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Verticillium wilt of cacao

Gaining a better understanding of a disease which is an increasing threat to cocoa production.

Verticillium wilt (VW) is caused by the soil-borne fungus *Verticillium dahliae* which is widely distributed in tropical regions. It is known to cause wilting and defoliation followed by stunting or death of the cocoa plant. It has been shown to be widespread and highly damaging in areas of Eastern DRC and Uganda, where it is a major risk to a thriving and expanding export trade and the small holder farmers in the region who depend on cocoa for their livelihoods. VW also has a severe impact in some areas of other cocoa producing countries, particularly in regions affected by droughts. With the threat that local and global climate change will result in drier conditions in many current cocoa growing areas, a better understanding of the disease and how to manage it is urgently needed.

Improved planting materials with resistance or tolerance to the disease are key to a sustainable management system. It is also essential to have a good understanding of the diversity of the pathogen and how different genetic groups interact with the plant host. This research examined the genetic diversity of 33 *V. dahliae* isolates taken from infected cocoa plants in Uganda, DRC and Peru. Four distinct genetic groups were found based on different molecular markers.These groups were also associated with differences in morphology and optimal growth conditions, with indications also of differences in virulence.

Progress in breeding for resistance can be accelerated through the development of efficient techniques to screen potential parental material and to identify promising individuals in breeding lines at an early stage. A range of laboratory- and field - based screening techniques were investigated, including new rapid preliminary screening methods based on plant tissues such as leaves and petioles rather than the more traditional approach using seedlings or rooted cuttings. Two new promising techniques based on electrolyte leakage from leaves and the re-isolation of the fungus from inoculated petioles were developed. These were shown to give a similar classification of genotypes as resistant or susceptible to the field results and offer good potential for screening genetic resources once they have been validated with further field data. Moreover, since the tests can also be undertaken in the laboratory with pathogen elicitors and effectors contained in fungal culture filtrates, rather than using the spores of *V. dahliae* in field/greenhouse conditions, they may offer an environmentally safer way to assess disease resistance potential of cacao accessions in genebanks and other field sites in cocoa producing areas.

Another approach to accelerating progress in breeding is through the use of marker-assisted selection. Preliminary work in this study identified a marker which can be used to discriminate between resistant and susceptible genotypes. This marker is associated with a gene with similarities to genes known to be important in resistance to *Verticillium* infections in cotton. Further research in this area, with a wider range of cocoa clones and genetic markers, is advocated to fulfil the potential of these techniques in breeding VW resistant planting materials.


Photo: A-S Bouchon


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ABSTRACT

Vascular wilt disease of Theobroma cacao in Uganda and DR Congo caused by Verticillium dahliae: studies on management using a genetic approach. Anne-Sophie Bouchon Verticillium wilt, a major disease damaging cacao (Theobroma cacao) in the North East of the Democratic Republic of Congo (DRC) and in West Uganda, is caused by the soil-borne fungus Verticillium dahliae. The most effective way to manage this problem in the long-term would be by growing resistant or tolerant varieties of cacao, as done against this pathogen for other crops. This aim of the research described in this thesis was to improve understanding of the disease and how it can be managed. Genetic diversity of 33 V. dahliae isolates taken from symptomatic cacao trees in Uganda, DRC and Peru was investigated. Four distinct genetic groups were found based on different molecular markers, these groups presented morphological variations in culture and possible different virulence. The possibility of using excised parts of cacao as suitable replacements for intact seedlings or rooted cuttings in preliminary screening of the relative susceptibility of clones to V. dahliae was studied for the first time in this work. Laboratory-based assays using leaf material to assess resistance/tolerance or susceptibility to V. dahliae were developed, including quantitation of epicatechin contents, enzyme assays, fungal re-isolation and electrolyte leakage. Among those examined, the assays based on electrolyte leakage and on re-isolation of the fungus from inoculated petioles showed promise for use in classifying clones as resistant or susceptible, further sampling optimization would improve their robustness. Similar clone classification was found with these two assays: clones PA70, SCA6, GU255/V, ICS95 and Pound 7 were found resistant; ICS6, GU114/P and SPEC54/1 were found susceptible in both assays. This agreed with known field reactions of cacao clones (ICS6 and Pound 7), in vivo testing would be required for the other clones. In addition to treatments with V. dahliae conidial suspensions, treatments with pathogen elicitors and effectors contained in fungal culture filtrate were tested and would enable environmentally safer assessments of cacao resistance. In addition to using in vitro tests, breeding could be accelerated thanks to marker-assisted selection. Genomic association between genetic markers and variation in Verticillium wilt disease susceptibility was studied. One cacao marker (TcSNP1111) enabled discrimination between resistance and susceptibility. It is located at a nucleotide binding site (NBS) gene. The NBS resistance genes are known to be important in resistance of cotton to Verticillium wilt. This genomic association study needs to be extended to include more cacao clones and more markers. The protocols developed and findings of this work contribute to the understanding of the potential use of in vitro tests and association study to enhance breeding activities in cacao producing countries.