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First report of *Peronospora variabilis* causing downy mildew disease in cañahua (*Chenopodium pallidicaule*) in Bolivia

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Short Report

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Abstract

Cañahua (*Chenopodium pallidicaule* Aellen) is a semi-domesticated grain cultivated in the Andean highlands for millennia. Cañahua seeds have high nutritional value and it has become attractive because of its high resistance to frost, drought and saline soils. In May 2018, cañahua plants showed symptoms of the downy mildew disease caused by *Peronospora variabilis* which is known to heavily affect its tetraploid-relative quinoa. Besides the typical symptoms in the plant, visual confirmation of *P. variabilis* reproductive structures by microscopy was achieved. In order to verify the ability of *P. variabilis* to infect cañahua, an artificial infection in three cañahua varieties was performed. The three cañahua varieties were infected by *P. variabilis* and developed downy mildew disease symptoms. The pathogen identity was confirmed by PCR and Sanger sequencing of the *PvCox2* and *PvITS region*. DNA sequence identification confirmed that the *P. variabilis* that usually infects quinoa can also infect cañahua plants. Therefore, cañahua when grown next to quinoa must be carefully watched for downy mildew disease symptoms because *P. variabilis* can be a potential threat for future large scale cañahua agriculture.

Brief Communication

Cañahua (Chenopodium pallidicaule Aellen) is a grain cultivated in the Bolivian Altiplano by Native American communities for thousands of years (Bruno and Whitehead 2003). Cañahua is important because of its nutritional value, resistance to frost, drought and saline soils at altitudes between 3600 and 4400 meters above sea level (Repo-Carrasco-Valencia et al. 2010; Rodriguez et al. 2016). Cañahua is usually cultivated in areas close to quinoa (*Chenopodium quinoa* Willd.) fields. Quinoa is typically affected by the downy mildew caused by Peronospora variabilis (Choi et al. 2010). Local farmers reported that cañahua plants can be affected by this disease in their cultivation handbooks (Gimenez, Mamani, and Canaviri 2017; Flores et al. 2008). P. variabilis has been detected in a cañahua seed and is known to infect *Chenopodium album* (Testen et al. 2014) and *Chenopodium murale* (Baiswar et al. 2010). In May 2018, at the greenhouse of the Chemical Research Institute, La Paz, cañahua plants showed chlorosis in the upper side of their leaves and heavy sporulation in the downside, typical symptoms of downy mildew (Rollano-Penaloza et al. 2019). The symptomatic cañahua plants were located near quinoa plants infected with P. variabilis. Microscopic analysis confirmed the presence of reproductive structures typical of the obligate parasite *P. variabilis* (Choi et al. 2010). In order to verify *P. variabilis* ability to infect cañahua, a pathogenicity test was performed in three-week-old cañahua plants that were sprayed with *P. variabilis* suspension $(1 \times 10^{6} \text{ spores/ml})$ supplemented with 25 µg/ml of propiconazole (Propilac, Guayaguil, Ecuador). Propiconazole inhibits fungi that may overgrow *P. variabilis* under high humidity growth conditions. After inoculation, plants were covered with polyethylene bags for 24 h and then incubated in a greenhouse at $17-25^{\circ}$ C under natural light conditions (12 h light/ 12 h darkness). Nine days post inoculation (dpi) localized chlorosis and necrosis were observed in the infected cañahua leaves and sporulation was observed at 11 dpi. Microscopic observations of the reproductive structures showed similar structures as the P. variabilis that infects quinoa (Rollano-Penaloza et al. 2019; Testen et al. 2014). The identification of *P. variabilis* was confirmed through PCR and Sanger sequencing with

specific primers for downy mildews (Hudspeth, Stenger, and Hudspeth 2003). The cytochrome oxidase II (*PvCox2*) mtDNA region and the ITS region (*PvITS*) were amplified and the product sequenced in both directions. The PCR product size of *PvCox2* was 505 bp and *PvITS* region had a size of 1158 bp. The consensus sequences were deposited in the NCBI GenBank as MK182604 and MT666070, respectively. The *PvCox2* sequence shared 99.8 % nucleotide sequence identity with *Peronospora variabilis* isolated from quinoa MK173058 and the *PvITS* sequence had a 100% nucleotide sequence similarity with the *P. variabilis* isolated from a quinoa host MH999837. Thus, confirming the infection of cañahua plants by *P. variabilis* isolate Kari (Rollano-Penaloza et al. 2019). To our knowledge this is the first peer review report of *P. variabilis* being able to infect cañahua plants. The downy mildew in cañahua can be considered a potential threat to the large-scale cultivation of cañahua in the near future. Due to the diploid genome of cañahua (Mangelson et al. 2019), it can be used to study the downy mildew disease at molecular level and benefit the whole Chenopodiaceae family.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Figures

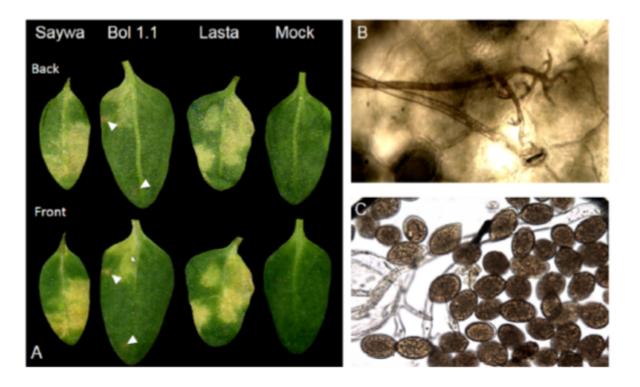


Figure 1

Infection of Peronospora variabilis in cañahua. A: Leaves of three cañahua cultivars infected with P. variabilis after 9 days of inoculation. Arrows indicate hypersensitive response in the cultivar Bol 1.1. The mock treatment was done with sterile water supplemented with propiconazole. All cultivars were tested but only Bol 1.1 is showed. B: Sporangiophores and C: sporangiospores of P. variabilis isolated from cañahua leaves and stained with I2/KI.