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PINE PITCH CANKER

NGS for diagnosis of *F. circinatum*

General information

Description	The use of Next-Generation Sequencing (NGS) techniques for diagnosis of *Fusarium circinatum*	
Geographical area	Spain, France, Portugal	
Group of tree species	All species. Vegetal material; trees, seeds, seedlings and soil samples.	
Date	June 2018	
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Tool type	Test reults/protocol	Survey results/protocol
Tool format	Machinery.	
Language	English	Spanish
Risk management plans to which the tools can be added	Risk management plan of Pitch Canker Disease	
Risk management plans link	https://plurifor.efi.int/wp-content/uploads/WP2/plans/Fusarium-risk-plan_ES.pdf	
This tool is…	☒ a new tool	☐ an improved tool
Original tool of which this one is an improvement	None	

Topic

Risk	Pine pitch canker		
Risk component	☒ hazard	☐ impact	☐ vulnerability
Risk area	Risk management		
Risk phase	Prevention		
Risk phase (alternative terms)	Preparedness		
Level	Global		
Sendai priorities	☐ Priority 1: Understanding disaster risk ☒ Priority 2: Strengthening disaster risk governance to manage disaster risk ☐ Priority 3: Investing in disaster risk reduction for resilience ☐ Priority 4: Enhancing disaster preparedness for effective response and to "Build Back Better" in recovery, rehabilitation and reconstruction		
Contribution to Sendai targets	☐ Reduce global disaster mortality ☐ Reduce the number of affected people ☒ Reduce the direct disaster economic loss ☐ Reduce disaster damage to critical infrastructure ☐ Increase the number of national and local disaster risk reduction strategies ☐ Enhance international cooperation to developing countries ☒ Increase availability of and access to multi-hazard early warning systems and disaster risk information and assessment		

Description and analysis

Summary

The use of Next Generation Sequening technologies for detection of *F. circinatum* provides a new and powerfull tool to speed up and improve the identification process of the pathogen. This tool is based on the amplification of different genes or regions of the genome of *F.circinatum*: (1) ITS, (2) LSU, (3) Elongation Factor.

Place in national/regional policy

Currently NGS is not used as identification tool for the pathogen, but it has been applied before to explore the composition of fungal communities of vegetal and soil samples. It has a great sensitivity and can detect presence of a fungal species even at low concentration and in asymptomatic plants.

Goals and achievements

The use of NGS for detection of *F. circinatum* represent for first time the employment of a powerfull tool to detect the pathogen.

Stakeholders involved

A workshop was held in Aveiro to present the new diagnosis tool to researchers, forests owners and representatives from Spain and Portugal. Furthermore, Cantabria government is involved as well as nursery owners and managers.

Implementation stage

The tool is being developed for the pathogen *F. circinatum*. At the moment, some sequencing has been done with the ITS region and we are actually developing the methodology for sequencing with other specific genes for *F. circinatum.*

State of technical knowledge

The NGS tool has been used before for exploration of fungal communities in vegetal and soil samples, however there is a need of improvement of the genes used for identification to be more specific for *F. circinatum*.

Regulatory and/or socio-economic contexts

There is no regulatory context at the moment. Socio-economic context is important since this tool can cheapen and speed up the identification process, limiting the spreading of asymptomatic seedlings in the forests.

Impacts of the tool

The use of NGS technologies for detection of *F. circinatum* provides a new and powerful tool to speed up and cheapen the identification process and limit the expansion of the pathogen. In addition to the DNA sequences, the use of NGS can provide the analysis of other biological components which will contribute to the knowledge of the host-pathogen interaction and will contribute on decision processes of management of the disease.

Implementation requirements and durability

Description of the implementation steps

1. Genes or regions defined for identification.
2. Run of NGS analysis *(in progress).*
3. Revision of results *(in progress).*
4. Application of the tool *(not done)*.

Governance

UVa is responsible for development, implementation and testing the tool. Cantabria government will contribute with technical support and assistance.

Regulatory framework

The tool will help to make decisions on vegetal trade and reforestation process.

Human resources requirements

Implementation of the tool will require communication between authorities and forest and nurseries owners. Qualified staff to carry out the analysis will be needed regarding the sample taking. The rest of the analysis can be done by specialized sequencing companieswhich are currently widespread and common.

Financial requirements

Despite NGS runs are expensive, this is a low-cost tool considering its high sequencing capacity compared to other laboratory detection techniques.

Technical requirements

Forest owners or authorities should only be in charge of taking samples. The following steps such as extraction of DNA, the use of NGS platform such as Illumina and interpretation of the results can be subcontrated as external services.

Priorities identified for successful implementation of the tool (political, technical, human, financial…)

The priorities are: (i) to incorporate the tool in the diagnosis protocol of *F.circinatum* to speed up the identification process and make decisions, specially regarding asymptomatic plants, (ii) to train staff to take samples properly, (iii) to find a region from the genome that allow the identification of a wide range of fungi and pathogens including *F. circinatum*.

Challenges or risk factors (legal, financial, safety…) expected during the implementation and solutions proposed

The main risk is that the tool does not detect the pathogen in asymptomatic plants; and a possible solution to this is to change the region sequenced to obtain a more accurate diagnosis.

Other risk is that the nurseries and administrations can not afford the use of this technique in their routines because of its cost although the analysis are becoming more a more affordables.

Additional and non-formal experiences to help the implementation of good practice

To help the implementation of the tool in the identification process of the pathogen, authorities and nursery owners should be trainned and informed to be more familiar with it.

SWOT analysis

Strengths	**Weaknesses**
• Detection of a the pathogen in asymptomatic plants. • Fast DNA sequencing. • High accuracy (99%). • Unbiased rather than pathogen-specific protocols. • Ability to detect co-infections. • Requires little or no prior knowledge of the pathogen.	• It is an expensive techinique. • Sequencing and interpretation of the results should be done by specialized staff. • The pathogen can not be detected with the universal primers ITS. • Needs specific primers for its detection.
Opportunities	**Threats**
• It can became a powerfull tool in routine inspections of asymtopatic plants in nurseries, reducing the potential threat of dispersion of the pathogen in the field.	• Not implemented in plant inspections because of the disadvantages and the costs. • That not detect the pathogen in asymptomatic plants.

Lessons learnt

Evaluation process, if exists (internal or external)

We are running the NGS analysis with different primers, so evaluation process will be performed shortly.

Assessment of results (quantitative and qualitative) and comparison with main goals

Until date, we have no success with the identification process with ITS universal primers so we are testing other regions of the genome.

Negative aspects identified

The universal primers from the ITS region do not work well for this pathogen.

The length of the region sequenced depends on the type og NGS machine used, so it can be a drawback.

Unexpected consequences (short- / mid- / long-term) and corrective measures implemented

The length of the region sequenced depends on the type of the NGS machine used, so other we will try other platformrs instead of Illumina to sequence a specific primer for the pathogen.

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