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Development and application of a genome-informed loop-mediated isothermal amplification (LAMP) assay for the detection of *Pseudomonas syringae* pathovar *pisi* and *syringae*

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The wide spread application of high throughput sequencing is being driven by low cost, ease of application, and advances in computational and bioinformatic capabilities. The vast amounts of data generated from sequencing technologies is facilitating the identification of novel targets for the design of specific and robust molecular diagnostics. This study reports the use of 10 draft genome sequences from the bacterial plant pathogen *Pseudomonas syringae* pathovar *pisi* (Ppi), the causal agent of bacterial blight in field pea, to identify unique diagnostic targets and design primers for a loop-mediated isothermal amplification (LAMP) assay. The assay reported here reliably differentiates strains of Ppi isolated from field pea from a range of other bacteria that are commonly associated with peas and other plants. The Ppi LAMP and a *Pseudomonas syringae* pathovar *syringae* (Psy) LAMP developed by collaborators, were validated with a range of *Pseudomonas* species and *P. syringae* pathovars including historical isolates from the VPRI collection and recent isolates from the field. LAMP assay for both Ppi and Psy proved to be highly sensitive, accurate and versatile for application on bacterial colonies, and extracts or exudates from infected host plant material. The LAMP assay is a suitable diagnostic tool for the glasshouse and laboratory and as well as for in-field surveys.

Molecular diagnosis of viruses causing yellowing and stunting symptoms affecting pulse crops in Central West Asia and North Africa Countries

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Viruses causing yellowing and stunting are the most important virus diseases affecting pulses in many regions of the world, and were considered for many years to be caused mainly by infection with beet western yellows virus (BWYV, genus *Polerovirus*, family *Luteoviridae*). Knowing the exact identity of a virus affecting pulse crops is essential for breeding for resistance and crop management purposes. More than 5000 pulse samples (faba bean, lentil, and chickpea) with symptoms typical of virus infection including stunting, yellowing, necrosis and reddening were collected during the last two decades from different countries in Central West Asia and North Africa (CWANA). All samples were tested serologically by tissue-blot immunoassay (TBIA) technique using specific luteoviridae monoclonal antibodies. Selected samples were further tested by reverse transcription polymerase chain reaction (RT-PCR) using different luteoviridae primer pairs (generic and specific) followed by amplicon sequencing. RT-PCR results revealed clearly that there was a greater variation in polerovirus species detected than was indicated by TBIA alone. Molecular diagnosis has clearly shown that there are a number of *Polerovirus* species, in addition to BWYV (detected in Algeria, Ethiopia, Lebanon, Morocco, Sudan, Tunisia and Uzbekistan), each of which can produce yellowing and stunting symptoms in pulses in CWANA. These viruses are cucurbit aphid-borne yellows virus (detected in Algeria, Lebanon, Syria, Sudan and Uzbekistan), chickpea chlorotic stunt virus (detected in Algeria, Ethiopia, Lebanon, Morocco, Syria, and Tunisia), pepper vein yellows virus (detected in Morocco and Sudan), pepo aphid-borne yellows virus (detected in Sudan), and cotton leafroll dwarf virus (detected in Sudan and Uzbekistan). This study clearly showed that molecular characterization is an essential tool for accurate identification of plant viruses, which is the first step towards better crop management.