# The Change of Peroxidase Activity in Three Cucumber Cultivars during Development of Powdery Mildew Infection

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## Abstract

A study carry out to determine the change of peroxidase activity throughout development of powdery mildew infection that caused by *Sphaerotheca fuliginea* to three cultivars of cucumber :Beit alpha ,Jabbar and Babylon. The relationship between peroxidase activity and susceptibility of cucumber cultivar to powdery mildew was investigated . The result showed that peroxidase activity in leaves of cucumber cultivars increased significantly after inoculating with *S. fuliginea*. Peroxidase activity were correlated positively with development of disease .The highest rate of peroxidase activity were after seven days from inoculation . Increase of peroxidase activity in cultivars Beit alpha ,Jabbar and Babylon were 202.7%, 108 % and 171 % in infected tissues respectively compared with that of control of same cultivar . Also peroxidase activity in Jabbar cultivar tissue significantly higher than other cultivars. The Results of sporulation (conidia and conidiophores) of *S. fuliginea* on leaves of cucumber cultivars indicated that all cultivars were susceptible to this fungus ,but there were variations in susceptibility of cucumber cultivars ,then Jabbar cultivar was significantly more susceptible than other cultivars , therefore number of conidiophores of *S.fuliginea* was 7.2 (conidiophore /$mm^2$ leaves) on Jabbar cultivar leaves ,its significantly higher than Beit Alpha and Babylon (4.7 and 4 conidiophores /$mm^2$ leaves) respectively.Also number of conidia of *S. fuliginea* on Jabbar leaves were higher than other cultivars .The result of conidia supported result of conidiophores that Jabbar cultivar more susceptible from other cultivars .

## الخلاصة

نفذت هذه الدراسة لتحديد التغير في فعالية إنزيم البيروكسديز خلال تطور الإصابة بمرض البياض ألدقيقي المتسبب عن الفطر *Sphaerotheca fuliginea* لثلاثة أصناف من الخيار هي Beit alpha و Jabbar و Babylon والعلاقة بين فعالية إنزيم البيروكسديز وحساسية الأصناف لمرض البياض ألدقيقي . أظهرت النتائج أن فعالية إنزيم البيروكسديز في أوراق أصناف الخيار تزداد بشكل معنوي بعد التلقيح بالفطر *S. fuliginea* . و فعالية إنزيم البيروكسديز ترتبط ارتباطا موجبا بتكشف المرض . وكانت اعلى فعالية للإنزيم بعد سبعة أيام من التلقيح . أن زيادة فعالية إنزيم البيروكسديز في الأصناف Beit alpha و Jabbar و Babylon كانت ٢٠٢% و١٠٨% و ١٧١% على التوالي في الأنسجة المصابة مقارنة بمعاملة المقارنة لنفس الصنف . كذلك فعالية إنزيم البيروكسديز في أنسجة صنف Jabbar أعلى بشكل معنوي من الأصناف الأخرى . أشرت نتائج التجرثم (الكونيديات والحوامل الكونيدية ) للفطر *S. fuliginea* على أوراق أصناف الخيار بان جميع الأصناف كانت حساسة لهذا الفطر ولكن هناك تباينات في حساسية أصناف الخيار إذ كان الصنف Jabbar أكثر حساسية بشكل معنوي من الأصناف لذلك كان عدد الحوامل الكونيدية للفطر ٧,٢( حامل كونيدي/ مليمتر مربع من سطح الورقة ) في الصنف Jabbar وهو أعلى بشكل معنوي من الصنفين Beit alpha و Babylon (٤,٧ و ٤ حامل كونيدي /مليمتر مربع من سطح الورقة على التوالي). كذلك عدد السبورات الكونيدي للفطر على أوراق الصنف Jabbar كانت أعلى من الأصناف الأخرى .كما أن نتيجة السبورات الكونيدية تسند نتائج الحوامل الكونيدية بان الصنف Jabbar أكثر حساسية من الأصناف الأخرى .

## Introduction

In general, plant defend themselves against pathogens by a combination of weapons from two arsenals : (1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and (2) biochemical reaction that take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant .The combinations of structural characteristics and biochemical reactions employed in the defense of plants are different in different host-pathogen systems. In addition , even within the same host and pathogen ,the combinations vary with the age of the plant ,the

kind of plant organ and tissue attacked ,the nutritional condition of the plant ,and the weather conditions(Agrios,2005)

Resistance is defined as an enhancement of the plants defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation.Pre-treatment of plants with a virulent pathogens (biotic inducers) or chemical compounds (a biotic inducers)can enhance resistance to subsequent attack not only at the site of treatment,but also in tissues distant from the initial infection sites.Typically,this inducible resistance system as systemic acquired resistance (SAR) is effective against diverse pathogens including viruses, bacteria and fungi (Ryals *et al*.,1996).Defense related genes encode a variety of proteins including enzymes controlling secondary metabolism, pathogenesis related proteins (PR) and regulatory proteins that control the expression of other defense related genes (Dixon *et al.*,1994).The defense gene products include polyphenol oxidase (PPO), peroxidase (POD) that catalyzes the formation of lignin, and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis.Other defense enzymes include pathogenesis-related proteins (PRs) such as β-1, 3-glucanases (PR-2 family) and chitinases (PR-3 family), which degrade the fungal cell wall and cause lysis of fungal cell. Chitin and glucan oligomers released during degradation of fungal cell wall act as an elicitor that elicit various defense mechanisms in plants ( Frindlender *et al.*, 1993). Induction of defense proteins makes the plant resistant to pathogen invasion (Van Loon , 1997), and has been correlated with defense against pathogen invasion in cucumber (Rasmussen, 1991).In present study a change of peroxidase activity after infection with powdery mildew in three cultivars of cucumber and its developing during time after infection with the pathogen were investigated, in addition the relationship between peroxidase activity and susceptibility of cucumber cultivar to powdery mildew .

## Materials and Methods

### Inoculation of pathogen

Isolate of *Sphaerotheca fuliginea* was obtained from single spore lesions on leaves of orka. Isolate were maintained *in-vitro* on the cotyledons of squash. Artificial infected leaves of squash were Intensely colonized areas with *S. fuliginea* were used as inoculation source.

### Detection of peroxidase activity

. Seeds of cucumber cultivars :Beit alpha ,Jabbar and Babylon were sown in plastic pots (15×15×10 cm diameter ) containing compost and soil(1:4) ,five seeds of each cucumber cultivar per pot with three replicate per each treatment were sown and kept at greenhouse condition(temperature 21±3,humidity 70%) .When the second leaf was fully expanded plants were placed in the settling tower(diameter 60 cm, height 80cm ) and inoculated by exposing them to an even distribution of *S.fuliginea* conidia from heavily infected leaves of squash with 6 leaf per 10 pots ,which had been shaken 6 h previously to remove old spores (Eyal *et al*.1968 ;Abood *et al*. 1992) . after 0, 2 and 7 days inoculation samplies 0.5 g from healthy and infected treatments leaves were taken, graded by Ceramic mortar with 2ml phosphate buffer (PH=6 , 0.1 M) and centrifuging at 6000 for 10 min.Mixture contained phosphate buffer(PH=6 , 0.1 M), quaiacol ( 0.05M) , $H_2O_2$ (0.02M) and distilled water with percentages 1:1:1:7.

2.8 ml from Mixture solution was added to cell spectrophotometer and initial of reaction with 0.2 ml plant extract . The activity of peroxidase was measured by Spectrophotometer through observation the change in light absorption at 420 nanometer /min/g fresh leaf tissues during 3 min. The spectrophotometer was adjusted by reaction mixture without root extract according to modified procedure ( Whitakar and Berhard, 1972).

**Sporulation (conidia and conidiophores) of *Sphaertheca fuliginea***

Seeds of cucumber cultivars as previous experiment were sown in plastic pots containing compost and soil(1:4),five seeds of each cucumber cultivar per pot with three replicate per each treatment in greenhouse .When the second leaf was fully, expanded plants were placed in the settling tower(diameter 60 cm , height 80cm ) and inoculated by exposing them to an even distribution of *Sphaerotheca fuliginea* conidia from heavily infected leaves of squash with 6 leaf per 10 pots ,which had been shaken 6 h previously to remove old spores (Eyal *et al.*1968 ;Abood *et al.* 1992).Five leaf discs(10mm in diameter ), one from the centre of the leaf and two from each half ,from corresponding leaves from each three replicate plants ,were sampled at 11day after inoculation by a cork borer .Leaf discs were placed in vials containing 5ml fixative solution (ethanol :acetic acid :formalin 8:1:1volume) and shaken to detach the conidia ,which were counted with the aid of a haemocytometer ,using the procedure of Bashi and Aust (1980). Number of conidiophores were counted in later experiments to provide an assessment of sporulation .Leaf discs sampled from the positions described above were cleared and used stained in trypan blue (5g/l) (Carver and Carr,1977) then examined using ×10 objective .The number of conidiophores that crossed atransect ,provided by the scale line of an eyepiece graticule were recorded .

**Statistical analysis**

All experiments were designed complete randomized design and some data analyzed by using least squares analysis of variance (ANOVA), least significant difference (L. S. D.) test was used in some data at the 1% and 5% levels of significance (Steel and Torrie,1960) and other by regression of Excel program 2003 .

## Results

**peroxidase activity in three cucumber cultivars during 0 , 2 and 7 days after the infection with powdery mildew**

The result showed that peroxidase activity in leaves of cucumber cultivars increased significantly after infection with powdery mildew disease compared with control treatments(Fig.1,Fig.2&Fig.3). These are increases of peroxidase activity were varied according to a time after inoculation with *Sphaerotheca fuliginea* ,then the highest activity of peroxidase were after seven days of inoculation ,but increase of peroxidase activity in cultivars Beit alpha ,Jabbar and Babylon were 202.7%, 108 % and 171 % in infected tissues respectively compared with control of same cultivar . Also peroxidase activity in Jabbar cultivar tissue significantly higher than other cultivars (Table 1).

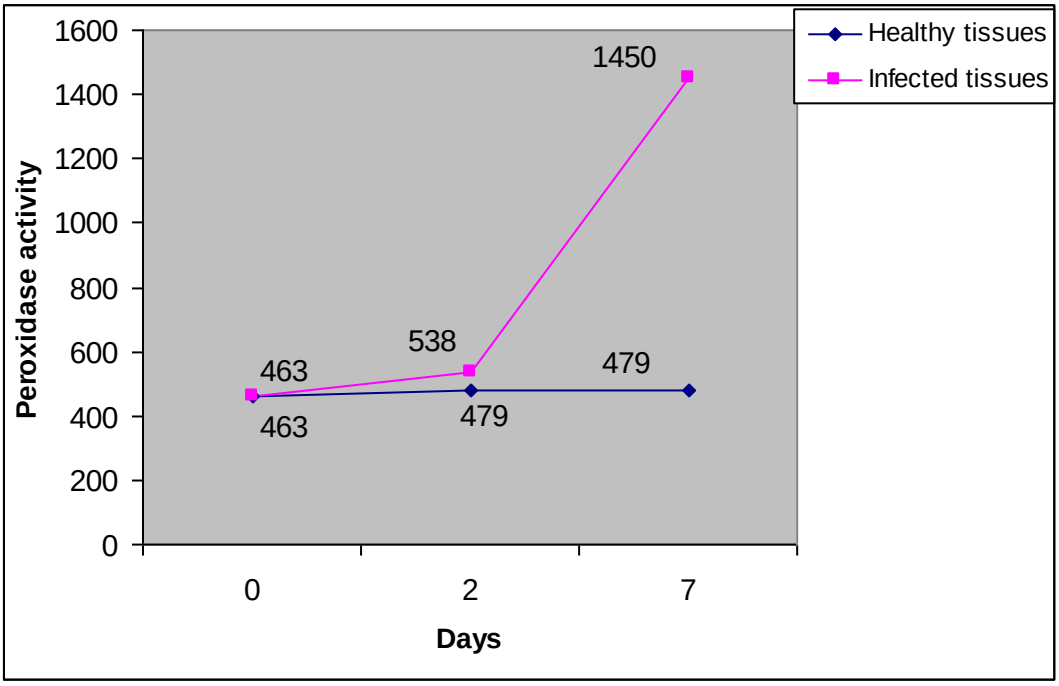


**Fig.1.Peroxidase activity in cucumber leaves of Beit Alpha cultivar after 0,2 and 7 days of inoculation with powdery mildew .**

L.S.D.$_{0.01}$= 231

*Each number is mean of trireplicate

****** Peroxidase activity was calculated on the basis of light absorption at 420 nanometer/min/g fresh leaf tissues

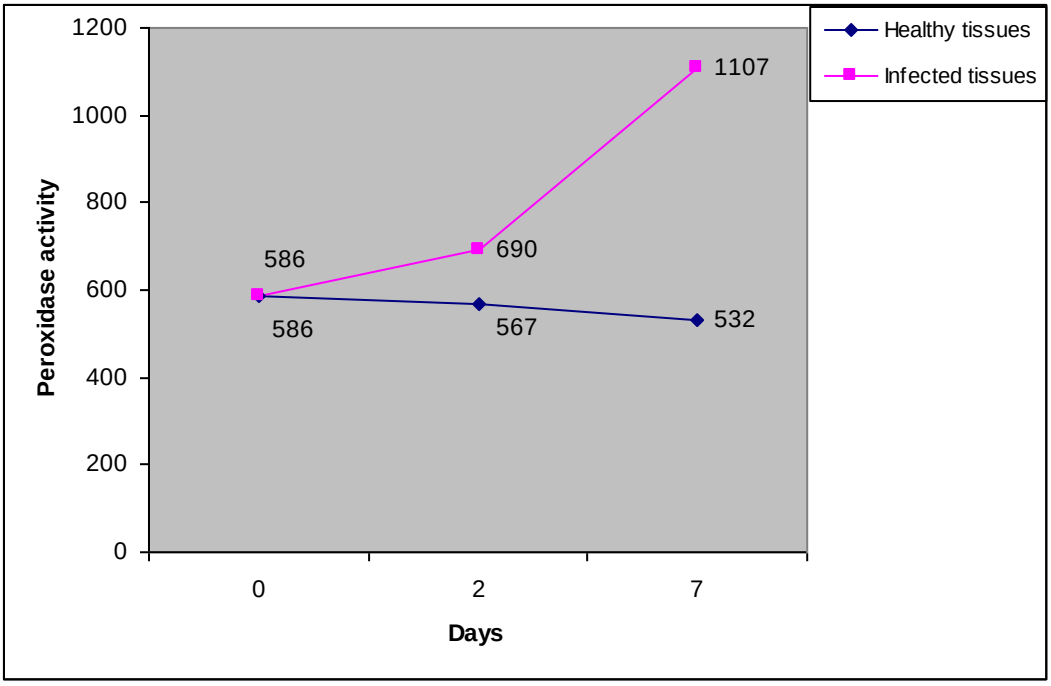


**Fig.2.Peroxidase activity in cucumber leaves of Babylon cultivar after 0,2 and 7 days of inoculation with powdery mildew .**

L.S.D.$_{0.01}$= 168

*Each number is mean of trireplicate

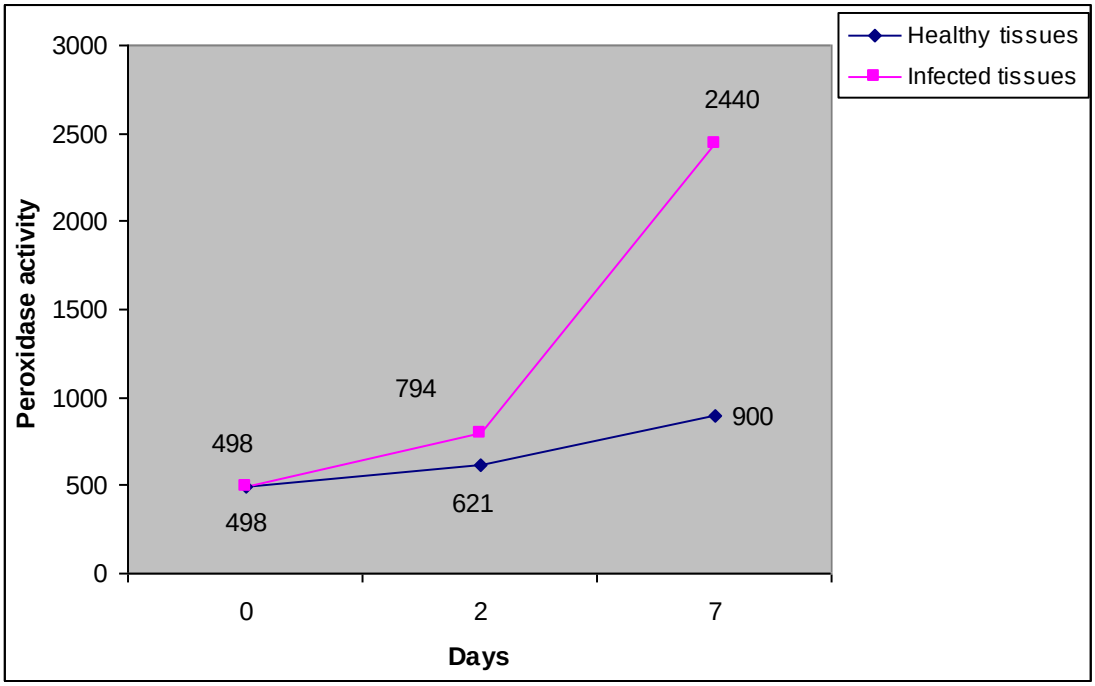


**Fig.3.Peroxidase activity in cucumber leaves of Jabbar cultivar) after 0,2 and 7 days of inoculation with powdery mildew .**
**L.S.D.$_{0.01}$= 353**
*Each number is mean of trireplicate

**Table.1. Peroxidase activity in leaves of three cucumber cultivars tissues After 7 days of inoculation with** *Sphaerotheca fuliginea.*

| Cultivars | Plant tissues | | Means |
|---|---|---|---|
| | Healthy tissues | Infected tissues | |
| Beit Alpha | 479 | 1450 | 965 |
| Babylon | 532 | 1107 | 820 |
| Jabbar | 900 | 2440 | 1670 |
| Means | 637 | 1666 | |

L.S.D. 0.01cultivar = 268
L.S.D. 0.01 infection=219
L.S.D.0.01 Interaction =379
*Each number is mean of trireplicate

The Results of sporulation (conidia and conidiophores) of *Sphaertheca fuliginea* on leaves of cucumber cultivars indicate that all cultivars were susceptible to infection with powdery mildew ,but there were variations in susceptibility of cucumber cultivars toward powdery mildew infection ,then Jabbar cultivar was significantly more susceptibliy than other cultivars ,therefore number of conidiophores of *S.fuliginea* was 7.2 (conidiophores /mm$^2$ leaves) on Jabbar cultivar leaves ,its significantly higher than Beit Alpha and Babylon (4.7 and 4 conidiophores /mm$^2$ leaves respectively)(Fig.4).Also number of conidia of *S. fuliginea* on Jabbar leaves were higher than other cultivars(Fig.5) .The result of conidia supported result of conidiophores that Jabbar cultivar more susceptible from other cultivars .

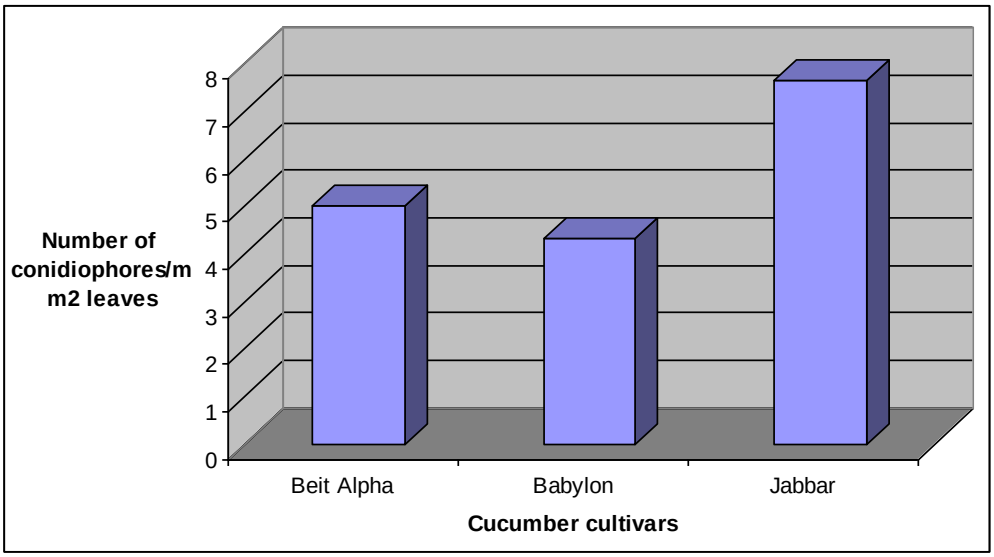


Fig.4.Number of conidiophores of *Sphaertheca fuliginea* per $mm^2$ leaves of cucumber cultivars after 11days of inoculation with *S. fuliginea*.

L.S.D.0.01=1.8

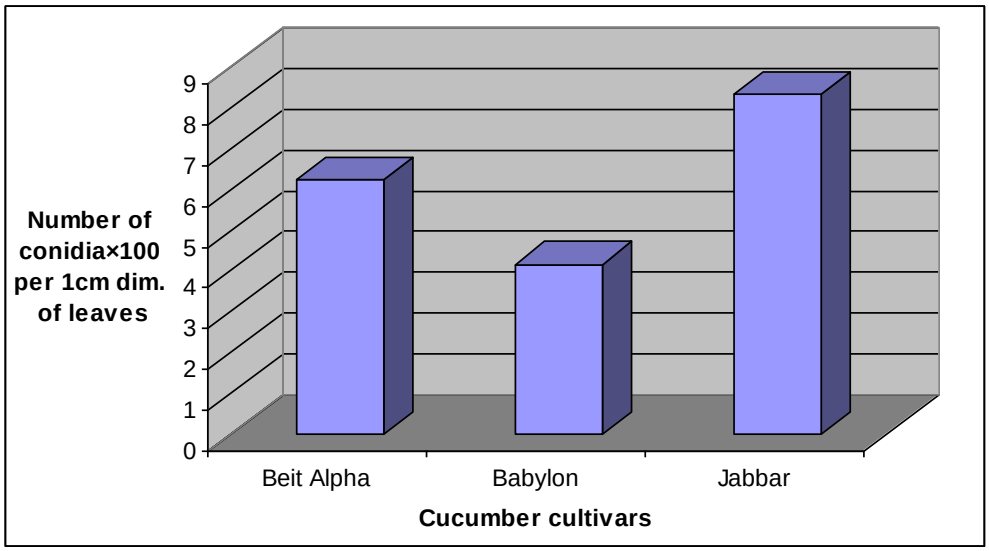


Fig.5.Number of conidia of *Sphaertheca fuliginea* per disc (1cm dim.) leaves of cucumber cultivars after 11days of inoculation with *S. fuliginea***.**

## Discussion

The findings of this study supported the results of previous studies that peroxidase (POX) are important enzyme involved in plant defense system, and its activity increases by the attack of pathogens and injuries (Avdiushko *et al.* 1993; Slusarenko 1996). Induction of defense proteins makes the plant resistant to pathogen invasion (VanLoon , 1997), and has been correlated with defense against pathogen invasion in cucumber (Rasmussen, 1991).The variation of peroxidase activity changes in cucumber cultivars after infection with *Sphaertheca fuliginea* a specially after seven days from inoculation with the pathogen may be attributed to much of mycelium of *S. fuliginea* in tissues of more susceptible cultivar (Jabbar cultivar) more than that of little susceptible cultivar as Babylon cultivar or may be attributed to many of peroxidase isoenzyme in tissues of more susceptible cultivar after infection more than that of little susceptible cultivar.

The anti-fungal properties of peroxidases are not well characterized, although their oxidative products such as indoles, oxygen radicals and halides are well described as having cytotoxic effects on host cells and pathogens (Nappi and Ottaviani, 2000).
The mechanism of peroxidase cytotoxic activity may be direct or indirect. The commercial horseradish peroxidases and partially purified sea fan peroxidases may be directly toxic to *A. sydowii* in culture. Caruso *et al.* (2001) isolated a basic peroxidase 36 kDa in size, from wheat kernels, that slowed growth in three fungal species. Their experiments demonstrated that the peroxidase inhibited germ tube elongation and conferred direct anti-fungal activity. Alternatively, the oxygen radicals generated by peroxidase may indirectly inhibit the fungus. In tobacco plants peroxidase-generated hydrogen peroxide was shown to prevent germination of fungal spores *in vitro* (Peng and Kuc, 1992).This result revealed that peroxidase activity could be a good indicator for plant cultivar resistance to disease .

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