

# Tree and Forestry Science and Biotechnology

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### Guest Editors

**Dr. Paula Tennant**

**Dr. Noureddine Benkeblia**

**University of the West Indies, Jamaica**

**In co-operation with**

**University of the West Indies, Jamaica**



**Cover photos:** Top, left and center: GUS-positive calli and embryos, respectively (Duan *et al.*, pp 12-17). Top, right: Citrus leaves with variegated chlorosis symptoms (de Souza *et al.*, pp 73-80). Center, right: A *p23*-trifoliate orange plant showing developmental aberrations (Fagoaga *et al.*, pp 23-29). Center, left (photos + graph) Stages of flavedo coloration and TDFs distribution of down- and up-expression in FJWC compared with FJ72-1 at different ripening points (Liu *et al.*, pp 5-11). Bottom, left: Canker symptoms on lower leaf surfaces; Bottom, center: Adult winged forms and nymphs of the brown citrus aphid, *Toxoptera citricidus*; Bottom, right: Nymphs and eggs of the psyllid, *Diaphorina citri* (Tennant *et al.*, pp 81-107).

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## The Guest Editors

**Dr. Paula Tennant** is a research plant pathologist working with virus diseases of fruit crops at the University of the West Indies, Mona, Jamaica. She received a BSc degree in Botany from the University of the West Indies and her PhD in Plant Pathology at Cornell University. Post doctoral experience working with *Papaya ringspot virus* and the efficacy of pathogen-derived resistance for the control of the virus in Jamaica was at Cornell University and the Biotechnology Centre, University of the West Indies, Mona. These efforts and close collaboration with the Jamaica Agricultural Development Foundation (JADF) have led to the establishment of the appropriate regulatory structures to oversee the use and importation of agricultural biotechnology products in Jamaica and the development of a bio-engineered agricultural product of the Caribbean. She joined the Staff in the Department of Life Sciences in 2001, continuing work with papaya and more recently initiating work on the characterization of *Citrus tristeza virus* isolates and citrus viroids in Jamaica. In 2001, Dr. Tennant received the Young Agriculturist Award from the Inter-American Institute for Cooperation on Agriculture (IICA) for her work on papaya. She co-teaches a graduate course, Plant Diseases and their control, and undergraduate courses in Molecular Biology, Virology and Plant Biotechnology at the University of the West Indies, Mona.



**Dr. Noureddine Benkeblia** is a scientist involved in postharvest plant biochemistry and physiology, including preservation technologies for horticultural crops. His work is mainly devoted to the metabolism of the carbohydrate, fructooligosaccharides (FOS), during plant development and storage periods. A few years ago, he introduced the new concept of system biology – Metabolomics – to investigate the mechanisms of biosynthesis and accumulation of FOS in Liliaceous plants. Dr. Benkeblia first received his BSc, MSc and Doctorate (PhD) from INA (Algeria), and Doctor in Agriculture (PhD) from Kagoshima University (Japan). After few years teaching in Algeria, he joined INRA, Avignon (France) as a Postdoctoral Scientist from 2001. From 2002 to 2008, he worked as a Visiting Professor in the University of Rakuno Gakuen, Ebetsu (Japan) and also as a Research Associate in Hokkaido University from 2005 to 2007. Dr. Benkeblia joined the Staff in the Department of Life Sciences University of the West Indies, Mona, Jamaica in 2008, continuing his work on the physiology, the biochemistry and metabolomic of fructan-containing plants in Jamaica. He also works on the postharvest physiology and biochemistry of local fruits such as ackee and sorrel. Dr. N. Benkeblia is teaching plant physiology, horticulture and postharvest management of crops.



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**SPECIAL ISSUES: CITRUS III (Guest Editors: Paula Tennant, Nouredine Benkeblia (University of the West Indies, Jamaica)) ~ March, 2009**

**Special Issue 2**

**Viviana De Luca, Serena Rizza, Giuliana Catara, Clemente Capasso, Antonio Capasso, Vincenzo Carginale (Italy)** The Application of Differential Display as a Gene Profiling Tool in *Citrus* (pp 1-4)

**ABSTRACT**

**Invited Mini-Review:** *Citrus* (*Citrus* spp.) is one of the most important and widespread fruit crops, with great economic and health value. However, it is among the most difficult plants to improve through traditional breeding approaches. In this context, the recent advent of genomic technology and of powerful new tools, such as differential display (DD-PCR), serial analysis of gene expression (SAGE), and cDNA microarray, have greatly increased the study of biological processes relevant to citriculture, ranging from developmental biology, to biotic and abiotic stresses, and post-harvest processes. This mini-review deals with the application of the DD-PCR technique to investigate gene expression in *Citrus*. The findings of various research groups on the isolation and profiling of genes expressed in *Citrus* during physiological events, and biotic and abiotic stress conditions with this methodology are described. Even genes expressed at very low levels, such as transcriptional factors, membrane proteins, and rare enzymes have been successfully isolated using this approach. These results suggest that DD-PCR is a very suitable method for investigating rare genes involved in life cycle of plants, especially when genomic sequence information of the species is not available.

**Yong-Zhong Liu, An-Dan Zhu, Ying Lei, Xiu-Xin Deng (China)** Identifying Differently Expressed Transcripts between a Novel Late-Ripening *Citrus sinensis* Mutant and its Wild Variety during Fruit Ripening using cDNA-AFLP (pp 5-11)

**ABSTRACT**

**Original Research Paper:** 'Fengjiewancheng' (FJWC) is a late-ripening bud sport of 'Fengjie72-1' navel orange (FJ72-1). To investigate transcript differences during fruit ripening between the two cultivars and to get clues as to the mechanisms monitoring the late-ripening traits, we compared their transcript profiles during fruit ripening using cDNA-AFLP. As a result, 144 transcript-derived fragments (TDFs) were found differently expressed in FJWC compared with FJ72-1 of which 129 TDFs were recovered and assembled into 51 uniTDFs. Blast annotation indicated that 39 uniTDFs were assigned with putative function in signal transduction, control of gene expression and carbohydrate biosynthesis. Some TDFs shared relatively high identities with known functional genes and showed differential expression patterns in fruit pulp or peel between FJWC and FJ72-1 during fruit ripening, implying a potential role in the formation of the late-ripening phenotype.

**Yanxin Duan, Dingli Li, Jing Fan, Xiuxin Deng, Wenwu Guo (China)** *Citrus* Callus Browning Influences Transformation Efficiency (pp 12-17)

**ABSTRACT**

**Original Research Paper:** Our previous experiments on *Agrobacterium*-mediated citrus callus transformation showed that transformant regeneration is often accompanied by callus browning. To further evaluate the effect of citrus callus browning on transformation, embryogenic calli from 13 citrus species/varieties (including *Citrus reticulata*, *C. paradisi*, *Fortunella crassifolia*, *C. sinensis*, etc.) were inoculated with *Agrobacterium* and assessed for their transformation potentials. Transformation results showed that all but one succeeded in regenerating kanamycin resistant tissues, and among them six produced whole transgenic plants. Histochemical  $\beta$ -glucuronidase (GUS) analysis and southern blot hybridization of resistant tissues confirmed transgene integration. In fact, more than 91% of transformed calli were regenerated from browning callus. Moreover, the transformation efficiency was higher in severe browning species/varieties, suggesting that callus browning was closely related to transformation efficiency. A further study showed that the total phenolic content of citrus callus affected callus browning and was positively correlated with transformation efficiency. Taken together, our data indicate that the effect of citrus callus browning on transformation efficiency might correlate with its content of polyphenols.

**Sergio Fatta Del Bosco, Mirko Siragusa, Anna Mariani (Italy)** Cytomixis in Pollen Mother Cells of *Citrus* Genotypes (pp

**ABSTRACT**

**Original Research Paper:** A microsporogenesis analysis performed in several *Citrus* populations revealed the presence of cytomicis (i.e., chromatin migration between meiocytes) in pollen mother cells of diploid and tetraploid genotypes. Single and double chromatin bridges between adjacent meiocytes were mainly observed at prophase I, but also in the subsequent phases of first division and in the second meiotic division. The percentage of cytomictic cells in the genotypes examined was quite variable. In addition to cytomicis, other meiotic abnormalities were observed, in both the first and second meiotic divisions. The cytomictic plants showed lower values of pollen viability compared to the control plants. A possible relationship between the occurrence of cytomicis, pollen viability and the reduced fertility of *Citrus* populations is discussed.

**Carmen Fagoaga, Carmelo López, Pedro Moreno, Luis Navarro, Ricardo Flores, Leandro Peña (Spain)** The p23 Protein from *Citrus tristeza virus* is a Pathogenicity Determinant in Transgenic Citrus Hosts (pp 23-29)

**ABSTRACT**

**Invited Mini-Review:** The 23 kDa protein (p23) coded by the 3'-terminal gene of *Citrus tristeza virus* (CTV), is a RNA-binding protein that contains a motif rich in cysteine and histidine residues in the core of a putative zinc-finger domain. Transgenic Mexican lime plants were generated carrying a p23 transgene, or a truncated version thereof, under the control of the *Cauliflower mosaic virus* (CaMV) 35S promoter. Ectopic expression of the p23 gene from a severe (T36) strain of CTV induced phenotypic aberrations that resembled symptoms incited by CTV in non-transgenic lime plants, whereas transgenic plants expressing the p23 truncated version were normal. The intensity of the CTV-like symptoms in p23-transgenic plants was associated with the p23 accumulation level. Besides, expressing the same gene from a mild strain (T-317) induced similar symptoms irrespective of the source CTV strain. Transformation of CTV-susceptible sweet and sour orange and CTV-resistant trifoliate orange with p23-T36 also led to CTV-like symptoms that did not develop when plants were transformed with a truncated p23 version. In these transgenic citrus species, symptom intensity correlated with levels of p23 transcripts, as protein accumulation was barely detectable. Conversely, transgenic expression of p23 in CTV non-host *Nicotiana* spp. led to accumulation of p23 without phenotypic aberrations, indicating that p23 interferes with plant development only in *Citrus* species and relatives. This was the first case in which a protein encoded by a woody plant infecting RNA virus was identified as being directly involved in pathogenesis in its natural host. In this note, we summarize our results on the role of p23 as a pathogenicity determinant of CTV in citrus hosts.

**Vicente J. Febres, Abeer Khalaf, Frederick G. Gmitter Jr., Gloria A. Moore (USA)** Production of Disease Resistance in Citrus by Understanding Natural Defense Pathways and Pathogen Interactions (pp 30-39)

**ABSTRACT**

**Invited Review:** Molecular tools have facilitated the discovery and study of genes associated with natural defense pathways in a number of model systems. In our laboratories, citrus homologues of key genes have been identified (for instance, *NPR1* and *PR1*) using comparative analysis and their expression characterized. In addition, differential gene expression during infection with citrus canker has been examined. Both approaches have facilitated the study of defense responses in citrus. The improved understanding of these natural defense pathways in model species has allowed plant-derived genes to be used to induce disease resistance. These recent discoveries as well as strategies for their practical application in citrus breeding are discussed in this review.

**Paulo Teixeira Lacava, João Lúcio Azevedo (Brazil), Thomas Albert Miller, John Stephen Hartung (USA)** Interactions of *Xylella fastidiosa* and Endophytic Bacteria in Citrus: A Review (pp 40-48)

**ABSTRACT**

**Invited Review:** Citrus variegated chlorosis (CVC) is a disease of sweet orange [*Citrus sinensis* (L.)] caused by *Xylella fastidiosa* subsp. *pauca*, a phytopathogenic bacterium that has been shown to infect all sweet orange cultivars. *Xylella fastidiosa* is a fastidious Gram-negative, xylem-limited bacterium which was rapidly disseminated by infected nursery trees and by several xylem-feeding sharpshooter insect vectors. In Brazil, CVC is the most economically important of several plant diseases caused by *X. fastidiosa*. One factor that may confer apparent resistance to CVC is the endophytic microbial community colonizing individual *C. sinensis* plants. Endophytes are microorganisms that do not visibly harm the host plant, but

can be isolated from the internal tissues of surface-disinfected plants. Furthermore, as they colonize an ecological niche similar to that of certain plant pathogens, they are likely candidates for biocontrol agents. There is evidence that *X. fastidiosa* interacts with endophytic bacteria present in the xylem of sweet orange, and that these interactions, particularly with *Methylobacterium mesophilicum* and *Curtobacterium flaccumfaciens*, may affect disease progress. Studies of endophytic bacterial populations in sweet orange suggest that symptoms of CVC in sweet orange could be influenced by the relative populations of *Methylobacterium* spp., *C. flaccumfaciens* and *X. fastidiosa* subsp. *pauca*. Symbiotic control is a new strategy that uses symbiotic endophytes as biological control agents to antagonize or displace pathogens. Candidate endophytes for use in symbiotic control of CVC must occupy the xylem of host plants and attach to the precibarium of sharpshooter insects in order to have access to the pathogen. In the present review, we focus on interactions between endophytic bacteria from sweet orange plants and *X. fastidiosa* subsp. *pauca*, especially those that could result in some strategy for symbiotic control of CVC.

**Ana Ortuño, José Antonio Del Río (Spain)** Role of *Citrus* Phenolic Compounds in the Resistance Mechanism against Pathogenic Fungi (pp 49-53)

#### ABSTRACT

**Original Research Paper:** Fungi of the genera *Penicillium* and *Alternaria* are responsible for substantial post and pre-harvest losses in *Citrus*. To prevent their development and reduce commercial losses, chemical fungicides are used. However, such treatments can result in serious problems, such as residues on the fruit and the development of fungicide resistant strains. For this reason, it was of pressing interest to investigate alternatives to chemicals, such as flavonoids, that are believed to be involved in the defence mechanism of the genus, *Citrus*. The results obtained show that in the case of infection by *Penicillium digitatum*, certain citric flavonoids may well be involved in the defence mechanism, and may be considered as a phytoalexin against this fungus and to act as a chemical barrier. Although these secondary compounds exhibited phytotoxic activity against *Alternaria alternata* pv. *citri* in *in vitro* studies, the effect was not sufficient to slow down the development of the fungus. This fungus has developed a mechanism to metabolise these phenolic compounds which actually promotes the development of necrotic areas in the fruit, through which the fungus to spread more easily.

**Satish Kumar Sharma, Ram Rosham Sharma (India)** Citrus Canker Approaching Century: A Review (pp 54-65)

#### ABSTRACT

**Invited Mini-Review:** Citrus canker was recognized in 1912 in Florida, USA, and it became so severe that mass eradication of diseased plants was undertaken in the United States to prevent further spread. The campaign to eradicate citrus canker in the USA began in 1915 and the disease was declared eradicated from these areas by 1947. The pathogen originated in the tropical areas of Asia, such as South China, Indonesia and India, where *Citrus* species are presumed to have originated. The disease is presently prevalent in Africa, Asia, Australia, Oceania and South America. Citrus canker causes heavy losses when the infection occurs at early stages of plant growth. The causal bacterium, *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin, has three distinct forms (A, B and C) based on geographical distribution and host range. This review focuses primarily on historical developments of canker disease, host-pathogen interactions, variability, and latest achievements in the management of the disease through quarantine, cultural means, resistance sources, biocontrol techniques and biotechnological approaches. It also takes stock of the situation where restricted chemicals are still being used in some countries for managing the disease and will be a source of information for researchers and extension workers.

**Nian Wang, Wenbin Li (USA), Mike Irey (China), Gene Albrigo (USA), Kong Bo (China), Jeong-soon Kim (USA)** Citrus Huanglongbing (pp 66-72)

#### ABSTRACT

**Invited Mini-Review:** Citrus Huanglongbing is one of the most devastating diseases of citrus. This disease is associated with a phloem-limited, non-cultured, Gram-negative alpha-proteobacteria: *Candidatus Liberibacter* spp. The pathogen causes blotchy mottle on the leaves, yellow shoots, stunted growth, tree decline, as well as lop-sided fruits. In this review, we attempt to present a compilation of the current research activities on the diagnosis, *in planta* distribution of the bacterium and symptomology, and host response to HLB pathogen infection.

**Alessandra Alves de Souza, Marco Aurélio Takita, Alexandre Morais do Amaral, Helvécio Della Coletta-Filho, Marcos A. Machado (Brazil)** Citrus Responses to *Xylella fastidiosa* Infection, the Causal Agent of Citrus Variegated Chlorosis (pp 73-80)

#### ABSTRACT

**Invited Mini-Review:** Among citrus diseases, citrus variegated chlorosis is one of the most important in Brazil. It causes annual losses of US\$100 million to the citrus industry with chemical control of the vectors, pruning and roguing of diseased trees. The production of severely infected trees is heavily affected since they end up with reduced fruit size with hard rind, and not useful for industry or direct consume. The disease is caused by *Xylella fastidiosa*, a Gram-negative bacterium that lives inside the xylem vessels. First identified as a pathogen of vine, this bacterium has its pathogenicity associated with the colonization of the vessels, forming a dense biofilm that blocks the flow of the sap, generating a strong water deficit in the upper part of the affected plants. The tree physiology is heavily affected with a misbalance in different processes of the susceptible plants. The spectrum of susceptibility/resistance varies widely among the citrus group and genetic factors seems to be associated with the resistance in particular species. The understanding of the resistance mechanisms in citrus is deeper and points to an active response mediated by recognition elements that signalize the presence of the pathogen leading to the production of defense weapons that kill the bacterium. This chapter reports the complex responses associated with the presence of *X. fastidiosa* both at the physiological and genetic levels that allowed a better understanding of this host-pathogen interaction in a search for resistant varieties with increased agronomical value.

**Paula F. Tennant, Dwight Robinson, Latanya Fisher, Stacy-Marie Bennett, Dave Hutton, Phyllis Coates-Beckford, Wayne Mc Laughlin (Jamaica)** Diseases and Pests of Citrus (*Citrus* spp.) (pp 81-107)

#### ABSTRACT

**Invited Review:** *Citrus* spp. are susceptible to a number of destructive diseases that are continuously emerging and which can severely limit production or totally decimate an industry of a country. There is the well known tristeza caused by the *Citrus tristeza virus*, the crippling bacterial citrus huanglongbing (*Candidatus Liberibacter*) and canker (*Xanthomonas axonopodis* pv. *citri*). Among fungal diseases, soil-borne infections caused by *Phytophthora* and postharvest pathogens are widespread and lead to heavy yield losses. Several species of phytonematodes have been detected in citrus rhizosphere, but the economic importance is known for only a few species. *Tylenchulus semipenetrans* occurs worldwide and can reduce yields when populations are dense. Other nematodes of local or regional importance include *Radopholus citrophilus*, *Belonolaimus longicaudatus* and species of *Pratylenchus* and *Meloidogyne*. Numerous species of insects have attained pest status at various locations at different times because of the damage they cause on citrus trees and fruits and the pathogens they transmit. Lepidopterans of major importance are *Papilio* spp., particularly the lime swallowtail butterfly, *Papilio demoleus*, which has recently invaded the western hemisphere. Larvae of coleopterans, *Exophthalmus* and *Pachnaeus* spp. cause extensive root damage, while adults damage young leaves. Of the aphid and psyllid species, *Toxoptera citricida* and *Diaphorina citri*, are considered as potentially the most dangerous at this time because they serve as vectors for *Citrus tristeza virus* and huanglongbing. This paper presents an overview of the important pathogens and pests limiting citrus production, diseases they elicit, genetic structure and diversity, and factors contributing to their emergence.

**Kouhei Ohtani, Takeshi Fukumoto, Satoshi Nishimura, Yoko Miyamoto, Kenji Gomi, Kazuya Akimitsu (Japan)** Alternaria Pathosystems for Study of Citrus Diseases (pp 108-115)

#### ABSTRACT

**Invited Mini-Review:** Leaf spot disease of rough lemon and brown spot disease of tangerines caused by distinct pathotypes of *Alternaria alternata*, and postharvest fruit black rot disease caused by *A. citri*, are the three major fungal diseases of citrus. The pathogenicity and specificity for the pathogens of Alternaria leaf spot and brown spot diseases are associated with the production of different host-specific toxins designated as ACR- and ACT-toxins. Production of endopolygalacturonase plays a role in causing disease symptoms of Black rot pathogen of *A. citri*. This review summarizes the recent progress on these citrus pathomodels.

**Carla A. Nunes, Teresa Manso, M<sup>a</sup> Emília Lima-Costa (Portugal)** Postharvest Biological Control of Citrus Fruit (pp 116-126)

#### ABSTRACT

**Invited Review:** Economical losses due to postharvest decays are very important worldwide, and fungicides are the primary means to control these losses. Public concern in food safety and the increase of pathogen resistant population has enhanced the interest in developing alternatives fungicides to control postharvest fruit diseases. The research in biological control using

antagonistic microorganisms has been developed as an important food safety alternative. Biocontrol of postharvest products has the advantage to be in a controlled environment which can be manipulated to favor the biocontrol agent. Actually there are already in the market three biofungicides to control postharvest diseases of fruits, including citrus fruit. It is likely that several more products will enter the market in the near future, as the result of the biological control research programs worldwide. The development of a biocontrol system requires several steps in order to isolate, test and select a potential biocontrol agent. Bioassays at a pilot and commercial scale must be addressed; the antagonistic mechanism of the microorganism has to be understood. For commercial application, biocontrol agent has to be produced and formulated at an industrial scale, maintaining its biocontrol activity. This paper presents an overview of postharvest biological control approaches especially of citrus fruit and explores new possibilities of research to improve biocontrol activity.

**Lluís Palou (Spain)** Control of Citrus Postharvest Diseases by Physical Means (pp 127-142)

#### **ABSTRACT**

**Invited Review:** Economic losses due to fungal postharvest diseases are among the most important concerns of the citrus industry worldwide. Typically, these diseases have been successfully controlled by the application of synthetic chemical fungicides. However, human health risks and environmental contamination associated with chemical residues and the proliferation of resistant strains of the pathogens are major problems associated with the continuous and widespread use of conventional postharvest fungicides. There is, therefore, an increasing need to find and implement alternatives such as physical, chemical, or biological postharvest treatments as part of integrated management programs for disease control. In this article, extensive research work based on the evaluation of physical means used alone or in combination with other control methods for citrus decay control is reviewed. Efficacy, general performance, direct and indirect modes of action, potential benefits, advantages and disadvantages, and commercial feasibility of direct antifungal physical treatments, such as heat (curing, hot water dips, and hot water rinsing and brushing) and irradiation (UV-C illumination and ionizing radiation), are discussed. The role of complementary physical means such as storage at low temperatures or in controlled atmospheres to minimize decay losses is also described.