AGRONOMY

Anne-Laure Boutigny

Stellenbosch University

Mentor: A Viljoen

Broad research area: Agronomy

Specific research field: Plant pathology

Purpose of study:

My research focus area involves an important plant disease called Fusarium head blight (FHB) of small grains. FHB results from the infection of cereal grains by microscopic fungi of the genus Fusarium and leads to drastic reductions in crop yield. Various species of Fusarium can produce toxic secondary metabolites, referred to as mycotoxins, which accumulate in the grain. This causes a reduction in grain quality, leading to significant economic losses. In addition, the occurrence of mycotoxins in grains constitutes an important food safety issue. My research project aims at identifying the *Fusarium spp.* involved in the FHB of wheat and barley fields of South Africa in order to develop management strategies for the disease. Characterising these Fusarium isolates by means of molecular and biochemical analysis in terms of their ability to produce mycotoxins is one of my primary objectives, as mycotoxin contamination of cereals and their derived products can lead to significant economic and health consequences in South Africa.

Contact: aboutigny@sun.ac.za

Charlotte Sawyer

University of KwaZulu-Natal

Mentor: Prof M D Laing

Broad research area: Plant pathology

Specific research field: Post-harvest bio control of citrus

Purpose of study:

Globally, the greatest post-harvest loss of citrus fruit is as a result of decay caused by the postharvest pathogens *Penicillium digitatum* (green mould), *P. italicum* (blue mould) and *Geotrichum candidum* (sour rot) and may be as high as 40% in export crops (Palou et al, 2001). Post -harvest damage as a result of infection by these pathogens is controlled using fungicides imazalil and guazatine. However, resistance problems, cost and international objections to fungicide residues restricts the effective use of these post-harvest fungicides. The need for environmentally and consumer-friendly alternatives is therefore a priority. Prior research suggests that the application of a potassium silicate (K2SiO3) fertiliser in the orchard prior to harvest, combined with novel treatments in the packhouse may provide a competitive alternative to fungicides. The system involves a hot K2SiO3 bath, followed by application of a yeast biocontrol agent to fruit picked from citrus trees treated with silicon fertiliser in the orchard.

Soluble silicon fertiliser applications pre-harvest improves the ability of citrus fruit to withstand chilling injury (Confidential: pers. comm. JP Bower). This is important where fruit is exported to markets under cold sterilisation conditions (21 days at -0.5°C), to kill fruit fly larvae. The possible improvement of resistance to chilling injury is important to lemon and grapefruit farmers because these cultivars are more susceptible to chilling injury. Interestingly, heat treatments have been recognised as a method for inducing resistance to chilling injury (Lafuente et al, 2005).

There has been a trend towards increased awareness of consumers of the usage of fungicides on their fruit, and many retailers demand that chemical usage be minimised. In addition, resistance to commonly used fungicides by key post-harvest pathogens is becoming apparent and while currently multiple resistance between old and new post-harvest fungicides has not been detected (Kanetis et al, 2008), strains with resistance to multiple fungicides are certain to develop.

Contact: 209540444@ukzn.ac.za

Julia Meitz
Stellenbosch University
Mentor: Dr A McLeod

Broad research area: Agronomy
Specific research field: Plant pathology

Purpose of study:

The main *Phytophthora* species known to occur on citrus include *P. nicotianae, P. citrophthora* and *P. palmivora*. Of these species *Phytophthora palmivora* has not yet been reported from citrus in South Africa. The objectives of the study were to determine which *Phytophthora* species are present in different citrus production regions and to evaluate real-time PCR methods for detecting Phytophthora species from soil samples.

A *Phytophthora* survey was conducted in 64 citrus orchards and nurseries in seven different South African citrus production regions (Eastern Cape, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, Western Cape). In total 172 *Phytophthora* isolates were collected that were identified to the species level using PCR-RFLP of the ITS region. The species that was most frequently found was *P. nicotianae* (72%), followed by *P. citrophthora* (24%). *Phytophthora citricola* was only found in one orchard in the Western Cape. *Phytophthora nicotianae* was found in all seven provinces, whereas *P. citrophthora* was found in four of the provinces (Eastern Cape, Limpopo, Mpumalanga and Western Cape. In addition to the aforementioned three species, a few *Phytophthora* isolates were also found that may represent new species, and these are being characterized further. No *P. palmivora* isolates were found in the survey. We further developed a more sensitive method (Real time PCR) for detection of the pathogens. We used one published primer pair for detection of the genus *Phytophthora*, as well as one newly developed species specific primer for *P. palmivora* using SYBR GreenTM. The *Phytophthora* genus specific primer pair amplification products were analyzed using high resolution melting (HRM) curve analyses, which was able to distinguish among some of the species. The real-time PCR method revealed the presence of some of the inoculated species that could not be detected using the culture based method.

Contact: juliam@sun.ac.za

Maina Muniafu
Tshwane University of Technology
Mentor: Prof F D Dakora

Broad research area: Agronomy
Specific research field: Assessing water stress tolerance in Cowpea (*Vigna unguiculata*)

Purpose of study:

The project involves a study in which a wide range of cowpea cultivars are measured for their tolerance to water stress using 13C and 15N isotopes. Stable isotopes have now been widely accepted as one of the methods that can be used to measure drought tolerance of crops growing in marginal environments. The method integrates many physiological variables that cannot be measured individually in a practical manner. Twenty one varieties of cowpea (*Vigna unguiculata*) are grown in a split-plot randomised design in polythene pots in a controlled environment where the main plots consist of three treatments namely; unstressed (well watered), medium stressed (moderately watered) and severely stressed conditions, replicated three times. Five seeds are sown, after inoculation with rhizobium, in each pot but the plants are thinned to two 14 days after sowing. Stressed treatments received water once a week while medium stressed plants received a half of the amount received in the unstressed treatment weekly. Severely stressed plants also received half of the amount given to unstressed plants but only once every two weeks. Soil moisture content is measured gravimetrically weekly. Humidity, temperature, soil nutrient content and soil texture were also scored weekly. Plant sampling is done near peak biomass whereas 13C and 15N measurements were measured from whole plants, shoots and roots and then correlated with soil moisture regimes. From the results it is possible to assess which cowpea cultivars present a higher tolerance to soil water deficits.

Contact: MuniafuMM@tut.ac.za

Rashid Ishmael Ibrahim
University of KwaZulu-Natal

Broad research area: Genetics
Specific research field: A transformation vector for the chloroplast genome of cotton (*Gossypium barbadense L.*)

Purpose of study:

Most genes of the chloroplast genome are polycistronic. Therefore, an operon of multiple trangenes can be introduced in a single transformation process. For optimal expression and translation, promoters and terminators of trangenes must be of endogenous chloroplast origin. Integration of transgenes into an intergenic spacer in the inverted repeat of the cotton chloroplast genome, secures two transgenes per genome.

The expression cassette in this vector composed of promoter and terminator sequences of the rbcL gene as expression control elements for a selection marker gene, as well as promoter and terminator sequences of the psbA gene as expression control elements for transgene. Here, the aadA gene was used as a selection marker because it produces more transplastomic plants than other gene markers (Svab and Maliga, 1993).

Flanking sequences are rrn23-trnA and rrn16-trnI will be incorporated on both ends of the expression cassette to enable its targeted insertion into the chloroplast genome via homologous recombination. Prompters and terminators of rbcL and psbA as well as rrn23-trnA and rrn16-trnI were PCR-amplified from cotton, cloned and sequenced to confirm that they are the right sequences.

All primers contained overlap restriction sites so as to facilitate vector assembly, which is now underway. Upon construction, biolistic bombardment delivery of this vector into cotton leaf tissues will be attempted. Rounds of selection under spectinomycin/streptomycin pressure will ensure transplastomic callus, which will be induced to grow into mature plants up to flower and seed setting. Leaf extracts will be tested for the presence of trangenes using PCR and Southern blotting.

Contact: nagrash29@hotmail.com

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