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Tomato Varietal Response to *Alternaria solani* and *Fusarium solani* Infection

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Abstract: Some tomato cultivars were tested for their response to infection with early blight (*Alternaria solani*) and root rot (*Fusarium solani*). Castele Rock cv. proved to be the most susceptible among all the tested cultivars to both early blight and root rot diseases, while Tezier cv. was the most resistant against fungal infection. On the orher hand Riogrand cv. showed high tolerance against root rot infection. Histopathological studies confirmed these findings concerning the susceptibility of Castele Rock cv. to *F. solani*, whereas the massive colonization of host tissues by the fungal hyphae was observed. The pathogen ingresses toward the vascular stele and severe cell wall deteriorations were also noticed. In contrast, resistant Riogrand cv. showed restricted invasion by the pathogen.

Key words: Early blight · root rot · *Alternaria solani* · *Fusarium solani* · Histopathological study · plant cultivars

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.), is considered the most widely cultivated vegetable crop in Egypt. Total cultivated area 194000 Ha yielded 7.550.000 tons [1].Root rot disease caused by *Fusarium solani* and early blight caused by *Alternaria solani* are among the most destructive diseases of tomato, causing hazardous losses in yield [2-5]. Host resistance was proved to be the only sustainable disease management strategies. The introduction of resistant cultivars would be a desirable addition to other management strategies for the control of these diseases [6].Many efforts directed toward breeding resistance tomato cultivars, the lack of the single-gene resistance and the complex patterns of inheritance have resulted in the availability of no commercial tomato cultivar that possesses adequate levels of resistance to *A. solani* [7]. Recently, two new sources of resistance were identified in the lines IHR1939 (*L. pimpinellifolium* L4394) and IHR1816 (*L. esculentum* NCEBR-I); the genetic nature of these sources has not been reported by Thirthamallapa and Lohithaswa [8].

Fusarium diseases constitute most of the loss in tomato production worldwide, because it spread on all geographic areas. In cultivar developing, molecular marker assisted techniques replaced traditional breeding techniques which are high cost and time consuming for breeders [9].

Therefore, this study was carried out to: (1) evaluate the efficacy of some methods enhancing the sporulation induction by *A. solani*. (2) Determine the varietal response of some tomato cultivars to *A. solani* and *F. solani*, early blight and root rot agents. (3) Carry out comparative histopathological study between tomato cultivars susceptible and resistant to root rot disease caused by *F. solani*.

MATERIALS AND METHODS

Plant Cultivars: Tomato cultivars namely; Roma, Castle Rock, Ac55VF, Super marmand, Super strain B, Peto86, (obtained from Bakus and El-Hadara commercial seed markets) and Teizer and Riogrand cultivars (obtained from Libyan commercial seed markets).

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Planting under Greenhouse Conditions

A. *Alternaria solani* Experiment: Tomato seeds from Six cultivars namely; Castle Rock, Ac55VF, Super marmand, Peto86, Super strain B, Tezeir, were surface sterilized with 2% sodium hypochlorite solution for 2 min. then rinsed in sterile distilled water and dried between folds of sterilized filter paper and then sowed in foam trays contained sterilized peat: sand: clay, 1:1:1 for three weeks. Nursery was irrigated when needed. Pots 25 cm diameter were sterilized by sub-emerging in 7% formaldehyde solution for a few hours and left to aeration. Sterilized pots were filled with the autoclaved soil mixture of 1:2:1 sand: clay: peatmoos. After that the seedlings were transferred pots and planted at the rate of 5 seedlings per pot and which were placed in the greenhouse at 12:12 hours light: dark cycle, with 24-26 °C: 16-18°C day: night and about 65% relative humidity.

B. *Fusarium solani* Experiment: Tomato seeds from five cultivars namely; Riogrand, Ac55VF, Roma, Castle Rock, Super strain B, were surface sterilized using NaOCl, rinsed in sterile distilled water, dried and then sowed in the inoculated and uninoculated pots. Cultivated pots were placed in the greenhouse under the same above mentioned conditions.

Fungal Isolates

Two *F. Solani* Isolates Were Applied: isolate (1) from Assiut University and isolate (2) from El-Sabhia Research Station. Two *A. solani* pathogenic isolates were applied: isolate (1) isolated from fresh tomato fruits obtained from El-Behira governorate and isolate (2) from Mycological Center, Assiut University.

Activation of *A. solani* Sporulation: The purified fungal isolates were maintained on PDA slants and used when needed. It was difficult throughout this investigation to obtain *A. solani* conidia enough for inoculation experiments. Therefore, an experiment was designed to test many environmental stresses for sporulation induction, including growing on different growth media, addition of some chemicals to the media, exposure to different dark-light growth periods, exposure to UV light for different exposure time periods and scratching of the growing mycelium. More than one of these stresses may be included once in the same treatment. Therefore, experimental treatments include:

- Growth on PDA and V-8 juice agar medium. After the mycelium growth covered 1/2 to 3/4 of petri dish bottom it was exposed to ultraviolet light 1,1.5,2,2.5,3,4,5,6,7,8 min intervals.
- Growth on PDA medium. Ten days later, the mycelium was scratched and exposed for 12 hours to fluorescent light for 3 days [10].
- Cultivation. The growing mycelium was then scratched and exposed sunlight and aeration [11].
- Growth on PDA media enriched with thiamin.
- Growth on PDA mixed with β-aminobutyric acid (BABA).
- Growth on Dextrose Agar (DA) free from potato cooking liquid [12].
- Growth on S-medium [13].

Inoculation of Host Plant: Inoculum of *F. solani* was prepared according to the technique described by Baraka *et al.* [14]. *F. solani* isolates were grown for 3 weeks at 28°C on sterilized barley grain medium. Soil infestation was carried out, using isolate No.1, which proved throughout the experiment to be more active and gave dense growth on the medium, compared with isolate No.2, at the rate of 3% of soil (w/w) before planting. Pots served as control were filled with the soil mixed with the same amount of sterilized barley grain medium without inoculation. Sets of five pots each, with ten seeds were used for each tested cultivar and fungal isolate used.

Inoculation with *A. solani* was carried out on 45 days age tomato plants using a mixture of spores from the isolates 1, 2 at the rate of 5 pots for each cultivar, containing 5 plants each and five pots were leaved as control. To harvest the spores, 10-day old cultures were brushed gently to separate the spores on mycelium surface and then rinsed with a 0.01% Tween 20 solution. The resulting spore suspension was quantified using a haemocytometer to 10^4 spores/ ml. Plant leaves were dusting with Carborundum crystals and inoculated by spraying the spore suspension until run-off to ensure good spore germination, the plants were covered with transparent plastic bags for 24 hours to increase the relative humidity.

Assessment of Disease Severity: *F. solani*: The tomato plants were daily irrigated and kept under observation to determine the number of pre- and post-emergence root rot and survival seedlings, check treatment also. Percentage of pre, post-emergence root rot and plant survival were

calculated; 15, 20, 30 days after sowing, respectively. While severity of root rot was determined after 40 days according to Datnoff *et al.* [15] using a rating scale of 0 to 3, on the basis of the area of infected lesions of root, where 0= no damage or lesions, 1= small lesions on tap root or secondary roots, less than 25% of the root and hypocotyls tissues covered with lesions, 2= 26-50% lesions areas, moderate discoloration of crown or root tap and 3= 51-100% lesions area, severe damage and death of seedlings. The percentage of disease severity was calculated according to Trabulsi *et al.*, [16] as follows:

$$DS\ \% = \sum (n \times r) \times 100/ 3N$$

Where:
n = number of seedlings of a given disease rating,
r = disease severity rating
N = total number of seedlings rated.

***A. solani*:** The disease incidence and severity were tested on the tomato cultivars as mentioned. Two weeks after inoculation disease incidence was estimated according to El-Farnawany [4] using the following ratings: 0 = free of infection, 1= trace -25% leaf area spoted, 2= 26-50%, 3= 51-75%, 4= 76-100% leaf areas killed. Disease severity was calculated according to the following equation [16]: $\%DS= \sum (n \times r) \times 100/ 4N$. Where 4= higher rating value.

Histopathological Studies

Light Microscope: The technique of slide section preparation followed the method described by Shama[17] and Waked [18].Preparations of the slide sections of tomato roots, inoculated with *F.solani*, were carried out in the electron microscope unit at Faculty of science, Alexandria University, Shatby. The stained slides were, then, mounted in Canada balsam, microscopically examined and photomicrographed using digital camera at Plant Pathology Laboratory, Faculty of Agriculture, Saba Basha.

Transmission Electron Microscopy (TEM): Samples of tomato roots, inoculated with *F.solani*, were cut into suitable parts, fixed in glutaldehide solution for two days and then washed in Koradelt buffer solution followed by washing in sodium sulphate buffer for 30 minutes. Pieces were fixed in osmium tetroxide then dehydrated in ascending grades of ethanol followed by adding propylene and kept for 30 minutes at 4°C. The pieces were poured on the plate and incubated at 20°C for 4 hours and followed by ultramicrotome with dimond knife to have section with 1 mm thickness.

Stain gold and silver sections with 2% uranyl acetate acetone (1:1) for 10 minutes, then stained with Reynold's lead citrate for 20 minutes, then washing with double distilled water for several times and drying on filter paper [19]. Finally, the ultra sections were then examined with Electron microscope (JEO-100CX), in the Electron Microscope Center, Faculty of Science, Alexandria University.

Statistical Analysis: All data were subjected to ANOVA and the means were tested for significant differences using the Least Significant Difference LSD test [20].

RESULTS

Induction of Sporulation in *Alternaria Solani*: Seven treatments were tested to induce sporulation by *Alternaria solani* mycelia sown *In vitro* on axenic media. According to results, profuse sporulation was obtained only on PDA medium enriched by thiamine, or by scratched mycelia sown on PDA medium and exposed to fluorescent light. Moreover the other tested treatments failed to induce any sporulation, however, scarce sporulation was obtained on PDA-BABA media. The description of the cultivation methods and its results were shown in Table 1.

Pathogenicity Tests

A-Root Rot: *Fusarium solani* root rot incidence at pre- and post-emergence stages as well as disease severity were determined using five tomato cultivars namely; Super strain B, Roma, Ac55VF, Riogrand and Castle rock. Data presented in Table 2 revealed that inoculation of the tested cultivars with *F. solani* resulted in the incidence of pre- and post-emergence root rot symptoms; however tomato cultivars significantly differed according to their response. Highest pre-emergence root rot values were obtained by Castle Rock, Ac55VF and Roma cvs as 80, 76 and 74%, respectively. On the contrary, Riogrand expressed the least pre-emergence root rot value which recorded as 54% (Fig. 1 a, b). The recorded root rot infection at post-emergence growth stage among the tested cultivars showed insignificant differences, ranging from 8 to 16%. However,

Table 1: *In vitro* production of *A. solani* spores in response to different growth techniques

Cultivation technique	Spore production
PDA & V-8 juice agar + exposure to UV light	-
PDA + mycelium scratching + fluorescent light	Profuse
PDA + mycelium scratching + sunlight and aeration	-
Mixture of PDA + 10 ml thiamin	Profuse
Mixture of PDA + 10 ml β-ABA	Scarce
DA medium without potato liquid	-
S- medium	-

Profuse Profuse sporulation
- No sporulation
Scarce Rarely sporulation

Table 2: Pathological response of some tomato cultivars to inoculation with *F. solani,* root rot agent under greenhouse conditions

	% Root rot			
Cultivar	Pre-emergence	Post-emergence	% Plant survival	% Disease severity
Riogrand	54^{b}	12^{ab}	34^{b}	7.55^{b}
Ac 55 VF	76^{a}	14^{ab}	10^{a}	30.66^{a}
Roma	74^{a}	8^{a}	18^{a}	13.77^{b}
Castle Rock	80^{a}	12^{ab}	8^{a}	28.4^{a}
Super strain B	68^{a}	16^{b}	16^{a}	30.21^{a}
L.S.D. α=0.05	8.2	6.5	13.2	10.08

Values followed by the same letter(s) in each column don't differ significantly ($P \leq 0.05$)

Table 3: Disease incidence and severity of early blight of some tested tomato cultivars under greenhouse conditions

	Disease incidence		
Cultivar	%	Mean	% Disease severity
Castele rock	72^{a}	3.60^{a}	73.80^{a}
Ac 55 VF	68^{a}	3.40^{ba}	43.70^{b}
Super marmand	48^{b}	2.40^{ba}	49.75^{ab}
Peto 86	40^{b}	2.00^{bc}	27.09^{b}
Super strain B	44^{b}	2.20^{abc}	34.77^{b}
Tezier	16^{c}	0.80^{c}	26.70^{b}
LSD α= 0.05	9.02	1.45	24.82

Values followed by the same letter(s) in each column don't differ significantly ($P \leq 0.05$)

post-emergence root rot in Roma cultivar (8%) was less than that of Super Strain B (16%), in significant order which indicating that Roma cv. was more resistant than Super Strain B. Meanwhile the highest plant survival value was attained in Riogrand cv. (34%) compared with the other tested cultivars significantly. Also data in Table 2 showed that disease severity values in Riogrand and Roma cvs 7.55 and 13.77% respectively were significantly lower than those of the other tested cultivars.

B-Early Blight: Inoculation of the six tested tomato cultivars was carried out using the tested isolate of *A. solani.* Disease incidence and disease severity were determined and data was presented in Table 3. According to the obtained results, it was evident that all the inoculated tested cultivars showed early blight disease symptoms; however, they varied in disease incidence and severity. The highest disease incidence records for both Castle Rock and Ac55VF cultivars were 72 and 68%, whereas the least value was attained with Tezier cv. as 16% (Fig. 2). The rest of the tested cultivars were ranged between 40-48 %.Disease severity of Castle Rock attains significantly the highest values compared with the other tested cultivars. Moreover, differences in disease severity among the other tested cultivars were insignificant (Fig. 3).

Assessment of disease severity showed the same response of all tested cultivars as occurred in their disease incidence. Tezier exhibited lower disease severity compared with the other cultivar, but it was significantly with Castle rock only (Fig. 2). Other cultivar were varied in their disease severity, where Castele rock has the maximum value; 73.80 (Fig. 3).The results of early blight caused by *A. solani* showed that Castele rock was the most susceptible cultivar due to its high values obtained from disease incidence and severity; 72, 73.8% respectively, while Tezier was the most tolerant cultivar which has lower values in disease incidence and severity as 16. 26.7%, respectively.

Histopathological Observations: Light and Electron microscope observations of cross section of the relatively susceptible Castle Rock tomato cultivar, showed that fungal hyphae invaded cells both inter and intracellularly (Fig. 4 B), leading to complete colonization, discoloration and destruction of epidermal and outer layers of cortical cells (Fig. 4 C). The fungus may extend deeper to colonize inner layers of cortex (Fig. 5 A, B). Invasion of vascular tissues and pith was also observed (Fig. 4 E).In relatively resistant Riogrand cv. hyphae colonization of cells was limited and invasion was confined to limited areas of both epidermal and cortical cells. Partial discoloration and destruction of cortical cells were also observed. In most cases, cells maintained their normal shape (Fig. 5 C, D).

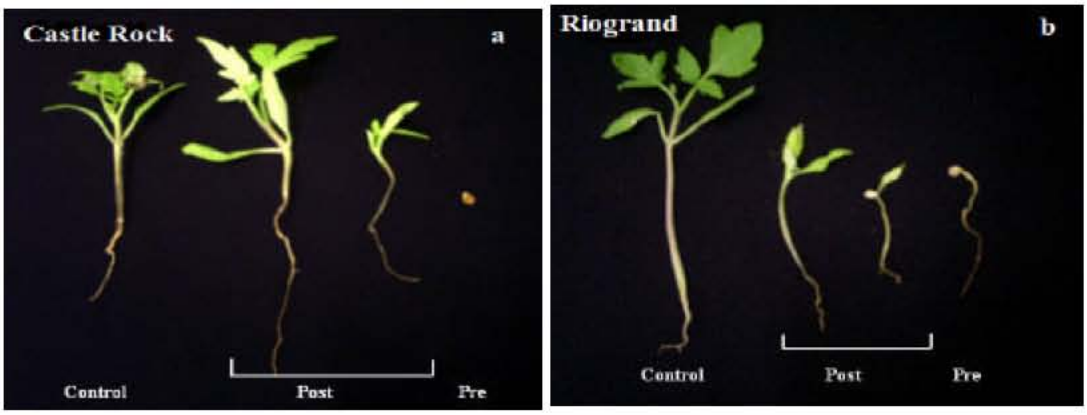

Fig. 1: Susceptible (Castle Rock) and resistant (Riogrand) cvs to *F. solani*, pre- and post-emergence damping-off agent

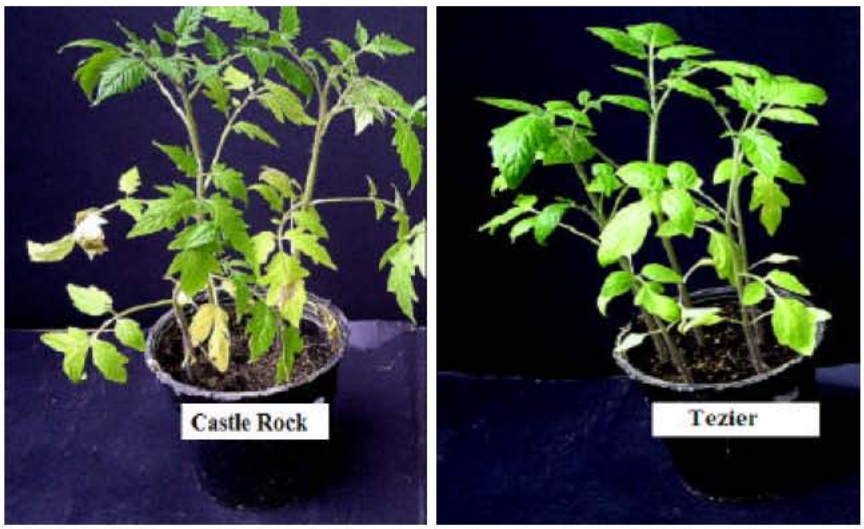

Fig. 2: Susceptible (Castle Rock) and resistant (Tezier) cvs to A. solani, early blight agent

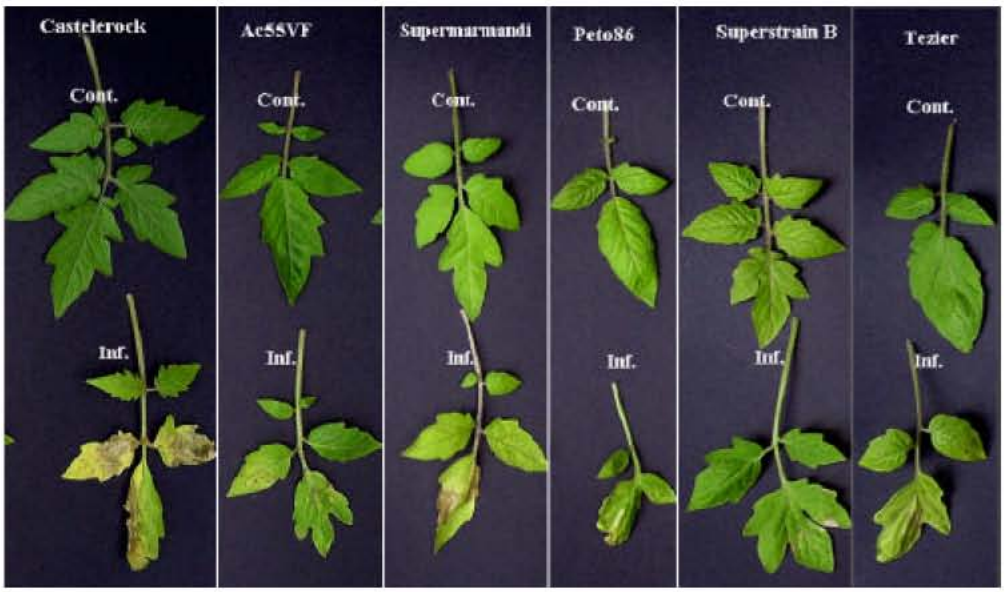

Fig. 3: Tomato varaietal response to A. solani on six cultivars

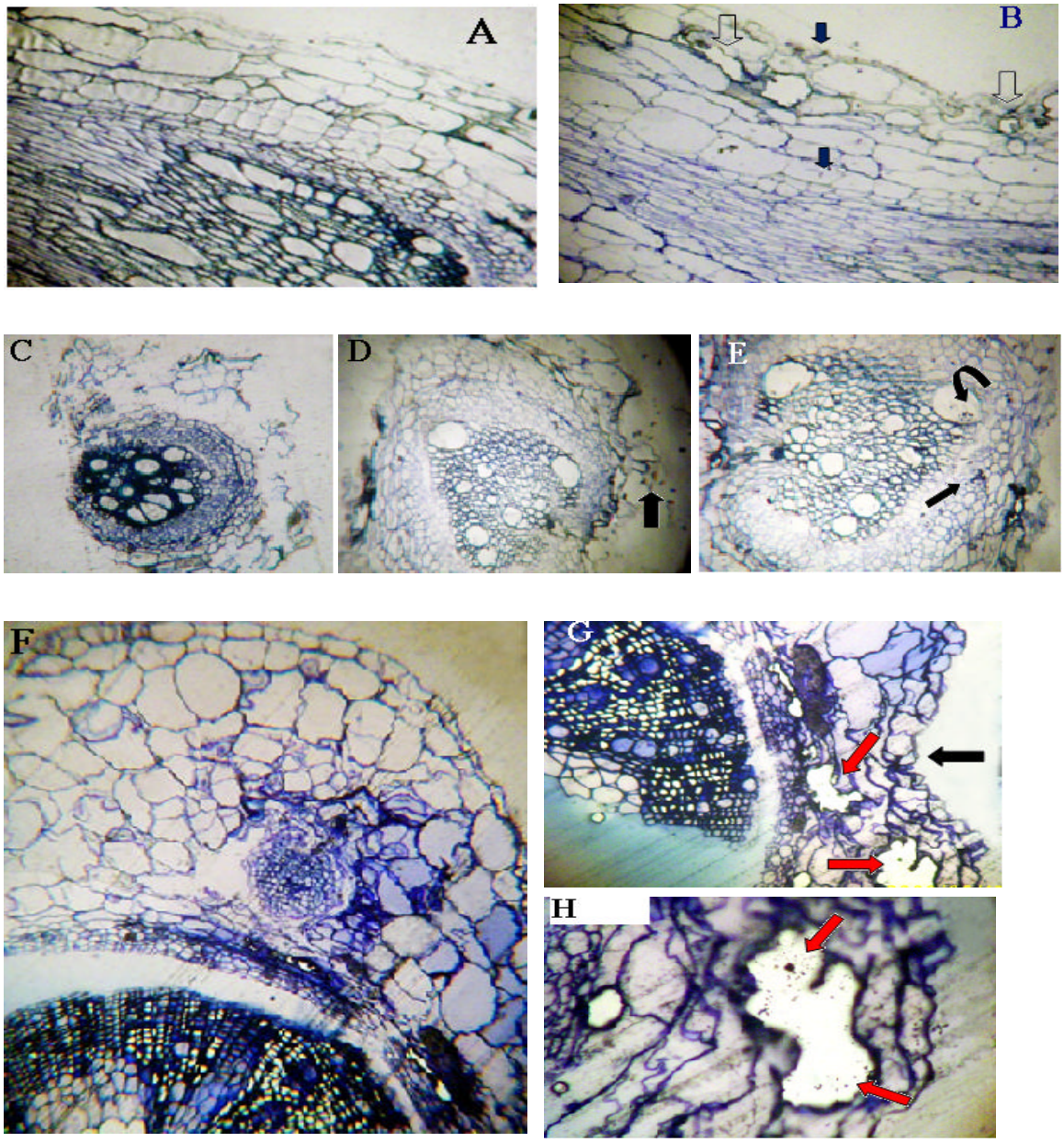

Fig. 4: Light micrograph of samples from tomato roots 6 d after inoculation with *F. solani*. A, healthy susceptible variety "Castele rock" (control). B to D, Fungal invasion of epidermal and cortical cells. E, fungus invasion was also extended to the pith and vascular tissue fungus (dark arrows), destructive cells (hyaline arrows). F, healthy resistant variety "Riogrand" (control). G, destruction was restricted to the epidermis and cortex (arrows). H, fungal invasion of some cortical cells (arrows).

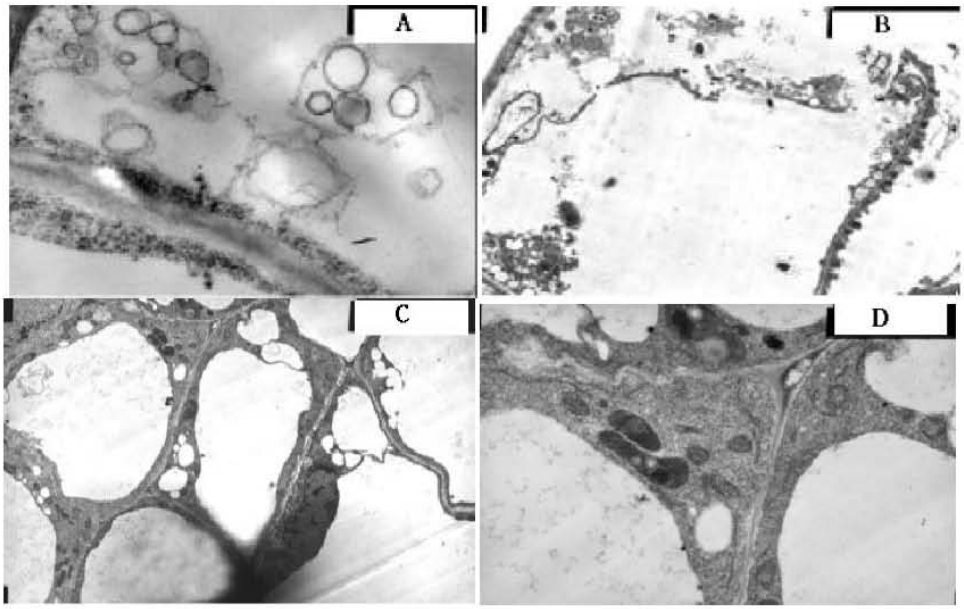

Fig. 5: Transmission electron micrographs of susceptible variety "Castele rock" (A and B) and resistant variety "Riogrand" (C and D) tomato root tissues collected 6 d after challenge with F. solani. A, showed fungus hyphae colonize the root tissue rapidly; cell invasion occurs through direct host cell wall penetration. B, The invasion causes extensive cell damage and host cell wall alterations. C and D, in roots from resistant showed clear cells free from fungus invasion, the cells kept its typical shape and normal organelles.

DISCUSSION

Among various methods used in our study, addition of chemical substance (thiamin) and scratching the mycelium followed by exposure to fluorescent light were the only two methods proved to be effective in induction of sporulation of the tested *A. solani* isolates. Similar results were obtained by Kumar *et al.* [21], using V-8 juice agar medium. Moreover, many reports concerning spore induction of *A. solani* were also reported. Commonly used methods for sporulation induction include exposure to sunlight or ultraviolet light [10,22], mycelia scratching [23], fluorescent light [10], duration and different colors of light[11] and medium dehydration or the use of chemical additives [22]. The applied method of sporulation induction in *A. solani* culture was found to be varied according to the fungal isolate [24, 25].

In *F. solani* root rot trial, pathogenicity tests showed that all the tested cultivars exhibited root rot symptoms. However, cultivars significantly differed in their response to infection. According to pre-emergence damping-off, plant survival and disease severity determinations, Castle Rock was relatively the most susceptible, whereas Riogrand was relatively the most resistant. Labate *et al.* [26] tested 198 tomato cultivars and found tremendous variations in root rot incidence among cultivars. He concluded that their variations were mainly of genetically origin. On the other hand, all the tested cultivars, inoculated with *A. solani* isolates, showed different percentage of disease incidence percentage, ranging between 16-72%. Our data revealed that, Castle Rock cv. was also relatively the most susceptible among the other six tested cultivars, whereas Tezier cv. was again the most tolerant. However, Castle Rock cv. according to El-Farnawany [4] proved to be less susceptible than the other tested Strain-B and Super Strain cultivars, based on stomatal index and wax content determinations.

According to the available literature, limited success has been attained in incorporating resistance to early blight disease [27]. However, recent investigations succsseded in developing new inbreeding lines of early blight resistance [28]. Host-pathogen compatibility appears to be highly dependent on genetic features [29].

Light and transmission electron microscope observations on tomato roots inoculated with *F. solani*, root rot agent showed that there were a great difference between Castle Rock susceptible tomato cv. and the resistant cv., Riogrand in both, fungal behavior or defense-host structures. Restricted epidermal and cortical invasion in resistant cv. may be due to rapid recognition

of the pathogen [30] and the inability of the pathogen to grow or multiply and spread inside the resistant host [31]. Cell wall strength may be of great significance in host resistance [32]. On the other hand Southerton and Devarall [33] considered that resistance may due partly to accumulation of phenolic compounds in resistant cultivar. In contrast to that, inoculated Castle Rock root section showed rapid invasion and colonization of epidermal, cortical, which may be extended to vascular and pith tissues, this data was in agreement with that obtained by Rodriguez-Molina *et al.* [34]. These observations may be attributing to slow recognition of the pathogen, lack of host defense structures, or failure to produce active compounds responsible of hindering pathogen parasitic activities. These exploitations were mostly confirmed by Deese and Stahmann [35] and Kobayashi *et al.* [36].

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