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Variation in downy mildew (*Peronospora variabilis* Gäum) resistance of some quinoa (*Chenopodium quinoa* Willd) cultivars under Egyptian conditions

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ABSTRACT

This study was designed to evaluate and compare the response of three quinoa cultivars (*Hualhuas*, *CICA* and *Real*) to downy mildew, a major restrictive factor for plant growth and productivity, under field conditions in Egypt. These cultivars showed genotypic variability in their resistance as indicated by disease incidence, severity, susceptibility indices and PCR detection. Whereas, no disease symptoms were observed on the leaves of "*Hualhuas*" plants, disease incidence was 36.8% and 63.6% for "*CICA*" and "*Real*" plants, respectively. Symptomatic lesions were obvious, particularly on "*Real*" leaves, where typical lesions covering up to 90% of the leaf area were recorded. Microscopic inspections of these lesions revealed the presence of dichotomously branched sporangiophores, bearing ellipsoidal, light brown sporangia, typical for *Peronospora variabilis*. Susceptibility indices varied between cultivars, being zero for *Hualhuas*, 60.7% for *CICA* and 94.4% for *Real*. PCR results revealed the presence of amplicons (866 bp), representing the internal transcribed spacer (ITS) of *P. variabilis* only in diseased leaves of "*CICA*" and "*Real*". Further, several oospores were observed in the seed wash-fragments of both cultivars, suggesting that seedborne oospores are probably the main source of inoculum in Egypt. These findings allow for the speculation that "*Hualhuas*" is not only a resistant quinoa cultivar suitable for Egyptian conditions, but also through a deep understanding of its physiological and molecular resistance mechanisms, would provide a possible route to enhance mildew resistance in other quinoa genotypes.

Key words: *Peronospora variabilis, Chenopodium quinoa,* Disease resistance, Disease incidence and severity, Oospores.

Introduction

Quinoa (*Chenopodium quinoa* Willd), a traditional Andean crop, is increasingly garnering worldwide attention, owing to its high nutritional value and robust adaptability to hostile environments (Cocozza *et al.,* 2013; González *et al.,* 2015; Razzaghi *et al.,* 2015). This species has been grown in the Andes region since 5000 – 7000 years ago in various agro-ecological zones from 5° North Latitude in southern Colombia to 43° South Latitude, with altitudinal distribution ranges from sea level to 4000 masl (Ruiz *et al.,* 2015). Due to a broad diversification in terms of its native habitats, quinoa is characterized by an extraordinary resistance to environmental abiotic and biotic stresses (González *et al.,* 2015; Pulvento *et al.,* 2015; Razzaghi *et al.,* 2015; Hussin *et al.,* 2017). Quinoa seeds are rich in a wide range of important minerals (Ca, P, Mg, Fe and Zn), vitamins (B1, B9, C and E), oil (containing large amounts of linoleate, linolenate and natural antioxidants), and protein (containing ample amounts of essential amino acids such as lysine and methionine) (Repo-Carrasco *et al.,* 2003; Gordillo-Bastidas *et al.,* 2016). Its potential as a nutritious and resistant crop was recognized by the United Nations Food and Agriculture Organization (FAO), which declared the year 2013 as the International Year of Quinoa (www.iyq2013.org) (FAO, 2013; Bazile *et al.,* 2015). Because of its nutritional richness and the high level of adaptability in marginal environments, quinoa was introduced in several areas outside its origin as non-conventional cash crop, with reports demonstrating an acceptable adaptation in United States, Canada, Italy, Morocco, India and Egypt (Jellen *et al.,* 2005; Bhargava *et al.,* 2007; Pulvento *et al.,* 2010; Munir *et al.,* 2011; Shams, 2011;

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Bazile *et al.,* 2015; Eisa *et al.,* 2017). However, this was accompanied by sustained seed transportation between countries, which did not comply, in some cases, with phytosanitary standards, leading inevitably to the spread of some seedborne diseases (Danielsen *et al.,* 2004; Testen *et al.,* 2014). Among these, downy mildew (*Peronospora variabilis* Gäum, formerly *Peronospora farinosa* f.sp. *chenopodii* Byford), the most serious and well-known disease, severely influences quinoa growth and productivity at a global level (Danielsen *et al.,* 2004; Choi *et al.,* 2010). This pathogen belongs to the family *Peronosporaceae*, whose members are highly specialized obligate parasites (biotrophs) that parasitize vascular plants causing downy mildew in a limited range of species (Danielsen and Ames, 2004).

Downy mildew of quinoa was initially recorded in Peru in 1947 (Garcia, 1947), and has since been reported in several countries world over (Tewari and Boyetchko, 1990; Danielsen *et al.,* 2004; Kumar *et al.,* 2006; Choi *et al.,* 2010; Testen *et al.,* 2012; Mhada *et al.,* 2015). The disease symptoms include sporulation on the leaf undersides, chlorosis, necrosis, and in extreme cases eventually up to 100% defoliation, depending on the genotype (Danielsen and Ames, 2004; Kitz, 2008). During crop growth season and when the environmental conditions are conducive to downy mildew development, infection is mainly via wind-dispersed sporangia. It proliferates under relative humidity above 80% and temperatures between 15 and 20°C (Danielsen and Ames, 2004). However, under dry and warm conditions, the inoculum remains dormant as oospores (sexual reproduction structures) on the seeds, old leaf tissues, and surrounding soil (Danielsen, 2001). Oospores can survive inside host tissues until environmental conditions are favorable for germination (Danielsen, 2001). Yield losses due to downy mildew vary and may reach up to 100% under favorable conditions in highly susceptible cultivars (Danielsen *et al.,* 2004; Testen *et al.,* 2014), indicating that this disease is detrimental for quinoa production. The incidence and severity depend on the prevailing environmental conditions, crop management approaches, phenological stage of the plant when infected and the cultivar's degree of resistance. Controlling downy mildew traditionally using fungicides is a non-sustainable measure (due to its environmental hazards) and may eventually be overcome by resistant isolates, as the pathogen is sexually recombinant (Albourie *et al.,* 1998; Danielsen and Munk, 2004), and shows high levels of genetic diversity among populations (Ochoa *et al.,* 1999; Swenson, 2006). Within a scenario of a rapidly spreading and sexually reproducing pathogen, the use of durable host plant resistance seems to be one of the most reliable and efficient approaches in managing downy mildew. Reportedly, *C. quinoa* exhibits a broad intra-specific range of resistance to mildew, as revealed by comparative studies on many different accessions, landraces, and cultivars. For example, valley ecotypes growing in regions where humidity is high and the disease is rampant, often display high to moderate mildew resistance, whereas, southern altiplano ecotypes growing in drier regions show more susceptibility (Bonifacio, 2003; Fuentes *et al.,* 2009). However, reports from Denmark indicate that Dutch and Danish quinoa cultivars with Chilean lowland background showed drastic levels of susceptibility (Danielsen *et al.,* 2000). Quinoa is a relatively new cash crop in Egypt, with cultivation started 10 years ago (Shams, 2011), thus downy mildew is not known yet, but this disease has potential to cause extensive loss. Although some downy mildew symptoms were recorded in two quinoa trials in Egypt (El-Assiuty *et al.,* 2014), precise knowledge about disease incidence, severity and the level and mechanism(s) of downy mildew resistance of the available quinoa germplasm under Egyptian conditions is lacking so far. This information is of paramount importance and considered as a prerequisite for rational incorporation of *C. quinoa* as new non-conventional cash crop into the Egyptian agricultural production system. In this context, the present study was designed to evaluate and compare three quinoa cultivars, namely: *C. quinoa* cv. *Hualhuas*, *C. quinoa* cv. *CICA* and *C. quinoa* cv. *Real* for downy mildew resistance under field conditions. These cultivars originate from different agro-ecological zones and are expected to exhibit various levels of adaptability and downy mildew resistance. Our intent was to (i) identify the causal organism of downy mildew in these quinoa cultivars, (ii) to adopt a simple and reliable disease assessment method easy to use under natural epiphytotic conditions in Egypt, (iii) to determine the level of mildew resistance in these closely related quinoa cultivars. Comparing the response of these cultivars to *P. variabilis* may give an opportunity for elucidating key mechanism(s) involved in mildew resistance in quinoa and open prospects to select the most suitable cultivar for comprehensive and commercial field trials under Egyptian conditions.

Material and Methods

Plant materials and experimental set-up

A coastal lowland quinoa cultivar "*Hualhuas*" (origin: International Potato Center, CIP, Lima, Peru) and two Altiplano quinoa cultivars namely: "*CICA*" (origin: Perú, Puno region) and "*Real*" (origin: Salar de Uyuni, Bolivia) were screened for downy mildew resistance under experimental field conditions at the Faculty of Agriculture, Ain Shams University, Qalyubia Governorate, Egypt (30° 06′ 42″ N 31° 14′ 46″ E), during the 2012 to 2015 growing seasons (from November to February). This region is characterized by a continental climate with dry hot summer and relatively wet winter. During the growing seasons, the mean temperatures were 21.4 ± 2.9 °C (day) and 12.1 ± 2.5 (night), mean relative humidity was 81.7 ± 3.1 % and the maximum amount of rainfall was 12.1 mm/month. After soil preparation, the seeds of each cultivar were sown separately in plots, with five rows of four-meter length (approx. 12 m^2), with row-to-row and plant-to-plant distance of 60 and 15 cm, respectively. The trials were conducted in a complete randomized block design with five plots (replicates) for each cultivar. The plant density was kept at 18 plants per square meter. The plants were left untreated to allow the disease to develop and spread naturally.

Assessment of disease incidence, severity and susceptibility index

Disease incidence and severity under natural epidemics were measured on the leaves of all screened quinoa cultivars, when the disease symptoms were fully developed (8 weeks after sowing date). Disease incidence was evaluated on the leaves of five randomly selected plants per replicate (as a percentage of infection based on the number of sporulating leaves per plant). In addition, the leaves of the selected plants were rated for the evaluation of disease severity as described by Mhada *et al.* (2015). The symptoms on each leaf were scored from 0 to 5, where 0= no lesion; 1= small lesions with diameter less than 1 mm without sporulation on the underside of the leaves; 2= clearly individual lesions, with higher number and larger size (0.5 – 1 cm), without sporulation; 3= lesions covering less than 50% of the leaf surface with a beginning of sporulation at the lower side; 4= lesions of larger size, covering more than 50% of the leaf area; 5= lesions covering more than 90% of the leaf area, with high sporulation on the lower and the upper leaf surfaces. Disease severity was calculated and expressed as the percentage of leaves in each category. Based on the disease severity measurements, susceptibility index (SI) for each quinoa cultivar was calculated according to Wan *et al.* (2007) using the following equation:

$$\text{Susuceptibility index (SI)} = \frac{\Sigma\ (\text{grade value} * \text{no. of leaves in that grade})}{\text{Total leaf number} * \text{the highest grade value}} * 100$$

The resistance level of downy mildew of each cultivar was scored based on the SI value after the following scheme: 0 – 5: extremely resistant (ER); 5 – 25: highly resistant (HR); 25 – 50: resistant (R); 50 – 75: susceptible (S), and > 75: highly susceptible (HS) (Staudt and Kassemeyer, 1995).

Identification of causal agent based on morphological features

Fresh symptomatic leaves were collected from the field trials. Leaf epidermis peels from healthy and diseased leaves of each cultivar were prepared and investigated using a light microscope (Leica DM 2500), equipped with a digital camera (Leica, Wetzlar).

Detection of oospore on the seeds

At harvest time, about 200 – 300 g seeds from each quinoa cultivar under the evaluation were collected. Seed samples (three replicates per each cultivar) of about 1.5 ml volume (about 500 - 1000 seeds, depending on the cultivar) were soaked in 50 ml distilled water and shaken for 5 min using a benchtop shaker (300 rpm). The seeds were then removed by pouring the solution through one layer of cheesecloth. Washing water (suspension) were then centrifuged at 3000 rpm for 5 min and the pellet was re-suspended in 5 ml distilled water. Ten drops (each of 10 µl) from the suspension were examined for the presence of oospores using a light microscope (Leica DM 2500), equipped with a

digital camera (Leica, Wetzlar). Additionally, transverse sections in the seeds from both infected and non-infected plants from each cultivar were prepared using the paraffin method as described by Johanson (1940). The paraffin blocks were then sectioned at 10 µm thickness using a rotary microtome (MR 2258). The sections were mounted on slides, stained with safranin-fast green and examined for the presence of oospores with a light microscope (Leica DM 2500), equipped with a digital camera (Leica, Wetzlar).

Detection of causal agent based on conventional PCR

Total DNA of the fungal hypha was isolated from the leaf tissues of each cultivar using the ZR plant/seed DNA MiniPrep TM (ZYMO Research) kit according to the manufacturer's instructions. A T100^{TM} thermal cycler (BIO-RAD) was used for conventional PCR to detect the DNA of *P. variabilis*, using a specific ribosomal internal transcribed spacer (ITS) primer set P1: 5′-GAACCTGCGGAAGGATCA-3′ and P2: 5′-AGTTCAGCGGGTAATCTTGC-3′ (Kitz, 2008). PCR reactions were carried out using the following temperature cycles: 1 cycle at 94ºC for 5 min; 40 cycles at 94ºC for 30 s, 52ºC for 30 s, 72ºC for 1 min; and a final extension cycle at 72ºC for 7 min. The final PCR product was separated and visualized on agarose gel 1%, stained with ethidium bromide and visualized under UV light (Kitz, 2008).

Statistical analysis

As the trends of the data in the three seasons were similar, the test of homogeneity was performed according to Bartlett's and the combined analysis of the data was applied as described by Snedecor and Cochran (1982). All data sets were then subjected to analyses of variance (ANOVA) using Tukey's HSD of the SPSS 16.0 statistical package (SPSS, Chicago, USA) to find a posteriori homogeneous sub-groups of means that differ significantly at $P \leq 0.05$.

Results

Disease symptoms

Typical downy mildew symptoms were clearly observed in "*CICA*" and "*Real*" plants (Fig. 1B and C) in December and January. The symptoms started as irregularly-shaped chlorotic lesions on the upper surface of the older leaves (low in the canopy), later turned light to dark brown, with diameters up to 5 cm. The corresponding lower leaf surface showed typical grayish black patches characteristic of downy mildew. As the infection progressed, spore formation led to a yellowish or reddish appearance of the leaves, which eventually dropped, depending on the genotype. Interestingly, no downy mildew symptoms were detected on the leaves of "*Hualhaus*" plants (Fig. 1A).

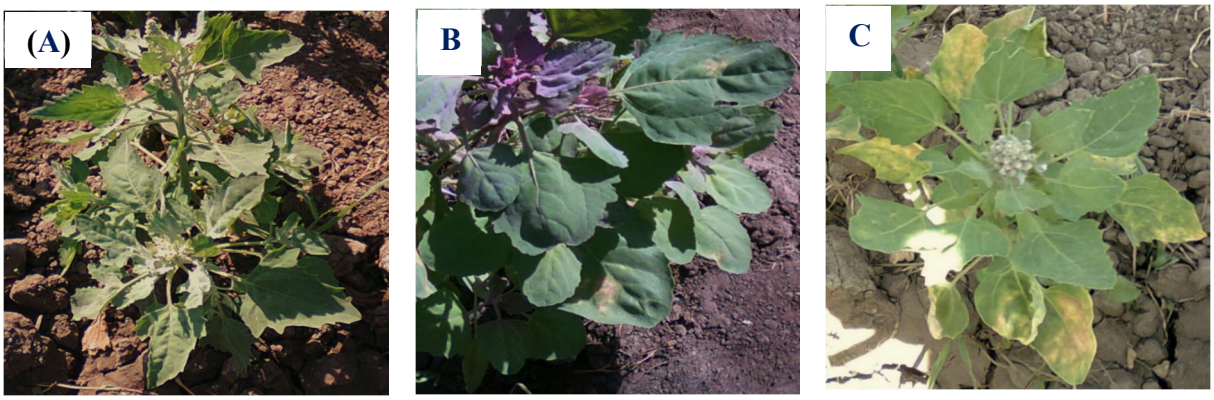

Fig. 1: Variations in downy mildew symptoms among three quinoa cultivars under natural field conditions. No symptoms of downy mildew were observed on the leaves of "*Hualhaus*" plants (A), moderate symptoms were noted on the leaves of "*CICA*" plants (B), while typical symptoms (pale or yellow chlorotic lesions on the upper leaf surface and grey patches of sporangia usually emerge on the underside of the leaves) were found on the leaves of "*Real*" plants (C).

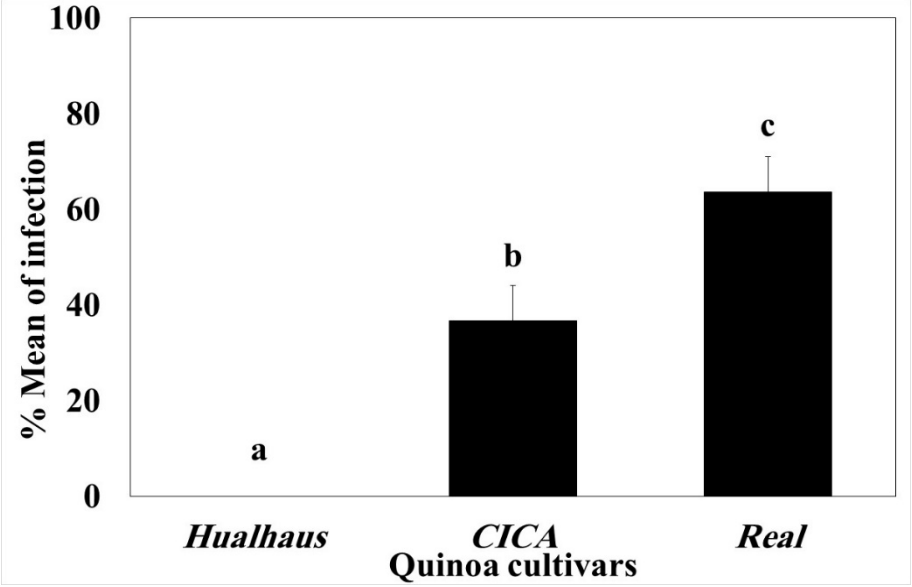

Fig. 2: Disease incidence calculated as the percentage of infection based on the number of sporulating leaves per plant. Each column represents the mean values of 15 replicates and the bars represent the standard errors. Columns with the same letters are not statistically different ($P \leq 0.05$), Tukey's HSD test.

Disease incidence, severity, and susceptibility index

Data in Figure (2) showed that disease incidence (expressed as % of leaves with sporulation/plant) was 0% in "*Hualhuas*" plants and reached 36.8 ± 7.5% and 63.6 ± 7.3% in "*CICA*" and "*Real*" plants, respectively. Disease severity among the cultivars screened was significantly different (Fig. 3). While no downy mildew symptoms were found on the leaves of "*Hualhuas*", symptomatic lesions of different size, colour and sporulation level were observed on the leaves of both "*CICA*" and "*Real*" plants. As a general trend, "*Real*" plants displayed a significantly higher disease severity compared with "*CICA*" ones (Fig. 3). The majority of the diseased leaves in "*Real*" plants showed typical lesions of grade 4 and 5 that covered up to 90% of the leaf area, with high sporulation degree on the lower and upper leaf surfaces (Fig. 3). Calculated susceptibility indices (SI) varied also among quinoa cultivars under the study (Fig. 4), being zero for "*Hualhuas*" plants and ranging between 60.7 % for "*CICA*" and 94.4 % for "*Real*" plants.

Identification of causal agent based on morphological features

Light microscopic examination of the lesions on symptomatic leaves of both quinoa cultivars "*CICA*" and "*Real*" showed the presence of asexual reproductive structures of the causal agent *P. variabilis* on the undersides of leaves. Multiple colorless, straight to slightly curved dichotomously branched sporangiophores extruded onto the leaf surface via stomata (Fig. 5A and B). The sporangiophores measured between 160 – 247 µm (length) and 8 – 12 µm (width) at the bases, and ended with sterigmata at acute angles bearing single sporangium (Fig. 5A and B). Mature sporangia were light brown in color, with very distinct oval shape, measuring about 21 – 30 µm in length and 16 – 19 µm in width, with smooth walls (Fig. 5A and B).

Detection of oospore on the seeds

Oospores and sporangia of *P. variabilis* were detected in the seed wash of both "*CICA*" and "*Real*" cultivars (Fig. 6), but not in that of the cultivar "*Hualhaus*". These oospores were dark brownish in color with thick walls, averaging 30 – 55 µm in diameter. In addition, some relatively transparent oospores were found in the seed wash of "*Real*" plants (Fig. 6). Transverse sections in the mature seeds of diseased plants of both "*CICA*" and "*Real*" cultivars revealed the presence of dark

brownish oospores embedded into the cells of the pericarp (Fig. 7). No oospores were detected in the tissues of the seeds of "*Hualhaus*" plants (Fig. 7).

Detection of causal agent based on conventional PCR

Amplicons of 866 bp, representing the ITS of *P. variabilis* were detected from diseased leaf tissues of both quinoa cultivars "*CICA*" and "*Real*" (Fig. 8). By contrast, no amplicons were generated from "*Hualhaus*" plants (Fig. 8).

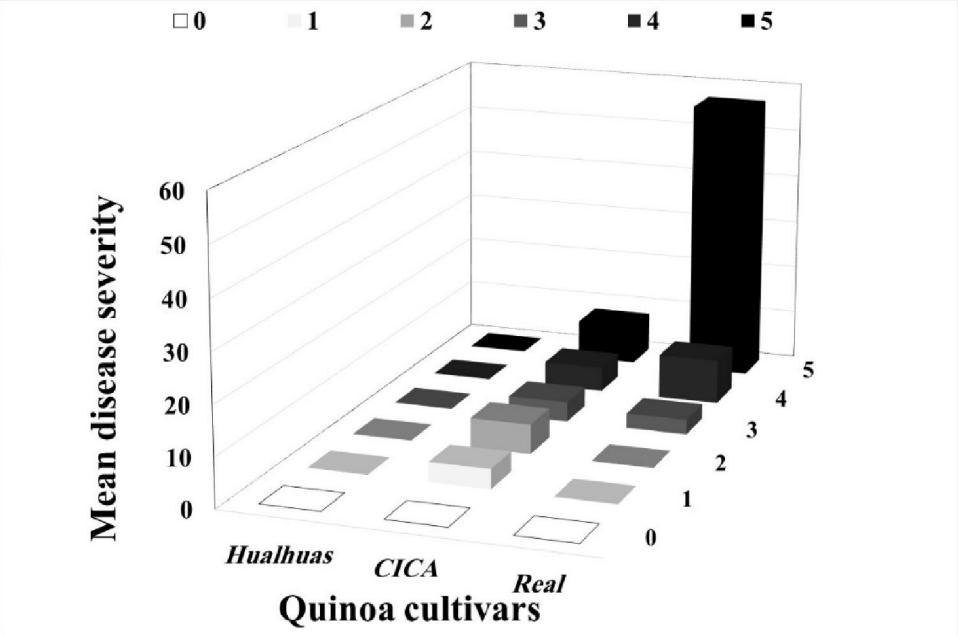

Fig. 3: Assessment of disease severity of downy mildew in different quinoa cultivars. The symptoms on the leaves were rated from 0 to 5 as described by Mhada *et al.* (2015). Disease severity was calculated as a percentage of leaves in each disease category.

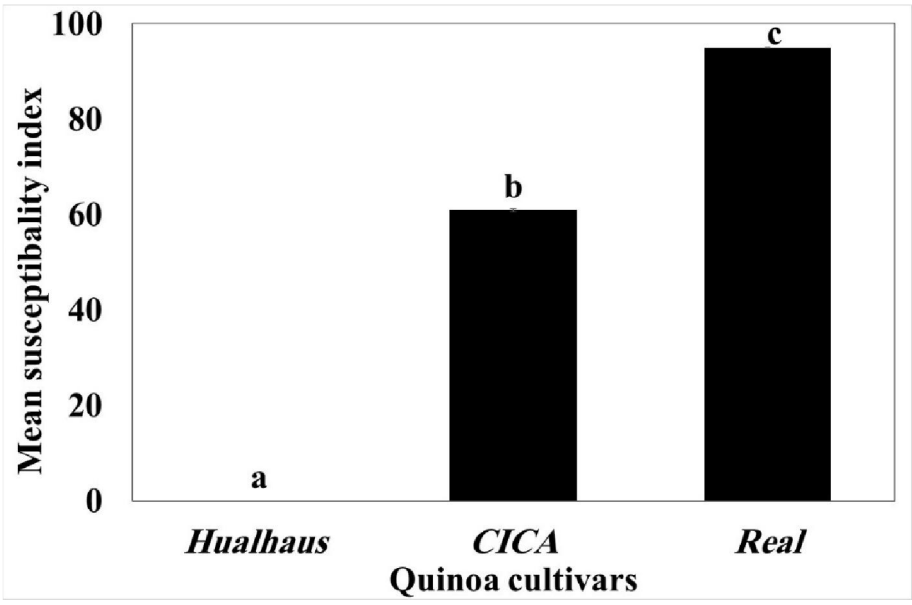

Fig. 4: Susceptibility indices of different quinoa cultivars to downy mildew calculated according to Wan *et al.* (2007). Each column represents the mean values of 15 replicates and the bars represent standard errors. Columns with the same letters are not statistically different ($P \leq 0.05$), Tukey's HSD test.

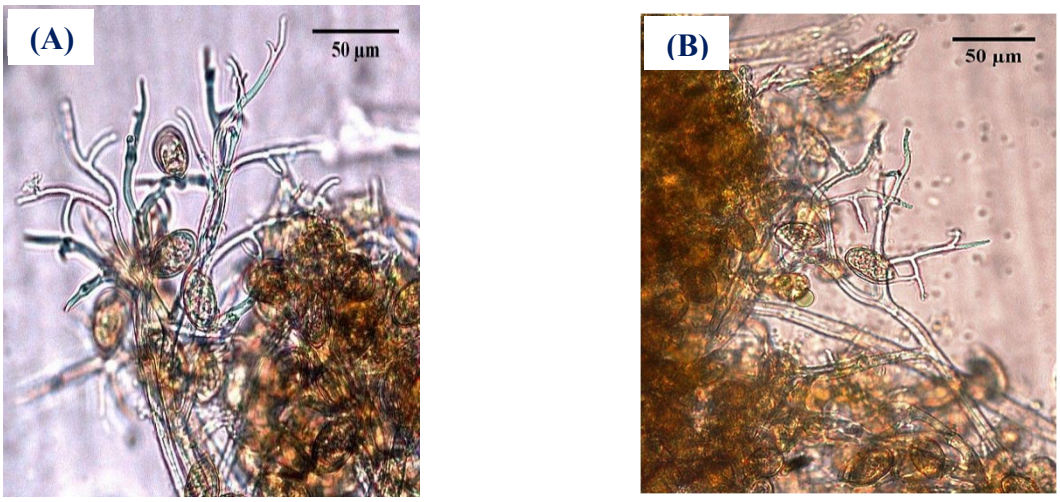

Fig. 5: Representative light microscopic graphs of *P. variabilis* grown on the leaves of "*Real*" plants. (A), dichotomously branched sporangiophores with slightly curved sterigamata; (B), the branches of sporangiophore and pyriform sporangia.

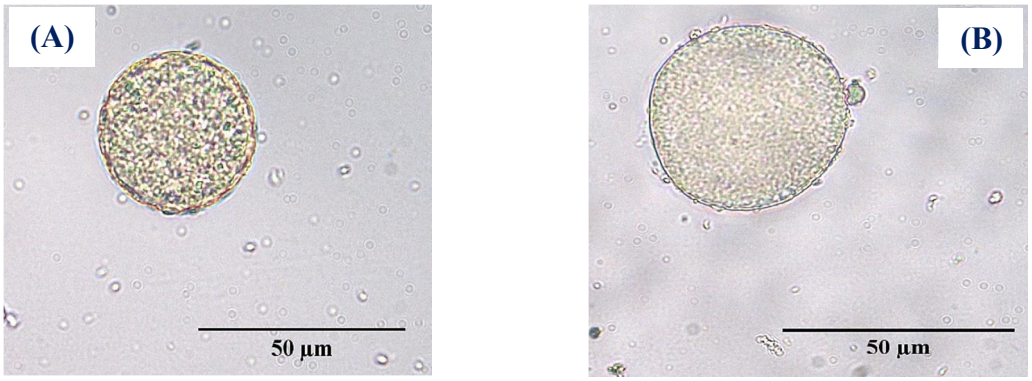

Fig. 6: Representative micrographs of mature oospores of *P. variabilis* detected in the seed wash of susceptible quinoa cultivars "*CICA*" and "*Real*" (A). Immature oospore characterized by relatively clear contents and thin walls observed in the seed wash of "*Real*" plants (B).

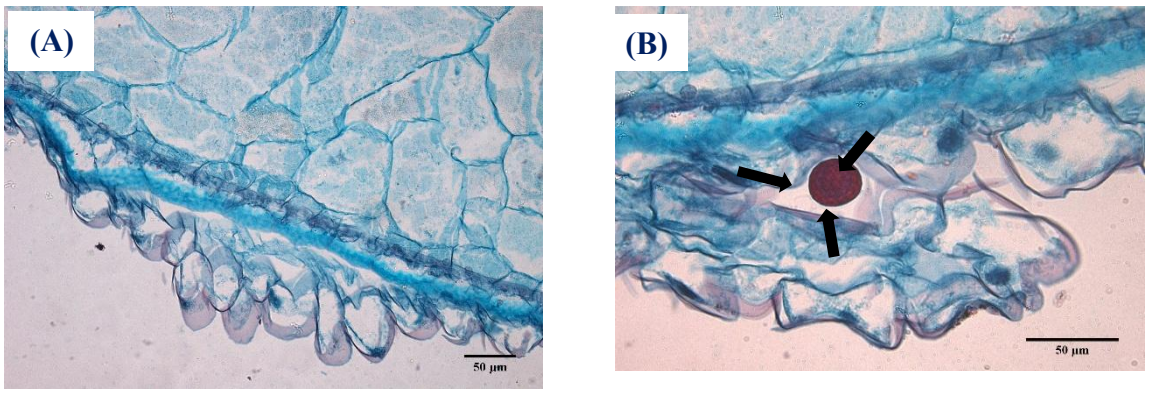

Fig. 7: Transverse sections in the seeds of the cultivar "*Hualhuas*" (A) and "*CICA*" (B). Note the presence of mature oospore (arrows) of *P. variabilis* in the pericarp layers in "*CICA*".

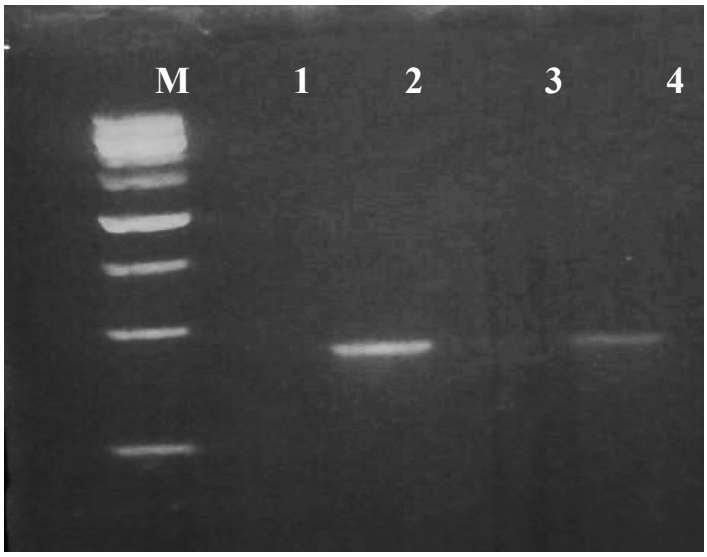

Fig. 8: Detection of *P. variabilis* ITS region in naturally infected tissues of different quinoa cultivars using conventional PCR with *P. variabilis* specific primers. Product size is approximately 866 bp. M, 1-kb ladder DNA marker; lane 1, negative control; lane 2, DNA from leaf tissues of *C. quinoa* cv. *Real*; lane 3, DNA from leaf tissues of *C. quinoa* cv. *Hualhaus*; lane 4, DNA from leaf tissues of *C. quinoa* cv. *CICA*.

Discussion

Utilization of quinoa cultivars, having durable resistance to downy mildew, represents an effective and economical strategy for sustainable quinoa production in Egypt. Hence, the present study aimed to evaluate and screen downy mildew resistance in three commercial quinoa cultivars grown under Egyptian field conditions. Our results confirm the occurrence of downy mildew infection and the diseased plants showed a variety of symptoms, depending on the cultivar and environmental conditions. These symptoms include pale, yellow to brown lesions of different size on the upper leaf surface with corresponding grayish sporulating patches on the underside of the diseased leaves (Fig. 1). Light microscopic inspections of these lesions revealed the presence of dichotomously branched sporangiophores, which tapered to a blunt point and produced ellipsoidal, light brown sporangia (Fig. 5). Based on these morphological features and in accordance with previous findings (Choi *et al.,* 2010), the pathogen was identified as *P. variabilis*. Furthermore, the morphological characteristics of the sporangia and sporangiophores were also in agreement with previous records of the causal agent of quinoa downy mildew (Tewari and Boyetchko, 1990; Danielsen and Ames, 2004; Choi *et al.,* 2010; Mhada *et al.,* 2015).

In the present study, symptoms of downy mildew started to occur in December and intensified in January, due to the prevailing cool and damp conditions at the experimental field. These climatic conditions favor spore production and dissemination and promote disease development, as has been previously reported (Danielsen *et al.,* 2004). Even under these conducive conditions, the screened quinoa cultivars showed genotypic variability in their resistance to downy mildew. While "*Hualhus*" plants appeared to be resistant, both "*CICA*" and "*Real*" cultivars seem to be susceptible, displayed typical downy mildew symptoms on the leaves of diseased plants (Fig. 1). This is supported by the trends of disease incidence, severity, and susceptibility indices. Our data showed that "*Hualhuas*" plants displayed the lowest level of disease incidence (0%), compared to the other cultivars. Furthermore, the degree of disease incidence for "*CICA*" plants was significantly ($P \leq 0.05$) less than that of "*Real*" plants (Fig. 2). Disease severity is another pertinent criterion to assess the intensity of infection and disease development. A variety of assessment methods and scales have been proposed for the evaluation of downy mildew severity in quinoa and examined for their effectiveness and accuracy (Danielsen *et al.,* 2004). The 0-5 scale (Mhada *et al.,* 2015), adopted in this study to assess

downy mildew severity revealed that "*Real*" plants displayed consistently higher disease severity compared to "*CICA*" plants. The majority of the diseased leaves in "*Real*" plants exhibited typical lesions of grade 4 and 5 (Fig. 3), covering up to 90% of the total leaf area, with high sporulation degree on the underside of the leaves. In some "*Real*" plants, severe infection was obvious leading to extensive premature leaf loss. As can be seen in Figure (4), susceptibility index (SI) was high, reached approx. 60.7% and 94.4% for "*CICA*" and "*Real*", respectively, but very low, being 0% for "*Hualhuas*" plants. This implies that "*Hualhuas*" could be considered as an extremely resistant (ER) cultivar, whereas "*Real*" could be ranked as a highly susceptible (HS) cultivar to downy mildew (Staudt and Kassemeyer, 1995). A considerable amount of evidence has accumulated, proving that the response of quinoa to downy mildew is strongly genotype dependent (Danielsen *et al.,* 2000, 2004; Kumar *et al.,* 2006). Lowland cultivars, originating from areas where humidity is high and the disease is rampant, often show high to moderate downy mildew resistance, while ecotypes of southern high plateau growing in drier regions are more susceptible (Bonifacio, 2003). This might be either because the resistance genes in quinoa varieties from dry regions have been "lost" during evolution, or because varieties without resistance have an evolutionary advantage over resistant ones in these areas (Danielsen *et al.,* 2003). This may explain, at least in part, the high downy mildew resistance observed in "*Hualhuas*" (coastal lowland cultivar) as compared to "*CICA*" and "*Real*" (adapted to harsh and dry conditions of high elevations). Although the type and nature of mechanisms behind downy mildew resistance in quinoa have not been thoroughly studied (Ochoa *et al.,* 1999), genotypic variations in quinoa reaction to downy mildew suggest that intrinsic factors are involved in these mechanisms. According to Gandarillas *et al.* (2015), downy mildew resistance in quinoa is controlled by major genes (vertical resistance), minor genes (horizontal resistance) or by a combination of both major and minor genes, resulting in partial or durable resistance. They added that horizontal resistance is the widely common type and the degree of resistance varies from highly susceptible to resistant, depending on the number of resistance genes that the variety exhibits. Reportedly, plant resistance to biotrophic pathogens is complex and involves some pre-existing physical barriers as well as inducible and constitutive substances which act as fungicide-like molecules (Dangl and Jones, 2001; Wink, 2003). The presence of different types of saponins, some of which have potent antifungal activity, might be involved in downy mildew resistance in quinoa (Osbourn, 1996; Fuentes *et al.,* 2009; Kuljanabhagavad and Wink 2009; Miranda *et al.,* 2013). However, the potential antifungal activity of saponins against *P. variabilis* remains to be elucidated. It seems also interesting to investigate the relationship between the degree of downy mildew resistance and the saponin content within quinoa germplasm.

Right now, the source of inoculum for quinoa downy mildew infection in Egypt is not certain. As has been demonstrated by Choi *et al.* (2008), downy mildew exhibits a high level of host specificity, so that no threat is posed from strains of downy mildew that infect other related chenopods, as these strains belong to *Peronospora farinosa*, a separate species from *P. variabilis*. It is therefore tempting to speculate that seedborne oospores might constitute an important inoculum source for downy mildew infection in quinoa trials under Egyptian conditions. Seedborne oospores have been previously demonstrated to act as a primary source of inoculum of downy mildew in quinoa (Risi and Galwey, 1984; Tewari and Boyetchko, 1990; Danielsen *et al.,* 2004; Testen *et al.,* 2014) and several other pathosystems (Vulsteke *et al.,* 1997; Adenle and Cardwell, 2000). Here, we could observe several oospores of *P. variabilis* (in different physiological stages) only in the seed washing-fragments of both susceptible cultivars "*CICA*" and "*Real*" (Fig. 6). The density of oospores in the seed washing-fragments varied between 300 and 1000 oospores/ml, with "*Real*" seeds having the highest density. Oospores of many plant pathogenic oomycetes were also observed in different parts of the seed tissues. In line with other investigations (i.e. Danielsen *et al.,* 2004), oospores were also observed in the pericarp tissues of the fixed quinoa seeds from both "*CICA*" and "*Real*" diseased plants (Fig. 7). In contrast, oospores were never detected in the seed tissues of "*Hualhuas*" plants in the present study.

In this study, the presence of *P. variabilis* is further confirmed by PCR-based method. Our results revealed the presence of 866-bp amplicons, which represent the ITS of *P. variabilis* only in the diseased leaf tissues of both quinoa cultivars "*CICA*" and "*Real*" (Fig. 8). *P. variabilis* was not, however, detected in "*Hualhuas*" plants, indicating that this cultivar is resistant, at least, to *P. variabilis* strains currently present in Egypt. However, further sequencing-based markers (i.e., ITS

and *COX2* sequences) are needed to validate the results of PCR-based detection methods. Additionally, comparison of ITS and *COX2* sequences from this local *P. variabilis* isolate with those from South America and Denmark would allow to infer more about the pathogen population in Egypt. It should be mentioned that more pathogenicity studies on "*Hualhuas*" cultivar under controlled conditions (with known pathogen populations and consistently high disease pressure) would help in understanding the nature of the resistance in this cultivar and aid in finding and characterizing resistance gene(s) for breeding programs.

In summary, we could detect typical symptoms of downy mildew caused by *P. variabilis* only on the leaves of Altiplano quinoa cultivars "*CICA*" and "*Real*", while no disease symptoms were found on the leaves of the sea level cultivar "*Hualhuas*". Trends of disease incidence, severity, and susceptibility indices showed clearly that the lowland cultivar "*Hualhuas*" was extremely resistant to downy mildew compared to both Altiplano cultivars "*CICA*" and "*Real*". The disease assessment methods used in this study were suitable and easily utilized for resistance screening under natural field conditions. Furthermore, PCR results revealed the presence of specific ribosomal internal transcribed spacer amplicons of 866-bp only in the diseased leaves of both "*CICA*" and "*Real*" plants. Together, these findings confirm the high degree of downy mildew resistance of "*Hualhuas*" plants, at least to the isolate currently present in Egypt. These allow for the speculation that this cultivar is a promising candidate in terms of downy mildew resistance under Egyptian conditions. Finally, more investigations on downy mildew under controlled conditions (artificial inoculation) would help in understanding the nature of the resistance in this cultivar and aid in finding and characterizing resistance genes for quinoa breeding programs.

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