Mycopath (2011) 9(2)**:** 95-104

Research Article

Induction of systemic defenses in plants under the activity of dynamic inducers

Sobiya Shafique[1], Tehmina Anjum[1], Shazia Shafique[1], *Aqeel Ahmad[1], Waheed Akram[1] and Zoobia Bashir[2]

[1]Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan.
[2]Department of Physics, Bahauddin Zakariya University, Multan.
**Corresponding author's e. mail*: *aqeelahmad1@gmail.com*

Abstract

It's a commonly known phenomenon that the physical and biotic factors modulate the metabolic pathways in plants. They result into triggering of defense responses in plants against detrimental factors i.e. physical stresses or pathogens. This phenomenon is collectively called as resistance induction and the agent responsible for this phenomenon is termed as resistance inducer or inducer. Resistance induction varies greatly with reference to types of inducers and their mode of interaction. This is entirely dependent upon the plant inducer combination and other physical factors prevailing in the local environment i.e. radiations temperature etc. Resistance has been induced either by using biotic or abiotic stimuli. Variation of abiotic factors such as temperature, radiations and chemicals may induce a number of defense compounds in plants. Biotic factors namely bacteria, fungi and nematodes have also potential to alter biochemical profile of plants. All these factors directly alter the rate of transcription and translation processes in plant cells. A number of studies have concluded that during stress conditions, transcription of genes coding for defence related enzymes and chemicals have been accelerated significantly.

Keywords: Biochemical profile, induced systemic resistance, metabolic modulation, physical inducers, systemic acquired resistance.

Introduction

Plants generally have some constitutive (pre-existing) defense mechanisms against their pathogens. Plants having strong basal defenses have quick energy production and transmission systems. These defenses can be triggered with the help of external stimuli, called induced resistance. Induced resistance may be Systemic acquired resistance (SAR) or induced systemic resistance (ISR). They precondition plant internal environment for better cope against pathogen and result into disease suppression in a specific host. SAR is hormonally produced throughout the plant body (Ryals *et al.,* 1996; Durner *et al.,* 1997; Sticher *et al.,* 1997). It generally enhances both the quantitative and qualitative defenses of plants and is triggered by weak or incompatible pathogens (Kuc, 1990) or by chemical inducers (Kessmann *et al.,* 1994). These elevated defenses have a substantial importance in plant disease control and post-harvest pathology. Here is the brief view of scientific studies conducted to discover IR and its impact and it will lead researchers towards better understanding and development of resistance in plants of human interests.

Resistance induction

Defense responses or resistance can be induced in plants by using chemical (Porat *et al.,* 2002; Venditti *et al.,* 2005), physical (Arcas *et al.,* 2000; Ben-Yehoshua *et al.,* 2003), antagonistic microbes (Fajardo *et al.,* 1998; Droby *et al.,* 2002) and avirulent strains treatments (Anwar *et al*., 2000; Anwar et al., 2008). Extent of induced resistance depends upon the type of treatment, dose of treatment, age of plant and method of treatment. Plants can also be induced for increased cellular defenses when their physical barriers have been breached by invading pathogens (Ballester *et al*., 2010). The understanding of mechanisms of resistance induced may helpful for developing alternative methods of disease control.

Defense reactions which are induced by some stimulus are termed as active defense reactions as they are involved in inhibition of actively invading pathogen (Keen, 1992). These reactions are accelerated by over production of defense related proteins than routine (Jackson and Taylor, 1996; Anwar and McKenry, 2000). Activation of defense responses directly resulted into disease suppression or resistance induction in plant body. This resistance induction is somehow related with SAR. Keen (1990) also relates

resistance induction with hypersensitive responses (HR) in plants occurred during incompatible host-pathogen combinations.

A lot of strides over the biochemical and physical basis of such type of disease suppression have been revealed a wide range biochemical spectrum involved in plant defense activities. A number of these phytochemicals have been recognized and their chemical structure has been elucidated. Some of these chemicals are commercially available in market and provide an easy and effective approach to the farmer for better disease control (Wisniewski *et al.*, 2007). There are three main types of resistance inducers used by investigators i.e. chemical inducers, Physical inducers and biological inducers. All of these inducers have specific biochemical profile triggered by them. Moreover, the extent of a biochemical triggered, also varies by varying different inducers and intensity/dose of a single inducer.

Chemical inducers

A number of chemicals have been investigated for their potential in suppressing the disease and some of chemical inducers with significant results are also available in market for their use in conventional agriculture (Vallad and Goodman, 2004). The effectiveness of these inducers has been confirmed by a number of researchers e.g. Segarra *et al.* (2006) conducted a study on induction of resistance under the activity of chemical inducers against root rot diseases and found ISR rapidly developed in plants making the plants resistant. Chemicals playing role behind this resistance induction were accumulation of phytoalexins, lignifications of phenols, and activation of chitinase, polyphenoloxidase and peroxidase.

Use of Chemicals as seed treatment

Seeds of the test plant are dipped/ soaked in the solution of chemical inducer of definite strength for some minutes and then are allowed to germinate. Biochemical profile of non-treated and treated plants is recorded and compared for analyzing the biochemical changes occurred in plant tissue under the activity of seed treatment. This technique has been adopted in number of studies. A study designed by Ragab *et al.* (2009) concluded that oxalic acid may be the more efficient resistance inducer against root rot than salicylic acid while ascorbic acid plays no role in this regard. Maximum number of plants can survive by applying oxalic acid. Basil soaking of seeds in to salt (KCl, K_2HPO_4 and Na_2HPO_4) solution can prevent crops from pre-emergance damping off. While, treatment with KCl is best for prevention against root rot (Ragab *et al*., 2009). It has also been concluded that seed treatment with KCl increase chitinase activity in plant body followed by SA and ascorbic acid respectively.

Use of chemicals as plant treatment

In this technique plants are grown under controlled recommended conditions and then they are treated with some chemical inducer either by vegetative means or by roots. Chemical solution is vegetatively sprayed on plant tissue or plant roots are soaked in chemical solution and then replanted. In both of the cases comparison of treated and non-treated plants discover the biochemical changes induced in plant body. Studies have shown that these means of resistance induction have brought about significant control root rots and other diseases. If Oxalic acid is applied to plants then it may increase the activity of polyphenoloxidase and peroxidase (Ragab *et al.*, 2009). In plant treatments oxalic acid triggers the activity of defense related enzymes most efficiently while ascorbic acid shows very little effect.

Use of Growth Regulators

Growth regulators (GRs) are generally used in commercial farming to direct the plant growth in particular dimension. Plant pathogens also disturb the balance of these growth vitals and cause abnormal growth. Although GRs e.g. prohexadione-Ca are inactive with respect to pesticides (Rademacher, 2000; Rademacher and Bucci, 2002), it had been reported that plants do not only show better growth under the action of growth regulators but also show reduced occurrence of disease (Fernando and Jones, 1999; Momol *et al*., 1999; Roemmelt *et al*., 1999, 2003a; Yoder *et al*., 1999; Costa *et al*., 2001; Maxson and Jones, 2002).

Indole Acetic Acid (IAA) showed best prevention results in case of pre and post emergence damping off (Ragab *et al*., 2009), followed by Indole Butyric Acid (IBA) which provided second best prevention under the identical circumstances. Bazzi *et al.* (2003a; 2003b) investigated bacterial wilts and downy mildews of different crop plants and reported that application of GRs is also a beneficially preventive measure against them. Halbwirth *et al.* (2003) concluded that use of growth regulators may be a

considerable alternative of antibiotics against bacterial blights.

Physical Inducers

Use of physical factors in controlling plant diseases is considered environmental friendly and safe for human health. They have been preferentially and successfully used against plant diseases and agricultural produce. Heat treatment and radiation are major types of physical inducers which have been used to induce resistance in plants against different diseases.

Heat Treatment

Heat treatment has been successfully used in controlling plant diseases as well as post diseases of agricultural commodities (Fallik, 2004). Plants and their produce are subjected to heat treatment either by hot water or by hot air for few minutes to few days to control plant and fruits diseases. Heat treatment is responsible for the activation of two major groups of proteins (Heat Shock Proteins and Pathogenesis Related Proteins) in host tissue which are responsible for its increased defense. Group of heat shock proteins (HSPs) comprise of a large number of proteins ranging from 15-115 kDa in molecular weight. They are believed to be expressed in all organisms and are responsible for thermotolerance (Sabehat *et al*., 1998). Pathogenesis related proteins (PRPs) are coded by host genes after receiving pathogen elicitors and other stress conditions and play important role in plant defense against pathogens. Defense related enzymes with β-1,3-glucanase activity and chitinase activity are also induced by pathogens and stress conditions (heat) in plant tissues (Van-Loon and Van-Strien, 1999) and these enzymes inhibit the growth of fungal pathogens by catalyzing the breakdown of chitin and cleavage of 1,3-β-D Glucosidic linkages in β-1,3-glucan, which are the components of fungal cell wall (Porat *et al*., 2002; Wang *et al*., 2003).

Pre-storage dips of fruits in hot water with temperatures ≥40 °C have been used successfully against storage decays. Hot water not only reduces the pathogen inoculum present in fruits lots but it also enhances resistance of fruit tissue and influences its metabolism (Barkai-Golan and Philips, 1991; Nafussi *et al*., 2001). In the same way hot air treatment of fruits can also be used as tool for resistance induction in fruits prior to storage. Increased antimicrobial activity has been shown in each different part of fruit peel separately, when subjected to hot air (Ballester *et al*., 2010).

Duration for heat treatment varies according to the layer of fruit bearing pathogen inoculum e.g. treatment of only few minutes is required if the control of pathogen inoculum is needed on the surface and in the few upper cell layers of fruits (Lurie, 1998). Heat treatment can remove incipient infections and can kill spores present in wounds and act directly to induce defense responses in host tissue (Schirra *et al*., 2000).

A lot of research work has been carried out by a number of researchers on the role of heat in controlling pathogen infections. Schirra and D'hallewin (1997), Porat *et al.* (2000), Palou *et al*. (2001) and Ben-Yehoshua (2003) used hot water for controlling Penicillium decays on citrus fruits and reported favorable results for using hot water treatment (HWT). Fruits are dipped in hot water for curing (controlling wounds and incipient infections). Success of infection management on fruits by hot water dips depends upon (i) water temperature and (ii) Duration of dip. Above mentioned two factors are experimentally evaluated for each fruit by considering (i) pathogenic species present and (ii) layer of incipient infection in fruit. For curing of citrus fruits by HWT, dips for 2-3 minutes for 50-53 °C is equally effective as dips for 72 hours at 36 °C. Such treatments are also used for controlling chilling injuries and are less expensive due to shorter duration and easy availability of heat sources. In a study by Ben-Yehoshua (2003) hot water dip ceased the growth of *P. digitatum* infecting citrus fruits for 24-48 hours and during this phase of no further growth, resistance against fungal pathogen is developed in citrus peel under the combined action of pathogen and hot water dip. In another study Inkha and Boonyakiat (2010) reported that HWT at different temperatures induced resistance in tangerine fruit against *Penicillium digitatum*. It also induced the accumulation of HSPs and enhanced peroxidase, β-1,3-glucanase and chitinase activities in fruit peel which controlled the green mold infection effectively. Hot water treatment of fruits is also helpful in preventing infections by rearranging the wax components present on fruit surface. Due to increased temperatures wax on fruit cuticle melts and fills the barely visible cracks and openings of fruits thus minimizing the number of entry sites for pathogen (Rodov *et al*., 1995; Schirra and D'hallewin, 1997; Porat *et al*., 2000). Ballester *et al.* (2010) inoculated oranges with *P. digitatum* and one day later treated the same fruits with hot air at 37 °C for three days. Results were recorded with the remarkable increase in resistance and enzyme activities in albedo (inner white part of

orange peel). Flavedo (Upper colored part of orange peel) also showed significant increase in enzyme activities. It was also observed that enzyme activity and resistance were directly proportional to each other and significant increase in expression of gene encoding basic isoforms of PRPs was also observed. Besides all studies favouring the efficiency of heat treatment, it has also been observed that cured fruits with heat treatment show more infection than non-cured which means that heat treatment only eradicates the already existing infections leaving the fruit more susceptible for subsequent pathogen attacks which occur during handling after heat treatment (Ballester *et al*., 2010).

Radiations

Exposure to radiation had been resulted into development of resistant plants from previously susceptible ones against biotic and abiotic stresses. The regulating mechanism behind such type of resistance induction is mutation in genes dealing with stresses. High frequency radiations are generally involved in the alteration of genetic materials of individual plant cells. So, selection of the resistant genotype from the pool of randomly mutated genotypes is necessary to develop and introduce abiotic stress tolerant plants. Although, a number of studies have been carried out on plant mutations resulted from irradiations, but a very little of those are with respect to resistance induction. Gosal *et al.* (2001) successfully induced resistance in potatoes by gamma rays against late blight. That resistance was successively increased from lab to field trials and from first progeny to subsequent progenies. He also tested irradiated potato plants for high temperature tolerance and recorded healthy results.

Biological Inducers

Living entities are applied to plant tissues for disease suppression in many host pathogen systems (Narisawa *et al*., 1998). Plant endophytes are the microbes living in plant intercellular spaces. They have got attention due to their unique biochemical nature and pharmaceutical potential (Wagenaar and Clardy, 2001). These diverse chemicals produced by endophytes enable them to suppress diseases in plants.

Endophytic Microbes

Plant endophytes are the microbes which colonize intercellular spaces of plant tissues. Fungi and bacteria are the major members of beneficial endophytes with more relative fungal species than bacteria. They are the promising group of microbes in terms of producing pharmaceutically important chemicals (Wagenaar and Clardy, 2001). It is assumed that archaebacteria and mycoplasmas live in plant tissues endophytically but no significant evidence has been presented in this regard.

Disease suppression may of many factors involved e.g. direct and indirect. In the direct mechanism of disease suppression biocontrol agent (an endophyte) directly inhibit the pathogenicity of the pathogen by competing with it in term of food resources and space inside the plant tissue or by antibiosis. In indirect way of disease suppression biocontrol agent induce some way of resistance in plant (M'Piga *et al*., 1997).

Endophytes live in plant tissue without developing any significant symptoms (Petrini, 1991). They mostly colonize the above ground tissues of plants but also have been isolated from roots (Mandyam and Jumpponen, 2005; Leho-Tedersoo *et al*., 2009). Endophytic bacteria penetrate and systemically disseminate by colonizing apoplast actively (Quadt-Hallmann *et al*., 1997b) and conducting through vessels (Hallmann *et al*., 1997) and intracellular spaces (Quadt-Hallmann *et al*., 1997a). Endophytes systemically colonize tissues of broad leaf higher plants. According to Yang *et al.* (1994), endophytes develop mutualistic relationship with plants in which plant provides nutrients to endophytic microbe and receives some factors in turn that protect it from attack of microbes, insects and animals. Main advantage to endophytic microbes for their antagonistic activities is that they occupy the same ecological niche as plant pathogens. So, compete with pathogens for nutrients space and other vital resources. Plant endophytes exhibit a great phenotypic plasticity than the plant pathogens (Schulz and Boyle, 2005) which make them more competent than plant pathogens. Disease suppression under the action of endophytic microbes has been described in several pathosystems (Narisawa *et al*., 1998).

It has also been investigated that endophytic microbes exhibit enhanced ability for degradation of pollutants (Doty, 2008). This enhanced degradation ability ensure their better survival during the lethal changes in plant metabolism. Huang *et al.* (2001) reported that endophytic microbes can produce a number of bioactives which are pharmaceutically important. And scientists have molecular markers which help them for developing tools to determine the fate of bioactives produced by endophytic microbes. Shimanuki, (1987) was first researcher who

described the effect of endophytes on plant diseases. He used *Phleum pretense* in his study and give a way for several recent studies on endophytes. Although, endophytic and mycorrhizal fungal species have been known for enhancing MEP pathway metabolic flux in plants; Mandyam and Jumpponen, (2005) and Leho-Tedersoo *et al*. (2009) described the phenotypic differences between these two groups. It was concluded by Deshmukh *et al*. (2006) that some endophytic fungi kill some of the host cells for establishing and proliferating mutualistic relation with host tissue. But still it is assumed that endophytes exhibit a balanced activity of antagonism and mutualism. Strobel *et al*. (2001) concluded that endophytes kill the pathogenic fungi and bacteria by producing volatile compounds.

Microbial elicitors derived from fungal endophytes enhance production of biomass and biosynthesis of terpenoids (Wang *et al*., 2006). It is hypothesized that endophytes may be the governing factor for the production of essential oils and unique bioactives by plant as Wang *et al*. (2006) concluded that *Mentha piperita* produced different profile of essential oil when infected with a leaf fungal endophyte.

A study conducted by Hasegawa *et al*. (2006) revealed that endophytic actinomycetes may affect plant growth either by enhaced secondary metabolites or nutrient assimilation. Endophytes induce the mycelial-choked heads of plants to produce specific antifungal compounds including sesquiterpenes, phenolic glycerides, hydroxyl-unsaturated fats, chokols and an aromatic sterol (Koshino *et al*., 1989). Van-Wees *et al.* (1999) reported that endophytes control plant diseases indirectly by modulating the mechanisms of plant immune systems including the induction of SAR.

Avirulent strains

Most of the time defense reactions are induced by pathogens itself or by some entities strongly related with pathogens. Plant viruses, nematodes, bacteria and fungi may be responsible for increased active responses in plants (Anwar and McKenry, 2006; McKenry and Anwar, 2007). These responses are more significant where an avirulent strain of a pathogen suppresses the pathogenicity of virulent strain when applied prior to it and develop resistance in plants. This phenomenon is equally true for viruses (Ross, 1964), bacteria (Hopkins, 2005), nematodes (Anwar *et al.*, 2008) and fungi (Kuc, 1983). SAR can be developed in tomato and pyrethrum plants by inoculating them with incompatible species of *Meloidogyne incognita* (Ogallo and McClure, 1995) and this resistance can reduce the pathogenic population of *M.halpa* up to 84% on tomato and 72% on pyrethrum (Ogallo and McClure, 1995). In the same way McKenry and Anwar (2007) developed SAR in grapes against virulent species of *M.arenaria* by prior inoculation of *M.incognita*. Another proof of SAR was provided by Eisenback (1983) when tobacco plant resistant to *M.incognita* lost their resistance when inoculated with *M.arenaria* or *M.halpa* three weeks earlier.

Many pathogens have their avirulent and virulent strains which differ in their reproduction rate and amount of caused disease (Anwar *et al*., 2000). Difference in reproduction rate is also used as distinguishing character between virulent and avirulent strains of same species (Zhou *et al*., 2000). Avirulent strains not only induce resistance in host plants but they also reduce the reproduction rate of virulent strain (Anwar and McKenry, 2006). Studies have also been revealed that curing of infected tissues results into higher degree of resistance induction than the curing of wounded tissues (Ballester *et al*., 2010) indicating that elicitors originated from pathogen and pathogen related entities are capable to induce strong defense responses when combined with heat treatment.

Other inducers

Resistance in plants can also be induced by subjecting plants to water stresses and varying availability of plant nutrients at specific time during their development. Scientific investigations in this direction revealed that drought stress had induced resistances in plants. Takahashi *et al.* (1994) induced resistance in plants previously susceptible to chilling stresses by subjecting them to water stress; and he was not successful in this regard by applying chemical treatments. Water stress also triggered drought responding genes and resulted significant variation in transcriptome analysis.

Enzymes are also involved in triggering the complex spectrum of defensive compounds in plants. Basic mechanism behind RI under the action of these enzymes is very simple; as they are responsible for the production of physical defense barriers or the production of biochemical defenses. Umesha and Kavitha (2011) studied the involvement of an enzyme (Cinnamyl alcohol dehydrogenase) in the resistance of tomato against bacterial disease. He recorded that the enzyme was directly involved in the biosynthesis of lignin and

thus localize pathogen infection by depositing higher quantities of lignin around infection site.

Molecular and biochemical basis for resistance induction in plants

It is universally known phenomenon that there is always biochemical warfare between host plants and their related pathogens. Plants have to defend themselves by using biochemical weapons and these weapons enable plants for making effective combat against pathogens. Increase in resistance always means the increase in biochemicals produced by plants. Many studies have been conducted about antimicrobial activities of individual biochemicals. All these antimicrobial metabolites have some molecular or genetic origin from which they are encoded e.g. PRPs are the proteins with antimicrobial activities and are directly encoded by their respective mRNA. In the same way activity of defense related enzymes e.g. peroxidase and chitinase etc. depends upon their respective gene expression. So, all biochemical weapons responsible for resistance in plants are the result of transcription and translation processes occurring in plant cell. It is very important for us to recognize the biochemical basis and molecular origin of these plant metabolites to improve plant resistance against different diseases.

Induction of PRPs and phytoalexins, and expression of chitinase and β-1,3-glucanase genes have been analyzed in some studies related to resistance in plants. Porat *et al.* (1999, 2001) generally relates the induction of PRPs, chitinase and β-1, 3-glucanase with the treatment of hot water, UV irradiation and biocontrol yeast. But it can be supposed as strict rule as Fajardo *et al.* (1998) reported no induction of PRPs against *P. digitatum* in the peel of oranges when treated with different biological derived elicitors.

Some researchers have reported that lignification and accumulation of phenolics is associated with the infection of fruits with fungal pathogen itself (Angioni *et al*., 1998; Ortuno *et al*., 2006). But only pathogen infection brings small increase in accumulation of antifungal compounds. It can be enhanced up to the fungicidal levels by heat treatment of fruits. Study of Kim *et al*. (1991) supports the same phenomenon in which the levels of antifungal scoparone were tremendously increased when fruits were subsequently heat treated after inoculation with fungal pathogen. The only pathogen infection was unable to bring such high increase in that study. Induction of same phytoalexins was also observed by Rodov *et al*. (1992) when fruits were UV irradiated. Droby *et al*. (2002) induced resistance in fruits by using yeast biocontrol and observed the increase in same phytoalexins. All previous studies conclude that each inducer stimulate a specific set of responses in plant body which have been finally compiled in Table 1. Droby *et al*. (2002) concluded that yeast biocontrol and UV irradiation are capable for induction of Phenyl ammonia lyase (PAL) and peroxidase activity in grape fruits. PAL catalyses the phenylpropanoid pathway from which scopoletin and scoparone are produced which exhibit the antimicrobial activity. Peroxidase is involved in the process of lignin synthesis which reinforces the cell wall against attacks of different pathogens and may also alter the antioxidant ability of fruits to cope with fungal pathogens (Ballester *et al*., 2006). So, in this way, both of these enzymes can said to be defense related enzymes due to their important role in increased resistance of plants. In citrus fruits, increased defense responses of albedo against pathogenic attack are barely studied by the researchers up till now (Venditti *et al*., 2005). But, it is interesting to know that albedo is more susceptible to fungal pathogens than flavedo (Kavanagh and wood, 1967; Ballester *et al*., 2006) which clearly indicates the more importance of resistance induction in inner tissues than the outer. Ballester *et al*. (2010) concluded that induction of chitinase activity was greater than the all other defense related enzymes under the action of physical elicitors. Same results were recorded in case of gene expression level.

Conclusion

Sytemic resistance can be induced in plants in a number of ways. The type of inducer regulates the biochemical behavior of plant. One can modulate plant metabolism according to his desire by applying right kind of inducer. All these metabolic alterations make plant resistant against biotic and abiotic factors. Inducer affect the plant at all stages of its life but the type of defenses triggered may vary according to the age of plant. Induced resistance is a kind of stress metabolism; which, persists in plants for longer periods and provide protection against a large number detrimental factors. By applying right kind of inducer at right time, plants can be protected against a variety of pathogens in the most cost effective way.

Table. 1: Plant resistance inducers with their respective types of induced resistance

Type of resistance induced	Potential Elicitors				
PRPs	Hot Water	UV irradition	*Yeast biocontrol		
Phytoalexins	Hot water	Pathogen attack	Pathogen attack+ Hot water	UV irradiation	Yeast Biocontrol
Defense related enzymes	Hot water	Pathogen attack	Yeast biocontrol	UV irradiation	
Phenolics	Heat treatment	Pathogen attack	Pathogen attack+ Heat treatment	UV irradiation	Yeast Biocontrol
Lignification	Heat treatment	Pathogen attack	Pathogen attack+ Heat treatment		

(*) indicates the contradictions among researchers about the role of inducer during resistance induction.

References

Angioni A, Cabras P, D'hallewin G, Pirisi FM, Reniero F, Schirra M, 1998. Synthesis and inhibitory activity of 7-geranoxycoumarin against *Penicillium* species in *Citrus* fruit. *Phytochemistry*, **47:**1521-1525.

Anwar SA, McKenry MV, 2000. Penetration, development and reproduction of *Meloidogyne arenaria* on two new resistant *Vitis* spp. *Nematropica*, **30:**9-17.

Anwar SA, McKenry MV, 2006. Induction of systemic acquuired resistance and susceptibility in tomato by two *Meloidogyne incognita* populations. *J. Nematol.*, **38:** 259.

Anwar SA, McKenry MV, Faddoul J, 2000. Reproductive variability of field populations of Meloidogyne spp. on grape rootstocks. *J. Nematol*., **32:** 265-270.

Anwar SA, Mckenry MV, Yasin SI, 2008. Occurrence of rice-root nematode, *Hirschmaniella oryzae* among 11 rice and 10 weed selections. 5th International Congress of Nematology Brisbane, Australia. pp. 198.

Arcas MC, Botia JM, Ortuño AM, Del Río JA, 2000. UV irradiation alters the levels of flavonoids involved in the defence mechanism of *Citrus aurantium* fruits against *Penicillium digitatum*. *Eur. J. Plant Pathol.*, **106:**617-622.

Ballester AR, Izquierdo A, Lafuente MT, González-Candelas L, 2010. Biochemical and molecular characterization of induced resistance against *Penicillium digitatum* in citrus fruit. *Postharvest Biol. Technol*., **56:**31-38.

Ballester AR, Lafuente MT, González-Candelas L, 2006. Spatial study of antioxidant enzymes, peroxidase and phenylalanine ammonia-lyase in the citrus fruit-*Penicillium digitatum* interaction. *Postharvest Biol. Technol.*, **39:**115-124.

Barkai-Golan R, Phillips DS, 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. *Plant Dis.*, **75:**1085-1089.

Bazzi C, Messina C, Tortoreto L, Bini F, Cecca GS, Stefani E, 2003a. Investigations on the possible use of abiotic and biotic elicitors in defence-related responses in plants. *Eur. J. Hort. Sci.*, **68:**115-122.

Bazzi C, Messina C, Tortoreto L, Stefani E, Bini F, Bru- nelli A, Andreotti C, Sabatini E, Spinelli F, Costa G, Hauptmann S, Stammler G, Doerr S, Marr J, Ra-demacher W, 2003b. Control of pathogen incidence in pome fruits and other horticultural crop plants with prohexadione-Ca. *Eur. J. Hort. Sci*., **68:**108-114.

Ben-Yehoshua S, 2003. Effect of postharvest heat and UV applications on decay, chilling injury and resistance against pathogens of citrus and other fruits and vegetable. *Acta Hort.*, **59:** 159-173.

Costa G, Andreotti C, Bucchi F, Sabatini E, Bazzi C, Malaguti S, Rademacher W, 2001. Prohexa- dione-Ca (Apogee(r)): Growth regulation and reduced fire blight incidence in pear. *Hort. Sci.*, **36:**931-933.

Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH, 2006. The root endophytic fungus *Piriformos-pora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. *Proc. Natl. Acad. Sci.*, **103:**18450-18457.

Doty SL, 2008. Tansley review: enhancing phytoremediation through the use of

transgenics and endophytes. *New Phytol.*, **179:**318-333.

Droby S, Vinokur V, Weiss B, Cohen L, Daus A, Goldschmidt EE, Porat R, 2002. Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*. *Phytopathology*, **92:** 393-399.

Durner J, Shah J, Klessig DF, 1997. Salicylic acid and disease resistance in plants. *Trends in Plant Science*, **2:**266-274.

Eisenback JD, 1983. Loss of resistance in tobacco cultivar NC 95 by infection of *Meloidogyne arenaria* or *M. hapla*. *J. Nematol*., **15:**478.

Fajardo JE, McCollum TG, McDonald RE, Mayer RT, 1998. Differential induction of proteins in orange flavedo by biologically based elicitors and challenged by *Penicillium digitatum* Sacc. *Biol. Control,* **13:**143-151.

Fallik E, 2004. Prestorage hot water treatments (immersion, rinsing and brushing). Postharvest Biol. Technol., **32:**125-134.

Fernando WGD, Jones AL, 1999. Prohexa-dione Calcium Ð a tool for reducing secondary fire blight infection. *Acta Hort.,* **489:**597-600.

Gosal SS, Das A, Gopal J, Minocha JL, Chopra HR, Dhaliwa HS, 2001. In vitro induction of variability through radiation for late blight resistance and heat tolerance in potato. In vitro techniques for selection of radiation induced mutations adapted to adverse environmental conditions. *International Atomic Energy Agency* (IAEA)., IAEA-TECDOC-1227.

Halbwirth H, Martens S, Wienand U, Forkmann G, Stich K, 2003. Biochemical formation of antho- cyanins in silk tissue of *Zea mays*. *Plant Sci*., **164:**489-495.

Hallmann J, Quadt-Hallmann A, Maha!ee WF, Kloepper JW, 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol*., **43:** 895-914.

Hasegawa S, Meguro A, Shimizu M, Nishimura T, Kunoh H, 2006. Endophytic actinomycetes and their interactions with host plants. *Actinomycetologica*, **20:**72-81.

Hopkins DL, 2005. Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. *Plant Dis.*, **89:**1348-1352.

Huang Y, Wang J, Li G, Zheng Z, Su W, 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants Taxus mairei, Cephalotaxus fortunei and Torreya grandis. *FEMS Immunol. Med. Microbiol*., **31:**163-167.

Inkha S, Boonyakiat D, 2010. Induction of resistance to *Penicillium digitatum* in tangerine fruit cv. Sai Num Phung flavedo by hot water treatment. *Songklanakarin J. Sci. Technol*., **32:**5:445-451.

Jackson AO, Taylor CB, 1996. Plant-microbe interactions: life and death at the interface. *Plant Cell*, **8:**1651-1668.

Kavanagh JA, Wood RKS, 1967. The role of wounds in the infection of oranges by *Penicillium digitatum* Sacc. *Ann. Appl. Biol.*, **60:**375-383.

Keen NT, 1990. Gene-for-gene complementarity in plantpathogen interactions. *Annu. Rev. Genet*., **24:**447-463.

Keen NT, 1992. The molecular biology of disease resistance. *Plant Mol. Biol.*, **19:**1:109-122.

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzog J, 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annu. Rev. Phytopathol*., **32:**439-459.

Kim JJ, Ben Yehoshua S, Shapiro B, Henis Y, Carmeli S, 1991. Accumulation of scoparone in heat-treated lemon fruit inoculated with *Penicillium digitatum* Sacc. *Plant Physiol*., **97:**880-885.

Koshino H, Yoshihara T, Sakamura Y, Shimanuki S, Sato T, Tajimi A, 1989. A ring B aromatic sterol from stromata of *Epichloe typhina. Phytochemistry*, **28:**771-772.

Kuc J, 1983. Induced resistance in plants to diseases caused by fungi and bacteria. J. A. Bailey and B. J. Deverall, eds., The dynamics of host defense. *London: AcademicPress*. pp. 146-170.

Kuc J, 1990. A case for self defense in plants against disease. *Phytoparasitica,* **18:**3-8.

Leho-Tedersoo, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. *Environ. Microbiol.,* **11:**3166-3178.

Lurie S, 1998. Postharvest heat treatments. *Postharvest Biol. Technol.,* **14:**257-269.

M'Piga P, Belanger RR, Paulitz TC, Benhamou N, 1997. Increased resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas yuorescens* strain 63-28. *Physiol. Mol. Plant Pathol*., **50:**301-320.

Mandyam K, Jumpponen A, 2005. Abundance and possible functions of the root-colonising dark septate endophytic fungi. In: The Missing Lineages: Phylogeny and ecology of endophytic and other enigmatic root-associated fungi, ed. Summerbell, R. Currah, R.S. and Sigler, L. *Studies Mycol.*, **53:**173-189.

Maxson KL, Jones AL, 2002. Management of fire blight with gibberellin inhibitors and SAR inducers. *Acta Hort.*, **590:**217-223.

McKenry MV, Anwar SA, 2007) Virulence of Meloidogyne spp. and induced resistance in grape rootstocks. *J. Nematol*., 39:50-54.

Momol MT, Ugine JD, Norelli JL, Aldwinckle HS, 1999. The effect of prohexadione calcium, SAR inducers and calcium on the control of shoot blight caused by *Erwinia amylovora* on apple. *Acta Hort*., **489:**601-605.

Nafussi B, Ben Yehoshua S, Rodov V, Peretz J, Ozer BK, D'hallewin G, 2001. Mode of action of hot-water dip in reducing decay of lemon fruit. *J. Agric. Food Chem*., **49:**107-113.

Narisawa K, Tokumasu S, Hashiba T, 1998. Suppression of clubroot formation in chinese cabbage by the root endophytic fungus, *Heteroconium chaetospira*. *Plant Pathol.*, **47:**206-210.

Ogallo JL, McClure MA, 1995. Induced resistance to *Meloidogyne hapla* by other Meloidogyne species in tomato and pyrethrum plants. *J. Nematol*., **27:**441-447.

Ortuno MF, García-Orellana Y, Conejero W, Ruiz-Sánchez MC, Alarcón JJ, Torrecillas A, 2006. Stem and leaf water potentials, gas exchange, sap flow and trunk diameter fluctuations for detecting water stress in lemon trees. *Trees*. **20:**1-8.

Palou L, Smilanick JL, Usall J, Vihas I, 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. *Plant Dis.*, **85:**4:371-376.

Petrini O, 1991. Fungal endophytes of tree leaves. In Microbial Ecology of Leaves (ed. j. H. Andrews & S. S. Hirano). Springer Verlag: New York. 179-197.

Porat R, Daus A, Weiss B, Cohen L, Fallik E, Droby S, 2000. Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. *Postharvest Biology and Technology*. **18:**151-157.

Porat R, Lers A, Dori S, Cohen L, Weiss B, Daus A, Wilson CL, Droby S, 1999. Induction of chitinase and beta-1,3-endoglucanase proteins by UV irradiation and wounding in grapefruit peel tissue. *Phytoparasitica* **27:**1-6.

Porat R, McCollum TG, Vinokur V, Droby S, 2002. Effects of various elicitors on the transcription of a ß-1,3-endoglucanase gene in citrus fruit. *J. Phytopathol*., **150:**70-75.

Porat R, Vinokur V, Holland D, McCollum TG, Droby S, 2001. Isolation of a citrus chitinase cDNA and characterization of its expression in response to elicitation of fruit pathogen resistance. *J. Plant. Physiol.*, **158:**1585-1590.

Quadt-Hallmann A, Benhamou N, Kloepper JW, 1997b. Bacterial endophytes in cotton: mechanisms of entering the plant. *Can. J. Microbiol*., **43:**577-582.

Quadt-Hallmann A, Hallmann J, Kloepper JW, 1997a. Bacterial endophytes in cotton: localization and interaction with other plant-associated bacteria. *Can. J. Microbiol*., **43:**254-259.

Rademacher W, 2000. Growth retardants: Effects ongibberellin biosynthesis and other metabolic pathways. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **51:**501-531.

Rademacher W, Bucci T, 2002. New plant growth regulators: High risk investment? *Hort. Technol*.. **12:**64-67.

Ragab EA, Hosny M, Kadry HA, Ammar HA, 2009. Flavanone Glycosides from *Gleditsia caspi., J. Nat. Prod*.,

Rodov V, Ben Yehoshua S, Kim JJ, Shapiro B, Ittah Y, 1992. Ultraviolet illumination induces scoparone production in kumquat and orange fruit and improves decay resistance. *J. Am. Soc. Hort. Sci.*, **117:**788-792.

Rodov V, Ben-Yehoshua S, Fang DQ, Kim JJ, Ashkenazi R, 1995. Preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance. *J. Agric. Food Chem*., **43:**1057-1061.

Roemmelt S, Treutter D, Speakman JB, Rademacher W, 1999. Effects of prohexadione-Ca on the flavonoid metabolism of apple with respect to plant resistance against fire blight. *Acta Hort.*, **489:**359-363.

Roemmelt S, Zimmermann N, Rademacher W, Treutter D, 2003a. Unusual flavonoid routes induced by the dioxygenase inhibitor prohexadione-Ca in apple (*Malus domestica*). *Phytochemistry*, **64:**709-716.

Ross AF, 1964. Systemic resistance induced by localized virus infections in beans and cowpea. *Phytopathology*. **4:** 436.

Ryals J, Neuenschwander U, Willits M, Molina A, Steiner HY, Hunt M, 1996. Systemic acquired resistance. *Plant Cell*, **8:**1809-1819.

Sabehat A, Weiss D, Lurie S, 1998. Heat-shock proteins and cross-tolerance in plants. *Physiol. Plantarum*, **103:**437-441.

Schirra M, D'hallewin G, 1997. Storage performance of fortune mandarins following hot water dips. *Postharvest Biol. Technol.*, **10:**229-238.

Schirra M, D'hallewin G, Ben-Yeboshua S, Fallik E, 2000. Host-pathogen interactions modulated by heat treatment. *Postharvest Biol. Technol.*, **21:**71-85.

Schulz B, Boyle C, 2005. The endophytic continuum. *Mycol. Res.*, **109:**661-686.

Segarra G, Jauregui O, Casanova E, Trillas I, 2006. Simultaneous quantitative LC-ESI-MS/MS analysis of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. *Phytochemistry*, **67:**4: 395-401.

Sticher L, Mauch-Mani B, Metraux JP, 1997. Systemic acquired resistance. *Annu. Rev. Phytopathol.*, **35:**235-270.

Strobel GA, Dirksie E, Sears J, Markworth C, 2001. Volatile antimicrobials from a novel endophytic fungus. *Microbiology*, **147:** 2943-2950.

Takahashi R, Joshee N, Kitagawa Y, 1994. Induction of chilling resistance by water stress, and cDNA sequence analysis and expression of water stress-regulated genes in rice. *Plant Mol. Biol*., **26:**339-352.

Umesha S, Kavitha R, 2011. Induction of cinnamyl alcohol dehydrogenase in bacterial spot disease resistance of tomato., *J. Bacteriol. Res*., **3:**16-27.

Vallad GE, Goodman RM, 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Sci*., **44:**1920-1934.

Van Loon LC, Van Strien EA, 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mole. Plant Pathol*., **55:**85-97.

Van-Wees SC, Luijendijk M, Smoorenburg I, van Loon LC Pieterse CM, 1999. Rhizobacteria mediated induced systemic resistance (ISR) in *Arabidopsis* Is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. *Plant Mol. Biol*., **41:**537-549.

Venditti T, Molinu MG, Dore A, Agabbio M, D'hallewin G, 2005. Sodium carbonate treatment induces scoparone accumulation, structural changes, and alkalinization in the albedo of wounded *Citrus* fruits. *J. Agric. Food Chem*., **53:**3510-3518.

Wagenaar MM, Clardy J, 2001. Dicerandrols, new antibiotics and cytotoxic dimmers produced by the fungus *Phomopsis longicolla* isolated from an endangered mint. *J. Nat. Prod.*, **64:** 1006-1009.

Wang JW, Zheng LP, Tan RX, 2006. The Preparation of an elicitor from a fungal endophyte to enhance artemisinin production in hairy root Cultures of *Artemisia annua* L. Chin. *J. Biotechnol.*, **22:**829-834.

Wang Y, Kausch AP, Chandlee JM, Luo H, Ruemmele BA, Browning M, Jackson N, Goldsmith MR, 2003. Co-transfer and expression of chitinase, glucanase, and *bar* genes in creeping bentgrass for conferring fungal disease resistance. *Plant Sci*., **165:**497-506.

Wisniewski M, Wilson C, Droby S, Chalutz E, Ghaouth AE, Stevens C, 2007. Postharvest Biocontrol: New Concepts and Applications. Chapter 29. Biological control: a global perspective. *CAB International*, pp. 262.

Yang X, Strobel G, Stierle A, Hess WM, Lee J, Clardy J, 1994. A fungal endophyte-tree relationship: Phoma sp. in Taxus wallachiana. *Plant Sci*., **102:**1-9.

Yoder KS, Miller SS, Byers RE, 1999. Suppression of fire blight in apple shoots by prohexadione-calcium following experimental and natural inoculation. *Hort. Sci*., **34:** 1202-1204.

Zhou E, Wheeler TA, Starr JL, 2000. Root galling and reproduction of *Meloidogyne incognita* isolates from Texas on resistant cotton genotypes. *Suppl. J. Nematol*., **32:**513-518.