Session 10B

Natural Resistance of Plants

Arms Race or Balancing Selection?

Chairman & Session Organiser:	Professor Dr Pierre de Wit *Wageningen University, The Netherlands*
Platform Papers:	10B-1 to 10B-5
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Adaptive evolution of fungal avirulence genes imposed by plant resistance genes

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For many decades resistance (*R)* genes have been introduced into crop plants as a sustainable method to protect them against pathogens. However, as a result of selection pressure imposed by *R* genes pathogens are eventually able to overcome *R* genes resulting in outbreaks of epidemics known as the 'boom and bust' cycle. Resistant cultivars that were once booming eventually busted, forcing breeders to introduce anew *R* gene. Genetic studies on the model flax–*Melampsora lini* pathosystem by Flor has led to the development of the gene-for-gene model, which states that for every dominant *R* gene in the plant there is a matching dominant avirulence (*Avr*) gene in the pathogen.

R and *Avr* genes

The last decades many *R* and *Avr* genes have been cloned from different pathosystems. From these studies it has become evident that R proteins often show some homology among each other, whereas Avr proteins do not. The gene-for-gene model assumes that an *R* gene encodes a receptor protein that directly interacts with the product of matching *Avr* gene leading to elicitation of plant defense responses including the hypersensitive response (HR) and resistance. Most R proteins carry leucine-rich repeats, which are thought to provide a versatile binding domain for proteins including Avr proteins. However, direct interaction between R and Avr proteins has not often been observed and is the exception rather than the rule. This had led to the formulation of the guard hypothesis which states that an Avr proteins functions as an effector that interacts with and manipulates a host target in order to enable the pathogen to cause disease. These host targets are assumed to be guarded by R proteins. Manipulation of a host target by an Avr protein is sensed by the R protein that leads to triggering of defense responses [1].

Avr genes encode effectors whose activities are sensed by R proteins

Recent discoveries show that the plants require an innate immune system consisting of a primary and a secondary immune response to defend themselves against pathogens. With the primary immune response, plants recognize microbial-associated molecular patterns (MAMPs) of pathogens through pattern recognition receptors (PRRs) that subsequently mediate a basal defense response. The primary function of an Avr protein is assumed to be an effector that suppresses this basal defense enabling the pathogen to cause disease. With the secondary immune response, plants have gained the ability to recognize effector-induced perturbations of host targets through R proteins that subsequently mediate an HR that causes pathogen growth arrest at the site of penetration [1].

Functions of effectors and their adaptive evolution

As indicated above, it is now evident that an Avr protein is an effector whose primary function is to manipulate a host target in order to enable the pathogen to cause disease. The functions and mechanisms of delivery of fungal and oomycete effectors is not well understood. Of the eight effectors that are secreted by the extracellular fungus *Cladosporium fulvum* into the intercellular space of tomato, the Avr2 effector binds to and inhibits the plant cysteine protease Rcr3, whereas the Avr4 effector protects the fungus against the deleterious effects of plant chitinases.

Other fungal and oomycete effectors act inside host cells as has been shown for the effectors of the flax rust fungus *M lini*, the rice blast fungus *Magnaporthe grisea* , the potato late blight oomycete pathogen *Phytophthora infestans*, and the downy mildew oomycete pathogen of *A. thaliana, Hyaloperonospora parasitica*. The mechanism of delivery of fungal effectors into plant cells is still unknown, although oomycete effectors all carry an RXLR motif that has been suggested to facilitate effector uptake into the plant cell. This motif has reported to be crucial for the malaria parasite *Plasmodium falciparum* where it facilitates entry of its effectors into mammalian host cells [1].

Effectors are specific for particular strains of plant pathogens and sometimes target different domains of the same host component that is monitored by different R proteins. Indirect recognition of effectors by R proteins is observed more frequently than direct recognition. Interaction of Avr2 of *C. fulvum* with tomato Rcr3 which is sensed by the R proteins Cf-2 is an example of indirect recognition [2]. The arms race between plants and their pathogens has led to different modes of adaptation to primary and secondary defense responses. Co-evolution between plants and their pathogens has led to the generation of novel effectors that no longer directly interact with R proteins or perturb host targets in ways that are no longer sensed by R proteins. The type of mutations in effector genes depends on the importance of a particular effector for the pathogen's virulence and/or competitive abilities outside the host when it has to cope with antagonistic micro-organisms or other hostile environments. Thus, depending on their importance and mode of action, some effector genes can either be completely removed from the pathogen genome by deletion, thus preventing their recognition by R proteins, whereas others are mutated and produce effectors carrying subtle amino acid changes that decrease or avoid direct recognition by R proteins but still retain virulence functions. Loss of an effector has often been observed in cases where indirect interactions between R protein and effector-modified host target, complying with the guard hypothesis, occur. In these cases it is presumed that loss of the effector does not strongly affect pathogen's virulence as its function is presumably covered by functionally related effectors. Indeed, loss or partial deletion of effector genes has frequently been observed for fungal pathogens that are indirectly recognized by R proteins (*Avr2* of *C. fulvum* is non-functional in virulent strains), whereas subtle amino acid changes in effectors that presumably do not affect virulence function but avoid R protein recognition have been observed for effectors that directly interact with R proteins (interaction between several Avr proteins of *M. lini* with flax R proteins) [3]. For some pathogenic bacteria loss of effector genes occurs extremely quickly on resistant plants that contain the matching R proteins by genome rearrangements, including excision of the effector gene. Also here it is assumed that the modes of adaptive evolution depend not only on the virulence function of an effector but also on the lifestyle of a pathogen carrying that effector (biotrophic or necrotrophic pathogen).

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Can information on the mode of pathogen attack be used to formulate novel crop protection strategies?

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Fungicide application and the deployment of more resistant cultivars represent the major approaches used to limit crop diseases caused by fungal pathogens. However complete immunity to disease is rarely achieved as pathogens have demonstrated an ability to evolve insensitivity to fungicides and to overcome the protection provided by specific resistance genes. So the question remains - how can plants (and crop growers) ever hope to win the war against these enemies? One possible way of interfering with the development of diseases in the future may come from taking a much closer look at what exactly makes a plant species a host to a particular pathogen.

Several levels of host resistance must be overcome for a pathogen to infect and colonize a plant. Firstly, the pathogen must find the environment where it makes initial contact amenable for its further development. An inability to do so gives rise to the broadest and most widespread form of plant disease resistance, which is referred to as non-host (or species) resistance. A second tier of non-host resistance can also be brought into play by plants when a potential invader that had the initial ability to develop on the tissues is recognised via characteristic molecular features that serve to betray its presence and trigger an inducible plant resistance response. This is often associated with localised plant cell death referred to as the hypersensitive response (HR) and is a feature of some forms of non-host resistance. A further level of resistance operates between individual races of a pathogen and individual cultivars of a host plant and is controlled in accordance with the classic gene-for-gene hypothesis. This type of resistance is often referred to as race-specific resistance and involves interaction between pathogen encoded avirulence (Avr) factors with cognate plant resistance (R) factors. Race-cultivar resistance has been widely studied for host plant interactions with pathogenic fungi, bacteria and viruses and in many of these examples the HR has been shown to be an associated and perhaps necessary feature of the resistance reaction.

All of the above is widely accepted for interactions of plants with pathogens that proliferate within living tissue, for example biotrophic rust and powdery mildew fungi. What is less clear is how well this scheme fits host resistance mechanisms against necrotrophic pathogens which ultimately reproduce in dead or dying tissue? In contrast to the major *R* genes and resistance mechanisms characterised for interactions with biotrophic fungi, comparatively little is known about the molecular identity of *R* genes that are effective against individual races of necrotrophic pathogens, or how they operate mechanistically to provide protection.

Many studies have suggested that necrotrophic fungi use either secreted cell wall degrading enzymes and / or toxins to promote the death of plant tissues. This is frequently associated with pathogens that cause post-harvest diseases of fruits and vegetables including, for example, species of *Botrytis* which often have a broad host range. However many important necrotrophic fungi attack crop plants with very high specificity and there are numerous

examples where pathogens of this type have only a single known host. An example of this is *Septoria tritici* (*Mycosphaerella graminicola*) which only infects wheat leaves and is the causal agent of leaf blotch disease; economically the most important disease of wheat in the United Kingdom and Western Europe. In recent years *Septoria tritici* has evolved insensitivity towards many of the commonly applied fungicides. New wheat cultivars bred to have improved resistance to disease have often been seen to be insufficiently durable in the field. Both QTL and major gene resistance is known to operate against *Septoria* but none of the *R* genes (referred to as *Stb* genes) have been cloned, and it is therefore unclear how they function in providing resistance. However, an associated and important fundamental question is- why does *Septoria tritici* only infect wheat leaves? This is particularly curious when you consider that airborne spores of the fungus appear to germinate and form the initial penetration hyphae (which enter via leaf stomata) on all tested plants. Two possibilities exist for why all these other plants remain resistant to disease - (1) they all have very effective QTL and / or specific resistance genes to all known races of this sexually very active fungus, or, (2) wheat leaves are inherently and specifically sensitive to something that *Septoria tritici* does to them. Recent molecular and biochemical studies of the wheat leaf - *Septoria tritici* infection mechanism would favour the latter explanation. The cornerstone of this argument is that the leaf disease symptoms caused by this fungus appears to involve the activation of host responses that are more commonly associated with disease resistance towards biotrophic pathogens. The model therefore suggests that 'sensitivity factor(s)' exist in much of the current cultivated wheat germplasm. In this context the isolate-specific major *R* genes (*Stb* genes) appear to provide selective 'insensitivity' to infection.

So where is the potential for new targets for intervention in systems such as these? The emergence of full genome sequences of plant infecting fungi should facilitate the identification of determinants of host specificity including toxins, elicitors, effectors and avirulence factors and even more importantly allow the study of how their production is regulated. Efforts are also required to identify putative host sensitivity and resistance genes from crop plants that function during interactions with necrotrophic fungi. This is necessary in order to evaluate what other processes may be useful as targets for breeding strategies aimed at providing more durable disease control. In summary, if we are ever to win the war against these invaders (as opposed to winning the occasional battle) we may benefit from a better understanding of the fundamental biology of these important host-pathogen interactions.

New strategies for deployment of plant resistance in cereals

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Biotypic variation among populations of greenbug, *Schizaphis graminum* (Rondani), has been a driving force behind breeding programs for wheat, barley, and sorghum since the designation of the first biotype in 1961. Greenbug biotypes have been defined by their virulence relationship to a select group of plant genotypes. As more resistant genotypes of crop plants were developed, new biotypes of greenbugs were discovered. A gene-for-gene model was assumed, whereby newly developed resistant cultivars exerted selective pressure on the current predominant biotype, which in turn led to the development of a new virulent biotype. However, a detailed historic comparison of the chronologies of greenbug-resistant cereal deployments and greenbug biotype status reports clearly demonstrate that resistant cultivars could not have significantly contributed to the development of new biotypes (Porter *et al.*, 1997).

Phylogenetic studies, based on mitochondrial DNA divergence (COI haplotypes), have shown that biotypes of greenbugs identified solely from response by plant differentials are not discrete populations and the classification of biotype has neither an evolutionary nor taxonomic basis. Instead, it was concluded that the greenbug species complex is made up of host-adapted races that have diverged on non-cultivated grass species well before the advent of modern agriculture, and biotypes are comprised of genetically diverse individuals among different host races that merely share similar virulence genes (Shufran *et al.*, 2000; Anstead *et al.*, 2002). A regional assessment of the composition of greenbug biotypes on wheat, sorghum, and non-cultivated grasses confirmed that overall biotypic diversity was much greater among greenbugs collected from non-cultivated grasses, which would be expected if greenbugs diverged on grasses. The most virulent biotypes were not present on wheat or sorghum, but they were collected from non-cultivated grass hosts, thus providing further evidence that these hosts, not cultivated crops, are key to the development and maintenance of genetic diversity for virulence in greenbugs (Burd & Porter, 2006). Consequently, the putative relationship between greenbug virulence and reproductive fitness, which is assumed by the gene-for-gene model, is dubious because the more virulent biotypes were rarely found infesting cultivated crops.

We conclude that greenbug biotype ≠ genotype. A greenbug biotype is merely a plant phenotypic expression elicited by an indefinite number of genotypes. We define a greenbug biotype as an infra-specific population, independent of geographic distribution, which is able to injure a plant containing specific resistant gene(s) that are resistant to other infra-specific populations. Moreover, there is no presumption of the genetic basis within the greenbug for the ability to cause injury, nor is any evolutionary or taxonomic status implied. Obviously, there are genetic differences among greenbug biotypes, which affect feeding behaviour and the phenotypic response of the plant. However, the term biotype

does not describe those differences, nor does it require knowledge of the biotype-specific traits that induce the damage symptoms.

We also conclude that virulence ≠ greenbug fitness. The most virulent biotypes rarely occur in cultivated crops. Moreover, non-cultivated grasses play the most significant role in shaping and harbouring genotypic diversity. Biotypes, by definition, do not occur on non-cultivated grasses (visible damage is very limited and rare), instead there are host-specific races (i.e., populations can or cannot successfully colonize the host).

Finally, we conclude that resistance ≠ biotype selection. Quite simply, the greenbug species complex is comprised of host-adapted races that have diverged on non-cultivated grass species well before the advent of agriculture. There is no apparent correspondence between the deployment of resistance and the development of new greenbug biotypes.

Summary
Strategies for deploying crop cultivars with durable resistance to insects have been for years the focus of considerable debate and speculation. Interpretation of elaborate simulation models used to predict endurance of resistance has lead to the generally accepted paradigm that the widespread use of an insect-resistant cultivar with a single, major gene for resistance will be selective for new, virulent biotypes. Our research has shown that this breeding tenet does not hold for the greenbug.

Plant resistance to greenbugs will continue to be an important strategy in pest management; however, we question the tenet that places an emphasis on releasing tolerant, multi-genic cultivars and de-emphasizes antibiotic, simply inherited greenbug resistance.

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The association of differentially expressed proteins with maize resistance to *Curvularia lunata* (Wakker) Boed in China

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Curvularia leaf spot in maize caused by *Curvularia lunata* distributes widely in the world. To search the resistant genes against the disease in maize, we focused on our effort on exploring differential expressed proteins from different inbred lines through proteomic approach, which subsequently provided a potential clue for cloning resistance-related genes. All samples were taken from four inbred lines inoculated with the pathogen at presence or absence of *Trichoderma atroviride* in rhizosphere soil. We have used high-resolution two-dimensional gel electrophoresis (2-DE) and mass spectrometry to identify proteins that are differentially expressed in response to fungal infection in maize leaf.

Among over 300 spots, thirty eight spots were selected and subjected to tryptic digestion followed by identification using matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF MS/MS) and nanospray ion-trap tandem mass. The differentially-expressed proteins could be grouped into four groups according their known functions. The first group mainly involved in photosynthesis were Rubsico, chlorophyll a/b-binding protein, the second group associated with respiration metabolism included PEPase, P-pyruvate carboxylase, fructose 1, 6-bisphosphate aldolase, glyceraldehyde 3-phosphate dehydrogenase. The third group dedicated to bioenergy metabolism were ATP synthase and ATPase etc. The fourth group with a series of stress-mediated proteins included germin-like protein, 22kDa drought-inducible protein, translation initiation factor 5A, glutathione peroxidase, ascorbate peroxidase, ABA-and ripening-inducible-like protein and oxygen evolving enhancer protein1(OE1) etc. Besides, some unknown proteins also might participate the regulation of host defense response.

Comparatively, proteins in the fourth group seemed to be most associated with the maize leaf defense response to *C. lunata* infection in which germin-like protein (GLP),putative glutathione peroxidase (GPX), 22kDa drought-inducible protein and translation initiation factor 5A (eIF-5A) were only shared among resistant inbred lines (Luyuan 92 and 78599-1), similar like the finding from other corn disease research reported (Chen *et al*, 2004) GLPs has been already assured with activity of superoxide dismutase (SOD) or oxalate oxidase (OXOX) (Bernier & Berna, 2001). those enzymes functioned as scavenger to remove the accumulation of active oxygen radicals during pathogen invasion. Therefore, putative glutathione peroxidase (GPX) was not only just a common component of anti-oxidation system in host plant but also a protein to mediate host defense response to pathogen invasion, as a result, GPX and GLP might probably play a role in coordinated way to reinforce maize defense response against *C. lunata* infection.

22kDa drought-inducible protein was another essential protein probably involved in the host responses against the pathogen infection particularly when biocontrol agent *Trichoderma atroviride* was present in corn rhizosphere.

Proteins related to host tolerance to drought have been confirmed with contribution to host resistance to fungal pathogen infection (Chen, 2004). It has been observed that in Northern China frequent occurrence of drought climates in past decay has resulted in cultivars more susceptible to Curvularia leaf spot and moreover upon the pathogen infection, diseased leaf would lose water faster and larger from leaf surface through over-evaporation.

Eukaryotic translation initiation factor 5A (eIF-5A) is one of the factors necessary for the initiation of eukaryotic cellular protein biosynthesis and mRNA selective transport across to membrane in plant developmental and environmental response. In our studies, the expression levels of eIF-5A (spots 2, 31) were increased specifically in resistant inbred lines upon pathogen infection.

With exception of specifically induced proteins in resistant inbred lines, some proteins were common in resistance and susceptible inbred lines, however, those proteins were up-regulated in resistant inbred lines, such as OEE1. The protein has proved with function to eliminate reactive oxygen species (ROS) if accumulated over a certain concentration n maize leaf. ABA- and ripening-inducible-like protein was up-regulated in resistant inbred line78599-1, and down-regulated in susceptible inbred line Huangzao 4. Numerous evidences have assured the significance of ABA in regulation of plant stomata against drought stress and host defense response. The coordinated role of ABA- and drought-inducible proteins would devote higher contribution to improved host tolerance than single component. Similarly ascorbate peroxidase (APX) appeared commonly in both kinds of inbred lines, but its expression was much higher in resistant inbred lines. APX is actually an antioxidant similar like GPX with function of removal of active oxygen radicals. Furthermore, GLX1 as a member of glyoxalase system was specifically induced by *Trichoderma atroviride* in the presence of pathogen which made glutathione (GSH)-based detoxification more effectively.

Taken together, the proteins in the fourth group are induced in resistant inbred lines or at present of *Trichoderma atroviride* in a coordinated manner to remove ROS and increase host tolerance to drought stress which subsequently would reinforce maize leaf defense response against *Curvularia lunata* infection, therefore it would be reasonable to view them as genetic markers of host resistance. Other three groups of proteins were inferred to support in indirect way the action of unique proteins in the regulation of complex defense mechanism.

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Virulence genes and population studies of *Magnaporthe grisea* in Fujian Province, China

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The population studies on the *M. grisea* in Fujian Province

In order to know population structure of *M. grisea* from Fujian province of China, pathogenicity of *M. grisea* single spore isolates isolated from Fujian Province in recent years was analyzed on 3 sets of differential rice varieties. The analysis showed that 97 of 300 isolates could be differentiated into 15, 14 and 22 pathogenic types by the Chinese rice differentials, CO39 near-isogenic lines (NILs) and LTH NILs. The dominant pathogenic types were ZB13, I34.1 and J76.2 that occurred in frequencies of 55.7%, 52.6% and 28.9%, respectively. The pathogenic types ZB14, I35.1 and J72.2 were less dominant and occurred at frequencies of 13.4%, 22.7% and 14.4%, respectively.

It was also found that diversity index of *M. grisea* population in Fujian Province was 0.6697, 0.6692 and 0.8660 by the three sets of differentials, among which Chinese race differentials and CO39 NILs could be of interest as differentials to study the population structure of *M. grisea* in Fujian.

The virulence genes present in the *M. grisea* population in Fujian Province

Based on Flor's gene-for-gene hypothesis (Flor, 1956) and the virulence of isolates on 41 rice varieties with known resistance genes, we found that all the virulence genes that could overcome corresponding resistance genes were detected in Fujian isolates Table 1). The 41 virulence genes occurred in frequencies range of 2.3%-98.9% and the average frequency was 53.3%. Among those 16 virulence genes, including *Av-d2*(C), *Av- k*(1), *Av-k*^{m} , *Av-1*(1), *Av-k*^{h}, *Av-z*^{5}(1) , *Av-1*(2) , *Av-z*^{5}(2), *Av-ta2* , *Av-7(t)* , *Av-2*(C) , *Av-k*(2), *Av-k*^{p} , *Av-9(t)* , *Av-ta*^{2}(C) and *Av-2*(2), occurred below a frequency of 30%, 5 virulence genes, including *Av-k*(C), *Av-i* , *Av-z*^{t}, *Av-5*(t) and *Av -b*(C), occurred at frequencies between 30% and 70%, and 20 virulence genes, including *Av-a(2)*, *Av-3*,*Av-k*^{s},*Av-4b*, *Av-b* occurred at a frequency above 70%.

The results indicated that Fujian isolates contain multiple virulence genes with a high frequency. In addition, it has been suggested in the past that some resistance genes which could be overcome by corresponding virulence genes. However some resistance genes including *Pi-d2*(C), *Pi- k*(1), *Pi-k*^{m} , *Pi-1*(1), *Pi-k*^{h}, *Pi-z*^{5}(1) , *Pi-z*^{5}(2) and *Pi-1*(2) could be used as a resistance source in rice breeding programs in Fujian Province.

The virulence genes combination of *M. grisea* isolates in Fujian Province

We found that the tested isolates showed different combinations and frequencies of virulence genes. Most of isolates contained 24, 27, 18, 23, 25 virulence genes, and their frequency was 13.8% , 10.3%, 9.2%, 8.0% and 8.0%, respectively

Table 1. Virulence frequency of *M. grisea* isolates in Fujian Province

Known virulence genes	Donor*	Frequency (%)	Known virulence genes	Donor	Frequency (%)
Av-d2(C)	DiguB	2.30	*Av-sh*	Shin 2	71.26
Av- k(1)	Tetep	2.30	*Av-z*	Fukunishiki	74.12
Av-k^{m}	Tsuyuake	3.45	*Av-ta*(1)	C105TTP2L9	75.86
Av-1(1)	C101LAC	5.75	*Av-ta*	C101PKT	74.71
Av-k^{h}	K3	4.60	*Av-3*	C104PKT	75.86
Av-z^{5}(1)	C101A51	6.90	*Av-a*(1)	Aichi Asahi	79.31
Av-z^{5}(2)	C101A51 (R)	8.05	*Av-20*	IR24	78.16
Av-1(2)	C101LAC	9.20	*Av-12(t)*	Moroberekan (RIL10)	78.16
Av-ta2	Pi4	13.79	*Av-t*	K59	83.91
Av-7(t)	Moroberekan (RIL29)	13.79	*Av-19(t)*	Aichi Asahi	83.91
Av-2(C)	YC57	14.94	*Av-11*(C)	ZYQ 8	85.06
Av-k(2)	Kanto51	14.94	*Av-ta*(C)	F124-1 (Tadukan)	88.51
Av-k^{p}	K60	17.44	*Av-4a*	C101PKT	87.21
Av-9(t)	WHD-1S-75-1-127	13.79	*Av-k*^{m}(C)	F129-1 (Pusur)	90.80
Av-ta^{2}(C)	F128-1 (Tadukan)	21.84	*Av-k*^{p}(C)	F98-7	91.95
Av-2(2)	C101A51	27.59	*Av-b*	BL1 (BCHL01)	93.02
Av-k(C)	F80-1	56.32	*Av-4b*	C105TTP-4-L-23	93.10
Av-i	Fujisaka5	57.47	*Av-k*^{s}	Fujisaka 5	96.55
Av-z^{t}	Toride 1	65.52	*Av-3*	C104PKT	98.85
Av-5(t)	Moroberekan (RIL249)	60.92	*Av-a*(2)	CO39	98.85
Av -b(C)	F145-2 (Tijahaja)	65.52	Average		53.31

*Known donor resistance gene corresponding to virulence gene.

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Response of bird pepper (*Capsicum frutescens* L) genotypes to leaf curl virus

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Pepper (*Capsicum* spp.) is an important spice cum vegetable, providing green and dry fruits. Leaf curl virus (LCV) disease is one of the most serious and devastating biotic stresses in pepper causing severe loss in yield particularly in summer (Peter, 1998). It is caused by Tomato Yellow Leaf Curl Virus (TYLCV) which is a gemini virus spread solely by *Bemisia tabaci*, the silverleaf whitefly (SLW). Controlling the vector population using chemicals could reduce the severity of the disease but is costly and perilous to man and environment. Developing resistant cultivars would be an effective proposition. In the most cultivated *Capsicum annuum*, most genotypes are highly susceptible, whereas bird pepper (*Capsicum frutescens*) possesses considerable resistance to this disease. Inter-specific crosses between these two species are viable though with some difficulty. Hence, resistant lines of bird pepper identified can be utilized to transfer resistant genes into more economic *C. annuum* cultivars.

One hundred and fifteen genotypes of bird pepper collected from various agro-climatic regions of India were evaluated in two separate field experiments (experiment I and experiment II) each in RBD with two replications under irrigated conditions. Ten plants were maintained in each plot. All the recommended cultivation practices were followed in both the experiments but for any control measure against SLW in Experiment II where infected seedlings of susceptible *C. annuum* plants after acquisition feeding (Nene, 1972) with SLW for 24 hours were grown along the border. From two months after transplanting viruliferous SLWs were released to the experiment II field, every four days for two months. Observations on disease reaction and green fruit yield (g) per plant were recorded on five plants each at random in each plot. Scoring (0-4 scale) for the disease was done at 60^{th}, 90^{th} and 120^{th} DAP. Disease reaction at 90th day was used for computing vulnerability index (VI) taking individual plant scores of disease to measure the degree of resistance adopting equation suggested by Bos (1982). Analysis of variance (ANOVA) for green fruit yield per plant and VI was carried out; yield and VI in experiment I and II were compared.

Green fruit yield per plant and VI in both the experiments showed significant variation. The genotype, Nenmara Local (T_{51}) exhibited complete resistance to LCV in both the experiments (Table). This was on par with Mavelikkara Local (T_{50}), Thavanur Local-3 (T_{15}), and Kayamkulam Local 3 (T_{33}) in experiment I and only with T_{50} in experiment II. Genotypes, Karumukku Local (T_{11}) and (T_{50}) recorded the highest values for green fruit yield per plant and were superior to all other genotypes in experiment II. In experiment I, T_{11} and T_{15} were the highest yielding genotypes.

The genotypes were classified as resistant, tolerant, susceptible and highly susceptible based on their vulnerability index values in experiment II. Two genotypes, T_{51} and T_{50} were resistant (almost immune) to the disease (VI < 1); eight genotypes (*viz.*, T_{15}, T_{33}, T_{11}, Kayamkulam Local-2 (T_{35}), Mangalapuram Local (T_{10}), Paady Local (T_{49}), Vamanapuram Local (T_{81}) and Uliyathadka Local (T_{115}) showed tolerance to the disease (VI 1 to 25). They exhibited mild symptoms such as slight curling of a few terminal leaves. Forty two

genotypes were susceptible (VI >25 to 50) many of them showing curling of terminal and adjacent leaves and with blisters on leaves. The rest (63 numbers) were highly susceptible to the disease (VI >50) with severe curling of leaves and stunting of plants. In some cases, small clusters of leaves were produced due to proliferation of auxiliary buds.

Table 1: Mean vulnerability index for LCV disease and green fruit yield per plant in bird pepper genotypes (only 5 best and least are given)

	Vulnerability index				Green fruit yield/plant (g)		
Genotypes	Expt II	Expt I	Pooled	Genotypes	Expt II	Expt I	Pooled
T_{51} Nenmara L	0	0	0	T_{11} Karumukku L	272.0	309.1	290.55
T_{50} Mavelikkara L	1.099	0	0.55	T_{50} Mavelikkara L	239.6	250.3	244.95
T_{15} Thavannur L	4.148	0	2.07	T_{15} Thavanur L	236.0	300.1	268.05
T_{33} Kayamkulam L	5.499	0	2.75	T_{86} Peringamala L	197.85	227.2	212.52
T_{11} Karumukku L	6.243	6.65	6.45	T_{19} Meenachil L	194.1	244.8	219.47
T_{97} Kannur L	82.64	31.91	57.27	T_{37} Mulleria L	3.10	14.5	8.8
T_{79} Uliyathadka L	83.58	44.49	64.03	T_{111} Kollambadi L	3.05	4.50	3.77
T_{16} Ambalawayal L	85.10	51.56	68.33	T_{105} Kowdiar L	2.85	4.85	3.85
T_{102} Purakkad L	86.11	49.60	67.85	T_{78} Yethadka L	2.55	10.6	6.57
T_{86} Peringamala L	86.21	50.88	68.54	T_{68} Kozhikkod L	2.40	12.3	
CD (0.05)	*1.429*	*1.116*	*1.270*		*35.559*	*29.937*	*23.138*

Pooled analysis report showed that T_{11} and T_{15} gave the highest fruit yield per plant and were significantly superior to all others. T_{51} and T_{50} were superior to all others in LCV resistance reaction. Yield reduction in experiment II in resistant/tolerant genotypes was comparatively lesser than that in susceptible genotypes. Yield showed high and positive correlation among two experiments. Vulnerability index also exhibited very similar results. These indicated the existence of inherent genetic difference among genotypes for yield potential and reaction to LCV resistance. Green fruit yield per plant was negatively correlated with VI in both the experiments suggesting that greater susceptibility leads to reduction in yield.

Leaf curl virus resistant (T_{51} and T_{50}) and high yielding (T_{11} and T_{15}) genotypes could be used as superior genotypes in crop improvement to evolve high yielding LCV resistant pepper varieties. They are to be confirmed that they are not symptom-less carriers through methods like PCR amplification with appropriate primers. The genotype T_{50} is the one which needed special attention as it possessed both the desirable characters in appreciable intensity.

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Screening of tea varieties for susceptibility to *Lasiodiplodia theobromae* by serological techniques and induction of resistance by botanicals

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Several elite varieties of tea cultivated in the Darjeeling hills are susceptible to *Lasiodiplodia theobromae* (Pat.) Griffon & Mauble, a common pathogen causing diplodia disease. The present study was framed with the following objectives in two phases. In the first phase, we studied the susceptibility of seven commercially cultivated elite varieties of tea for their susceptibility to *L. theobromae* by determining levels of cross reactive antigens (CRA) between the host and pathogen by immuno-diffusion (ID), immuno-electrophoresis (IEP), and ELISA. Immunogold labeling followed by silver enhancement was performed to study *in situ* localization of CRA by light microscopy. The most susceptible variety was then used to study induction of systemic resistance by application of extracts from potential plants that do not have direct antifungal activity.

Several authors have shown that the extent of compatibility and susceptibility of a plant cultivar to a pathogen correlates to levels of common antigens present in both host and pathogen. In the present study, pathogenicity was initially tested in two-month-old seedlings in pots by inoculating with mycelial plugs of the pathogen. Results showed that TS-449 was most susceptible and TS-491 was most resistant. These two varieties were selected for the serological tests. ID and IEP revealed strong precipitin reactions with multiple arcs between homologous antigen-antisera combinations. In cross reactions, weak precipitin lines (maximum one) were visible when antiserum of the pathogen *L. theobromae* was allowed to react with antigens of TS-449 and TS-520, both recorded as susceptible varieties. Reciprocal cross-reactions between antiserum of TS-449 and antigen of the pathogen also produced single weak precipitin lines. In contrast, such cross reactions could not be detected when antiserum of the resistant variety was reacted with antigen of the pathogen. In indirect ELISA formats, the susceptible varieties showed higher absorbance values while the resistant varieties showed lower absorbance values when antigens of all the test varieties were cross reacted with antiserum of the pathogen. Thus for compatible host-pathogen interaction, presence of common antigens at a certain minimum level seems to be essential. Presence of common antigens was also evident through microscopic observations when leaf sections of both resistant and susceptible varieties were treated with pathogen antiserum labelled by the immunogold labelling technique followed by silver enhancement. Intense labelling indicating an overall distribution of common antigens mainly in the epidermis was observed in the cross reaction of leaves of the susceptible varieties. It is therefore evident from our results that serological similarity involving one or more antigenic determinants exists between pathogen and compatible host. This resemblance may be utilized in grading the different varieties according to their susceptibility to the pathogen by ELISA. Information on susceptibility factors of compatible hosts or plant genes required for susceptibility of a particular cultivar to a pathogen is limited. Hence, it is difficult to clearly define the role of CRA in host pathogen interaction. It may directly act in invasion and growth of the pathogen leading to

disease establishment or indirectly be involved as immuno-suppressor by interfering with innate host defense machinery.

Several synthetic as well as natural compounds without any antimicrobial activity have been described which can control a large variety of diseases in several crops primarily by inducing the defense related activity in plants against several pathogens through the development of systemic acquired resistance (SAR). However, little is known about defense responses of tea and the underlying mechanisms. In the present study, seedlings of TS-449 (susceptible variety) were sprayed with aqueous extract prepared from fresh leaves (0.5 g ml^{-1}) of *Azadirachta indica, Acalypha indica, Catharanthus roseus* and *Jasminum jasminoides*. The species were selected based on previous reports on their elicitor potential or due to presence of specific bioactive components that might contribute towards their anticipated eliciting property. After 24 hours, one set each of treated and control plants (twenty plants in each set) were inoculated with *L. theobromae*, and chitinase, peroxidase and β-1,3-glucanase activity was estimated every 24 h until four days for four sets categorized as untreated-uninoculated (control), untreated-inoculated, treated-inoculated and treated-uninoculated. Results showed that all the tested extracts induced significant protection against diplodia disease. There was a general two to three-fold increase in levels of peroxidase, chitinase and β-1,3-glucanase over control after 72 hours in all treatments. The plants treated with leaf extract of *Acalypha indica* showed maximum induction of resistance among the tested extracts as almost all enzymes recorded maximum increase in activity. Highest activities were recorded in treated-inoculated sets. Disease incidence was also found to be least in plants pretreated with *Acalypha indica* and challenge inoculated with *L. theobromae*. The induction and accumulation of PR proteins like β-1,3-glucanase (PR-2), chitinase (PR-3) and peroxidase (PR-9) is well documented. In the present study, higher levels of both chitinase and β-1,3-glucanase indicates that these acted synergistically in the treated tea seedlings to counter fungal attack and induced further defense response by the products generated. Peroxidase enzymes may be involved in the biochemical reaction necessary for lignification that protected the plants from invasion by *L. theobromae*. The peroxidase isozyme analysis was performed 72 hours after challenge inoculation with *L. theobromae* using leaf samples from the tea seedlings pretreated with *Acalypha indica* extract. Two major bands (approx mass 38KDa and 33KDa) were observed in all treatments. Staining intensity was more for the slower migrating isozyme in case of untreated-inoculated plants. While both isozymes were less intense in control and treated-uninoculated sets, a particular band of molecular mass approx 33KDa showed maximum intensity in the treated-inoculated leaf samples. The peroxidase isozyme induced by pathogen infection appeared to be different from that induced following treatment with extract and then challenge inoculated with pathogen. Expression of the 33KDa isozyme did not increase much following treatment with phyto-extract but increased dramatically when these treated plants were inoculated with *L. theobromae*. Literature reports indicate that the enhanced ability of the SAR tissue to better activate cellular defense responses does not become obvious until pathogen attack of that tissue. Similarly our results showed increased peroxidase activity in treated tea seedlings following pathogen inoculation. Western blot analysis of the proteins extracted from leaf samples of tea seedlings using antiserum raised against the 33KDa protein showed very weak positive reaction in untreated-inoculated sets or sets that were treated but not inoculated by the pathogen. In leaf samples from plants treated with *Acalypha indica* extracts and challenge inoculated with *L. theobromae*, a positive 33KDa band was clearly observed. This further indicates the involvement of this isozyme with induced resistance in tea against diplodia disease.

Resistance to silverleaf whitefly, *Bemisia argentifolii* (Hemiptera: Aleyrodidae) in *Gossypium thurberi*, a wild cotton species

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The whitefly species, *Bemisia argentifolii* Bellows & Perring and *B. tabaci* (Gennadius), are worldwide cotton pests causing economic losses via yield reduction, lint contamination, and by viral disease transmission. Most studies searching for host plant resistance to whiteflies in cotton focused on two morphological traits, smooth-leaf and okra-leaf shape. The smooth-leaf trait is consistently associated with lowered whitefly susceptibility. Okra-leaf trait results are mixed; it is associated with lowered whitefly susceptibility in some studies, while a slight effect, no effect, or even the opposite effect occurred in other studies. Neither of these traits provides whitefly resistance levels that can be solely relied upon in commercial cotton production. The wild cotton species, *Gossypium thurberi* Todaro, demonstrated a high level of whitefly resistance in an Arizona trial (Wilson *et al.* 1993).

The objectives of this study were to first confirm the presence of high levels of whitefly resistance in *G. thurberi*, and secondly, since *G. thurberi* possesses both smooth-leaf and okra-leaf traits, we compared whitefly susceptibility between *G. thurberi* and a commercial cultivar with the same two traits to determine if resistance in *G. thurberi* is any greater than expected from simply having smooth-leaf and okra-leaf. Finally, we attempted to determine if resistance in *G. thurberi* is characterized as antibiosis and/or antixenosis.

G. thurberi seed was provided by the USDA ARS, Southern Crops Research Laboratory, College Station, Texas). The *G. hirsutum* varieties used included DP 5415 (Delta and Pine Seeds) which has smooth-leaf trait, Siokra L23 (Cotton Seed Distributors Ltd. of Australia) which has both smooth-leaf and okra-leaf, and Stoneville 474 (Stoneville Pedigree Seed Company) which has neither trait. Field tests were conducted at the University of California Desert Research and Extension Center in 2000, 2001, 2002 and 2003. Whiteflies were sampled semi-monthly between June and September by standard whitefly sampling methods in cotton. For each sample date, numbers of adults, eggs, and nymphs were compared between *G. thurberi* and each of the 3 aforementioned commercial cotton entries with three nonparametric Wilcoxon signed rank tests, pairing the data on the basis of experimental block. The family error rate for the three paired comparisons was kept at $\alpha = 0.05$ by using a significance level of $\alpha = 0.0167$ for each comparison.

Stage-specific survival tests: The first stage-specific survival test was conducted on young potted plants of *G. thurberi* and *G. hirsutum* cv. DP 5415 in a greenhouse. Female whiteflies were placed in leaf cages for a 1 day oviposition period. As eggs started to hatch, infested leaves were examined at two to three day intervals until all nymphs had either emerged as adults or died. At each examination date, every nymph was examined, and its instar and status (alive, dead, or missing) were recorded. Survivorship data was plotted, and the time from egg to adult was compared between *G. thurberi* and *G. hirsutum* cultivars

using a two-sample t-test for samples with unequal variances. Two additional stage-specific survival tests were conducted in the field in 2003 and 2005 using similar methods as used in the greenhouse test. The 2003 stage-specific survival field test compared *G. thurberi* and all of the aforementioned *G. hirsutum* cultivars. The 2005 stage-specific survival field test compared *G. thurberi* and cv. DP 5415.

Oviposition tests: Choice and no-choice oviposition tests to compare numbers of eggs oviposited by *B. argentifolii* on *G. thurberi* and *G. hirsutum* cv. DP 5415 were conducted in the greenhouse (2002) and field (2003). In the greenhouse no-choice test, a single unexpanded leaf per plant was fitted with a leaf cage that enclosed one. Adult female whitefly for a two day oviposition period. Numbers of eggs deposited on *G. thurberi* and *G. hirsutum* were compared using the Wilcoxon distribution-free rank sum test. In the choice tests in the greenhouse, pairs of young leaves, one of *G. thurberi* and one of *G. hirsutum*, were confined together in a plastic cylindrical leaf cage with five adult female whiteflies for an oviposition period of five to seven days. Numbers of eggs deposited on *G. thurberi* and *G. hirsutum* were compared using the Wilcoxon distribution-free signed rank test. The choice and no-choice oviposition tests in the field were conducted in a similar manner as described for the greenhouse test except that the whiteflies were contained within mesh tents that covered an entire young plant (no choice test) or pair of plants (choice test). For both choice and no-choice tests, approximately 100 adult whiteflies (mixed sex) were introduced into each tent for an oviposition period of four days. Numbers of eggs deposited on the third fully expanded mainstem leaf of each plant were compared between *G. thurberi* and *G. hirsutum* using the Wilcoxon distribution-free rank sum test for the no-choice tests and the Wilcoxon distribution-free signed rank test for the choice test.

In the field tests *G. thurberi* consistently demonstrated very high levels of resistance when compared to all the other entries. This was evident in adult, egg, and nymphal counts. In the stage-specific survival greenhouse study, survival to adult emergence on both *G. thurberi* and *G. hirsutum* was very high (> 87%), and the survivorship curves were similar between the two cottons with survival on *G. thurberi* slightly higher than on *G. hirsutum*. Survival in the two field tests was much lower than in the greenhouse test, and survival to fourth instar again did not differ greatly among the cotton entries. In the greenhouse test, time to adult emergence was significantly shorter on *G. thurberi* than on *G. hirsutum* (19.5 ± 2.7 days, n =245 versus 27.7 ± 12.9 days, n = 182 [mean ± SD] $P = 0.0001$; two sample t-test for samples with unequal variances). In both the choice and no-choice oviposition tests, there was no significant preference for oviposition on *G. hirsutum* over *G. thurberi*. While the field data conclusively demonstrate a very high level of whitefly resistance in *G. thurberi*, the mechanisms of the resistance remains an enigma. Despite the failure of the oviposition tests to demonstrate non-preference, we suspect that non-preference is the most likely candidate for the mechanism of resistance. The reason for this suspicion is that each year of the field plot tests, the number of adults and eggs early in the season, which would be the initial colonization, were much less on *G. thurberi* than on the other cotton entries. In our choice and no-choice oviposition experiments in this study, the whiteflies were in a confined space which may have interfered with their normal host selection behavior.

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Searching for resistance sources against the Mexican bean weevil (*Zabrotes subfasciatus*) in common bean (*Phaseolus vulgaris*) genotypes

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Introduction

The common bean *Phaseolus vulgaris* L. is the most important one of several related species in the Leguminosae family. Originally from the New World and first cultivated by American ancient civilizations, the crop is nowadays grown widely throughout all major continental areas under extremely variable cropping systems. Beans represent the major vegetable protein source for human consumption worldwide and, in some poor regions, are even the only protein source available to people. In Brazil, the Mexican bean weevil *Zabrotes subfasciatus* (Coleoptera: Bruchidae) is the most important pest of beans during storage, infesting warehouses and causing serious damages to seeds, causing its complete destruction and making them unsuitable for consumption. Bruchid control has been achieved in several ways, but the use of resistant varieties, an environmentally safe control method, must be boosted. Thus, the objective of this work was to find resistance sources against *Z. subfasciatus* among bean genotypes and to identify its resistance mechanisms.

Methods

Insects used in bioassays were obtained from a stock culture that has been kept for several generations on seeds of a susceptible *P. vulgaris* cultivar (Bolinha). All *P. vulgaris* genotypes tested were obtained from the Common Bean Germplasm Collection of the Instituto Agronômico, IAC, in Campinas – SP, Brazil. Both experiments and insect rearing were conducted under laboratory conditions (27ºC, 70% r.h. and natural photoperiod). In a first step, we screened 185 common bean genotypes. Samples of each genotype were infested with one couple of *Z. subfasciatus*, which was allowed to mate and oviposit on seeds for three days and than removed. Seeds were kept into plastic boxes under laboratory conditions and the number of adults emerged from each genotype 50 days after infestation was used as the resistance parameter. 'Bolinha' was also included as the susceptible control. Each treatment had five replicates and the seven most resistant genotypes were selected for the identification of their resistance mechanisms.

In a second experiment we performed two different tests. In a free-choice test we evaluated the weevil oviposition preference by using arenas containing a seed sample of each bean genotype. Each arena was infested with 80 couples of *Z. subfasciatus* which were allowed to mate and oviposit for four days. Adults were then removed and the number of eggs laid on each genotype was assessed. In a no-choice test, two couples of adult weevils were scattered into plastic boxes each one containing 20 seeds from one of the selected genotypes. Insects were allowed to mate and oviposit on seeds during one day and then eliminated, and the plastic boxes were kept under laboratory conditions for evaluation of the number of eggs laid, number and wt. of adults emerged, egg to adult developmental

time and adult longevity in each genotype. 'Bolinha' was included as the susceptible control and each treatment had six replicates.

Results and discussion

In our initial screening, the number of *Z. subfasciatus* emerged ranged from 0 to 18.8 adults/genotype (data not shown). The genotypes with accession numbers 570, 583, 584, 610, 816, 818 and 819 were selected for further evaluation due to the reduced number of adult weevils emerged from them. In our second experiment, the number of eggs laid in the free-choice test ranged significantly among genotypes (Table 1), suggesting sources of antixenosis for oviposition in some of them. The number of eggs laid in the no-choice test ranged from 13.8 (610) to 5.6 (570), but differences were not statistically significant (data not shown). Although there was no difference in this parameter, the difference found in the number of adults emerged can reflect antibiosis sources for genotypes in which this number was low.

The consequence to an insect feeding exclusively on an unsuitable food source is an abnormal development, yielding underweighted and undersized adults. Insect weight is also an indication of antibiosis. However, some insects can compensate food unsuitability by taking in larger amounts of food to reach a normal body size, which could explain the poor range in adult weight found in this research. In the case of weevils, this compensation could lead to an elongated developmental time, which also explains the variations observed. Adult longevity also reflects the insect nutritional status, but our values ranged poorly. Based on our results, the most resistant genotypes are 819, 818 and 816. These genotypes contain different arcelin isoforms, which has been shown to be an important resistance trait against other species.

Table 1. Means of number of eggs laid by *Zabrotes subfasciatus* (in a free choice test) and its number and weight of adults emerged, developmental time and adult longevity (in a no-choice test) in different *Phaseolus vulgaris* genotypes. Means followed by the same letters in a column do not differ by Tukey's test ($P \leq 0.05$).

Genotype	Eggs laid	Adult number	Adult weight (mg)	Developmental time (days)	Adult longevity (days)
610	237.50 a	11.00 a	2.37 ab	30.37 c	8.69 ab
584	233.33 a	3.50 bc	2.55 ab	40.93 ab	10.52 a
816	209.50 ab	1.80 c	2.00 b	44.34 a	8.77 ab
819	200.17 ab	3.00 c	2.23 ab	41.02 ab	8.32 ab
570	197.00 ab	4.80 bc	2.63 a	30.18 c	7.83 b
583	166.50 abc	5.00 bc	2.30 ab	37.98 ab	9.56 ab
818	110.83 bc	2.67 c	2.42 ab	35.65 bc	10.06 ab
Bolinha	71.83 c	8.50 ab	2.72 a	28.92 c	8.92 ab

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Development of defense gene expression monitoring systems by the bioluminescence reporter genes in higher plants

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Induced resistance of plant is mediated by defense gene expression in response to pathogen attack. In order to study the regulated expression of genes involved in the induced resistance, we developed a non-destructive and sensitive defense gene expression monitoring system by the bioluminescence reporter gene. Several defense gene promoter regions fused to the firefly luciferase gene (Fluc) were introduced into higher plant cells and tested for their mode of induction. Treatment with chemicals capable of inducing defense gene expression resulted in a dramatic increase in Fluc activity of transgenic plants harboring the promoter: Fluc fusion. The system also allows us to monitor the regulated gene expression pattern induced by the multiple chemical treatments in a multi-well format. In addition, the system is suitable for the observation of spatiotemporal response of defense genes.

Arabidopsis MPK3 (*Mitogen-activated protein kinase 3*) is an example of a functional gene induced by pathogen infection. It functions in MAP kinase cascade activated by bacterial flagellin that leads to the induction of disease resistance against the bacterium *Pseudomonas syringae* and the necrotrophic fungus *Botrytis cinerea*. Other investigators have speculated that expression of the *MPK3* gene is induced after inoculation with the virulent fungus *Alternaria brassicicola* (Narusaka *et al.* 2003). The *MPK3* gene has been reported to be induced by treatment with signaling compounds that induce defense gene expression (Schenk *et al.* 2000). However, the molecular mechanism involved in the *MPK3*-mediated pathway is not necessarily clear, and the manner of interaction between the MAPK cascade-mediated pathway and SA- or JA-dependent pathway is uncertain. In addition, transcriptional regulation of the *MPK3* gene promoter has not yet been analyzed in detail.

Therefore, study of the regulated expression of the *MPK3* is important for our understanding of the interactions among multiple signaling pathways involved in induced resistance. To further elucidate the mechanism of defense gene regulation in higher plants, we developed an *in vivo* assay system to study *MPK3* gene expression. Luciferase activity was detected spatiotemporally with an *in vivo* imaging system as described previously (Watakabe *et al.* 2001). Bioluminescence images were obtained with a VIM camera (Hamamatsu Photonics) and were subjected to area analysis for quantification of the luciferase activity. To investigate the kinetics of the *MPK3* promoter in response to chemical treatments, we monitored the sequential expression pattern *in vivo*. Fluc activity as determined by luminescence levels demonstrated that induction of the *MPK3* promoter was detectable by 12 h after SA treatment and reached a maximum at 72 h after the treatment. Fluc activity also was induced by 12 h after BTH treatment, but the activity at 72 h was lower compared with that of the SA treatment. *MPK3* promoter activities were not induced by 4HBA treatment, and expression levels were indistinguishable from those of control plants.

To test *MPK3* promoter responsiveness to plant pathogen infection, transgenic *Arabidopsis* plants harboring *MPK3*::Fluc were inoculated with *B. cinerea* by applying a drop of the spore suspension onto the leaf surface. To monitor defense gene expression levels *in planta* in time, we investigated the expression pattern of the *MPK3* promoter after inoculation with *B. cinerea*. Promoter activity in the inoculated leaf was maximal at 12 h after inoculation, and thereafter decreased gradually. The promoter activity in the non-inoculated leaf reached its maximum at 48 h after inoculation, and then tended to decrease over 72 h.

Because the plant defense response is under control of multiple signal transduction pathways, we conducted a comparative study with a different promoter-reporter system in *Arabidopsis*. To examine the expression of a gene that has a site of action different from that of *MPK3*, we performed expression analysis of the *PR-1a* gene promoter in response to *B. cinerea*. We have generated transgenic plants harboring the *PR-1a*::Fluc and could sensitively detect the induction of the *PR-1a* expression by SA or BTH treatment (Ono et al. 2004). To observe SA-mediated regulated gene expression in response to pathogen infection, we inoculated transgenic *Arabidopsis* plants harboring the *PR-1a*:Fluc with *B. cinerea*. The induction of *PR-1a* in the inoculated leaf was detected as enhancement of Fluc light emission. 48 h after inoculation, but, a higher increase in *PR-1a* promoter activity was observed in the inoculated leaf 72 h after inoculation. We also investigated the induction of tissue-specific expression of the *PR-1a* promoter after *B. cinerea* inoculation. The *PR-1a* promoter activity in the inoculated leaf clearly increased 48 h after inoculation, but its activity had decreased by 96 h. Unlike the *MPK3* promoter the non-inoculated leaf of the *PR-1a*::Fluc plants inoculated with *B. cinerea* was induced at a low level.

Results obtained in this study suggest that the luciferase assay system developed in this study is suitable to monitor defense gene expression *in planta*. Using the assay system described in this study, we may be able to obtain further information on the regulatory mechanisms that control the interaction between the defense system and signals that induce defense responses in plants.

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Bacterial acyl-homoserine lactones - signal molecules in quorum sensing and plant defense

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Introduction

Only since few years it is known that bacteria are able to sense their population density and regulate gene expression in a process termed quorum sensing. In Gram-negative bacteria, a wide range of quorum sensing systems is based on the emission of *N*-acyl homoserine lactone (AHL) structures. Upon a threshold concentration, AHL re-entering bacterial cells bind to transcription factors, which stimulate transcription of certain target genes. Thus, the quorum sensing system allows bacteria to regulate gene expression responsible for production of antibiotics or virulence factors, swarming, plasmid conjugal transfer and additional processes. Very recently it was suggested that AHL signal recognition is not restricted to bacteria, but may also affect roots of plants via the rhizosphere, a soil compartment representing a rather densely populated microbial habitat. Although there have been reports on the influence of AHLs on plants, and the production of structural mimics affecting bacterial communities, the fate of the signal compound itself in plants has scarcely been traced. Barley and Yam bean were model plants for our experiments. Based on the question whether signal molecules are taken up and transported in the plant, AHL uptake and translocation in these only far related species was examined.

Materials and methods

In our experiments, barley (*Hordeum vulgare*) and the tropical legugme yam bean (*Pachyrhizus erosus*) were grown as individual plants and were directly compared regarding their uptake and translocation of three AHLs: *N*-Hexanoyl- (C6-HSL), *N*-Octanoyl- (C8-HSL) and *N*-Decanoyl- (C10-HSL) homoserine lactone, which all represent naturally occurring signals in Gram-negative bacteria. Plants were grown in axenic culture in glass vessels on glass beads, and exposed to selected AHL via the roots after 17 and 21 days of growth. AHL concentrations in the system and in plants were followed by UPLC analysis. For questions regarding plant uptake and transport of AHL, radio-labelled compounds were synthezised. Tritiated products were prepared with a method utilizing a palladium catalyst and tritiation with gaseous tritium was applied. Total activity of the [^{3}H]-labelled AHLs enclosed in the mix was 3.7 MBq (100 µCi). Extraction from plants was done by thoroughly homogenizing plant parts in 1 mL of 25% (v/v) acetonitrile using a disperser. The suspension was then added to 5 mL of scintillation liquid, mixed and measured 5 min in a liquid scintillation counter.

Results and discussion

In this experimental setup, less than 10% of the AHLs underwent abiotic degradation processes like photo-catalyzed oxidation or alkaline hydrolysis.

Table 1: names, structures and elemental composition of the investigated AHL

Name	Structure	Elemental composition
C6-HSL		$C_{10}H_{17}NO_3$
C8-HSL		$C_{12}H_{21}NO_3$
C10-HSL		$C_{14}H_{25}NO_3$

Our experiments show clearly that uptake of AHL into plant roots occurs. In Yam bean, uptake seems to be more efficient, but cleavage processes have been described for legumes that might also play a role in this context. Uptake is dependent on the length of the aliphatic chain, and after 21 days of incubation, 72, 66 and 38% of the initial C6, C8 and C10 AHL were left in barley rhizosphere, whereas only 37, 17 and3 % were detectable in Yam bean root zones, as tested with UPLC. When the concentration of radio-labelled AHL was followed in experiments with barley plants, linear decline within 14 days was measured (Table 2).

Table 2: Disappearance of labelled AHL in media of barley experiments means ± Std. dev.

Time [d]	0	2	4	7	12	14
% C6-AHL	100	99	95	98	88	83
Std	1,6	1,8	2	1,9	2	2
% C8-AHL	100	92	84	88	70	63
Std	1,6	1,9	2	1,8	2	2

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The variation and distribution of rice blast physiological race of in Jilin province

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Rice blast, a serious rice disease in China, occurred in large area every year. Planting resistant varieties have been the preferred means to control it. Varieties resistance shown significantly difference in different locations in same year, even lost a few years later. Lots of research results indicated that host resistance variation and losing were correlated with pathogen physiological races. Therefore, monitor and evaluate the variation tend of physiological races are very important to resistance breeding, varieties distribution and delivery varieties. The paper gave out the variation and distribution results of rice blast physiological races in Jilin during 2002 to 2005.

Materials and methods

The samples collected and monoconidial isolates

332 rice blast specimens were collected from paddy fields in 14 locations in Jilin during 2002-2005, and 306 monoconidial isolates were obtained from the specimen.

Differential varieties

Differential rice varieties were 7 China varieties in the test, Tetebo, Zhenlong13, Sifeng43, Dongnong363, Guandong51, Hejiang18 and Lijiangxintuanheigu respectively.

Results and analysis

The types of physiological races:

306 mono-conidial isolates were classified into 7 groups and 29 physiological races by assessment on 7 Chinese differential varieties. They were ZA group (ZA_3, ZA_9, ZA_{26}, ZA_{33}, ZA_{35}, ZA_{41}, ZA_{57} and ZA_{61}), ZB group (ZB_1, ZB_9, ZB_{15}, ZB_{17}, ZB_{23} and ZB_{25}) , ZC group (ZC_1, ZC_3, ZC_5, ZC_9, ZC_{11}, ZC_{13} and ZC_{15}) , ZD group (ZD_1, ZD_3, ZD_5 and ZD_7) , ZE group (ZE_1 amdZE_3), ZF group (ZF_1) and Z group (ZG_1). The predominant groups were ZD and ZE, both occurrence frequencies (OF) were 21.9%. ZG and ZF were secondary (OF were 18.3% and 17.6% respectively), and OF of ZA, ZB and ZC were only 5.8%, 3.9% and 10.7%, respectively. The predominant physiological races were ZG_1, ZE_1 and ZF_1. OF were 18.3%, 17.65% and 17.32%, respectively.

The dynamic variation of predominant physiological races

Japonica physiological race was dominant in Jilin, but dominant groups often varied in different years. ZD was the dominant group in 2002 with an OF was 34.7%, but it became the secondary group in 2003 and 2004 with an OF of 25.6% and 18.9% respectively, whereas OF was only 3.9% in 2005. The OF variation trend of ZD had been decreased during 2003 to 2005. ZE group had a relatively higher OF in 2003 and 2005 when OF was 29.3% and 30.3%, respectively. ZF and ZG groups had higher OF (OF was 26.4%) in 2004. Of of ZC group was 22.3% in 2005.

Dominant physiological races also varied in different yeas. ZD_1 was the dominant race in 2002 (OF was 23.2%), but it became secondary race in 2003 (OF was 18.36%) and OF decreased to 1.3%. ZD_1 was more important in 2003 (OF was 21.9%), but its OF decreased to 9.4% in 2004 and increased to 26.7% in 2005 again. ZF_1 and ZG_1 had an OF (26.4%).

The distribution of race groups in Jilin province

There were seven groups and 29 physiological races in Jilin Province. The distribution of groups and races was: a. Jilin City had seven groups and 25 races. ZD, ZD_1 and ZF_1 were dominant; b. Tonghua had 7 groups and 14 races, ZD, ZF and ZF_1 were dominant; c. Siping had 7 groups and 13 races, ZE, ZG, ZG_1 and ZE_1 were dominant; d. Changchun had 5 groups and 6 races, ZE and ZE_1 were dominant; e. Songyuan had 4 groups and 5 races, ZE and ZE_3 were dominant; f. Liaoyuan and Baicheng had 3 groups and 6 races, ZG and ZG_1 were dominant

Concerning the whole province, there were more races in east part of Jilin, and strong pathogenicity races had higher OF. The races of the middle and west part of Jilin showed weak pathogenicity.

Conclusions

There were seven groups and 29 rice blast races in Jilin province. The dominant groups were ZD and ZE, secondary groups were ZG and ZF. ZG_1, ZE_1 and ZF_1 were dominant races. Major races were Japonica races. Dominant race varied with years and locations.

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Correlation between cyanogenic acid (HCN) content in cassava leaves and tolerance to anthracnosis caused by *Colletotrichum gloeosporioides*

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Abstract
A study carried out in Cameroon, permitted to characterize cassava cultivars (LMR, 81/00110, 92/0326 and W94/009) according to their HCN content in leaves and tolerance to anthracnosis. Results show that low cyanide cultivars: LMR (0.44 $mg.g^{-1}$ HCN) and 81/00110 (0.59 $mg.g^{-1}$ HCN) were more infected by anthracnosis, while cultivars 92/0326 and W94/009 containing high level of HCN (1.23 and 1.77 $mg.g^{-1}$) were more tolerant. Studies *in vitro* show a high inhibitory effect (> 51.41 %) of the HCN extract on *C. gloeosporioides* growth.

Introduction
Anthracnosis is a fungal disease which attacks plant stem and leaves. It is expressed by excessive defoliation leading to low yield and even death of plants. Many methods (chemical, cultural, genetic and biological) are used to fight against cassava diseases. The chemical method which is the most used, presents harmful consequences on the environment. Recourse to natural defense mechanisms is a priority in research programs for plant protection and sustainable agriculture. Cyanogenic substances (linamarin and lotaustralin) are a group of phytocides synthesized by cassava. These glycosides after hydrolysis can produce HCN which has a high inhibitory effect on aerobic respiration (Conn, 1973). In recent years, many researchers are interested at understanding the role of cyanogenic substances in cassava natural defense (Belloti & Riis, 1994). The aim of this work was to show the role of HCN in the reduction of cassava leaf spot in order to establish the correlation between cassava host resistance and HCN content in leaves.

Material and methods
Four cassava varieties with different sensitivity to diseases and different cyanide status were used (Var 1=LMR, Var 2 = 92/0326, Var 3 = 81/00110, Var 4 = W94/009). One strain of *C. gloeosporioides* was isolated on diseased cassava plants for the test *in vitro*. Cultivars (cuttings of 20 cm length) were planted on August 2005 in a random design blocks with four replicates. Anthracnosis symptoms appeared in natural conditions. HCN content in leaves, rate of leaves falling and severity of disease during growing cycle were assessed starting from two months after cuttings planting. The number of leaves was determined by counting all leaves per plant in any observation and then the percentage of leaf falling caused by disease was determined. The severity of anthracnosis was determined by using conventional phyto-pathological methods. The proportions of infected leaves occupied by disease symptoms were estimated by visual examination. HCN content in leaves was evaluated twice: 120 and 210 days after planting (DAP).Hence, 20 g of leaves were crushed and distilled. The distillate was added with a 20 ml of a solution of NaOH 2.5 %. The HCN content was determined by titration with ($AgNO_3$) 0.02 N (1 ml of $AgNO_3$ is equivalent to 1.08 mg of HCN). To evaluate the inhibition potential of HCN on *C. gloeosporioides*, two doses of HCN (75 and 100 µl) were used. The explant (6 mm in diameter) of the fungal inoculum was deposited on PDA medium at the centre of a Petri dish. Mycelial growth was

measured daily for six days in control and HCN treatments. Collected data were analysed statistical. Means in different treatments were compared using the test of Duncan ($P<0.05$).

Results

Two groups of cultivars were effectively distinguished at 210 DAP based on their HCN content. Var1 and var3 with low HCN contents were more sensitive to anthracnosis and showed strong disease severity (Table). This was contrary to var2 and var4 on which high concentrations of HCN were recorded with very low disease infection. Leaves fell more from less cyanide varieties than from more acid ones (Table). The biological test on PDA medium showed that the two concentrations (75 and 100 µl) of HCN extract gave inhibition rates of 51.16 and 51.96%, respectively after six days development of *C. gloeosporioides* compared to controls where the fungus completely covered the Petri dish from the 4th DAP.

Table. HCN content in cassava leaves and disease severity (DS) 120 and 210 DAP

Varieties	120 DAP		210 DAP		Rate of leaf falling (%)
	HCN($mg.g^{-1}$)	DS (%)	HCN ($mg.g^{-1}$)	DS (%)	
Var1	0.38 b	4.43 c	0.52 b	21.38 c	50.19 ± 6.21 a
Var2	0.90 a	2.44 a	1.23 a	5.50 a	43.56 ± 7.71 b
Var3	0.45 b	3.75 ab	0.79 b	17.79 bc	49.22 ± 8.25 a
Var4	0.49 ab	2.74 a	1.77 a	5.31 a	42.43 ± 6.45 b

Means followed by the same letter are not different according to the Duncan's test ($P<0.05$)

Discussion

HCN content recorded 120 DAP (dry season) was relatively low despite differences between cultivars. At this same period, the degree of cassava stems and leaf infection by anthracnosis was very low. 210 DAP (rainy season), the incidence and severity of anthracnosis were higher on all the cultivars, confirming the return of favorable conditions for the installation, propagation and evolution of the disease. Chemical analysis of leaves collected 210 DAP equally showed an increase in HCN content in the different cultivars. On the one hand, this leads to the thought that cyanogenic substances are best mobilized in the presence of parasites, hence participating in the defense of plants that synthesize them. On the other hand, atmospheric conditions play a role in this mobilization. This corroborates the results of Bala (1994); Belloti & Riis (1994). Var1 and var3 which had low HCN contents in leaves were more sensitive to the disease than var2 and var4 with high HCN contents which resisted strongly against anthracnosis. This allows to say that there is a positive correlation between HCN content in cassava leaves and resistance to leaf spot disease. The strong inhibition of *C. gloeosporioides* by HCN extracts observed *in vitro*, confirms the hypothesis which state that cyanogenic substances actively take part in the defense of plants. In this light, cyanogenic substances instead of witnessing discrimination should be exploited in selection programs as molecular markers for the protection of plants against diseases and other parasites.

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Transgenic resistance to *Heliothis/Helicoverpa*: implications for sustainable crop production

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There is a continuing need to increase food production, particularly in the developing countries of Asia, Africa, and Latin America. One practical means of increasing crop productivity is to minimize the losses caused by insect pests, which are estimated at 14% of the total agricultural production. Massive application of insecticides to minimize the losses due to insect pests has resulted in adverse effects on the environment, and it is in this context that insect resistant transgenic plants can play a major role in pest management for sustainable crop production (Sharma *et al.*, 2004).

Genetically modified plants with resistance to insects were developed in the mid 1980's (Barton *et al.*, 1987), and since then, there has been a rapid progress in developing transgenic plants with insect resistance (Sharma *et al.*, 2004). The area planted to transgenic crops has increased from 1.7 million ha in 1996 to over 100 million ha in 2006 (James, 2006). Nearly one quarter of the crop area planted to transgenic crops is grown in developing countries, and over 75% of the farmers are the small resource-poor farmers in the developing countries.

Development of transgenic crops for resistance to *Heliothis/Helicoverpa*

Transgenic cottons expressing *Bt* genes are effective against *Helicoverpa zea*, *Heliothis virescens* (Benedict *et al.*, 1996), and *Helicoverpa armigera* (Sharma and Pampapathy, 2006). In China, *Bt* cotton hybrids resulted in 20% increase in productivity over the conventional cultivars (Dong *et al.*, 2004). In India, transgenic hybrids suffered 20 to 40% less bollworm damage, and resulted in 25 to 45% increase in cottonseed yield than the non-transgenic hybrids (Sharma and Pampapathy, 2006). Under farmers' field conditions, the transgenic hybrid MECH 162 suffered lower damage by the bollworms, *H. armigera* and *P. gossypiella* (11.5%) as compared to the conventional cotton (29.4%) under integrated pest management (Bambawale *et al.*, 2004). Only three sprays were needed for pest control on the transgenic cotton compared to seven sprays on the conventional cotton.

Resistance to feeding by corn earworm, *H. zea* has been observed in *Bt* maize hybrid, Tex6 as compared to non-transgenic hybrid, B 73 (approximately three-fold) (Dowd and White, 2002). Plants expressing *Bt* genes have also been found to be resistant to *H. armigera* in chickpea (Sanyal et al., 2005) and pigeonpea (Surekha *et al.*, 2005; Sharma *et al.*, 2006).

Transgenic plants in *Heliothis/Helicoverpa* management

Transgenic crops have shown a major impact on *Heliothis/Helicoverpa* management worldwide (Sharma *et al.*, 2004). The major advantages of growing transgenic crops are: a significant reduction in insecticide sprays and increased activity of natural enemies, reduced exposure of non-target organisms to pesticides, and a reduction in pesticide residues in food and food products. Transgenic crops are not a panacea for solving all the pest problems. The major limitations of using transgenic plants are: emergence of secondary pest problems, evolution of insect populations resistant to the transgenic crops,

adverse effects on non-target organisms, gene escape into the environment, and social and ethical issues.

Conclusions

Incorporation of insecticidal genes in crop plants will have a tremendous effect on pest management. Emphasis should be placed on combining exotic genes with conventional host plant resistance, and also with traits conferring resistance to other insect pests and diseases of importance in the target region. There is a need to follow integrated pest management practices from the very beginning to make transgenic crops a viable technology for sustainable crop production.

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