AGING EFFECT TO ACCUMULATION OF CAMALEXIN IN *Arabidopsis thaliana* AFTER ELICITATION BY SILVER NITRATE

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ABSTRACT

The effect of plant age to accumulation of camalexin after elicited by silver nitrate in *Arabidopsis thaliana* was studied. Four, six, eight and 11-week plants were elicited by silver nitrate as an abiotic elicitor to induce the production of camalexin. Camalexin is a secondary metabolites compound that plays a role in *Arabidopsis* defence system. The camalexin was detected on TLC plate with a bright bluish purple fluorescence and has a maximum absorbance at 318 nm in spectrophotometer. The camalexin accumulation was significantly increased from week four to week eight. However, the camalexin concentration was decreased in week eleven. There is a strong relationship between plant age and accumulation of camalexin (R^2= 0.93632, $p<0.05$). Result from this study indicated that camalexin concentration decreased with aging when some healthy leaves became yellow indicating the senescence started. The data also suggested the accumulation of camalexin appeared to be dependent on plant age itself.

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ABSTRAK

Kesan umur pokok terhadap pengumpulan kamaleksin dalam *Arabidopsis thaliana* telah dikaji. Larutan silver nitrat telah disembur pada pokok dengan umur empat, enam, lapan dan 11 minggu untuk mengaruhkan penghasilan kamaleksin. Kamaleksin adalah kompaun metabolit sekunder yang memainkan peranan penting dalam sistem pertahanan tumbuhan. Kehadiran kamaleksin dikesan pada kepingan TLC dengan warna fluorensen iaitu ungu kebiruan terang dan mempunyai nilai serapan maximum pada 318 nm di bawah spektrofotometer. Pengumpulan kamaleksin adalah meningkat secara signifikan dari minggu keempat hingga minggu kelapan. Walaubagaimanapun, kepekatan kamaleksin menurun bagi minggu kesebelasan. Terdapat hubungan yang amat kuat antara umur pokok dan kepekatan kamaleksin (R^2= 0.93632, $p<0.05$). Keputusan dari kajian ini menunjukkan bahawa kepekatan kamaleksin berkurangan apabila pokok semakin berumur di mana beberapa daun sihat menjadi kekuningan menunjukkan proses penuaan telah pun bermula. Data juga menunjukkan bahawa pengumpulan kamaleksin adalah bergantung kepada umur pokok itu sendiri.

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LIST OF SYMBOLS

μg	Microgram
ml	Millilitre
$>$	Is greater than
$<$	Is less than
%	Percent
$=$	Equal
R^2	Regression Square
R_f	Retardation factor
A	Absorbance
ε	Extinction coefficient
$\int$	Sample path length
c	Concentration

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LIST OF ABBREVIATIONS

$AgNO_3$	Argentum nitrate
ANOVA	Analysis of variance
Cu (II)	Cuprum (II)
Fe	Ferum
H_2O_2	Hydrogen peroxide
IAA	Indole-3-acetic acid
IAO_X	Indole-3-acetaldoxine
IPM	Integrated Pest Management
NO_3	Nitrogen oxide
PDA	Potato Dextrose Agar
ROS	Reactive oxygen species
TLC	Thin Layer Chromotography
UV	Ultraviolet
Trp	Tryptophan
ARR	Age-Related Resistance

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LIST OF FORMULAS

3.1 Retardation factor

$R_f = a / b$

3.2 Beer-Lambert Law

$A = \varepsilon \int c$

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CHAPTER 1

INTRODUCTION

1.1 Preface

Crop products are main source of food supply for human. With the increasing of human capacity every year, the requirement for food from various crops such as paddy, corn, oat, potato, groundnut and other potential crops dramatically increased. However, there is some problems encounter in crop production that eliminates the production of healthy crops in a large quantity. The main problem is the poor production of crops caused by the various pathogens such as fungi, bacteria and viruses either infects plant in field or in postharvest storage.

In general, infected plants exhibit a various type of symptom, severity and often lead to the death. The symptom basically can be visually recognized through the appearance of necrosis, wilt, blight, canker and root rot on the plant part. This infected plant either caused by environmental stress or pathogen caused severe losses to human through the reduction of food supply, thus lead to starvation in certain country. An example to the tremendous impact of plant disease in human history is the potato late

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blight caused by the fungus *Phytophtora infestans* (Mehrotra and Aggarwal, 2003). Irish who had to depend on potatoes as their major source of food suffer from starvation for seven years from 1840 to 1847 and lead to the death of million people caused by this damage. Furthermore, million people are also force to migrates to other countries for survive. Direct economic losses through reduction of product price and quantities have been suffered among growers, thus affect the consumer food supplies (Mehrotra and Aggarwal, 2003).

Plant disease phenomenon contributes to the study of plant pathology where the defence mechanism is discovered in order to control the disease and economic losses. The studies of plant pathology provide information especially in plant defence mechanism. As the plants get infected, they will coordinate the rapid (active) and slower (passive) defence against invasion of pathogen, thus limit the pathogen growth in host plant. However, some defence response unable to inhabit the pathogen growth and contribute to severity and losses, indicating that pathogen has potential to infect even the plant is not its host (Agrios, 2005).

Severity and losses can be reduced when the growers recognized the disease symptom earlier. Early detection on infected part provides further curative action such as application of fungicide, pull out or burn the infected plant. However, spraying fungicide to infected plant can cause toxin problem as it always apply in high volume. Even by pulling out and burn the infected plant actually do not 100 percent terminated the disease (Agrios, 2005). The disease penetration will be occur to other plants as well as before the symptoms appear to that plant. Because the incubation and disease appearance may take a long period due to gene expression activities, it is difficult for

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us to identify which plant are already infected. Late detection may lead to the large damage or severity of the crops area. When this happen, lots of money will looses in a short while.

Now we really know that studies in plant disease is important in order to control and terminate the disease. We do not want starvation occur again just because of our failure in developing healthy crops in the future. To achieve this aim, widely studies on plant defence mechanism under variety of condition are necessary in order to increase the resistance of plant.

One of the fewest studies is the effect of aging in plant defence mechanism which is commonly known as age-related resistance. This age-related resistance is defined as the ability of whole plants or plant part to resist or tolerate disease as they matured (Ficke *et al.,* 2002). The level of resistance developed during plant aging may greatly affect disease severity and may even lead to lower susceptibility. The mechanism of age-related resistance was poorly understood even there is a few approach on it (Kus *et al*., 2002, Kim *et al*., 1989, Hwang *et al*., 1983 and Chongo and Gossen, 2001). Therefore, more study on this aging effect to plant defence response should be done especially in production of secondary metabolites compound, phytoalexin which is accumulated in plant cell in order to inhibit the infection and pathogen growth (Paxton, 1981). The gained knowledge would allow a better prediction of aging effect on disease progress, thus helps aligned the control measures to reduce the risk of yield loss. Furthermore, the knowledge may utilize in devising the strategies in Integrated Pest Management (IPM) in order to improve plant disease management especially in chemical input (Ficke *et al.,* 2002). For example in

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fungicide management where the timing of the fungicide application can be align between crops susceptibility based on the quantification of phytoalexin accumulated in plant cell during aging. Thus helps growers to reduce the frequency of fungicide application.

This aging effect to defence responses will be investigated in *Arabidopsis thaliana* which is known as a plant model system with a small amount of genetic material. Its relative ease in genetic manipulating and mutational analysis compared to other plant, provide some defence strategies that can be applied in economically important plants especially crops. For example, ones may be able to move useful defence genes from *Arabidopsis* which is responsible in resistance against certain fungal into plants that lack them (Glazebrook *et al*., 1997). Because of the time consuming and difficulties in manipulating genes for many crops species, it is important to built a simple model for plant defence system and it is only can be achieved through studies on *Arabidopsis thaliana* which has been completely sequenced in December 2000 (Mirza, 2001). Furthermore, an age-related resistance (ARR) model also can be established by using *Arabidopsis thaliana* which the information can then be applied to valuable crops especially in fungicide management.

1.2 Objective

The objective of this study is to quantify the amount of camalexin accumulated in *Arabidopsis thaliana* at different stage of growth after elicitation by silver nitrate.

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CHAPTER 2

LITERATURE REVIEW

2.1 *Arabidopsis thaliana*

Arabidopsis thaliana is a small cruciferous plant that has received considerable attention as a model plant system for investigating many aspect of higher plant biology. Its genetic system is significantly more tractable than those of other plant species and *Arabidopsis* exhibits all the major kinds of defence responses described in other plants (Glazebrook *et al.*, 1997). This small plant known commonly as mouse-ear cress, thale cress or wall cress (Wiersema and Leon, 1999) and has ability to self-fertilising diploid. It will produce large amount of seed approximately 10 000 seed or more per plant with a small genome size and is therefore well suited for genetic studies and molecular biology (Davis *et al.*, 1989). *Arabidopsis thaliana* is a member of mustard (Brassicaceae) family which includes cultivated species such as cabbage and radish. There are many ecotypes of *Arabidopsis thaliana* used in plant biological studies such as Columbia (Col-0), Wassilewskija (Ws-1), Niederzenz (Nd-1), (Merk-Turk *et al.*, 2003), Argentat (Ag-0), Brunn (Br-0) and Bayreuth (Bay-0) (Denby *et al.*, 2004).

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2.1.1 Scientific classification of *Arabidopsis thaliana*

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Dilleniidae

Order : Capparales

Family : Brassicaceae

Genus : *Arabidopsis* Heynh.

Species : *Arabidopsis thaliana* (L.) Heynh

(United States Department of Agriculture, http://plants.usda.gov.html)

2.2 Plant defence mechanism

Plants have been exposed to various threats from outside environment throughout their life cycle. Almost each plant is affected by different kinds of pathogenic microorganism such as fungi, bacteria and viruses thus trigger a defence response including the synthesis of reactive oxygen species and signal molecules such as salicylic acid, accumulation of antimicrobial compound and pathogenesis-related (PR) proteins. As plants are sessile, they have to develop a broad range of defence or stress response to protect themselves against biotic (fungus, bacterias and viruses) and abiotic stress (pesticides, heavy metal pollution, and excess fertilizer). All major

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aspects of plant defence response that have been described in other plant hosts have also been observed in *Arabidopsis* (Glazebrook e*t al*., 1997).

In general, plant can evoke one, two, many or even the combination of defence mechanism in an attempt to survive by restricting the pathogen growth and finally to destroy it (Agrios, 2005). The invading of pathogen in plant cell actually will activate a rapid and slower defence response that implicated in resistance. The rapid response occurs within minutes to a few hours and is not required gene transcription for their occurrence. One of the rapid responses is oxidative burst mechanism that releases the reactive oxygen species (ROS). Oxidative burst may function as a first line of defence in resistance plants by directly attacking the pathogen during earliest stages of infection (Strange, 2003). Enormous number of ROS such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) could serve as a signal to activate others defence response including hypersensitive reaction (HR) and phytoalexin (Wojtaszek, 1997). Others rapid response are generation of NO, cross-linking of cell wall protein and callose synthesis and deposition (Strange, 2003).

HR response, phytoalexin accumulation, pathogenesis related (PR) proteins and systemic acquired resistance (SAR) are among the slower response that required gene transcription and protein synthesis. Even though these defence responses are slow but they plays important role to inhibit and finally remove the pathogen. However, different plants will defence themselves against pathogen in different ways (Strange, 2003). *Arabidopsis* responds to pathogen attack by activating chemical and enzymatic defences and by displaying both gene-for-gene and systemic acquired resistance (Glazebrook *et al*., 1997).

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In general, plant defence responses can be divided into three mechanisms; the non-host resistance, host resistance and gene-for-gene resistance. These resistances directly or indirectly controlled by genes of host plant and the pathogen (Agrios, 2005).

2.2.1 Non-host resistance

Non-host resistance occur when the plant resists with the pathogen which the plant is not the host. This resistance is most common form of resistance in nature and represent the unsuccessful of plant-pathogen interaction. The non-host resistance is observed when all members of plant species exhibit resistance against all members of a pathogen species, thus indicate this type resistance is a non-specific resistance (Agrios, 2005). Furthermore, when the pathogen doest not recognised the plant as one of its host plants, it may not become attached to the plant or may not produce infection substance such as enzymes, appresoria, penetration pegs or haustoria. Lack of recognition factor basically leads to non-host resistance (Mehrotra and Aggarwal, 2003).

However, there are also some cases where the pathogen infects the non-host plant. For example, *Botrytis cinerea,* a major pathogen of grapes, pears, berries and vegetables infect the *Arabidopsis thaliana* and thus induced the camalexin to resist (Denby *et al*., 2004). This shows that non-host resistance in some pathogen-microbe interaction does not necessary need the recognition events but depends on genes carried by plant, which provide them with fully effective non-specific defence against pathogen (Agrios, 2005).

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2.2.2 Host resistance

Host plant resistance is a specific resistance, occur when the plant resists against pathogen which the plant is the host. The host plant resistance basically appears to be governed by a single or a small number of related genes encode the formation of compound that inhibits the advance invasion of pathogen. The pathogen recognised the host and try invading into the cell and soon disintegrates. The production of this compound was triggered by non-specific elicitors such as toxin, glycoprotein, carbohydrates, fatty acids, peptides or extracellular microbial enzymes that have been released from pathogen (Agrios, 2005).

Most of the plant will defence against their pathogen as described by the infection of *Alternaria brassicicola* to cruciferous species. A necrotrophic fungus *Alternaria brassicicola* interact with *Brassica* crops thus required specialized defence response in order to successfully eliminate the invasion (Kagan and Hammerschmidt, 2002). Highly specialized defence response is necessary against biotrophic fungi such as *Cochliobolus carbonum* (Kagan and Hammerschmidt, 2002) and *Peronospora parasitica* (Mert-Turk and Holub, 2002) which significantly infect *Arabidopsis* plant. This defense response against biotrophic pathogen required the presence of one or more resistance *(R)* genes or triggers a series of defence response such as hypersensitive response (HR), race specific or vertical resistance.

There are two types of host resistance; horizontal resistance, also called quantitative, polygenic or partial resistance and vertical resistance, also called race specific, R gene resistance or monogenic resistance. Vertical resistance is complete

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resistance to certain races where it depends on the race of the pathogen used to infect the variety of plant. The variety of plant may appear strongly resistance to one pathogen race and susceptible to another race. While horizontal resistance is partial resistance against all races pathogen where the plant just slow down the pathogen invasion (Agrios, 2005).

2.2.3 Gene-for- gene interaction

Compatible and incompatible are the two terms that are used to describe and understanding the gene-for-gene interaction. This compatibility and incompatibility are under the genetic control of both host and pathogen (Strange, 2003). When a pathogen interacts with the potential host, it may successfully colonize the host and cause disease in which case the pathogen is said to be virulent, the host is susceptible and the interaction is compatible. Alternatively, the plant may respond to the pathogen by rapidly activate the defence responses which interfering with the pathogen multiplication and thus prevent from disease. In this case, the pathogen is said to be avirulent, the host is resistant and the interaction is incompatible (Glazebrook *et al*., 1997).

Generally, but not always, gene for resistance in host is a dominant (*R*) where genes for susceptibility are recessive (*r*). While in pathogen, gene for avirulence is dominant (*Avr*) and recessive (*avr*) for virulence (Zhou *et al*., 1999; Agrios, 2005; Glazebrook *et al*., 1997). Plant will resist through incompatible interaction where the dominant gene correspond with others dominant gene. In other words, every allele specifying resistance in the host will correspond with the allele specifying avirulence

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REFERENCES

Agrios, G.N. 2005. *Plant Pathology*. 5th ed. Elsevier Academic Press, United States.

Bassey, J.S.E. 1998. *Basic Calculation for Chemical and Biological Analyses*. AOAC International, United States American.

Browne, L.M., Conn, K.L., Ayer, W.A.,and Tewan, J.P. 1991. The camalexin: New phytoalexins produced in the leaves of *Camelina sativa* (Cruciferae). *Tetrahedron.* **24** (47): 3909-3914.

Carnegie, S.F., and Colhoun, J. 1982. Susceptibility of potato leaves to Phytophora infestan in relation to plant age andleaves position. *Phytopathology Z.*, **104**: 157-167.

Chong, K.P. 2005. Role of phytoalexin in *Arabidopsis thaliana* and *Brassica oleracea* against *Botrytis cinerae*. *Master's Thesis*, Imperial College London.

Chongo, G., and Gossen, B.D. 2001. Effect of plant age on resistance to *Ascochyta rabiei* in chickpea. *Can. J. plant Pathol*. **23**:358-363.

Daniel, M., and Purkayastha, R.P. (edt.). 1995. *Handbook of Phytoalexin Metabolism and Action.* Marcel Dekker Inc., United State of America.

Davis, K.R., Schott, E., Dong, X., and Ausubel, F.A. 1989. *Arabidopsis thaliana* as a model system for studying plant-pathogen interactions. In: Lugtenberg, B.J.J. (edt.) *Signal Molecules in Plants and Plant-Microb Interaction.* Springer-Verlag Berlin Heidelberg, Germany.

Denby, K.J., Kumar, P., and Kliebenstein, D.J. 2004. Identification of *Botrytis cinerea* susceptibility loci in *Arabidopsis thaliana*. *The Plant Journal*. **38**: 473-486.

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Devlin, W.S., and Gustine, D.L. 1992. Involvement of the oxidative burst in phytoalexin accumulation and the hypersensitive reaction. *Plant Physiology.* **100**: 1189-1195.

Dzurilla, M., Kutchy, P., Zaletova, J., Ruzinsky, M., and Kovacik, V. 2001. Synthesis of camalexin. *Molecules*. **6**:716-720.

Essenberg, M. 2001. Prospect for strengthening plant defences through phytoalexin engineering. *Physiological and Molecular Plant Pathology*. **59**: 71-81.

Ficke, A., Gadoury, D. M., and Seem, R.C. 2002. Ontogenic resistance and plant disease management : A case study of grape powdery mildew. *The American Phytopathology Society*. **92** (6): 671-675.

Glawischnig, E., Hansen, B.G., Olsen, C.E., and Halkier, B.A. 2004. Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in *Arabidopsis*. *Proceeding of The National Academy of Science of The United States of America (PNAS)*. **101** (21): 8245-8250.

Glazebrook, J., and Ausubel, F.M. 1994. Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interaction with bacterial pathogens, *Proc. Natl. Acad. Sci. USA*. **91**. 8955-8959.

Glazebrook, J., Rogers, E. E., and Ausubel, F.M. 1996. Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics* **143**: 973-982.

Glazebrook, J., Rogers, E. E., and Ausubel, F.M. 1997. Use of *Arabidopsis* for genetic dissection of plant defense responses. *Annu. Rev. Genetics* **31**: 547-569.

Grene, R. 2002. Oxidative stress and acclimation mechanism in plants. In: *The Arabidopsis Book*. American Society of Plant Biologist, United States.

UMS
UNIVERSITI MALAYSIA SABAH

Hammerschmidt, R., Tsuji, J., Zook, M. and Somerville, S. 1993. A phytoalexin from *Arabidopsis thaliana* and its relationship to others phytoalexins of crucifers. In. Davis, K.R., Hammerschmidt, R. (edt.). *Arabidopsis thaliana as a Model for Plant-Pathogen Interaction.*

Hashmi, M.H., Abdus, S. A., Chughtai N.A., Chughtai F.R., and Shahid, M.A. 1968. Identification og twenty amino acids by circular Thin Layer Chromotography, *Mikrochimica Acta*. 291-296.

Hwang, B.K. 1995. Effects of age-related resistance and metalaxyl on capsidiol production in pepper plants infected with *Phytophthora capsici.* In: Daniel, M., and Purkayastha, R.P. (edt) *Handbook of phytoalexin metabolism and action.* Marcel Dekker Inc, United States of America. Pg: 503- 523.

Hwang, B.K., and Heitefuss, R. 1982. Characterization of adult plant resistance of spring barley to powdery mildew (Erysiphe graminis f.sp.bordei):1. Race specificity and expression of resistance. *Pytopathology Z.*, **104**:167-178.

Hwang, B.K., Ibenthal, W.D., and Heitefuss, R. 1983. Age, rate of growth, carbohydrate and amino acid contents of spring barley plants in relation to their resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*). *Physiological Plant Pathology.* **22**:1-14.

Kagan, L.A., and Hammerscmidt, R. 2002. *Arabidopsis* ecotype variability in camalexin production and reaction to infection by *Alternaria brassicicola. Journal of Chemical Ecology*. **28** (11): 2121-2140.

Kim, Y.J., Hwang, B.K., and Park, K.W. 1989. Expression of age-related resistance in pepper plants infected with *Phytophtora capsici*. *Plant Disease*. **73**:745-747.

Kliebenstein, D.J., 2004. Secondary metabolites and plant/environment interaction: a view through *Arabidopsis thaliana* tinged glasses. *Plant, Cell and Environment*. **27**, 675-684.

UMS
UNIVERSITI MALAYSIA SABAH

Kus, J.V, Zaton, K., Sarkar, R., and Cameron. R.K. 2002. Age-related resistance in *Arabidopsis* is a developmentally regulated defence response to *Pseudomonas syringae*. *The Plant Cell.* **14**: 479-490.

Leopold, A.C. 1980. Aging and Senescence. In: Thimann, K.V. (edt.) *Senescence in Plants*. CRC Press Inc., Florida.

Mehrotra, R.S. and Aggarwal, A. 2003. *Plant Pathology.* 5th ed. McGraw-Hill, India.

Mert-Turk, F., and Holub, E.B. 2002. Camalexin is not required for the function of *RPP1* and *RRP13* resistance genes in *Arabidopsis thaliana* inoculated with *Peronospora parasitica*. *Journal of Cell and Molecular Biology*. **1**: 93-99.

Mert-Turk, F., Bennett, M.H., Mansfield, J.W., and Holub, E.B. 2003. Camalexin accumulation in *Arabidopsis thaliana* following abiotic elicitation or inoculation with virulent or avirulent *Hyaloperonospora parasitica. Physiological and Molecular Plant Pathology.* **62**: 137-145.

Mirza, B. 2001. *Arabidopsis* a model system for plant research has been completely sequenced. *OnLine Journal of Biological Sciences*. **1** (4): 298.

Miyake, H., Mitsuya, S., and Md. Shahidur, R. 2006. Ultrastructural effects of salinity stress in higher plants. In: Ashwani, A.K., and Takabe, T. (edt). *Abiotic stress tolerance in plants.* Springer, Netherlands. Pg 215-226.

Morel, J.B., and Dangl, J.L. 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death and Differentiation*. **4**: 671-683.

Munne-Bosch, S., and Alegre, L. 2002. Plant aging increases oxidative in chloroplast. *Planta*. **214**: 608-616.

Paxton, J.D. 1981. Phytoalexin- A working redefinition. *Journal of Phytopathology.* **101**: 106-109.

UMS
UNIVERSITI MALAYSIA SABAH

Persley, D. 1994. *Disease of vegetables crops*. Department of Primary Industries, Queensland. Pg: 88.

Schutzendubel, A., and Polle, A. 2002. Plant responses to abiotic stress: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany*, **53** (372): 1351-1365.

Soylu, S. 2005. Accumulation of cell-wall bound phenolic compounds and phytoalexin in *Arabidopsis thaliana* leaves following inoculation with pathovars of *Peudomonas syringae. Plant Science* **170**: 942-945.

Soylu, S., Benett, M.H., and Mansfield, J.W. 2002. Induction of phytoalexin accumulation in broad bean (*Vicia faba* L.) cotyledons following treatments with biotic and abiotic elicitors. *Turk J. Agric For*. **26**: 343-348.

Strange, R.N. 2003. *Introduction to Plant Pathology.* John Wiley & Sons Ltd, England.

Subba , R. P.V., Wadia, K.D.R., and Strange, R.N. 1996. Biotic and abiotic elicitation of phytoalexin in leaves groundnut (*Arachis hypogea* L.) *Physiological and Molecular Plant Pathology.* **49**: 343-357.

Thomma, B.P.H.J., Nelissen, I., Eggermont, K., and Broekaert, W.F. 1999. Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola. The Plant Journal*. **19** (2) : 163-171.

Tsuji, J., Jackson, E.P., Gage, D.A, Hammerschmidt, R., and Somerville S.C. 1992. Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae pv syringae. Plant Physiology.* **98**: 1304 -1309.

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United States Department of Agriculture. 3/10/2006. Natural Resources Conservation Service, USA. http://plants.usda.gov.html.

Wiersema, J. and Leon, B. 1999. *World Economic Plants : A Standard reference*. CRC Press LLC, United States.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. *Biochem J.* **322** : 681-692.

Zhao, J., Williams, C.C., and Last, R.L. 1998. Induction of *Arabidopsis* tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress, and an abiotic elicitor. *The Plant Cell.* **10**: 359-370.

Zhou, N., Tootle, T.L., and Glazebrook, J. 1999. *Arabidopsis PAD3*, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. *The Plant Cell.* **11**: 2419-2428.

Zook, M. 1998. Biosynthesis of camalexin from tryptophan pathway intermediates in cell-susupension cultures of *Arabidopsis*. *Plant Physiol.* **118**: 1389-1393.

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