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HISTOPATHOLOGICAL STUDIES ON GRAIN SORGHUM PLANTS INOCULATED BY ARBUSCULAR MYCORRHIZAL FUNGI (AMF) TO CONTROL ACREMONIUM WILT DISEASE

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ABSTRACT

Anatomical structure variation in roots and stems of grain sorghum (*Sorghum bicolor* L. Moench) plants, cultivated in pot experiment during the two successive growing summer seasons of 2014 and 2015 as affected by cultivar susceptibility to acremonium wilt disease, *Acremonium strictum* infection and arbuscular mycorrhizal fungi (AMF) root colonization as biocontrol agent were investigated. Root of resistant cultivar (Dorado) has more epidermis and exodermis thickness, diameter of root and pith as well as average number of xylem arms/ vascular cylinder but less cortex thickness and metaxylem vessel diameter in comparison with those of the susceptible one (Giza 54). Infection of susceptible cultivar plants by *A. strictum* markedly reduced all tested anatomical measurements of roots and stems compared to uninfected one. Hyphae and spores of *A. strictum* were observed into metaxylem vessels. Marked disintegration and loosening of some cortex cells, pith and metaxylem vessels. The same trend was observed in ground tissue and vascular bundles of stem. Colonization of roots by AMF considerably increased all tested roots and stems anatomical measurements, except metaxylem vessel diameter compared to untreated control. These results may explain the role of AMF root colonization in inducing defense responses grain sorghum as mycorrhizal fungi plant. Inoculation of grain sorghum cv. (Giza 54) by AMF markedly enhanced plant growth expressed as dry weight of roots and shoot as well as soil rhizosphere dehydrogenase activity whereas, disease incidence was reduced by 65% compared to uninoculated plants.

Key words: Grain sorghum, histopathology, *Acremonium strictum*, arbuscular mycorrhizal fungi.

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Moench) is one of the most important Summer cereal crops in Egypt and allover the world, it ranks the fifth position among all cereal grains in extent of production after wheat, rice, maize and barley. It is mainly cultivated in Middle and Upper Egypt, with a total cultivated area of 352, 068 faddan, yielding 870, 946 ton with an average yield of 2.74 ton/faddan according to the Ministry of Agric. (Anonymous, 2015).

Acremonium wilt disease, caused by *Acremonium strictum* is one of the most important diseases that cause considerable losses in yield and drastically affected grain quality of sorghum (Ali *et al.*, 2005). Grain sorghum is considered the most important food and feed in developing countries, does not permit the use of costly inputs like chemical pesticides, host plant resistance and biological control strategy with good agricultural practices are the two best alternatives for disease management that help in keeping the environment free from pollution and health hazards due to chemicals (Kalappanavar and Hiremath, 1998).

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Arbuscular mycorrhizal fungi (AMF) play a key role in natural ecosystems and influence plant nutrition, resistance and productivity (Demir and Akkopru, 2007). It is credited with having launched plant growth promotion and biological control of soil-borne plant diseases. Host responses to AMF root colonization may involve complex physiological activities perhaps ultimately expressed in host cytological and anatomical alterations. Thus, there is a need for additional anatomical investigations for understanding more about those mechanisms which ultimately improved growth and resistance of mycorrhizal plants (Veerabhadraswamy and Garampalli, 2011and Pylro *et al*., 2013).

The foremost aim of the present study was to investigate the anatomical structure variations in roots and stems of grain sorghum plants as affected by grain sorghum cultivar, *A. strictum* infection and AMF inoculation as biocontrol agent compared to fungicide control of acremonium wilt disease.

MATERIALS AND METHODS

Sorghum Grains

Cultivars of grain sorghum (*Sorghum bicolor* L. Moench), Dorado (resistant) and Giza 54 (susceptible), were selected according to their response against acremonium wilt disease reported by El-Shafey *et al*. (1999) kindly supplied by Sorghum Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt. Initially germination rate was determined to be at least 92%.

Acremonium strictum Strain

An aggressive *A. strictum* strain was kindly provided by Maize, Sugar and Foliage Crops Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt.

Arbuscular Mycorrhizal Fungi (AMF)

AMF inoculum including carrier soil and root debris (200 spores/g) was kindly provided by Agric. Microbial. Dept., Soils, Water and Environment Res. Inst., ARC, Giza, Egypt.

Fungicide

Vitavax 200 (75% WP) consists of Carboxin (3,6 dihydro-2-methyl-1,4 oxathiline 3-carboxanilide) 37.5% WP and Thiram [tetramethyl thuram disulfide, bis (dimethyl- thiocarbamayl) disulfide] 37.5% WP was obtained from local company in Cairo, Egypt.

Fungal Inoculum Preparation

Phytopathogenic fungal inoculum was prepared by growing the aggressive *A. strictum* strain for 15 days at room temperature in sterilized glass bottles (500 ml capacity), each containing 100 g of sorghum grains moistened with 50 ml distilled water and inoculated with 3 discs (1cm in diameter), cut from 7 days-old culture. The ingredients in bottles were mixed thoroughly before incubation and bottles were shaken every two days to provide fast fungal distribution and obtain homogenous growth.

Pot Experiment

Pot experiment was conducted during the two successive Summer growing seasons of 2014 and 2015 of Maize, Sugar and Foliage Crops Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt to study the potentiality of AMF in management of acremonium wilt disease of grain sorghum and investigate anatomical measurement variations in both roots and stems of grain sorghum plants as affected by grain sorghum cultivar susceptibility to acremonium wilt disease where Giza 54 (susceptible) and Dorado (resistant) cultivars without infection by *Acremonium strictum* were used, *A. strictum* infection and AMF inoculation. Soil infestation was carried out by adding 200 g of the previously prepared 15 days old cultures of the aggressive *A. strictum* strain to each clay pot (25 cm in diameter) containing 6kg sterilized sandy- loam soil. The inoculum was mixed with the surface layer of the soil 3 days before sowing. Uninfested soil was used as control. Because AMF are symbiotic endophytes and colonize mycorrhizal plant roots therefore its inoculum was added just at sowing time 5 cm below the seed bed at the rate of 50 g to each pot to ensure good AMF root colonization according to El-Sharnoby (2013). Two and half g/kg seeds as seed coating of fungicide (Vitavax 200) was used as positive control while untreated grains were used as negative control. Sorghum grains were surface sterilized using sodium hypochlorite solution (5%) for 2 min. then washed in distilled water for 2-3 times. Sterilized grains were sown on the 20th May in both seasons at the rate of eight grains per pot and thinned after seedling emergence to five

seedlings. The experiment was conducted in 4 replicates. The pots were fertilized with super phosphate (15.5% P_2O_5) at the rate of 3.0 g per pot just before sowing. Plants were fertilized twice with urea (46.5% N) and potassium sulphate (48% K_2O) at a rate of 3:2 g/pot, respectively 21 days interval. Irrigation was done using tap water when necessary.

Anatomical Study

Specimens from adventitious roots and the middle part of the second lower internode of the healthy and diseased stem of grain sorghum plants representing all studied treatments were collected after 60 days of sowing during the second season of 2015 and subjected to microtechniqe practices given by Willey (1971) at Agricultural Botany Dept. Laboratory, Fac. Agric., Zagazig Univ., Zagazig, Egypt. The plant specimens were killed and fixed at least for 24 h in FAA solution (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). After fixation they were washed and dehydrated in ascending concentration series of ethyl alcohol then transferred to absolute alcohol before being embedded in paraffin wax (melting point 52-54ºC). Transverse sections were cut using a rotary microtome (EPMA) to a thickness of 14 microns. Paraffin ribbons were mounted on slides and sections were clarified in pure xylol for 10-20 min before transferring to absolute alcohol, stained with safranin/light green. Sections were mounted in Canada balsam. Selected sections were examined microscopically and photomicrographed using light microscope (Olympus) provided with digital camera (Canon power shot S80) connected to a computer. The photographs were taken by Zoom Browser Ex program. Dimensions of roots and stems were measured by using Corel Draw program ver.11.

Estimation of Shoot and Roots Dry Weight

Plant samples representing all treatments under study were gently uprooted 60 days after sowing and washed in running water to remove adhering soil particles. The collected plant shoot and root portions were oven dried at 70°C till a constant weight and their dry weight was recorded.

Assessment of AMF Root Colonization

The method of Philips and Hayman (1970) was used for staining root segments (0.5–1.0 cm) of grain sorghum plants 60 days after sowing. Colored segments were randomly chosen and microscopically inspected and AMF root colonization rate was estimated according to Trouvelot *et al.* (1986).

Assay of Soil Rhizosphere Dehydrogenase Activity

Soil rhizosphere dehydrogenase activity of grain sorghum plants (Giza 54) was assayed 60 days after sowing according to the procedure described by El-Sharnoby (2013) using 2,3,5-triphenyl tetrazolium chloride (TTC) as substrate. The produced 2,3,5-triphenyl formazan (TPF) concentration was determined spectorphotometrically (Perkin elmer-55E) at 450 nm.

Monitoring of Disease Incidence

It was calculated 90 days after sowing by using the following formula.

$$\text{Percentage of disease incidence} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total No. of plants}} \times 100$$

RESULTS AND DISCUSSION

Symptoms of Acremonium Wilt Disease of Grain Sorghum

The first visual symptoms appear as a pale green discoloration of the basal internodes with narrow, yellowish to reddish streaks extending longitudinally on one side of the stalk. Reddish to dark brown vascular bundles could be observed through the longitudinal section (Fig. 1a). Drying up of the leaf sheath and reddish to purple stripping along the leaf veins were also of the main symptoms observed (Fig. 1b). Most infected plants had no head formation and when formed, it was small with shrunken grains (Fig. 1c). The distribution of the pathogen in different parts of grain sorghum plant indicated that *A. strictum* develops systemically in the host plant. Such systemic development has been demonstrated by Bandyopadhyay *et al.* (1987). Browning of vascular bundles and adjacent tissues due to the deposition of dark pigments in vessels, disintegrated walls and surrounding paranchyma cells may indicate oxidized phenolic compounds induced by the pathogen mycelium in the xylem vessels (Tagne *et al*., 2002). These findings are in good agreement with those reported by Ali *et al.* (2005). Similar symptoms were early described by El-Shafey *et al*. (1999).

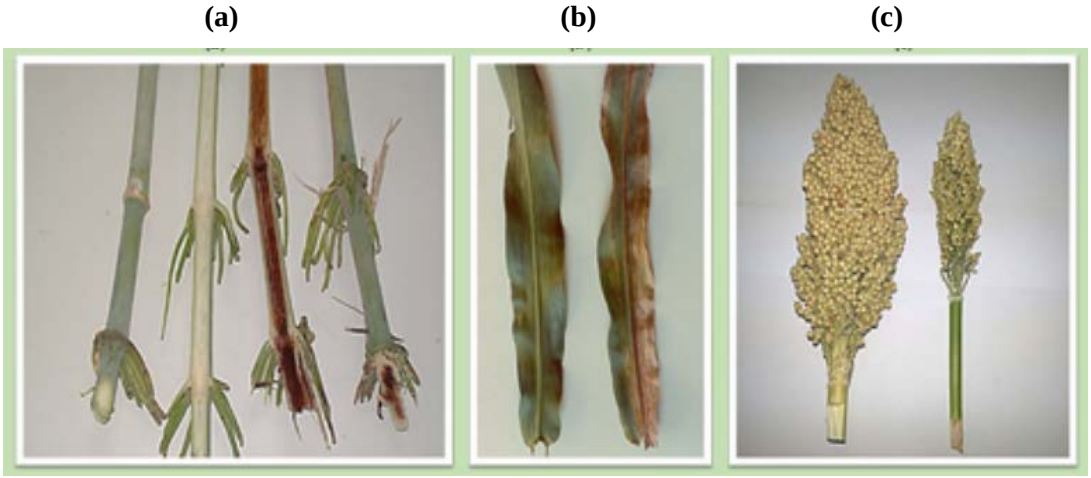

Fig. 1. Acremonium wilt disease symptoms on grain sorghum plant under field conditions where: (a) stalk basal internodes showing a pale green discoloration with narrow, yellowish to reddish streaks extending longitudinally on one side of the stalk. A longitudinal section shows reddish to dark brown vascular bundles; (b) leaf blade showing reddish to purple stripping along the leaf veins; (c) small head with shrunken grains. Healthy control on the left

Anatomical Structure Variations in Roots and Stems Transverse Sections of Grain Sorghum Plants as Affected by:

Grain sorghum cultivar susceptibility to acremonium wilt disease

Data presented in Table 1 and illustrated in Fig. 2 show that anatomical structure features of root (epidermis thickness, exodermis thickness, diameter of root and pith as well as average number of xylem arms per vascular cylinder) were larger in resistant cultivar (Dorado) which achieved 24.23, 89.31, 3150, 2291 µ and 52, respectively compared to susceptible one (Giza 54) which recorded 14.62, 39.85, 2447, 1532 µ and 36, respectively for aforementioned features. Contradictory, susceptible cultivar showed more cortex thickness and metaxylem vessel diameter (851.54 and 99.46µ, respectively) than the resistant one which registered 780.77 and 88.62µ, respectively for aforementioned features. Increments of epidermis and exodermis thickness, diameter of root and pith as well as average number of xylem arms/ vascular cylinder and decrement of cortex thickness and metaxylem vessel diameter in resistant cultivar explain the cultivar resistance to *A. strictum* infection. Anatomical structure features of stem (stem diameter, epidermis thickness, number of big and small vascular bundles were larger in resistant cultivar which achieved 10494, 20.77, 180 and 100, respectively. Also, big vascular bundle dimensions including length, width, phloem tissue thickness and metaxylem vessel diameter achieved 270.77, 217.85, 74.31 and 69.85 µ, respectively compared to susceptible one which recorded considerable decrement of stem diameter, 26%; epidermis thickness, 45%; number of big vascular bundles, 11%; number of small vascular bundles, 24% and big vascular bundle dimensions length, width, phloem tissue thickness and metaxylem vessel diameter by 55, 31, 47 and 19%, respectively compared to resistant cultivar.

It is obvious that root and stem anatomical structure of resistant cultivar enables the root to be more resistant to the phytopathogen *A. strictum* penetration and its development whereas, root and stem anatomical structure of susceptible cultivar reduced changes of resistance such phytopathogen compared to resistant cultivar. The anti-infection structures of epidermis and its outer surrounded layers in addition to the thick outer walls of epidermal cells play an important role in determining susceptibility of the host plant to phytopathogen invasion making certain varieties resistant to disease (Kalappanavar and Hiremath, 1998). The epidermis of the resistant cultivar adventitious roots was surrounded by a very dark thick layer,

Table 1. Anatomical structure counts and measurement variations between resistant cv. (Dorado) and susceptible one (Giza 54) in roots and stems of grain sorghum plants to Acremonium wilt disease under greenhouse conditions (60 days after sowing during the second growing season 2015)

Histological feature	Dorado resistant cultivar without infection	Giza 54 susceptible cultivar without infection	± (%) to resistant cultivar without infection
Root			
Root diameter (µ)	3150.00	2447.00	- 22
Epidermis thickness (µ)	24.23	14.62	- 40
Exodermis thickness (µ)	89.31	39.85	- 55
Cortex thickness (µ)	780.77	851.54	+ 9
Metaxylem vessel diameter (µ)	88.62	99.46	+ 12
Pith diameter (µ)	2291.00	1532.00	- 33
Number of xylem arms per vascular cylinder	52.00	36.00	- 31
Stem			
Stem diameter (µ)	10494.00	7782.00	– 26
Epidermis thickness (µ)	20.77	11.38	– 45
Number of big vascular bundles	180.00	160.00	– 11
Number of small vascular bundles	100.00	76.00	– 24
Big vascular bundle dimensions **Length (µ)**	270.77	123.15	– 55
Width (µ)	217.85	150.69	– 31
Phloem tissue thickness (µ)	74.31	39.38	– 47
Metaxylem vessel diameter (µ)	69.85	56.46	– 19

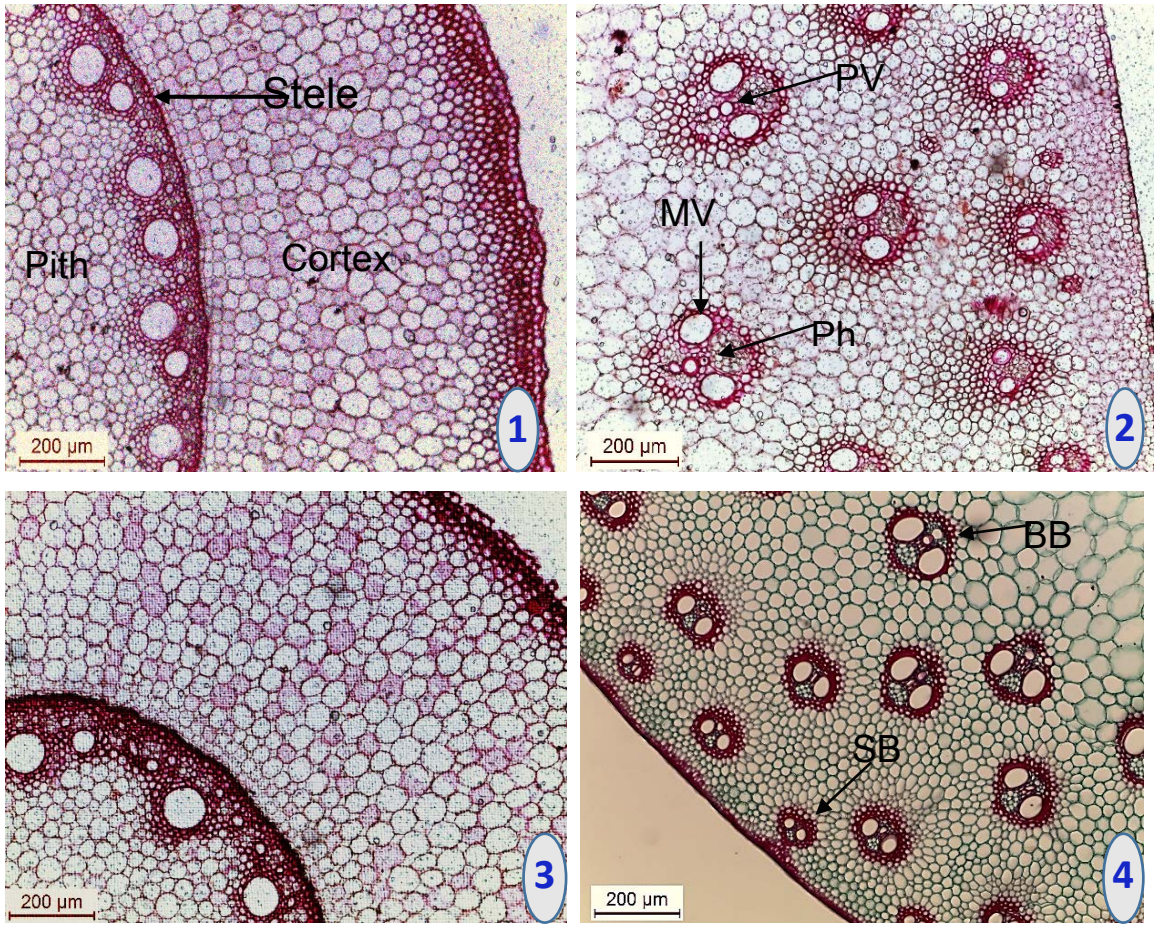

Fig. 2. Portions of transverse sections in adventitious roots and stems of grain sorghum plants 60 days after sowing under greenhouse conditions during the second season of 2015 showing anatomical structure variations between resistant cv (Dorado) and susceptible one (Giza 54) to *A. strictum* that causes acremonium wilt disease

1: Root of resistant cv. without infection. 2: Stem of resistant cv. without infection.
3: Root of susceptible cv. without infection. 4: Stem of susceptible cv. without infection.
PV, protoxylem vessel; MV, metaxylem vessel; Ph, phloem tissue; BB, big vascular bundle; SB, small vascular bundle.

this layer may be consisted of polysaccharides and proteins as described by Amarasinghe (1990) and it may interacts with phytopathogen through interfering with their recognition mechanisms or promoting production of compounds such as phytoalexins and plant defense enzymes (Darvill and Albarsheim, 1984). These findings are in consistence with those reported by Mahmoud (1998). Similar results were early described by Rushdi *et al*. (1975) and Saeed *et al*. (1990) for resistant and susceptible maize cultivars to *Cephalosporium maydis* the causative agent of late wilt disease of maize.

Eisa and El-Naggar (2015) reported that sclerenchyma cells of wheat plants infected by leaf rust pathogen are characterized by having uniformly thick lignified secondary walls. Loss of their protoplasm gave mechanical supporting to plants making them resistant to different pathogens attack. They also added that, the lowest values of sclerenchyma tissue thickness was observed in susceptible wheat cultivars. Meanwhile, leaves of resistant wheat cvs. (Misr-1 and Sids-12), recorded the highest values in this respect.

Acremonium strictum infection compared to uninfected ones

In order to investigate grain sorghum morbid anatomical structure variations as a result of *A. strictum* infection, transverse sections in both stems and adventitious roots of infected and uninfected susceptible cultivar (Giza 54) were prepared and examined. Fig. 3 and data presented in Table 2 and showed marked anatomical features variations between infected and uninfected root and stem transverse sections of grain sorghum plants. As regard to the infected root transverse section, hyphae and spores of the phytopathogen *A. strictum* were observed in metaxylem vessels causing marked disintegration and loosening of some cortex cells, metxylem cell walls and pith cells. Also, in stem *A. strictum* hyphae and spores into vascular bundles causing marked disintegration and loosening of some ground tissue cells and some vascular bundles were observed (Fig. 3). These observations indicated that *A. strictum* infection took place through young roots where the hyphae penetrate the cortex, spread towards the xylem vessels of the root. Later on, it reaches the vascular bundles of stalks and leaves causing true vascular wilt disease as a result of destruction of plant conducting system. These findings are in conformity with those reported by El-Shafey *et al.* (1979) and Khalefa (2000). Such systemic development of *A. strictum* in the host (maize) has been demonstrated by Tagne *et al.* (2002) who reported that the pathogen showed systemic development in the host tissue with inter- and intracellular colonization of the vascular bundle and adjacent tissues including protoxylem, metaxylem, sieve tubes and phloem. In addition, Arora *et al.* (2013) stated that, the infected grain sorghum plants with *Macrophomena phaseolina* showed darkening, decomposition and disintegration of different tissues of roots and stems. The vascular bundles showed development of sclerotia blocking xylem vessels and phloem causing destruction of the plant conducting system.

Root of infected cultivar with *A. strictum* exhibits marked decrement of root diameter (39%), epidermis and cortex thickness (47 and 41%, respectively) and number of xylem arms per vascular cylinder (11%). Whereas, exodermis thickness and metaxylem vessel diameter recorded slight decrement (4 and 2%, respectively) and pith diameter (39%) compared to uninfected root (Table 2). Also stem of infected cultivar shows considerable decrement of stem diameter (30%), epidermis thickness (13%), number of big vascular bundles (23%), number of small bundles (21%) and big vascular bundles length and phloem tissue (10 and 7%, respectively). Whereas, big vascular bundle width and metaxylem vessel diameter recorded slight decrement (3 and 4%, respectively). These findings coincide with those obtained by Khalefa (2000) who stated that *A. strictum* infection decreased the anatomical measurements of protoxylem diameter, metaxylem diameter and cell wall thickness by 7.7, 28 and 5.3%, respectively in stem of susceptible grain sorghum cultivar (Giza 15).

Arbuscular mycorhizal fungi inoculation for biological control of grain sorghum acremonium wilt disease caused by *A. strictum*

Arbuscular mycorhizal fungi inoculation increase host plant resistance directly and indirectly against several phytopathogen through various mechanisms which operate simultaneously. Host plant anatomical structure alteration is one of the most important mechanisms (Harrier and Watson, 2004 ; Abdel-Fattah *et al*., 2011). Results presented in Table 3 revealed that AMF root colonization of susceptible grain sorghum cultivar (Giza 54) as soil application or treated by fungicide (Vitavax 200) as grain coating exhibited increase of all root and stem anatomical measurements, except metaxylem vessel diameter compared to negative control (infected by *A. strictum* without AMF or Vitavax 200). Root of grain sorghum inoculated with AMF or treated by fungicide exhibit marked increment of epidermis thickness (189 and 149%), pith diameter (140 and 72%), root diameter (108 and 69%), exodermis thickness (84 and 27%), cortex thickness (53 and 68%) and number of xylem arms per vascular cylinder (38 and 31%) while, metaxylem vessel diameter showed slight decrement (8 and 1%, respectively)

Table 2. Anatomical structure counts and measurement variations between susceptible grain sorghum cultivar (Giza 54) plants uninfected and infected by *Acremonium strictum* in roots and stems under greenhouse conditions (60 days after sowing during the second growing season 2015)

Histological feature		Uninfected plants by *A. strictum*	Infected plants by *A. strictum*	Decrement (%) to uninfected by *A. strictum*
Root				
Root diameter (µ)		2447.00	1483.00	39
Epidermis thickness (µ)		14.62	7.69	47
Exodermis thickness (µ)		39.85	38.32	4
Cortex thickness (µ)		851.54	502.46	41
Metaxylem vessel diameter (µ)		99.46	97.15	2
Pith diameter (µ)		1532.00	934.00	39
Number of xylem arms per vascular cylinder		36.00	32.00	11
Stem				
Stem diameter (µ)		7782.00	5414.00	30
Epidermis thickness (µ)		11.38	9.85	13
Number of big vascular bundles		160.00	124.00	23
Number of small vascular bundles		76.00	60.00	21
Big vascular bundle dimensions	**Length (µ)**	123.15	111.00	10
	Width (µ)	150.69	146.15	3
Phloem tissue thickness (µ)		39.38	36.69	7
Metaxylem vessel diameter (µ)		56.46	54.00	4

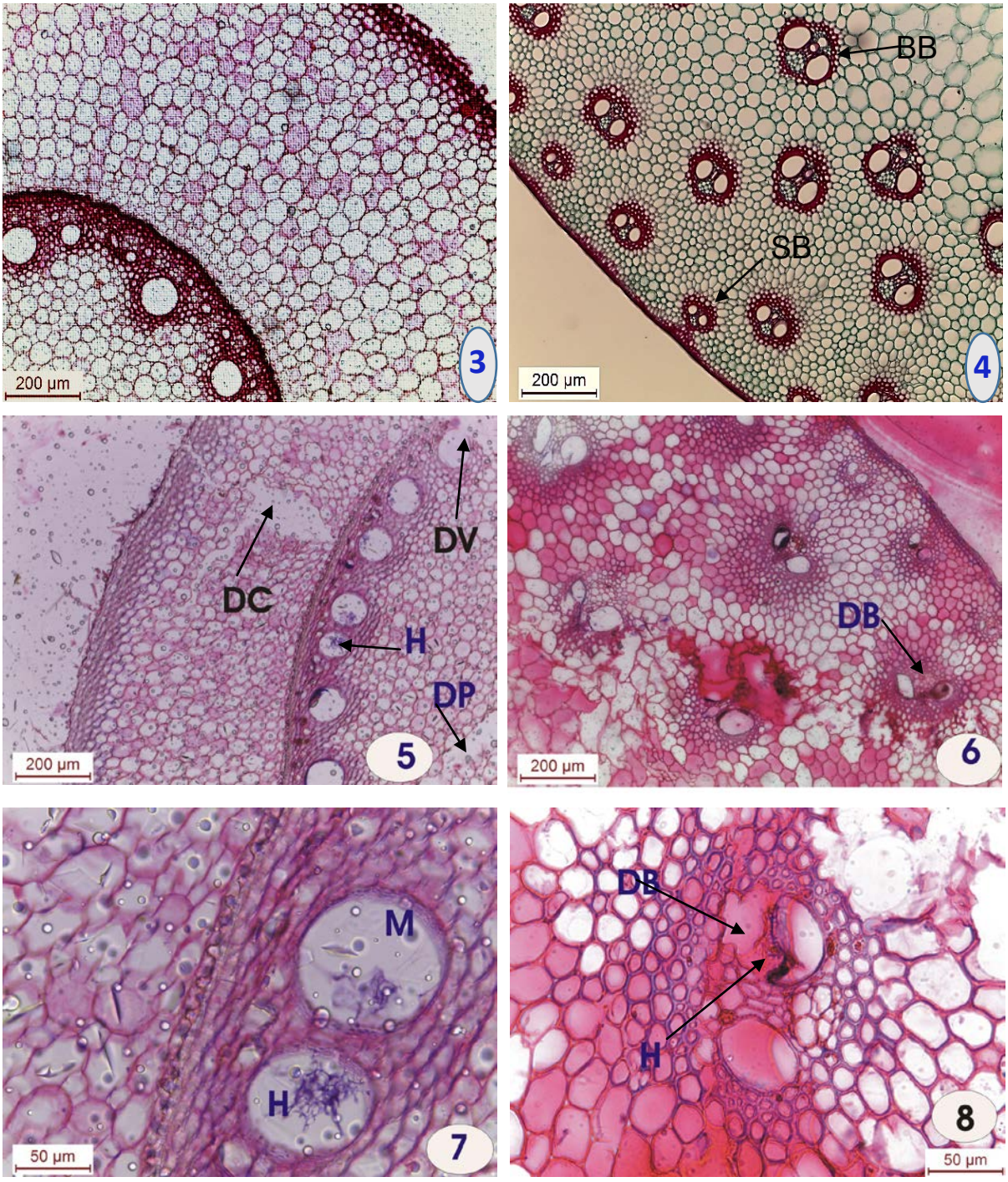

Fig. 3. Portions of transverse sections in adventitious roots and stems of susceptible grain sorghum cv. (Giza 54) plants 60 days after sowing under greenhouse conditions during the second season of 2015 showing anatomical structure variations between uninfected and infected plants with *A. strictum*

3: Root of susceptible cv. without infection. 4: Stem of susceptible cv. without infection.
5 and 7: Root of susceptible cv. infected by *A. strictum* (The bar for 5 plate = 0.2 mm but the bar for 7 plate = 0.05 mm).
6 and 8 : Stem of susceptible cv. infected by *A. strictum* (The bar for 6 plate = 0.2 mm but the bar for 8 plate = 0.05 mm).
DC, disintegrated cortex cells; DV, disintegrated vessel; H, *Acremonium strictum* hyphae;
DP, disintegrated pith cells; BB, big vascular bundle; SB, small vascular bundle; DB, disintegrated vascular bundle and M, metaxylem vessel

compared to untreated negative control. Also, stem of grain sorghum plant inoculated with AMF and treated with the fungicide showed considerable increment of stem diameter (79 and 51%), epidermis thickness (79 and 50%), number of small vascular bundles (73 and 40%) and number of big vascular bundles (52 and 32%, respectively). With respect to big vascular bundle dimentions, AMF inoculation showed considerable increment in length (58%), width (44%), phloem tissue thickness (57%) and metaxylem vessel diameter (46%) while fungicide treatment recorded slight increment in length, width and phloem tissue thickness (27, 10 and 9%, respectively). There was no alteration observed in metaxylem vessel diameter. It was noticed that inoculation of grain sorghum plants by mycorrhizal fungi obviously enhanced roots and stems anatomical measurements compared to those treated by fungicide. These results explain the importance of AMF as an efficient biocontrol agent, environment friendly and safe alternative in comparison with chemical fungicide.

In inconformity with Chandanie *et al*. (2006), Naher *et al*. (2013) and Rich *et al*. (2014), these findings may explain inducing defense responses in grain sorghum as mycorrhizal plant and subsequently, explain the decrement in susceptibility to the phytopathogen *A. strictum*. Moreover, the superiority of AMF in increasing anatomical structure measurements of both root and stem support the role of AMF as plant growth promoter and biological control agent as the vigorous plant is more potentially resistant to the phytopathogen attack than the weak one (Harrier and Watson, 2004). In this respect, Abdel-Fattah *et al*. (2011) revealed that AMF colonization led to an increase in cell wall thickness of common bean plant root. The intercellular spaces filled with dense materials, which may be act as a defense mechanism. The increase in lignification is thought to protect bean roots from penetration by *Rhizoctonia solani*.

Impact of AMF Inoculation and Treatment with Fungicide on Acremonium Wilt Disease Incidence, Plant Growth, Soil Rhizosphere Dehydrogenase Activity and AMF Root Colonization Percentage of Susceptible Grain Sorghum Cultivar Plants Infected by *A. strictum*

Data in Table 4 showed the average of two growing seasons 2014 and 2015. Both fungicide treatment and AMF inoculation improved plant growth and induced rhizosphere dehydrogenase activity with variable degrees compared to the check (negative control). As regard to plant growth the best performance was achieved by the fungicide Vitavax 200 treatment (positive control) followed by AMF inoculation. Fungicide treatment exhibiting 123 and 152% increment in shoot and root dry weight, respectively compared to negative control. While, AMF inoculation recorded 98 and 135% increment in both growth parameters, respectively. With respect to soil rhizosphere dehydrogenase activity, AMF inoculation supported the best performance (117% increment) followed by fungicide treatment (94% increment).

Concerning plant and AMF symbiosis, fungal hyphae invade thin roots and form intracellular coils (arbuscular) and colonize the cellular lumena of cortical cells. In this indo-symbiosis, AMF penetrate root cell wall of the host plant, maintaining the cytoplasm of the two partners separate during development of the symbiotic association (Rich *et al*., 2014). Figs. 5 and 6 illustrate AMF root colonization (x20) and AMF arbuscular (x40) as shown under light microscope. As expected, AMF root colonization showed the highest rate (95%), while both negative control and fungicide treatment recorded 0.0 %.

The higher disease incidence was observed in negative control pots (84% infection), while AMF inoculation as well as chemical fungicide varied in their effects and markedly restricted acremonium wilt disease incidence with superiority to the chemical fungicide resulting in disease incidence reduction of 65 and 99%, respectively (Table 4). These findings suggested that AMF inoculation could be considered as a promising biocontrol agent and plant growth promoter as well. These results coincide with those obtained by Abdel-Fattah *et al.* (2011) who demonstrated that AMF inoculation increased common bean plant growth parameters and markedly reduced Rhizoctonia root rot disease severity. Veerabhadraswamy and Garampalli (2011) also, found that colonization of AMF in root system of maize considerably reduced black bundle disease incidence by 75% compared to negative control and markedly enhanced maize plant growth.

Table 3. Anatomical structure counts and measurement variations between AMF inoculated and fungicide treated susceptible grain sorghum cultivar (Giza 54) plants roots and stems compared to negative control (infected by *A. strictum* without any treatment) under greenhouse conditions 60 days after sowing during the second growing season 2015

Histological feature	Infected plants by *A. strictum* only	Inoculated by AMF	± (%) measurement to Infected plants by *A. strictum* only	Treated by fungicide	± (%) measurement to Infected plants by *A. strictum* only
Root					
Root diameter (µ)	1483.00	3079.00	+ 108	2511.00	+ 69
Epidermis thickness (µ)	7.69	22.23	+ 189	19.15	+ 149
Exodermis thickness (µ)	38.32	70.38	+ 84	48.58	+ 27
Cortex thickness (µ)	502.46	766.69	+ 53	841.92	+ 68
Metaxylem vessel diameter (µ)	97.15	89.08	- 8	95.77	- 1
Pith diameter (µ)	934.00	2242.00	+ 140	1611.00	+ 72
Number of xylem arms per vascular cylinder	32.00	44.00	+ 38	42.00	+ 31
Stem					
Stem diameter (µ)	5414.00	9686.00	+ 79	8182.00	+ 51
Epidermis thickness (µ)	9.85	17.62	+ 79	14.77	+ 50
Number of big vascular bundles	124.00	188.00	+ 52	164.00	+ 32
Number of small vascular bundles	60.00	104.00	+ 73	84.00	+ 40
Big vascular bundle dimensions Length (µ)	111.00	175.00	+ 58	141.38	+ 27
Width (µ)	146.15	209.92	+ 44	160.54	+ 10
Phloem tissue thickness (µ)	36.69	57.77	+ 57	40.15	+ 9
Metaxylem vessel diameter (µ)	54.00	78.62	+ 46	54.12	+ 0.2

Table 4. Average impact of AMF inoculation and treatment with fungicide on acremonium wilt disease incidence, plant growth, soil rhizosphere dehydrogenase activity and AMF root colonization of susceptible grain sorghum cultivar (Giza 54) plants 60 days after sowing compared to negative control under greenhouse conditions during 2014 and 2015 growing season

Treatment	(%) Disease incidence	Plant growth (shoot and roots dry weight g/plant)		Soil rhizosphere dehydrogenase activity (µg TPF/g soil/ day)	(%) AMF root colonization
		Shoot	Root		
Untreated (Negative control)	83.80	58.50	8.10	95.00	0.0
Inoculated by AMF	29.50	115.80	19.0	206.00	95
± (%) to Negative control	- 65	+ 98	+ 135	+ 117	
Treated with fungicide (Positive control)	1.00	130.00	20.40	184.00	0.0
± (%) to Negative control	- 99	+ 123	+ 152	+ 94	

Each value represents average of the two season.
Negative control, infected by *A. strictum* without AMF inoculation or fungicide treatment
Positive control, treated with fungicide (Vitavax 200)
AMF, arbuscular mycorrhizal fungi
TPF, 2,3,5 triphenyl formazan.

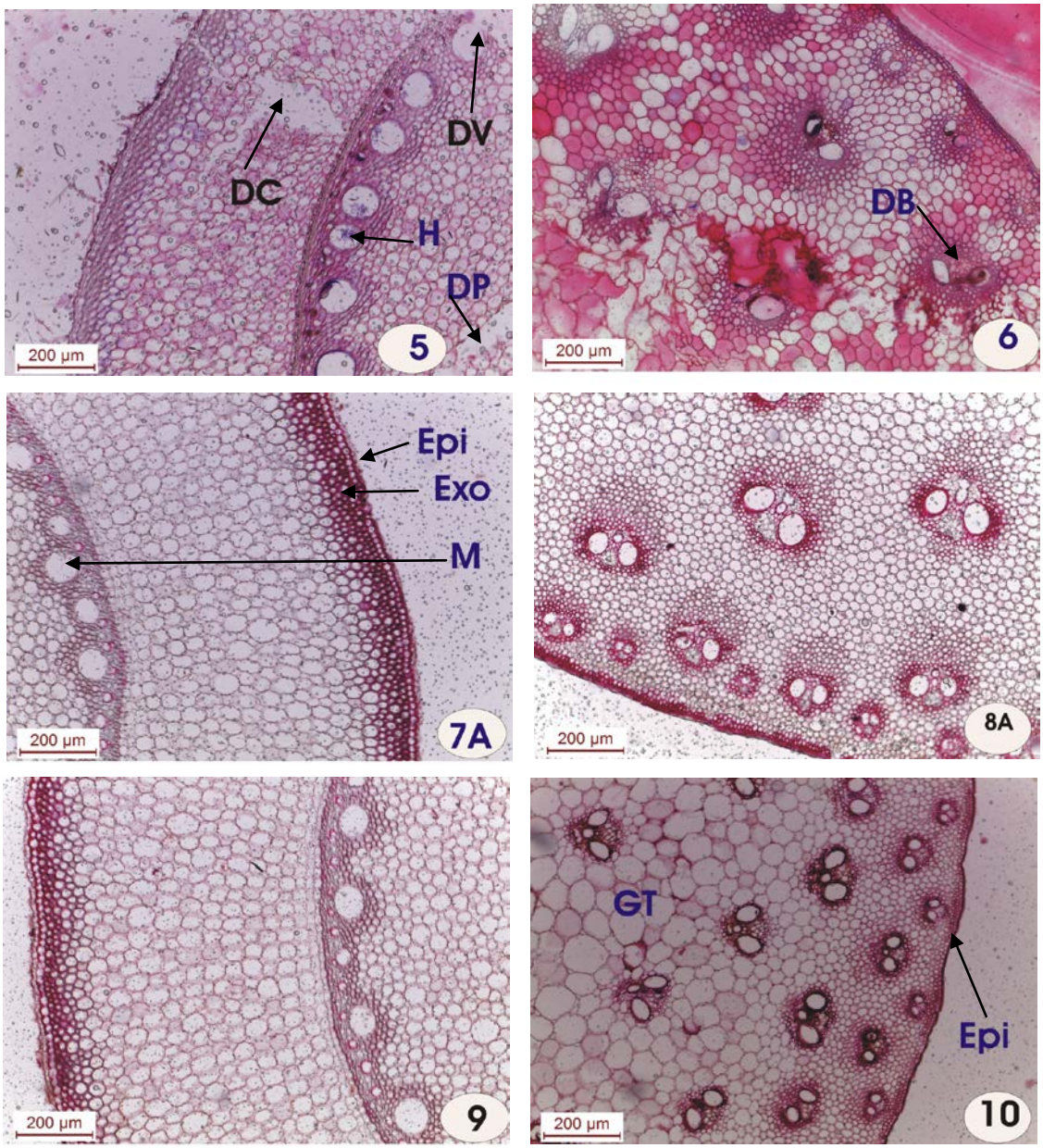

Fig. 4. Portions of transverse sections in adventitious roots and stems of susceptible grain sorghum cv. (Giza 54) plants 60 days after sowing under greenhouse conditions during the second season of 2015 showing anatomical structure variations of plants inoculated by AMF or treated by fungicide compared to control (infected by *A. strictum* without any treatment).

5 : Root of susceptible cv. infected by *A. strictum* without any treatment.
6 : Stem of susceptible cv. infected by *A. strictum* without any treatment.
7A: Root of susceptible cv. inoculated by AMF.
8A: Stem of susceptible cv. inoculated by AMF.
9: Root of susceptible cv. treated by fungicide.
10: Stem of susceptible cv. treated by fungicide.
DC, disintegrated cortex cells; DV, disintegrated vessel; H, *Acremonium strictum* hyphae;
DP, disintegrated pith cells; DB, disintegrated vascular bundle,Epi, epidermis; Exo, exodermis; M, metaxylem vessel and GT, ground tissue.

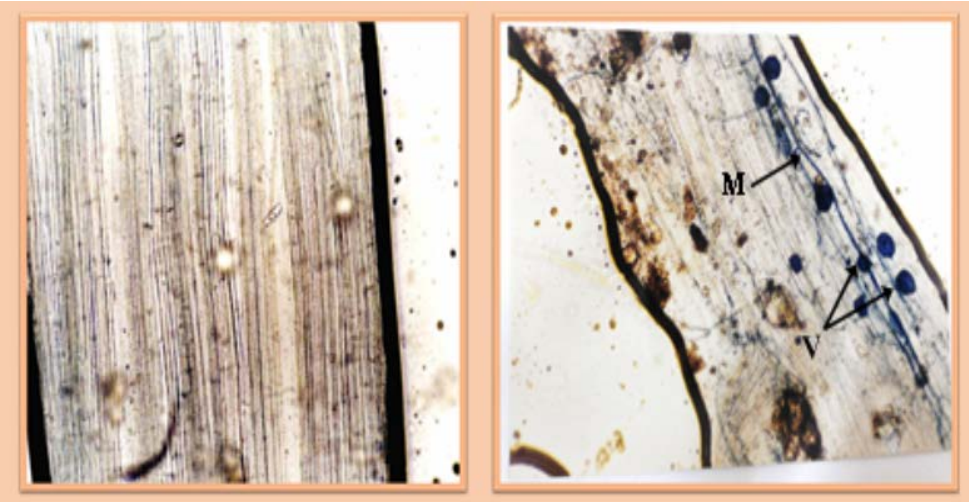

Fig. 5. Grain sorghum root segment, 60 days after sowing under greenhouse conditions, colonized by AMF as shown under light microscope (X20) exhibiting AMF vesicle (V) and mycelium (M). A segment without AMF colonization on the left

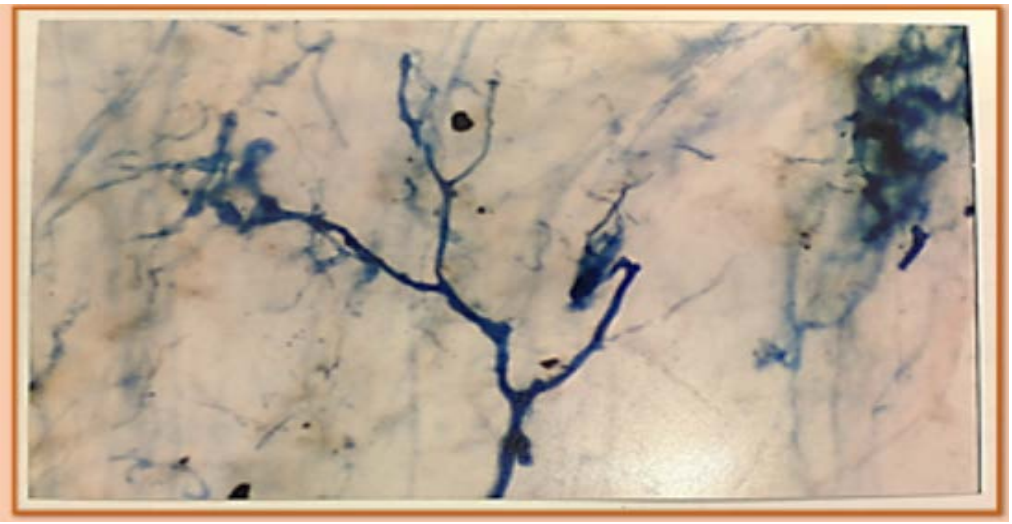

Fig. 6. AMF arbuscular as shown under light microscope (X40)

According to the aforementioned results it could be concluded that plant anatomical structure plays an important role in both host/pathogen and host/AMF interactions suggesting the important use of both resistant grain sorghum cv. and AMF as biological control agent are the best strategy alternatives against acremonium wilt disease incidence due to their help in keeping the environment free from pollution and health hazards due to chemical fungicides.

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دراسات تشريحية و مرضية على نباتات الذرة الرفيعة الملقحة بفطريات الميكورهيزا لمقاومة مرض الذبول الاكريمونيومى

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تم دراسة تغيرات التركيب التشريحى لجذور وسوق نباتات الذرة الرفيعة المنزرعة في تجربة أصص بالصوبة خلال صيف موسمين متعاقبين هما ٢٠١٤ و ٢٠١٥ لتقدير تأثير قابلية الصنف للإصابة بفطر اكريمونيوم استركتم المسبب لمرض الذبول الاكريمونيومى وكذا استعمار الجذور بفطريات الميكورهيزا كعامل مقاومة حيوية أو المعاملة بالمبيد الفطرى، أظهرت نتائج فحص القطاع العرضى لجذر الصنف المقاوم (دورادو) زيادة فى سمك البشرة والقشرة وقطر الجذر والنخاع وكذلك متوسط عدد أذرع الخشب للاسطوانة الوعائية فى حين حدث نقص في سمك القشرة وقطر وعاء الخشب الثانى عن الصنف القابل للإصابة (جيزة ٥٤) وهذا يفسر مقاومة الصنف لاختراق المسبب المرضى وانتشاره، إصابة نباتات الصنف القابل للإصابة بفطر اكريمونيوم استركتم خفض بشكل واضح جميع قياسات التركيب التشريحى التي تم اختبارها في الجذر والساق مقارنة بالنباتات غير المصابة، وظهرت فى جذور نباتات الصنف القابل للإصابة هيفات الفطر وجراثيمه داخل اوعية الخشب الثانى مسببة تحطم وانحلال بعض أوعية الخشب الثانى وخلايا القشرة والنخاع، وتلازم ذلك فى نتائج مشابهة فى النسيج الاساسى و بعض الحزم الوعائية للساق مما يدل على انتشار المسبب المرضى وتسببه في ذبول النبات، أدى استعمار جذور النبات بالميكورهيزا إلى زيادة واضحة في جميع قياسات التركيب التشريحى التي تم اختبارها في الجذور والسوق ما عدا قطر وعاء الخشب الثانى مقارنة بنباتات الكنترول (غير الملقح بالميكورهيزا والمصاب بفطر اكريمونيوم استركتم)، هذه النتائج ربما توضح دورالميكورهيزا فى تحفيز المقاومة المستحثة لنباتات الذرة الرفيعة كأحد عوائلها، أدى تلقيح نباتات الذرة الرفيعة صنف جيزة ٥٤ بالميكورهيزا إلى تشجيع نمو النبات (الوزن الجاف للجذور والمجموع الخضرى) بشكل ملحوظ وزيادة في نشاط انزيمات الديهيدروجينيز في الريزوسفير وخفض نسبة الإصابة بمعدل ٦٥% مقارنة بنباتات الكنترول.

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