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SHORT COMMUNICATION

Multiple large and small-spored *Alternaria* species causing leaf spot of potato in South Africa

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Abstract
Early blight and brown spot of potato in South Africa are caused by *Alternaria solani* and *A. alternata*, respectively. However, elsewhere in the world, other *Alternaria* species have also been associated with leaf spot of potato. These species include *A. grandis*, *A. protenta*, *A. arborescens*, *A. cantlous*, *A. infectoria* and *A. dumosa*. Potato growing regions in South Africa were surveyed to determine which *Alternaria* species cause disease on potato. Samples were collected from September 2018 to April 2021 from 13 potato production regions in South Africa. *Alternaria* isolates obtained from collected samples were identified based on morphology and phylogenetic analyses of *Alt a 1*, *tef 1-α*, *gapdh* and *rpb2* gene regions, depending on the morphological group the isolates were placed into. In addition to *A. alternata* and *A. solani*, two additional species were identified based on sequencing results, namely *A. arborescens* species complex (SC) and *A. grandis*. Virulent isolates were selected based on a detached leaf assay and inoculated onto potato plants in the greenhouse. Symptoms similar to those observed in the field were observed and the pathogens reisolated and identified. *Alternaria grandis* isolates caused symptoms similar to those of *A. solani* (early blight) and *A. arborescens* SC caused symptoms similar to those of *A. alternata* (brown spot). This is the first report of *A. grandis* and *A. arborescens* SC causing leaf spot of potato in South Africa.

Keywords Early blight · Brown spot · *Alternaria solani* · *Alternaria alternata* · *Alternaria grandis* · *Alternaria arborescens* species complex

The genus *Alternaria* contains various species that cause diseases on potato (*Solanum tuberosum* L.), such as *Alternaria alternata* causing brown spot on leaves and black pit on tubers (van der Waals et al. 2011), A. *arborescens*, *A. dumosa* and *A. protenta* were reported to be associated with leaf spots only (Simmons 1986), whereas *A. cantlous* (Amini et al. 2016), A. *grandis* and *A. infectoria* were reported to cause leaf spot (Ardestani et al. 2010; Rodrigues et al. 2010) and *A. solani* causes early blight of potato (van der Waals et al. 2001). In South Africa, brown spot and early blight are the most prominent potato diseases caused by *Alternaria* (van der Waals et al. 2001), but the occurrence and abundance of *Alternaria* species besides *A. alternata* and *A. solani* associated with potato in South Africa was unknown.

The aim of the study was therefore to survey potato production areas located in different climatic regions of South Africa. Samples were collected from September 2018 to April 2021 from 13 of the 16 production regions spread throughout South Africa. A total of 1237 *Alternaria* isolates were obtained and grown on potato carrot agar (PCA) under 12 h alternating light/dark conditions. Isolates were assigned to morphological groups based on conidial size, septation and chain formation according to guidelines by Simmons (1986) using a Zeiss Discovery.V8 stereo microscope (Carl Zeiss, Germany).

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Representative isolates from each group and region were selected and DNA extraction was performed with the Zymo Research fungal/bacterial DNA isolation kit (Zymo Research Corporation, Irvine CA, USA). Depending on the morphological group isolates were placed into, different gene regions were amplified for molecular identification. The glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene with primers gpd1 and gpd2 (Berbee et al. 1999) and the RNA polymerase second largest subunit (*rpb2*) with primers RPB2–5F2 (Sung et al. 2007) and fRPB2–7cR (Liu et al. 1999), were amplified for all large-spored isolates. Three gene regions were amplified for the small-spored isolates. The *Alt a 1* gene that encodes for the *Alternaria* main allergen was amplified using primers Alt-for and Alt-rev (Hong et al. 2005) and *rpb2* was amplified with primers mentioned above. The translation elongation factor 1α was amplified with primers EF1-728 F and EF1-986R (Carbone and Kohn 1999) or EF2 (O'Donnell et al. 1998). PCR reactions were conducted in a total volume of 25 µl that consisted of 12.5 µl of EmeraldAmp GT PCR Master Mix (Takara Bio Inc, Japan), 0.2 mM of each primer, 50 ng template DNA and PCR-grade water to make up the final volume. PCR conditions for *Alt a 1* were as described by Woudenberg et al. (2014) and for tef 1α, *gapdh* and *rpb2* as described by Woudenberg et al. (2013). PCR products were visualised on a 1% agarose gel stained with Novel Juice (GeneDireX Inc, Taoyuan, Taiwan).

PCR products were sequenced by Inqaba Biotec (Pretoria, South Africa), forward and reverse sequences were assembled, and consensus sequences generated using CLC Genomics Workbench 10.0 (https://www.qiagenbioinformatics.com/) and deposited in GenBank (Table 1). Sequences of type strains of *Alternaria* were downloaded from GenBank (https://blast.ncbi.nlm.nih.gov/) and aligned in MEGA version 11 (Tamura et al. 2021) using the Muscle algorithm. Phylogenetic analyses were done using PhyML 3.0 (Guindon and Gascuel 2003) using the Generalised Time Reversible (GTR) model and trees were visualised using MEGA version 11.

Spore suspensions were produced by growing *Alternaria* isolates on PCA under alternating light and dark cycles (12 h light, 12 h dark) for 14 days, whereafter cultures were flooded with sterile distilled water amended with 0.05% Tween 20. Spores was loosened by lightly scraping cultures with an L-shaped glass rod whereafter the resulting suspension was transferred to sterile McCartney bottles containing Tween 20 amended water. Spore suspensions were adjusted to 10^6 spores / ml and inoculated onto freshly detached potato leaves (cv. Mondial – the most popular ware potato cultivar in South Africa) and incubated on moist filter paper for five days in the light (adapted from method by Soleimani and Kirk, 2012). Water with 0.05% Tween 20 was inoculated onto control leaves. Five replicate leaves were included for each treatment. Lesions were measured (length and width) after five days and the most virulent isolates selected based on lesion size. Control leaves did not develop any symptoms. Spore suspensions (10^6 spores / ml) of the virulent isolates from each identified species were inoculated onto eight-week-old healthy potato plants (cv. Mondial) in a greenhouse by spraying the spore suspension on the leaves until run-off (approximately 3 ml per plant). Ten plants were inoculated with each of the selected isolates. Water with Tween 20 was sprayed onto ten control plants. Plants were covered with clear plastic bags for 48 h after inoculation, whereafter the bags were removed, and plants were maintained at day and night temperatures of 28 °C and 18 °C, respectively. Symptom development was monitored for six weeks and pathogens reisolated and identified. Control plants did not develop any symptoms.

Morphological investigations revealed small-spored isolates of 15–45 µm x 8–12 µm with 1–4 transvers and 1–2 longitudinal septa. Conidia are borne in chains of various lengths. Large-spored isolates (>100 µm) were mostly solitary, ovoid to narrow obclavate with long beaks, 11–19 transverse and 1–3 longitudinal septa. Morphological and phylogenetic analyses showed that two small-spored (Fig. 1) and two large-spored (Fig. 2) species were obtained from symptomatic plant material sampled in the different regions. In addition to the large-spored *A. solani*, *A. grandis* was also isolated from the early blight symptoms and no *A. protenta* (the third large-spored *Alternaria* species causing leaf spots on potato) was present. *Alternaria alternata* and *A. arborescens* SC were isolated from brown spot symptoms. All four of the species caused leaf spot on potatoes when inoculated onto healthy plants in the greenhouse (Fig. 3), and were reisolated from the symptoms observed, making this the first report of *A. grandis* and *A. arborescens* SC causing leaf spot of potato in South Africa.

This study highlights the importance of accurate identification of pathogens as a higher diversity of species may be involved in disease outbreaks than is at first apparent. The larger species diversity can hold implications for disease management as the different species may not have the same level of susceptibility to chemicals used to control them. Further work is needed to determine the host ranges of *A. grandis* and *A. arborescens* SC in South Africa.

Table 1 GenBank accession numbers of isolates included in phylogenetic analyses

Isolate	Species	GenBank accession number			
		rpb2	gapdh	Alt a1	tef 1α
EC7	*Alternaria alternata*	PQ189679		PQ475045	PX513184
EC206	*Alternaria alternata*	PQ189666		PQ475031	PX513187
EC221	*Alternaria alternata*	PQ189667		PQ475032	PX513188
EC226	*Alternaria alternata*	PQ189668		PQ475033	PX513189
EC252	*Alternaria alternata*	PQ189670		PQ475035	PX513191
EC300	*Alternaria alternata*	PQ189672		PQ475037	PX513193
EC341	*Alternaria alternata*	PQ189673		PQ475038	PX513194
EC483	*Alternaria alternata*	PQ189675		PQ475041	PX513196
EC627	*Alternaria alternata*	PQ189677		PQ475043	PX513198
EC873	*Alternaria alternata*	PQ189681		PQ475047	PX513200
EC86	*Alternaria arborescens* SC	PQ189680		PQ475046	PX513185
EC189	*Alternaria arborescens* SC	PQ189665		PQ475029	PX513186
EC248	*Alternaria arborescens* SC	PQ189669		PQ475034	PX513190
EC268	*Alternaria arborescens* SC	PQ189671		PQ475036	PX513192
EC445	*Alternaria arborescens* SC	PQ189674		PQ475040	PX513195
EC559	*Alternaria arborescens* SC	PQ189676		PQ475042	PX513197
EC629	*Alternaria arborescens* SC	PQ189678		PQ475044	PX513199
EC1205	*Alternaria arborescens* SC	PQ189662		PQ475027	PX513202
EC1288	*Alternaria arborescens* SC	PQ189663		PQ475028	PX513203
EC1431	*Alternaria arborescens* SC	PQ189664		PQ475030	PX513204
EC933	*Alternaria arborescens* SC	PQ189682		PQ475048	PX513201
EC45	*Alternaria grandis*		PQ252355		
EC220	*Alternaria grandis*		PQ252358		
EC249	*Alternaria grandis*		PQ252359		
EC264	*Alternaria grandis*		PQ252362		
EC274	*Alternaria grandis*		PQ252363		
EC600	*Alternaria grandis*		PQ252354		
EC885	*Alternaria grandis*		PQ252352		
EC998	*Alternaria grandis*		PQ252351		
EC15	*Alternaria solani*	PQ189702			
EC27	*Alternaria solani*	PQ189701			
EC42	*Alternaria solani*	PQ189700			
EC46	*Alternaria solani*	PQ189699			
EC152	*Alternaria solani*	PQ189698			
EC200	*Alternaria solani*	PQ189697	PQ252356		
EC207	*Alternaria solani*	PQ189696			
EC209	*Alternaria solani*	PQ189695			
EC213	*Alternaria solani*	PQ189694			
EC216	*Alternaria solani*	PQ189693			
EC217	*Alternaria solani*	PQ189692			
EC218	*Alternaria solani*	PQ189691	PQ252357		
EC250	*Alternaria solani*	PQ189690	PQ252360		
EC256	*Alternaria solani*	PQ189689	PQ252361		
EC257	*Alternaria solani*	PQ189688			
EC282	*Alternaria solani*	PQ189687			
EC285	*Alternaria solani*	PQ189686			
EC288	*Alternaria solani*	PQ189685			
EC312	*Alternaria solani*	PQ189684			
EC326	*Alternaria solani*	PQ189683			
EC640	*Alternaria solani*		PQ252353		

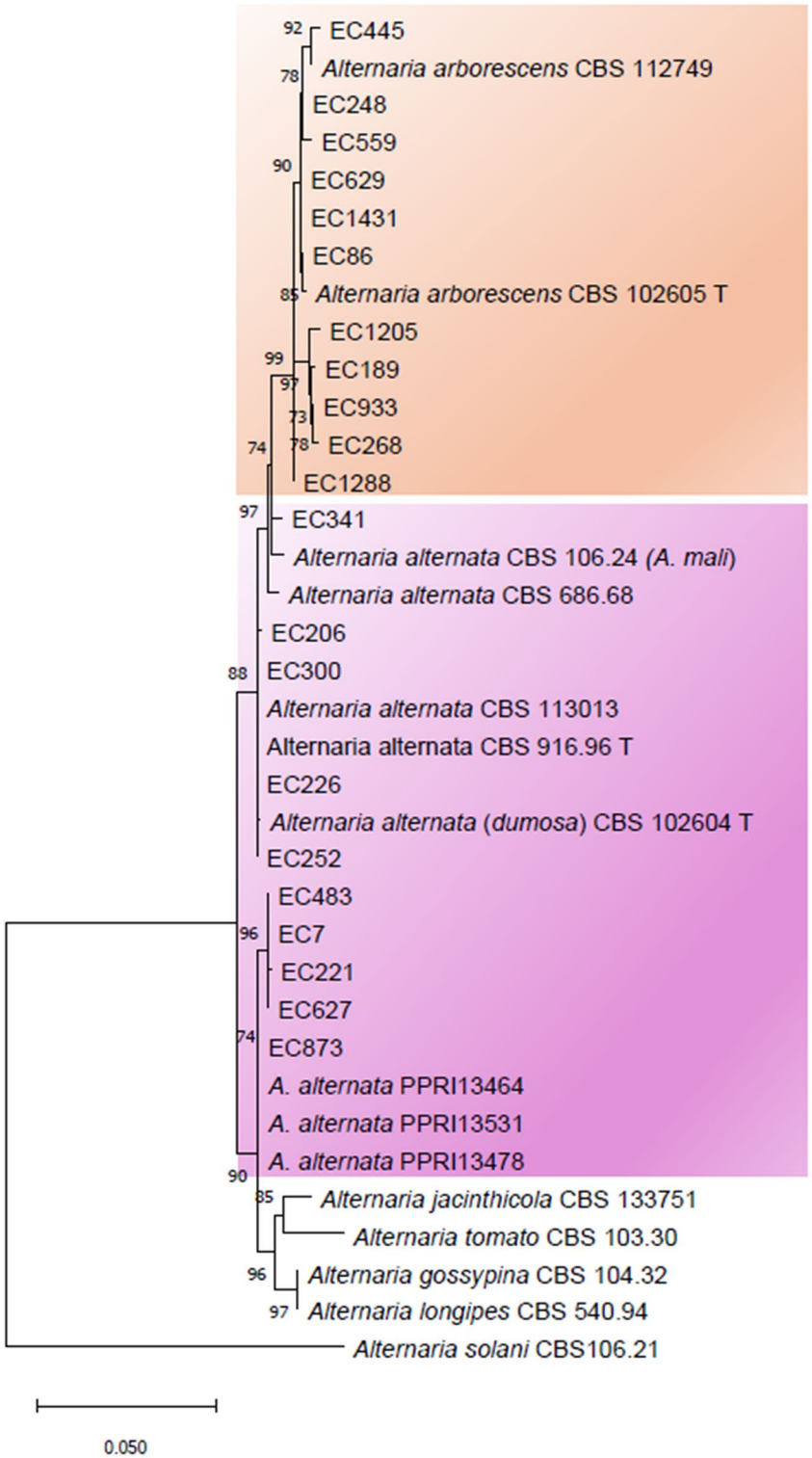

Fig. 1 Maximum likelihood tree of the combined *Alt a1*, *rpb2* and *tef-1α* gene regions of the small-spored *Alternaria* species obtained from diseased potato leaves. Isolates obtained from potato in South Africa are indicated with EC isolate numbers. Bootstrap values (1 000 replicates) of above 70 are displayed at supported nodes. Tree rooted to *A. solani*

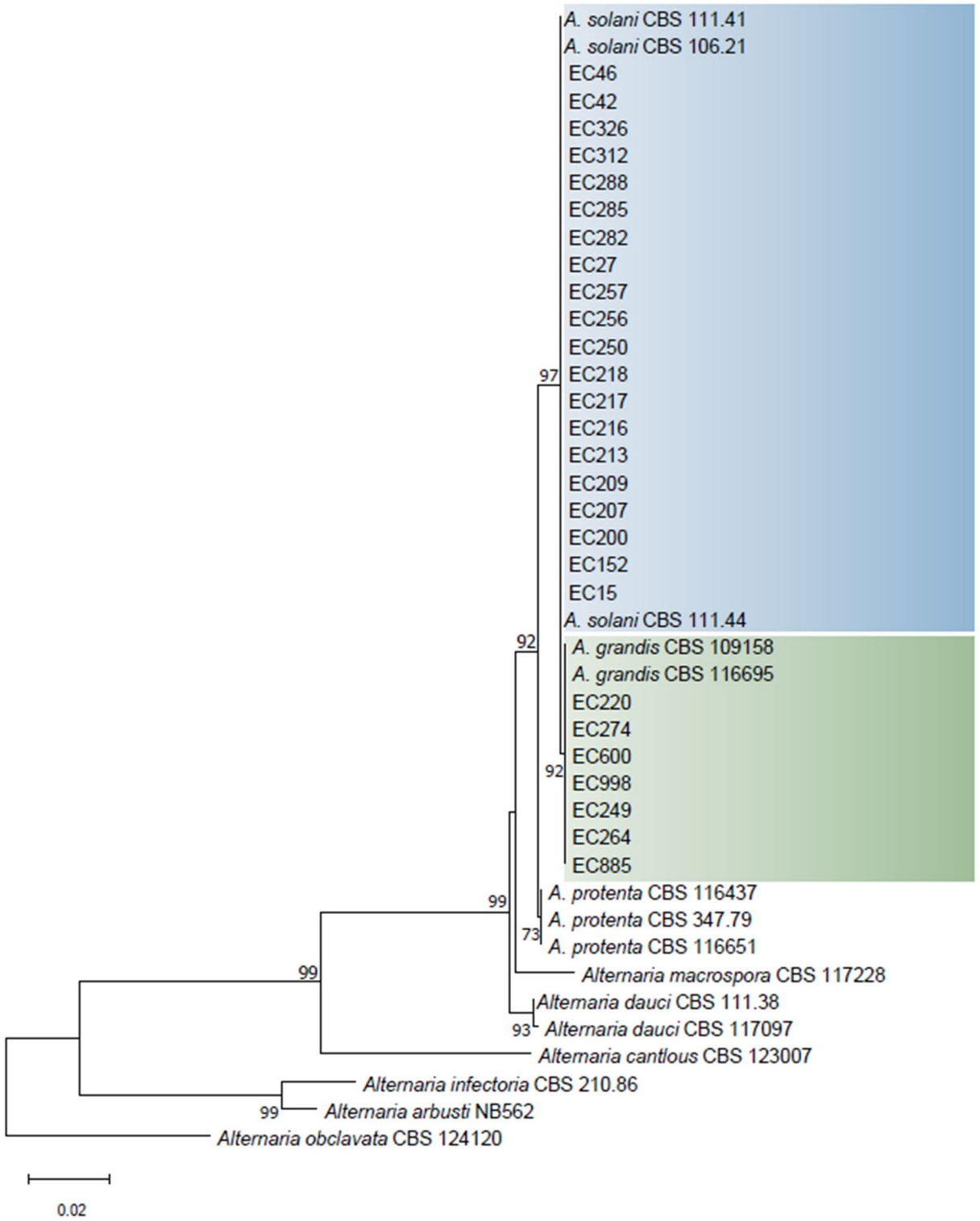

Fig. 2 Maximum likelihood tree of the combined GAPDH and *rpb2* gene regions of the large-spored *Alternaria* species obtained from diseased potato leaves. Isolates obtained from potato in South Africa are indicated with EC isolate numbers. Tree rooted to *A. obclavata*. Bootstrap values (1 000 replicates) of above 70 are displayed at supported nodes

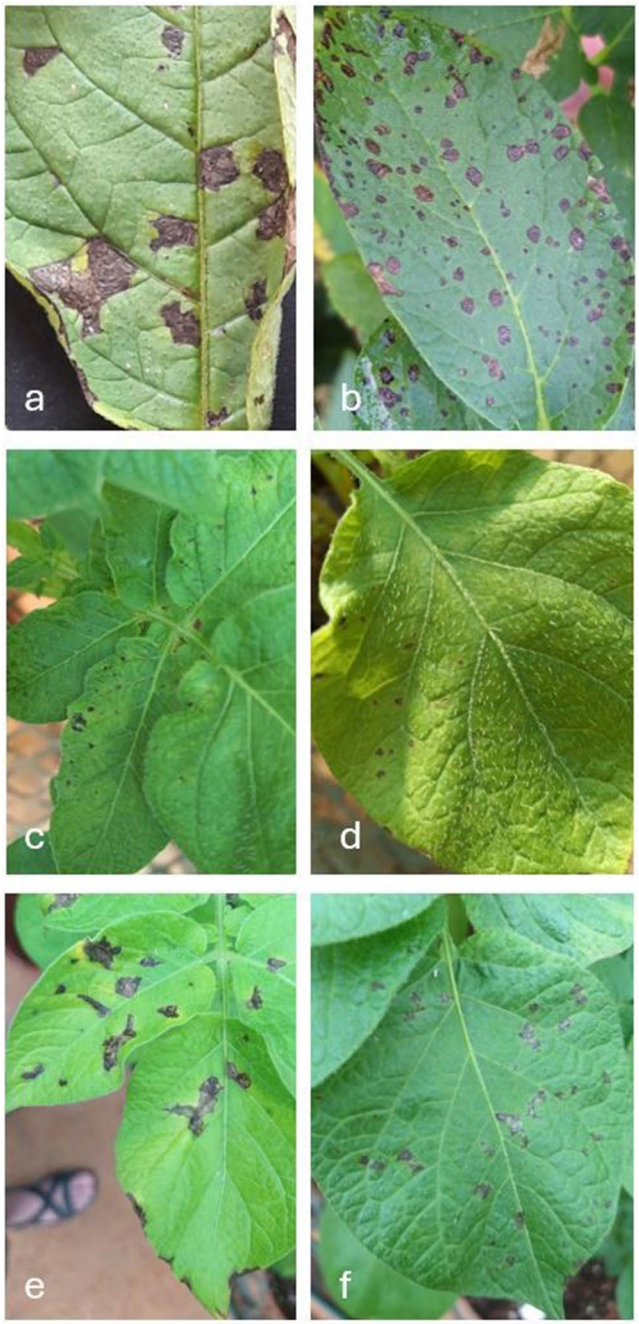

Fig. 3 Distinctive **a**) early blight and **b**) brown spot symptoms observed on potato plants in South Africa, **c**) *Alternaria alternata* and **d**) *A. arborescens* SC causing brown spot on potatoes in the greenhouse, **e**) *A. solani* and **f**) *A. grandis* causing early blight symptoms on potatoes in the greenhouse

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Data availability The datasets generated during and/or analysed during the current study are available in the Genbank repository, https://www.ncbi.nlm.nih.gov/genbank/about/.

Declarations

Ethical approval Not applicable.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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