Pak. J. Bot., 45(5): 1817-1824, 2013.

NATURE AND EFFECT OF *ALTERNARIA* SPP. COMPLEX FROM WHEAT GRAIN ON GERMINATION AND DISEASE TRANSMISSION

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Abstract

Diseases caused by *Alternaria* sp. are among the most common diseases of crops throughout the world. *Alternaria* sp. is a common component of the flora of wheat seed. Although isolation of *Alternaria* sp. from wheat (*Triticum aestivum*) seed has been reported in Argentina, development of the *Alternaria* blight in plants from infected seeds has not been demonstrated experimentally. Seed transmission of strains belonging to *Alternaria tenuissima, A. alternata, A. infectoria, A. triticina, A. chlamydospora* and related genera like *Embellisia* and *Ulocladium* sp. on wheat were investigated in the Argentinean growing area, on wheat cultivars Klein Escorpión and Buck Poncho. *A. tenuissima* was the dominant fungus in black pointed kernels. Transmission of all 42 seed-borne members of *Alternaria* complex from seeds to seedlings artificially inoculated was detected by trays seedling symptoms test. Among the fungi tested most isolates of *Alternaria*, *Embellisia* sp. and *Ulocladium* sp. produced distinct seed rot and seedling infection symptoms. This confirmed the seed-borne nature of these fungi. In each wheat cultivar tested inoculated seeds appreciably reduced their germination. The emerging coleoptile is externally infected by hyphal growth from the infected pericarp. Typical disease symptoms on the seedlings were exhibited. Recovery of the fungi from asymptomatic coleoptiles was also possible. Transmission efficiency varies with wheat cultivar and ranges from 0 to 92%. These results suggest that infected seed and seed transmission represents a mode of primary source of infection from which these microorganisms can start epidemics to the wheat crop and for dispersal of fungal strains to new areas.

Introduction

Wheat is an important cereal crop of Argentina grown extensively in different parts of Buenos Aires Province. From seed germination to harvest, wheat is attacked by a number of fungi, which under certain climatic conditions significantly reduce the yield and quality of the crop. Some of the fungal diseases are seed-borne and transmitted through seeds (Agarwal & Sinclair, 1987; Farr *et al*., 1989; Agrios, 2005; Fakhrunnisa *et al*., 2006; Anon., 2010). Of the various fungal organisms associated with wheat, members of *Alternaria* complex not only reduces germination and vigor of wheat seed that it also causes seedling blight disease in Argentina (Perelló, 2010a, b; Perelló *et al*., 1996, 2005, 2008; Perelló & Sisterna, 2006, 2008). Similarly in Pakistán, *Alternaria* spp were detected as predominant causing 82% reduction in germination of wheat seeds and also affecting seedling vigor (Rajput *et al*., 2005). From surveys carried out in the Province of Buenos Aires, Argentina, Perelló (2010a) founded that some samples were infected with isolates of the *Alternaria infectoria* complex to an extent of 6.0~79.5%. There is a lack of quantitative information on the disease prevalence and on the level of disease severity in the different agro-ecological locations of Buenos Aires Province. Neither it is known if these fungal pathogens were transmitted by seeds. During the present study, a severe infection on wheat kernel by *Alternaria* spp. was reported at some fields from the south-east of Buenos Aires Province. Literature review shows that no extensive work on the prevalence of the seed-borne fungi *Alternaria* and their transmission from seed to seedling of wheat seeds has been done in Argentina. Moreover, every year this seed-borne fungi cause heavy yield loss of the crop and some fungi of the complex could act as a primary source of infection. An accurate assessment of the relative importance of *Alternaria* wheat diseases nature is needed in order to help target research priorities and justify the use of resources. Considering the seriousness and common occurrence of kernel rot of wheat in Argentina by *Alternaria* spp., and the inadequate information regarding seed infection and transmission of *Alternaria* spp. this study was undertaken. The primary objective was to determine the members of the *Alternaria infectoria* and *A. alternata* species-group present in seed samples taken directly from fields at seven different growing areas in Buenos Aires and one location of Santa Fe, Argentina, just prior to harvest, and to examine samples to know the nature and level of other *Alternaria* species and related genus that may be wheat seed-borne. The present study is aimed to investigate and compare the *in vitro* germination rates of disinfected seeds of wheat to determine the recovery of seed-borne mycoflora belonging to *Alternaria* and related genera. The effect of seed-borne inoculums of the fungi on seedling emergence and transmission was also evaluated.

Materials and Methods

Isolation and identification of fungal pathogens: Twenty Wheat seeds samples obtained from different cultivars/localities of Buenos Aires Province and Santa Fe Province, Argentina, from 2009-2011 were collected and examined for the presence of fungal pathogens by blotter method as recommended by International Seed Testing Association (Anon., 1976, 1996b) (Table 1). The seeds samples were surface sterilized for 3 min in 1% sodium hypochlorite (NaOCl) solution and then washed thoroughly with sterile water and air dried in a laminar flow hood prior to planting on 9-cm Petri dishes containing potato dextrose agar 2%. Ten seeds were placed on each plate and then dishes were incubated under a combination of long-wave

ultraviolet and fluorescent light (12 h light: 12 h dark) for 7 days. Temperature was kept 20 ºC under the light and dark regimes, respectively. After 7 days of incubation, individual seeds were examined under a stereomicroscope for the presence and absence of fungi. Identification was confirmed by examining for the presence of mycelia and/or conidia under compound microscope. The fungal species present on each of the seeds were recorded and the percent incidence of each fungus per sample was computed. The agar plate method was used to further characterize the fungal growth that was observed in the plate. Each culture was re-isolated into pure culture using PCA (Potato Carrot Agar) as a medium. After 7 days of incubation, detailed examination was done by preparing semi-permanent slides and examining them under a compound microscope. The fungi were stored at 5ºC and maintained at the collection of cultures of CIDEFI till used.

Table 1. Procedence and effect on germination wheat seeds cultivar Klein Capricornio and Buck Guapo of 40 members of *Alternaria* complex.

Sample number	Cultivar and location of procedence	Seed colonization (%)	Seed germination (%)	
			Klein capricornio	Buck guapo
Aa1021	Buck Guapo-9 de Julio, Buenos Aires	20	99	50
Aa2	ProInta Puntal-Pla, Buenos Aires	25	8,33	33,3
Aa444	Buck Guapo-(1053)-9 de Julio, Buenos Aires	45	91,66	91,66
Aa17	ProInta Puntal-Pla, Buenos Aires	49	99	50
Aa112	Don Mario Onix-Pla, Buenos Aires	20	98	100
AaB	Klein Zorro-9 de Julio, Buenos Aires	45	83,33	66,66
Aa92	Don Mario Cronox-Bragado, Buenos Aires	47	100	33,33
Aa400	Don Mario Onix-Pla, Buenos Aires	34	91,66	16,66
Ate281	Don Mario Onix-pla, Buenos Aires	78	83,33	75
Ate1006	Baguette 11-Santa Isabel., Santa Fe	76	83,33	8,33
Ate205	Don Mario Cronox-Baradero, Buenos Aires	68	91,66	33,33
Ate6	ProInta Puntal-Pla, Buenos Aires	79	75	58,33
Ate220	Relmo Inia Tijereta. Pla, Buenos Aires	71	98	91,66
Ate312	Baguette 11-Santa Isabel, Santa Fe	67	83,33	50
Ate37	Don Mario Cronox-Baradero, Buenos Aires	69	58,33	50
Ate97	ACA 304 (1057)-Chacabuco, Buenos Aires	78	83,33	58,33
Ate1025	Baguette 11-Santa Isabel, Santa Fe	75	99	91,66
Ate44	SRM Nogal-Pla, Buenos Aires	76	58,33	8,33
AteB-888	Buck Baqueano, Buenos Aires	78	100	33,33
Ate94	Don Mario Cronox , Buenos Aires	75	91,66	50
Ate1088	Buck Baqueano-(1069) 9 de Julio, Buenos Aires	71	66,66	8,33
Ate1044	ACA 304 (1057)-Alberti, Buenos Aires	74	75	75
Ai80	Buck Baqueano (1069)-Chacabuco, Buenos Aires	69	83,33	25
Ai1008	Klein Gavilán- Bragado, Buenos Aires	56	91,66	33,33
Ai1010	Buck Guapo-9 de Julio, Buenos Aires	58	100	66,66
Ai700	Klein Gavilan-Bragado, Buenos Aires	60	75	25
Atm333	Buck Ranquel- Pla, Buenos Aires	45	99	58,33
Atm108	Baguette 11-Bragado, Buenos Aires	78	100	33,33
Atm98	Don Mario Cronox-Bragado, Buenos Aires	63	8,33	16,66
Atm82	Klein Escorpión-Rojas, Buenos Aires	67	98	100
AtD	Klein Escorpión-Pla, Buenos Aires	45	91,66	58,33
AtA	BioInta 3003-9 de Julio, Buenos Aires	40	100	33,33
At93	Don Mario Onix-Pla, Buenos Aires	47	83,33	41,66
At355	Buck Malevo-9 de Julio, Buenos Aires	56	98	16,66
At5	ProInta Puntal-Pla, Buenos Aires	53	75	50
Ach36	Buck Malevo-9 de Julio, Buenos Aires	27	33,33	25
Ach822	Klein 75 Aniversario-Rojas, Buenos Aires	34	41,66	66,66
Ach328	Klein Gavilán-Bragado, Buenos Aires	32	58,33	50
Pch16	Don Mario Onix-Pla, Buenos Aires	25	58,33	16,66
Pch009	Buck Guapo (1053)-Chacabuco, Buenos Aires	36	83,33	85
U1122	Baguette 11-Bragado, Buenos Aires	28	16,33	8,33
U70	Klein 75 Aniversario-Rojas, Buenos Aires	45	50	44,66

Germination test: *In vitro* germination rate tests were conducted in order to determine the effect of the 42 isolates from mycoflora associated with the seeds on K. Escorpión and Buck Guapo wheat cultivars. Seeds of both cultivars were surface disinfected with 1% sodium hypochlorite (NaOCl) solution for 3 min. and then they were washed three times with distilled water. The seeds were allowed to air dry for 24 h under a laminar flow hood. The seeds were inoculated by immersing the seeds in a standarized solution containing spores at a concentration of 3 x 10^5 spores/mL of the 42 species isolated from the agar plate method. A haemacytometer was used for determining the quantity of spores per mL. The seeds were sown in 9 cm diameter Petri dishes on three layers of moist papel towels. Each plate was moistened with 4 mL of distilled water. Forty seeds in each plate were spread at a regular distance on the surface of the paper. The Petri dishes were covered with a plastic bag to prevent drying and they were incubated. Incubation was at 20ºC for 7 days with 12 h of alternating cycles of NUV (near ultraviolet) light (Philips Black Light lamps TL 40W) and 12 h darkness. After incubation seeds were examined and germination percentages were recorded. Germination was considered present when the radical protrudes by 2-4 mm was observed (www.icrisat.org/what.../seed-processing-4.pdf).

Seedling emergence and evaluation of seed-to-seedling transmission: Simultaneously, the effects of the fungi isolated from the incubated wheat samples were tested on the seedling emergence and seedling transmission. Special plastic trays prepared with a layer of moist paper towel on a cotton layer previously sterilized and autoclaved for 10 minutes and 15 lb pressure at 121ºC were used. The seeds were arranged into each tray in rows of 10 seeds at regular intervals of 4 cm from the top edge and with leaving a 3-4 cm gap on the slides. The seeds were covered with another sheet of wet paper towel. Four hundred seeds for each of the two cultivars, Klein Capricornio and Buck Guapo were used to test the effect of the 42 strains of *Alternaria* spp. on the emerged seedlings. The tested trays were incubated in the growth chamber at temperature 20 ± 2 ºC and cycles of 12 h light-darkness to make accurate and repeatable estimates. The infected seedling was monitored for appearance of symptoms. Ten days after sowing, the condition (visual appearance) of seedlings in each treatment was recorded (Shafique *et al*., 2007).

The seedlings emergence and the seedling´s mortality was evaluated and the percent of emerged seedlings was calculated. The presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) caused by the microorganisms was registered by examining the seeds under stereo- binocular microscope.

To evaluate the seed-to-seedling asymptomatic transmission of the fungi, the technique of Carmona *et al*., (2004) with modifications was used. Ten seedlings from each treatment were cut at the level of the coleoptiles, disinfected in 70% ethanol for 2 min., and plated on moistened blotter papers in a plastic box for 5 days. The plants infected by the target fungus were counted using a stereomicroscope and the result was expressed as a percent.

Results and Discussion

Identification of fungal pathogens: The assessment of seed-borne mycoflora revealed 42 strains recovered from 21 wheat samples collected at Buenos Aires Province (18 samples) and Santa Fe Province (3 samples). From the total, 38 strains belonged to the *Alternaria* complex, 2 strains were identified as *Pithomyces* sp. and 2 strains as *Ulocladium* sp. Members of *Alternaria* species were clearly the dominant flora, with variation in the incidence of isolation between locations and cultivars and the taxa recovered. The identification of *Alternaria* species by examining morphological characters is not a simple task, requiring, culture on specific media for the production of patterns of growth and sporulation, conidiophore architecture and conidium size, shape and surface ornamentation (Dugan & Lupien, 2002; Simmons, 2007). Isolates were identified as *A. alternata (Aa), A. tenuissima (Ate), A. infectoria (Ai), A. triticimaculans (Atm), A. triticina (At), A. chlamydospora (Ach), Pithomyces chartarum (Pch),* and *Ulocladium* sp. *(U)* by observing their growth characteristics as well as morphological features of colony and conidia following the keys offered by different authors (Ellis, 1971; Neergaard, 1977; Rotem, 1994; Simmons, 1967, 1986, 1990, 1992, 1994, 1995, 2007) and on the basis of morphology and reaction to DRYES agar (Andersen & Thrane, 1996) after examining under stereo microscope and a compound microscope (Table 1). *A. tenuissima* was the dominant species with a seed colonization ranged from 68-79%, following by *A. infectoria* (56-69%) and *A. triticimaculans* (45-67%). Seed colonization ranged from 40-56% by *A. triticina* and from 20-49% by *A. alternata*, whereas infection was from 27-32% by *A. chlamydospora* and from 25-36% by *Pithomyces chartarum* and from 28-45% by *Ulocladium* sp. *A. tenuissima* can infect a high percentage of cereal grains (Andersen & Thrane, 1996; Gannibal, 2007; Kosiak *et al*., 2004) producing some toxins dangerous for plant, animals and human health (Andersen *et al*., 2002). Total nine species of *Alternaria* viz. *A. alternata, A. crassa, A. cichorii, A. chrysanthemi, A. dianthicola, A. longipes A. porri, A. tenuissima* and *A. triticina* were isolated from cereals like wheat in India (Rathod & Chavan, 2010). *Alternaria alternata* is ubiquitous and abundant especially during ripening and harvesting of cereal crops. Ripening ears of wheat are colonized by *A. alternata* soon after emergence, and it is also reported to be the most common subepidermal fungus of wheat grain (Christensen, 1958; Hyde & Gilleymore, 1951). *A. alternata* alone or with other fungi, e.g., *Alternaria triticina*, can cause a conspicuous black or brown discoloration of wheat kernels called black-point disease (Machacek & Greaney, 1938; Boyer, 1955; Bhowmik, 1969). This can result in decreased quality and yield of grain (Dickinson, 1981; Dash & Narain, 1989; Chalkley, 2012).

Some members of *A. infectoria* complex and *A. triticina* were previously isolated from wheat in Argentina (Perelló, 2010a, b; Perelló *et al*., 1996, 2006, 2008; Perelló & Sisterna, 2008) suggesting other pathogenic species different from *A. alternata*, are present with high prevalence in the Argentinian agroecological wheat area associated to this crop. Our results in this study agree with other reports on the vast majority of *Alternaria* strains

either *A. infectoria* or *A. tenuissima* (Dugan & Lupien, 2002) and rarely *A. alternata* as a dominant species on black pointed kernels (Özer, 2005). Discrimination between species of *Alternaria*, especially *A. alternata* and *A. tenuissima* on the one hand and *A. infectoria* on other, is very important for assessing the potential for contamination by mycotoxins. *A. infectoria* has diminished capacity for mycotoxin production compared to members of the *A. alternata* group (Andersen & Thrane, 1996). The distinction between *A. alternata* and *A. tenuisima* was made here primarily on the basis of sporulation patterns on V8 agar as described by Simmons (Simmons, 1995). This is the first report of *A. chlamydospora*, *Pithomyces chartarum* and *Ulocladium* sp. from wheat seeds in Argentina, only occasionally isolated in our studies. *Ulocladium* sp. was previously associated with the mycoflora of wheat (Dugan & Lupien, 2002) and barley (Richardson, 1983). Species in the genera posses characteristics of conidia that are morphologically reminiscent of both phragmosporic *Helminthosporium* species and dictyosporic *Alternaria* species. *Alternaria*, together with *Ulocladium* and *Stemphylium*, comprise a large group of closely related phaeodictyosporic Hyphomyetes whose members are plant pathogens (predominantly) or saprobes. Recent phylogenetic analyses, revealed that typical members of these genera comprised a monophyletic group with *Stemphylium* as the sister group of a large monophyletic blade of *Alternaria* and *Ulocladium* spp., (Pryor & Gilbertson, 2000).

Germination test: Significant differences were observed for seedling emergence of wheat as affected by inoculation of different seed-borne fungal organisms (Table 1). The controls without inoculations showed normal seedlings (100% germination) and had all the essential plant structures necessary for the plant to continue to grow normally under favorable conditions (Anon., 1996a; Anon., 1996b). The percent of seedling emergence was significantly affected by the inoculation of the seed-borne pathogens tested.

The reduction of germination (%) ranged between 0-92% according to the strain and cultivar tested. All the isolated tested (except Ai1010, Atm. 108, Aa112 and Atm82) reduced seeds germination and caused symptoms typical of seed-borne pathogens like necrosis of grain, root necrosis, shorter black roots and weakening of seedlings with less development. Inoculation of isolates Aa2, Ate1006, Ate44, Atm. 98 and U1122 exhibit the highest reduction of seedling emergence (91,67%) in both cultivars when compared with the rest of tested isolated (Table 1).

Seedling emergence and mortality and evaluation of seed-to-seedling transmission: results of transmission of the seed-borne fungi of wheat from seed to germinating seeds and seedlings are presented in Table 2. In both cultivars, K. Capricornio and B. Guapo, seed borne pathogens of the *Alternaria* complex were found to be seed transmitted from infected seed to seedlings with relatively high efficiency. Total values of seed transmission of 7, 14% and 28, 57% to coleoptile and plumule of cultivar B. Guapo were recorded. Higher values were found analyzing cultivar K. Capricornio (16, 66% and 54, 76% for coleoptile and plumule, respectively). In particular, isolates Ate1088 and Aa1021, presented the highest % of transmission. Most isolated tested shown the ability to establish on emerging seedlings and demonstrated to cause disease, affecting the seed germination at different rates, and pre-emergence and post emergence symptoms. Initial symptoms of common root rot in either two wheat cultivars originate on young seedlings from inoculum carried on the seeds or from infections originating from conidia near the seedling. Partially emerged seedlings were found rotted and blackened. Symptoms in seedlings were wilting, blackening of hypocotyls and brownish discolouration in root. Dark brown lesions with chlorosis appear on the coleoptile tissue and / or on the leaf base (Fig. 1). Sometimes roots of infected seedlings collapsed and finally died. Microscopic examination of infected seedlings provided information on the probable path followed by the fungus during seed-to-seedling transmission. *Alternaria* spp. infected externally the coleoptile as it emerged during germination by hyphal growth from the pericarp. Superficial mycelium was observed in several places along the first leaf blade, even in areas wheare no lesions were present, as well as along the edge of the leaf tip and on the leaf sheath. The first leaf apparently became infected by contact with hyphae from an infected coleoptile as the leaf pushed through its tip. No mycelium was observed on the blade or sheet of the second leaf. In most cases, the seedlings will survived but growth of the developing plant may be stunted. In an average, the reduction on seeds germination percentage was greater on K. Capricornio tan B. Guapo. By other hand, the rate of infection of the kernel rot pathogens from inoculated seed to germinating seeds causing pre emergence death or seed rot were always higher than that of transmission to seedling infection or seedling mortality (Table 2). The highest percentages of pre or post emergence death or seed rot (91.67%) and seedling infection (41, 7%), were recorded as same in both cultivars tested, depending on the isolate inoculated. Moreover, the asymptomatic transmission to coleoptile and plumule in cvs K. Capricornio was 45, 23% and 64, 28% respectively. In B. Guapo cultivar the percentages were 54.76 and 52.35% for coleoptile and plumule respectively. From these estudies, *A. alternata, A. tenuissima, A. infectoria, A. triticimaculans, A. triticina, A. chalmydospora, Pithomyces* sp. *and Ulocladium* sp. are seed-borne and seed transmission pathogens on wheat seeds. Twenty six of the 42 fungi tested (61, 90%) were found responsible for symptomatic transmission from seed to germinating seeds and seedlings and may provide initial inoculums in the field through root soils of the seedlings. On the other hand, 88.09% of the isolates tested showed asymptomatic transmission to any of both cultivars, K. Capricornio or B. Guapo. As was demonstrated from our results, *Alternaria* spp. complex as seed borne pathogens of wheat not only reduced the germination but also affected seedling vigor resulting in low yield. All these fungi under favorable environmental conditions can infect the growing plants and can also serve as source of inoculum for field crop in Argentina (Table 3).

Table 2. Symptomatic transmission of 40 isolates from *Alternaria* complex, *Pithomyces chartarum* and *Ulocladium* sp. from seed to seedlings wheat of cultivars Klein Capricornio and Buck Guapo after 14 days of inoculation.

Sample	Klein Capricornio		Buck Guapo	
	Coleoptile (%)	Plumule (%)	Coleoptile (%)	Plumule (%)
Aa1021	0,0	41,7	8,33	8,33
Aa2	0,0	16,7	8,33	0,00
Aa444	0,0	16,7	0,00	0,00
Aa17	0,0	16,7	0,00	8,33
Aa112	0,0	0,0	0,0	0,0
AaB	16,7	16,7	0,00	33,33
Aa92	0,0	8,3	0,00	8,33
Aa400	0,0	16,7	0,00	0,00
Ate281	0,0	0,0	0,00	0,00
Ate1006	0,0	8,3	0,00	0,00
Ate205	0,0	8,3	0,00	0,00
Ate6	0,0	0,0	0,0	0,0
Ate220	0,0	0,0	0,0	0,0
Ate312	0,0	8,3	0,0	16,67
Ate37	0,0	0,0	0,0	8,33
Ate97	16,7	25,0	0,0	16,67
Ate1025	16,7	0,0	0,0	0,0
Ate44	0,0	25,0	0,0	0,0
AteB-888	0,0	25,0	0,00	8,33
Ate94	0,0	0,0	0,00	0,0
Ate1088	41,7	16,7	0,0	0,0
Ate1044	0,0	0,0	0,00	0,00
Ai80	8,3	16,7	0,00	0,00
Ai1008	0,0	0,0	0,0	0,0
Ai1010	0,0	0,0	0,0	0,0
Ai700	0,0	8,3	0,00	25,00
Atm333	0,0	16,7	0,00	0,0
Atm108	0,0	0,0	0,00	0,00
Atm98	0,0	0,0	0,00	0,00
Atm82	0,0	0,0	0,0	0,00
AtD	25,0	16,7	0,0	8,33
AtA	0,0	0,0	0,0	0,0
At93	0,0	0,0	16,67	0,0
At355	0,0	0,0	0,0	0,0
At5	0,0	8,3	0,0	8,3
Ach36	0,0	0,0	0,0	0,0
Ach822	0,0	0,0	0,0	0,0
Ach328	0,0	16,7	0,0	8,33
Pch16	0,0	16,7	0,0	0,0
Pch1009	0,0	16,7	0,0	0,0
U1122	8,33	16,7	0,0	0,0
U70	0,0	0,0	0,0	0,0

Table 3. Asymptomatic transmission of 40 isolates from *Alternaria* complex, *Pithomyces chartarum* and *Ulocladium* sp. from seed to seedlings wheat of cultivars Klein Capricornio and Buck Guapo after 14 days of inoculation.

Sample number	Klein Capricornio		Buck Guapo	
	Coleoptile (%)	Plumule (%)	Coleoptile (%)	Plumule (%)
Aa1021	8,3	16,7	0,0	18,2
Aa2	8,3	66,7	8,33	0,00
Aa444	0,0	0,0	8,3	0,00
Aa17	18,2	18,2	12,5	25,0
Aa112	0,0	0,0	0,0	0,0
AaB	8,3	8,3	16,7	0,0
Aa92	0,0	0,0	0,00	16,7
Aa400	8,3	9,1	16,7	0,00
Ate281	0,0	0,0	0,00	0,00
Ate1006	0,0	27,3	0,00	0,00
Ate205	0,0	0,0	14,3	8,3
Ate6	0,0	18,2	11,1	0,0
Ate220	0,0	30,0	9,1	9,1
Ate312	8,3	0,0	8,3	33,3
Ate37	20,0	40,0	0,0	33,3
Ate97	25,0	25,0	16,7	14,3
Ate1025	0,0	0,0	14,3	8,3
Ate44	8,3	16,7	0,0	0,0
AteB-888	14,3	28,6	0,00	8,33
Ate94	45,5	45,5	22,2	9,1
Ate1088	8,3	0,0	8,3	11,1
Ate1044	0,0	0,0	0,00	0,00
Ai80	33,3	54,5	44,4	44,4
Ai1008	8,3	0,0	8,3	11,1
Ai1010	0,0	18,2	8,3	28,6
Ai700	0,0	8,3	11,1	10,00
Atm333	0,0	27,3	8,3	0,0
Atm108	0,0	0,0	0,00	0,00
Atm98	0,0	100,0	8,3	50,00
Atm82	16,7	25,0	0,0	0,00
AtD	0	16,7	0,0	25,0
AtA	0,0	18,2	0,0	0,0
At93	0,0	0,0	0,0	0,0
At355	0,0	0,0	8,3	0,0
At5	20,0	57,1	0,0	8,3
Ach36	16,7	40,0	33,3	14,3
Ach822	0,0	0,0	16,7	40,0
Ach328	8,3	0,0	0,0	0,0
Pch16	0,0	16,7	0,0	0,0
Pch1009	0,0	18,2	8,3	11,1
U1122	0,0	16,7	0,0	0,0
U70	8,3	27,3	0,0	0,0

Fig. 1a & b. Range of symptoms on wheat seedlings (cv B. Poncho) grown from *Alternaria*-infected seed, including necrotic lesions on the upper and lower coleoptile, a small foliar lesion with chlorosis and coleoptile distortion and craking, general stunting.

The presence of many members of *Alternaria* complex and related genera at high levels from various geographical areas indicates a clear need for field surveys for these fungi. They are common pathogens of different plants and are particularly difficult to distinguish with conventional methods (Nasim *et al*., 2012). There is also a need to increase public awareness on the aspect related to seed health and to develop suitable management for improving the quality of seeds. Testing the seed health of major crops as wheat should be introduced as a national seed quality control system. This necessity is specially highlight taking in account the great dispersion of several members of *Alternaria* complex with their potential production of toxic metabolites (Perelló *et al*., 2012) as emerging diseases of wheat in Argentina in the latest years.

Acknowledgements

We thanks Ing. N. Ballestra for technical assistance. This study was supported in part by CONICET Project PIP 114-200801-00535 period 2009-2011.

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(Received for publication 7 February 2012)