Detection and Incidence of *Peronospora variabilis* in Quinoa Seeds

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Quinoa

Quinoa, Chenopodium quinoa, is an important Andean crop with a seed that is a complete protein and high in other nutrients. The crop can tolerate nutrient poor and saline soils, drought, frost and intense UV radiation. Bolivia, Ecuador and Peru are the largest quinoa exporters, but research and breeding efforts are in progress to expand quinoa's range to countries such as Denmark and India.



Quinoa downy mildew, caused by the comycete Peronospora voriabilis, is the key pest of quinoa, with severe infections resulting in upwards of 90% crop loss. Quinoa downy mildew infections can originate from seedborne cospores or sporangia from related Chenopodium weed species that harbor the pathogen.

Quinoa downy mildew was described in the U.S. for the first time in 2011 in Pannsylvania. Symptoms of downy mildew were not observed on Chenopodium album (lambsquarters) in PA fields and Chenopodium weeds were not susceptible to U.S. isolates of P. variabilis under lab conditions. This suggests that the original inoculum was seedborne. A rapid, molecular method for screening quinoa plant material for P. variabilis has not been developed and is required for future disease management strategies.

Signs and Symptoms of Quinoa Downy Mildew



C) Sporulating leaves infected with P. variabilis

D) P variabilis isolate maintained in detached guinoa leaves

Research Question

Can R variabilis inoculum originate from quinoa seeds, especially imported, consumable seeds?



Objectives

- To identify potential sources of P. variabilis inoculum
- To determine if imported quinoa seeds are infested with P. variabilis
- To develop a rapid, molecular method for screening guinoa seeds for P. variabilis



For Sequencing

A semi-nested PCR with primers semispecific to the ITS region of Peronaspara and Pythium was used to amplify DNA. Obtained Sequences were then compared to GenBank P. variabilis sequences.

For Gel Electrophoresis

R variabilis specific primers were designed based on the ITS region of P. variabilis. Amplification was confirmed by running PCR products on agarose gels followed by EtBr staining.

Results

P. variabilis was detected based on sequencing results in 20 out of 22 quinoa seed lots, including seeds from 5 different countries (Bolivia, Ecuador, Peru, United States, and India).
 17 of 22 seeds lots tested positive when using P. variabilis specific primers in standard PCR.
 P. variabilis was not detected in seeds of Chengoodium album, a naturalized weed.

Colombia Series	Country of Origin	Conformition of E. particular	Angelitation of A meriodis with sandle primers	C-SHEET STREET	
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After Stockhood (ACM)	Berrie	Present	Tes :		me remark
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Wegman') Bulk Sia(k (Wegli)	Lifeth Females	Present	765	123	
Brisis (Organia Red (BOR)	1 Berria	Present	Vera .	100000000000000000000000000000000000000	
Bilen Singarita White (SDW)	- breaker	Present	tto	100	
Arresteed Milks White (Areste)	Mark Street	Present	765	17	
(Charte Granss Ourse Resi (ICN)	District Co.	Present	764		
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Greenhouse Grown Year' (Call)	United States	Present	- No		
Changesdaw album (CAL)	United States	6,0	160		

Discussion and Conclusions

- R variabilis was detected in nearly all seed lots tested, so imported, consumable seeds could serve as sources of inoculum for quinoa downy miles.

 The detection is a server of the se
- •The P. variabilis specific primers amplified DNA from the majority of infested seed lots.
- P. variabilis was detected in several sources from across the U.S., which indicates a broader distribution of the pathogen than expected.

Research Impacts

•The USDA APHIS New Pest Advisory Group is currently reviewing the status of *R. variabilis* in the United States, and this research supports that the pathogen is already established in the U.S. •Collaborators in Ecuador and Bolivia can use *R. variabilis* specific primers to detect the pathogen in quinoa plant material.

Future Research

Further primer specificity studies must be performed with other Peronospora sp. and related comycetes.
 Collaborators in Ecuador and Bolivia will validate these methods and provide samples for further studies on the diversity of P. variabilis from quinoaseeds and infected plant materials.



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