Automated primer design for DNA-based detection of the emerging potato pathogen *Dickeya dianthicola*

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Soft-rotting Enterobacteria (SRE) comprise a subset of bacterial plant pathogens including agronomically important genera *Pectobacterium* sp. and *Dickeya* sp. Several *Pectobacterium* and *Dickeya* species represent significant pathogens of potato, but identifying these species with PCR is made difficult by their close relatedness and frequent genetic exchange. Numerous SRE species continuously impact potato yields but the recent emergence of *Dickeya dianthicola*, an invasive pathogen from Europe, now challenges disease management and diagnostic procedures across North America. To address this concern, we developed an automated comparative primer design pipeline, Uniqprimer, and used it to design primers for DNA-based detection of *D. dianthicola*. Uniqprimer performed alignments of six *D. dianthicola* genomes to 74 non-target genomes, designed primers based on regions of divergence, and mapped the primer pairs back to the genomes to confirm specificity. To demonstrate the specificity and sensitivity of our primers, we performed conventional and quantitative PCR assays. All primers were evaluated and confirmed for specificity to *D. dianthicola* using diverse bacterial isolates and infected potato tissues. Our results demonstrate that specific and sensitive PCR assays designed using Uniqprimer can aid in diagnosis and disease management of the emerging potato pathogen *D. dianthicola*. Uniqprimer is being tested for public release in the Galaxy web platform.

Improvement of LCHV-1 detection by conventional RT-PCR and Real Time PCR protocols

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Sweet cherry is one of the most important fruit crops in economical income in Chile, placing the country in the first place in cherry production in the southern hemisphere. From October 2015 to March 2016, a survey was performed in the main sweet cherry producing regions of Chile, collecting samples from 223 trees. Several viruses were detected by RT-PCR, but not *Little cherry virus 1* (LChV-1), using primer pairs indicated in literature. Nineteen samples were randomly selected for small RNA sequencing in Illumina MiSeq platform and sample reads were trimmed using FASTX and assembled using VELVET. Four samples presented contigs that matched with LChV-1 Genbank references, indicating the presence of new variants of the virus in Chilean samples. As a consequence and in order to improve the detection of LCHV-1 by conventional RT-PCR and Real time PCR TaqMan, new specific primer pairs and a molecular probe were designed using the 3' untranslated region of the virus genome. Using the total nucleic acid extracts stored at –80°C, the same 223 samples were analyzed again, obtaining 15% and 30% of positive samples with conventional RT-PCR and Real time PCR TaqMan, respectively. These new detection tools allowed the improvement of the detection of LChV-1, being Real Time PCR the most sensitive protocol. In addition, this last method was developed for the first time for the detection of LChV-1.

Identification of *fungal pathogens* associated with cassava root rot in Thailand

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Cassava root rot (CRR) is the most serious diseases of cassava in Thailand, especially Northeastern region. The aim of this study was to identify the CRR causal agent. The study was carried out by collecting samples with root rot symptoms from various cassava planting areas including 3 districts of Nakorn-Ratchasima provinces, Thailand. The fungal pathogens were isolated from CRR lesions, the typical symptoms were often accompanied by wet, soft or dry rot symptom, and black pycnidia were found on the stalk and propagative stakes. The 139 representative samples were obtained into 5 genera according to the colony and spore morphology using a standard morphological study protocol. *Lasiodiplodia* spp. was the most frequently found fungus, constituting approximately 54% of the total, followed by *Fusarium* spp., *Neoscytalidium* sp., *Phytophthora* spp., *Sclerotium* sp. and other fungal genera which were found at 29, 7, 4, 1 and 5%, respectively. The pathogenicity test of representative 33 single-spore isolates showed that they could cause stem and root rot symptoms on the inoculated susceptible cassava cv. Rayong 72 under both moist-chamber and green house conditions, the isolate L11HSR2 was the most virulent. By using 3 primers including ITS1/ITS4, Ef1-688F/Ef1-1251R and Bt2a/Bt2b primers to amplify the DNAs from 8 representative isolates, only the primer of the EF1-α region was effective in differentiating the isolates at species level and had an agreeable result with that identified by the standard morphological technique. When these eight isolates was compared against GenBank's database using the Mega BLAST program, and the alignment data using the NJ, UPGMA and ML methods, could be identified as *L. theobromae, L. euphorbicola* and *N. hyalinum*. This is, so far, the first report of fungal pathogens complex species associated with CCR disease in Thailand.

Occurrence of Grapevine fanleaf virus in Russia

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Grapevine fanleaf virus (GFLV) is one of the most severe viruses of grapevine. It occurs worldwide, causes more than 50% yield loses and decrease sugar content and titratable acidity (Andret-Link et al., 2004). GFLV is transmitted by nematode *Xiphinema index*. To investigate the GFLV occurrence in Russia the survey of Crimea vineyards has been conducted in 2014-2017. Total of 937 leaf samples with virus symptoms were collected. Short internodes, zigzag growth of shoots, yellow discoloration of leaves were observed. Samples were analysed by RT-PCR with the specific to GFLV primers, followed by sequencing of the PCR products. GFLV was found in 2.3% of the samples. GFLV was detected in combination with RSPaV (0.51%). The combination of GFLV, GVA and GLRaV-1 occurs in 0.13% of samples and combination of GFLV, RSPaV and GLRaV-3 occurs in 0.13% of samples. Nowadays we develop immunochromatography assay for rapid GFLV detection in the vineyard.

NextRAD sequencing unravels the genetic diversity of cassava-colonizing *Bemisia tabaci*

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Bemisia tabaci is a vector of cassava mosaic begomoviruses and cassava brown streak ipomoviruses which cause significant cassava yield losses in Africa. Cassava-colonizing *B. tabaci* comprise several cryptic species that cannot be distinguished morphologically and so are separated by sequencing the mitochondrial DNA cytochrome oxidase I (mtCOI). The objectives of this study were to (i) determine the effectiveness of the mtCOI marker for delineating cassava *B. tabaci* haplogroups and (ii) determine if there is gene flow among these haplogroups. Ninety-five whitefly specimens collected from cassava in eight African countries were genotyped using NextRAD sequencing, and their phylogeny and population genetics were investigated

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