First report of *Alternaria alternata* causing discoloration on *Amaranthus* seeds in Argentina

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Abstract *Alternaria alternata* is recorded as the causal agent of seed discoloration of *Amaranthus caudatus* ssp. *mantegazzianus* for the first time in Argentina.

Keywords Amaranth · Seeds discoloration · Mycoflora *Alternaria alternata* · *Fusarium equiseti* · *Cladosporium cladosporioides* · *Penicillium* sp · *Phoma* sp · *Bipolaris* sp · *Rhizopus nigricans* · Blotter test · Agar test

Amaranthus caudatus ssp. *mantegazzianus* (amaranth) is an ancestral crop native to Bolivia and Argentina. Stems, leaves and seeds have a high nutritional value and contain proteins that act as antioxidants (Tironi and Añón 2010) and antitumorals (Barrio and Añón 2010). Among the fungal pathogens that affect amaranth, *Alternaria alternata* is the causal agent of spots and blights on leaves (Noelting et al. 2009).

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In April 2008, discolored seeds (Fig. 1c) from an amaranth crop (Fig. 1a), cultivated in Llavallol, Buenos Aires province, Argentina were observed. Standard sanitary methods viz blotter and agar tests (Neergaard 1979) were used to investigate the microorganisms present in these seeds and their effect on germination. Discolored and normal seeds were plated on Petri dishes and incubated for seven days at $25\pm 2^\circ\text{C}$ in a growth chamber under 12 h photoperiod.

The fungus most frequently isolated from the discolored seed was *Alternaria alternata* with a contamination percentage of 40% (blotter test) and 42% (agar test). The other common fungi recovered were *Fusarium equiseti*, *Penicillium* sp, *Bipolaris* sp, *Cladosporium cladosporioides*, *Phoma* and *Rhizopus nigricans*.

An isolate of *Alternaria alternata* was plated on Petri dishes with water agar and incubated in a growth chamber $25\pm 2^\circ\text{C}$ under 12 h photoperiod. The isolate exhibited a sporulation pattern with more than three branches of conidia per chain (Fig. 2).

Conidia were ovoid to ellipsoidal with a yellowish brown colour and transverse and longitudinal septa. Mature conidia measured $24.6\pm 3.5\times 14.6\pm 0.7\ \mu\text{m}$. Conidiophores were solitary and measured $32.4\pm 8.9\ \mu\text{m}$. Colonies on PDA had a felted appearance and were dark grey with a diameter of 45 mm after 7 days incubation. The isolates were identified belonging to *A. alternata* according to their sporulation pattern (Fig. 2a) and morphology of conidia and conidiophores (Simmond 1995). The isolate of *A. alternata* has been lodged in the culture collection of the Instituto Carlos Spegazzini, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina, with the accession number LPSC N° 1085.

Fig. 1 (a) Panicle of *Amaranthus caudatus* ssp. *mantegazzianus*; (b) seeds with normal appearance; (c) discolored seeds

Fig. 2 (a) Sporulation pattern of *Alternaria alternata*; (b) healthy seed; (c) seed inoculated with *A. alternata*

In order to determinate if *A. alternata* was the possible cause of discoloration, pathogenicity tests were carried out. Amaranth seeds with healthy appearance (Fig. 1b) were inoculated with a conidial suspension (1×10^5 conidia/ml) and incubated for seven days at $25 \pm 2^\circ\text{C}$ and $10 \pm 2^\circ\text{C}$ in a growth chamber under 12 h photoperiod. Untreated seeds were included as a control.

Pathogenicity tests showed the ability of *A. alternata* to produce seed discoloration at both incubation temperatures (Fig. 2b–c). At $25 \pm 2^\circ\text{C}$, discoloration integuments of germinated and ungerminated seeds were observed. A significant reduction in seed germination and an increase in the number of abnormal plants was also seen.

A. alternata was re-isolated from the seeds confirming Koch's Postulates and the pathogenicity of the fungus. Germination values were 37% for discolored seeds and 85% for seeds of normal appearance. No disease symptoms were observed in the controls (Fig. 1b).

This pathogen was also detected on discoloured amaranth seeds from La Pampa and Cordoba Provinces, Argentina. Although there are previous records of fungi on amaranth seeds (Noelting et al. 2004, 2006), this is the first report of *A. alternata* as the cause of discolored seeds on this crop in Argentina. Further studies are needed to

determine if this pathology affects the nutritional quality of the seeds.

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