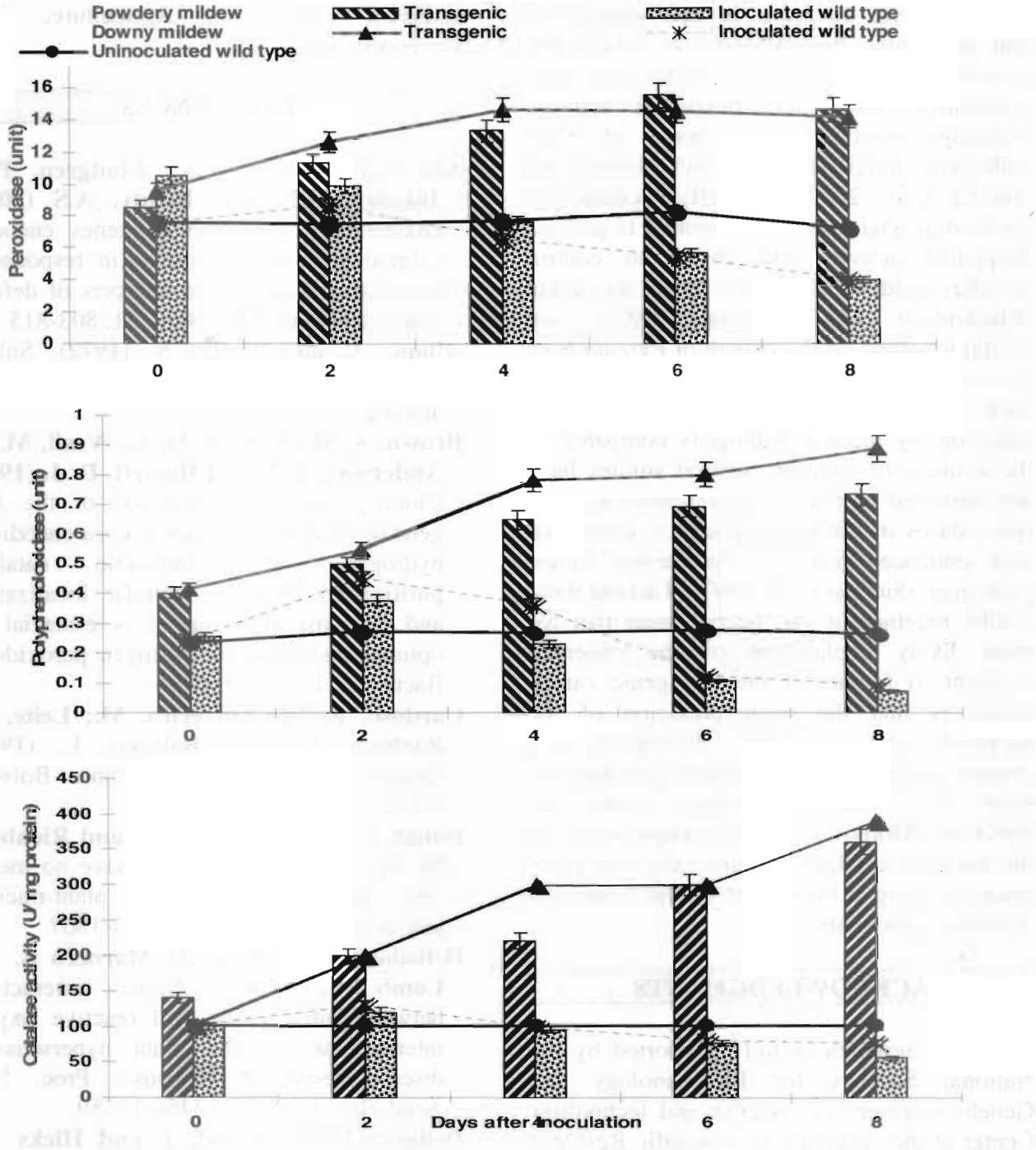

Fig (6): Peroxidase, pholyphenoloxidase and catalase activities in transgenic plants with the bacterial catalase (katE) gene and wild type canola inoculated with P. parasitica and E. polygonii.

(Walters, 2003). It is now established that polyamine biosynthesis can inhibit the growth of a wide range of fungi and these compounds also exert fungicidal activity, reducing infection by a range of plant pathogenic fungi (Haggag, 2005, Haggag and Abo-El Kair, 2007). Recently, commercial spermidine analogues were reported to possess fungicidal activity and ability to control powdery mildew infection in barley and apple (Mackintosh and Walters, 1997), and inhibitory effect on the growth of *Pyrenophora avenae* (Mackintosh *et al.*, 2001). Therefore, such leaves also exhibit greatly reduced infection by mildew pathogens compared to the control. In addition, several studies have demonstrated that overexpression of peroxidases in transgenic plants is associated with enhanced resistance to various fungal pathogens (Siegrist *et al.* 1997). Tacking these results together, it can be proposed that the most likely explanation of the observed resistant to fungus of the transgenic canola plants is that the overexpresseion of the bacterial catalase in the chloroplast may protect other enzymes, especially peroxidases, from the oxidative damages under the infection. Moreover, the overexpresseion of the bacterial catalase may help the transgenic plants to keep a high level of the fungicidal activity components.

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الملخص العربي

تحسين المقاومة للاصابة بالفطريات المسببة للبياض الدقيقي والبياض الزغبي في نباتات الكانولا المحولة وراثيا بجين الكتاليز البكتيري

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يلعب انزيم الكتاليز دوراً هاماً في نظام الدفاع الذاتي للنباتات ضد مسببات الامراض المختلفة مثل الفطريات والفيروسات والحشرات (الاجهادات الحيوية). تم في هذه الدراسة ادخال وتعبير الجين المسئول عن انتاج انزيم الكاتاليز في بكتيريا القولون (*KatE*) الي نباتات الكانولا وخضعت نباتات الجيل الثاني المحولة وراثيا لتجارب القياسات الحيوية (Bioassay) والعدوى الصناعية بالفطريات *Peronospora parasitica and Erysiphe polygoni* المسببة لمرضي البياض الزغبي والبياض الدقيقي لتقييم مدي مقاومتها للاصابة بهذه الفطريات. اوضحت صور المسح بالميكروسكوب الالكتروني وجود معدل اصابة اقل و عدد اقل من جراثيم كل من الفطرين في النباتات المحولة وراثيا مقارنة بالنباتات غير المحورة وراثيا .اوضحت نتائج التحليل الكيمياني وقياس النشاط الانزيمي انتاج النباتات المحولة وراثيا لتركيزات اعلي من بعض البولي فينولات الحرة مع زيادة نشاط انزيمات الكتاليز والبيروكسيديز والبولي فينول اوكسيديز بعد الاصابة بالفطريات ويحدث عكس ذلك في النباتات غير المحورة وراثيا. تشير هذة النتائج مجتمعة الي وجود تحسن معنوي واضح في مقاومة الاصابة بالبياض الزغبي والدقيقي في النباتات المحورة وراثيا بجين الكتاليز البكتيري مقارنة بالنباتات غير المحورة وراثيا.