Minufiya J. Agric. Res. Vol.35 No. 2:471-484 (2010) "http://agri.menofia.edu.eg/megla.html"

STRUCTURAL CHANGES IN RICE FLAG LEAF AS AFFECTED BY WHITE TIP NEMATODE DISEASE "*APHELENCHOIDES BESSEYI*"

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(Received: Feb. 18, 2010)

ABESTRACT: ***White tip disease of rice leaves caused by rice leaf nematode, Aphelenchoides besseyi Christie, 1942, is widespread and cause severe yield losses allover the world. To investigate the effect of the infection on anatomical structure changes of flag leaf in some rice susceptible cultivars (Reiho, Sakha 101 & Sakha 103) and resistant culivars (Giza 176, Giza 178, Sakha 104 & H1 hybrid)the current investigation was carried out at the experimental farm of Rice Research and Training Center, Sakha Agricultural Research Station during 2008 and 2009 rice seasons. The obtained results indicated that:***

a- Anatomical structure of flag leaf of some rice cultivars; Reiho, Sakha 101 and Sakha 103 significantly changed in response to white tip nematode infection. The flag leaf thickness, mesophyll layer thickness, bundle sheath thickness and midrib width significantly decreased compared with the healthy flag leaves of all cultivars. On the other hand, bundle diameter and midrib length was highly increased. White tip nematode infection was highly associated damage of motor cells, subsequently the lamina be twisted.

b- The response of rice cultivars to white tip nematode infection significantly differed according to their differences in anatomical structure. H1 hybrid as a highly resistant cultivar was superior in almost studied characters compared with other cultivars specially the susceptible ones. Also; Giza 178 and Sakha 104 surpassed in all studied characters compared with Reiho as a highly susceptible cultivar.

c- The deformation in flag leaf anatomical structure leads to reduction in chlorophyll content, leaf area and yield of susceptible rice cultivars.

Finally, Data indicate that relationships between the best anatomical characters on some leaf cultivar, and the most resistant my be cussed high yielding as flow :- H1 hybrid, Giza 178 and Sakha 104

Key words : ***Rice, Leaf structure, Aphelenchoides besseyi, white tip nematode.***

INTRODUCTION

White tip disease of rice induced by the rice leaf nematode, *Aphelenchoides besseyi* Christie, 1942, is widespread and presents nearly in rice ecosystems allover the world. The white-tip nematode, *A. besseyi* is an

ectoparasite on leaf primordia, leaves, ear primordia. florets and developing grains of rice (Israel *et al,* 1963). Nematode infestation at the heading stage results in twisting of apical part of boot-leaf poor , emergence of panicle, and reduction in panicle length and 1000-grain weight (Ichinohe 1973, Muthukrishnan *et al*. 1974). Feeding at leaf tips in rice results in whitening of the top 3-5 cm of the leaf, leading to necrosis (Yokoo, 1948). There is also distortion of the flag leaf that encloses the panicle. Diseased plants are stunted, lack vigor and produce small panicles. Affected panicles show high sterility, distorted glumes and small and distorted kernels (Ou, 1985). The loss in grain yield caused by this nematode has been reported to range from 20 to 40 per cent (Muthukrishnan *et al*. 1974, Nandakumar *et al.* 1975). Tsay *et al.* (1998), in Brazil, reported that white tip nematode caused losses of up to 50% in upland paddy. In Egypt, white tip nematode causes losses of up to 47% in highly susceptible cultivars; Giza 171 and Reiho, (El-Shafey, 2007). Artyukhova and Popova (1981) studied the ultrastructural changes caused by *A. besseyi* to leaves of susceptible rice plants. They reported that nematode causes crimping and chlorosis, considerable changes in the lamina, the structure of the epidermis, and misalignment, underdevelopment and deformation of the motor cells. Jairajpuri and Baqri (1991) reported that the injury due to *A. besseyi* caused by the nematode stylet leads to disintegration of phloem cells. Wan and Zhong (1981) found a positive correlation between leaf area index and dry matter production. Also, positive correlation was found between flag leaf area and panicle weight. Flag leaf area has a significant effect on grain yield through grains/panicle and panicle length Bashar, *et al.* (1991). Egyptian rice varieties significantly differ in their resistance to white tip nematode. Some of rice varieties are resistant such as; Giza 178, Giza 182, H1, H2, Giza195 and Giza176, while Sakha101 and Sakha 103 are moderately susceptible and Giza171and Reiho are highly susceptible , Abdel Hadi *et al*., 2005 and EL-Shafey (2007).

This study aimed to investigate:

1. **Effect of white tip nematode on growth characters of some rice cultivars.**
2. **The effect of infection with *Aphelenchiodes besseyi* on some anatomical changes in flag leaf structure of three susceptible rice cultivars; Reiho, Sakha 101 and Sakha 103.**
3. **Study the relationships between anatomical structure of flag leaf in some rice cultivars and their reactions to white tip nematode infection.**

MATERIALS AND METHODS

A- Evaluation of cultivars to field studies:

The current investigation was carried out at the experimental farm of Rice Research and Training Center, Sakha Agricultural Research Station during 2008 and 2009 rice seasons. Two field experiments were conducted to investigate the effect of white tip nematode on agronomic and anatomical characters of different rice cultivars. The cultivars were arranged in a

randomized complete block design with four replicates. All cultivars; Giza 159, Giza 176, Giza 178, Sakha 101, Sakha 102, Sakha 103, Sakha 104 and H1(hyprid) were evaluated for resistance to white tip nematode under artificial inoculation according to El-Shafey (2007). The highly and moderately susceptible cultivars; Rieho ; Sakha 101, Sakha 102 and Sakha 103 were used. The highly infected seeds were obtained from rice plants that showed severe infection symptoms of white tip nematode in the previous season. The resistant cultivars seeds were exposed to infection many successive seasons by cultivated with highly susceptible ones without any barriers among them. The healthy seeds of all cultivars were completely free from nematode.

All cultivars seeds were sown at nursery beds on May 15. One month later, three seedlings /hill were transplanted in plots measuring 3×3.5 m2 at 20 × 20 cm. The nitrogen fertilizer was added as Urea (46 % N) at recommended units of nitrogen per feddan for each cultivar. Two Thirds of nitrogen dose was incorporated to top 15cm of the dry soil as a basal application before transplanting, while one third of nitrogen dose was added thirty days after transplanting. Other cultural practices were undertaken as recommended.

A1- Effect of white tip nematode on growth characters of some rice cultivars:

a- Infection % of white tip nematode:

At booting stage, as white tip nematode symptoms becomes clear, total rice hills in one square meter were examined to record the infected hills and calculated the infection percentage according to the following formula:

$$\text{Percentage of infection} = \frac{\text{No. of infected hills/m}^2}{\text{Total no. of rice hills/m}^2} \times 100$$

b- Effect of white tip nematode on chlorophyll content.

One week after complete heading, chlorophyll content of flag leaf of rice cultivars was assessed using chlorophyll meter (Mod. Spad-502, Minolta Camera Co. Ltd., Japan). The chlorophyll content was assessed in the tip and a basal part of infected flag leaves and compared with that in healthy flag leaves. Thus, the reduction % in chlorophyll content was calculated as influenced by white tip nematode infection.

c- Effect of white tip nematode on flag leaf area (cm2).

To determine the effect of white tip nematode on rice leaves, flag leaf area of both healthy and infected cultivars was estimated using the following formula (Palaniswamy and Gomez, 1972):

$LA = K (L \times W)$. **Where :** $L =$ **leaf length**, $W =$ **maximum leaf width, and** $K =$ **constant (0.75)**

d- Yield (t/fed.):

Grain yield of each plot was estimated by harvesting all hills in the plot except one outer row from each side. Total weight was recorded for each plot and the weight was adjusted to 14% moisture content, then the yield was calculated as t/fed.

B- Anatomical studies:

1- **The effect of infection with *Aphelenchiodes besseyi* on some anatomical changes in flag leaf structure of three susceptible rice cultivars; Reiho, Sakha 101 and Sakha 103 was measured.**
2- **The relationships between anatomical structure of flag leaf in some rice cultivars and their reactions to white tip nematode infection were calculated.**

To investigate the effect of infection with *Aphelenchiodes besseyi* on some anatomical changes in flag leaf structure of three susceptible rice cultivars; Reiho, Sakha 101 and Sakha 103 and relationships between anatomical structure of flag leaf in some rice cultivars and their reactions of white tip nematode infection, anatomical studies were carried out only in the second season for preparing sections, as ten samples of flag leaf per plot were collected. Each sample measured 05cm of the tip portion of the flag leaf. All samples were killed and fixed for 48 hours in FAA (10 ml. formalin, 5 ml. glacial acetic acid, 50 ml. ethyl alcohol and 35 ml. water). The dehydrated samples were infiltrated and embedded in paraffin (52-54°C m.p.). The embedded samples were sectioned on a rotary microtome at a thickness of 8-10 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranine and light green, cleared in xylol and mounted in Canada balsam (Gerlach, 1977). Slides were microscopically examined and measurements and counts were taken and averages of 9 readings of 3 slides were calculated.

C-Statistical analysis:

Statistical analysis was performed using ANOVA technique by IRRISTAT computer software package

RESULTS AND DISCUSSION

Effect of white tip namtode infection on some growth characters of rice cultivars:

a. Infection % of white tip nematode:

Reiho recorded the highest infection (82.0%) followed by Sakha 103 (75.1%), while Sakha 101 recorded the lowest infection (48.0%), 2008 season Table (1).

The same trend was obtained in 2009 season. All cultivars including, Giza 176, Giza 178, Sakha 104 and Hybrid 1 did not exhibit nematode symptoms and performed as resistant to white tip nematode, Fig (1), and Table (1).

Table (1): Effect of white tip nematode infection on chlorophyll content .flag leaf area and Yield of some rice cultivars at 2008 and 2009 seasons .

Character / variety		Infection %		Chlorophyll content (SPAD)*		Reduction %	Flag leaf area (cm^2)*		Reduction %	Yield (t/fedd.)*		Reduction %
		2008	2009	H.	I.		H.	I.		H	I	
Susceptible	Reiho	82.0	86.7	31.1	3.1	90.03	25.9	4.2	83.78	3.6	2.1	41.7
	Sakha 101	48.0	46.0	46.3	5.0	89.20	37.5	13.5	64.00	4.8	3.9	18.8
	Sakha 103	75.1	73.2	38.1	3.8	90.03	28.9	8.2	71.63	4.0	2.9	27.5
Resistant	Sakha 104	No.S	No.S	35.1	35.0	0	30.7	31.0	0	4.5	4.5	-
	Giza 176	No.S	No.S	47.8	47.4	0	35.4	35.2	0	3.5	3.4	-
	Giza 178	No.S	No.S	42.1	42.0	0	34.4	35.1	0	4.7	4.6	-
	Hybrid 1	No.S	No.S	43.2	42.0	0	35.2	35.4	0	6.0	6.0	-

H = Healthy, I = Infected with white tip nematode. *average of 2008 and 2009 seasons No.S, No symptoms

b. Effect of white tip nematode on chlorophyll content of flag leaf:

Concerning Chlorophyll content (Table 2) there is no relationship between chlorophyll content and preference of white tip nematode to infect some cultivars. Some rice cultivars had high chlorophyll content and were susceptible to white tip nematode such as Sakha 101 (46.3). On the other hand, Some rice cultivars had high chlorophyll content and were resistant to white tip nematode such as Giza 176, H1 and Giza 178 (47.8, 43.2 and 42.1 SPAD value) respectively. While some rice cultivars have low chlorophyll content and susceptible to white tip nematode such as Reiho and Sakha 103 (31.1 and 38.1 SPAD value) respectively. On the other hand, Some rice cultivars have low chlorophyll content and resistant to white tip nematode such as Sakha 104 (35.1 SPAD value).

Chlorophyll content of the flag leaf was higly and negatively affected by white tip nematode infection in Reiho ; Sakha 101 and Sakha 103 cultivars.The reduction in chlorophyll content account fore 90.03;.89.20 and 90.03% for the three cultivars respectively, Fig (1), and Table (1)..

c. Effect of white tip nematode on flag leaf area (cm^2):

Flag leaf area of Sakha104; Giza 176, and Giza178 rice cultivars in healthy plants; were almost the same in nematode infected plants. The cultivar Reiho; Sakha 101and sakha 103 suffered high reduction in their flag leaf area due to nematode infection. These reduction were 83.78; 64.00 and 71.63% for the above mentiand cultivars respectively, Fig (1), and Table (1).

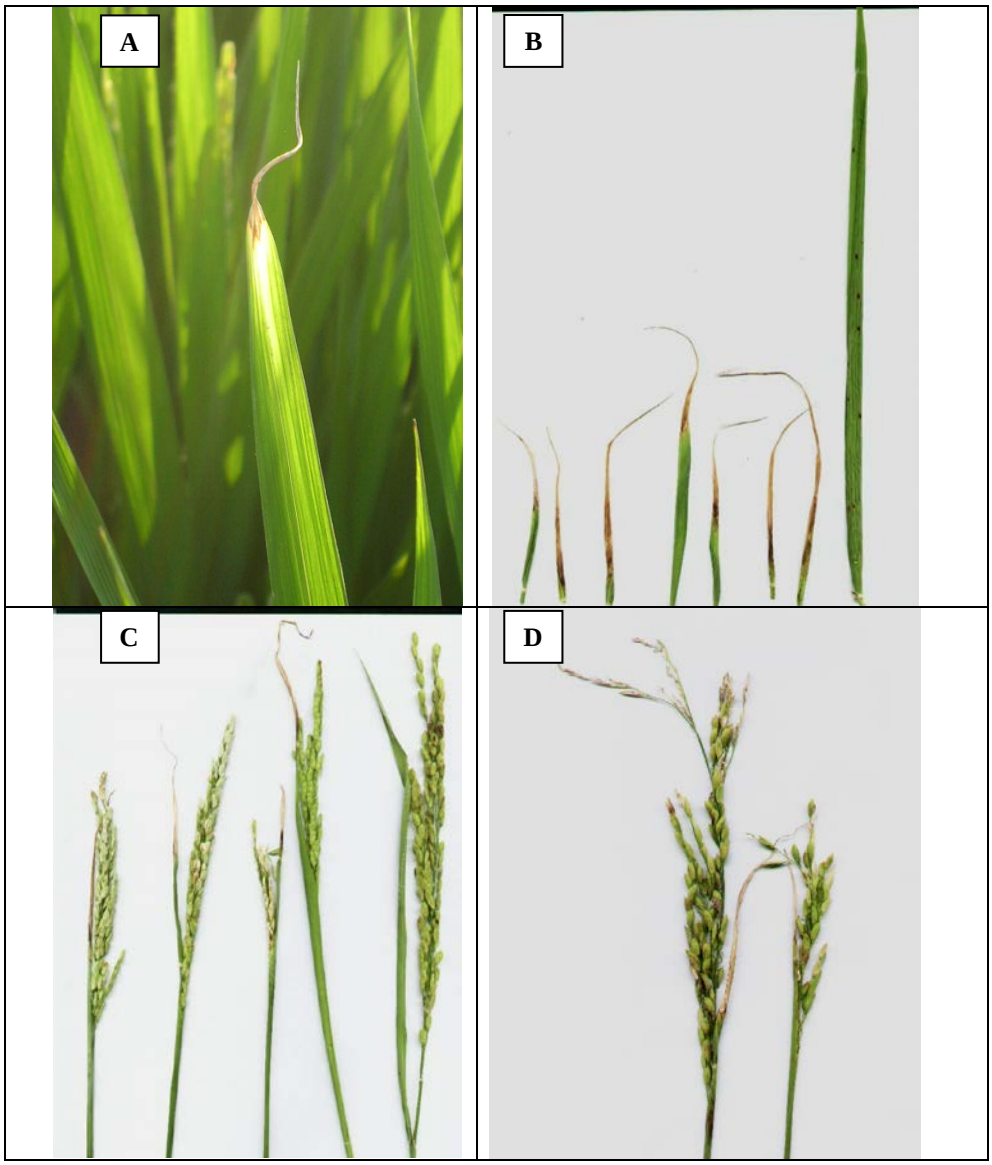

Fig. (1): A, Chlorosis and whitening of flag leaf tip due to white tip nematode
B, Damage of flag leaf area and deformation due to white tip nematode
C, Deformation and incomplete panicle exertion, twisting and necrotic area of flag leaf
D, Panicle branches degeneration and sterility of spikletes,

Table (2): Structure changes in flag leaf of rice cvs. Reiho, Sakha 101 and Sakha 103 due to white tip nematode infection.

Character / variety	Leaf thickness (µ)	Mesophyll µ	bundle sheath µ	bundle Ø	xylem Ø	vessel Ø	midrib length µ	midrib width µ
Reiho healthy	50.96	41.53	20.52	82.19	33.46	24.24	138.64	148.73
infected	47.97	24.43	13.99	86.52	35.14	35.14	127.38	132.62
S101 healthy	76.72	57.93	36.19	95.81	36.71	27.96	167.93	156.63
infected	37.02	21.15	14.79	118.02	23.71	23.71	203.02	126.93
S103 healthy	82.29	61.28	34.59	86.41	32.41	20.48	129.85	129.85
infected	34.81	17.97	21.54	108.29	36.33	36.33	170.36	140.14
L.S.D 5%	2.691	4.242	3.221	3.364	4.372	4.372	3.675	5.100

d. Effect of white tip nematode on Yield (t/fedd.):

For the response of yield to white tip nematode infection, yield was significantly reduced by infection of white tip nematode. The reduction % in grain yield reached to 41.7 %. Reiho, while the lowest reduction % was recorded with Sakha 101, 18.8 %. The yield reduction was highly associated and affected with the damage of flag leaf area and chlorophyll content, Table (1), Fig (1).

B- Anatomical studies:

1- The effect of infection with *Aphelenchiodes besseyi* on some anatomical changes in flag leaf structure of three susceptible rice cultivars; Reiho, Sakha 101 and Sakha 103 was studied (Table 2).

White tip nematode induced a significant change in the anatomical structure of flag leaf of rice cultivars; Reiho, Sakha 101 and Sakha 103 (Fig. 2 and Table 2). Data indicated that some tissues decreased, while the others increased in thickness or diameter as a clear response to white tip nematode infection. The flag leaf thickness, mesophyll thickness, bundle sheath thickness and midrib width, in the infected plants were significantly reduced compared with those of the healthy plants.

For leaf thickness, leaf thickness of all susceptible cultivars was significantly decreased with infection of white tip nematode. The highest value of leaf thickness was recorded with healthy flag leaves of Sakha 103, (82.29 µ), while the lowest are with the infected flag leaves of the same cultivar, 34.81 µ. The same range of reduction was recorded with the bundle sheath thickness and the midrib width.

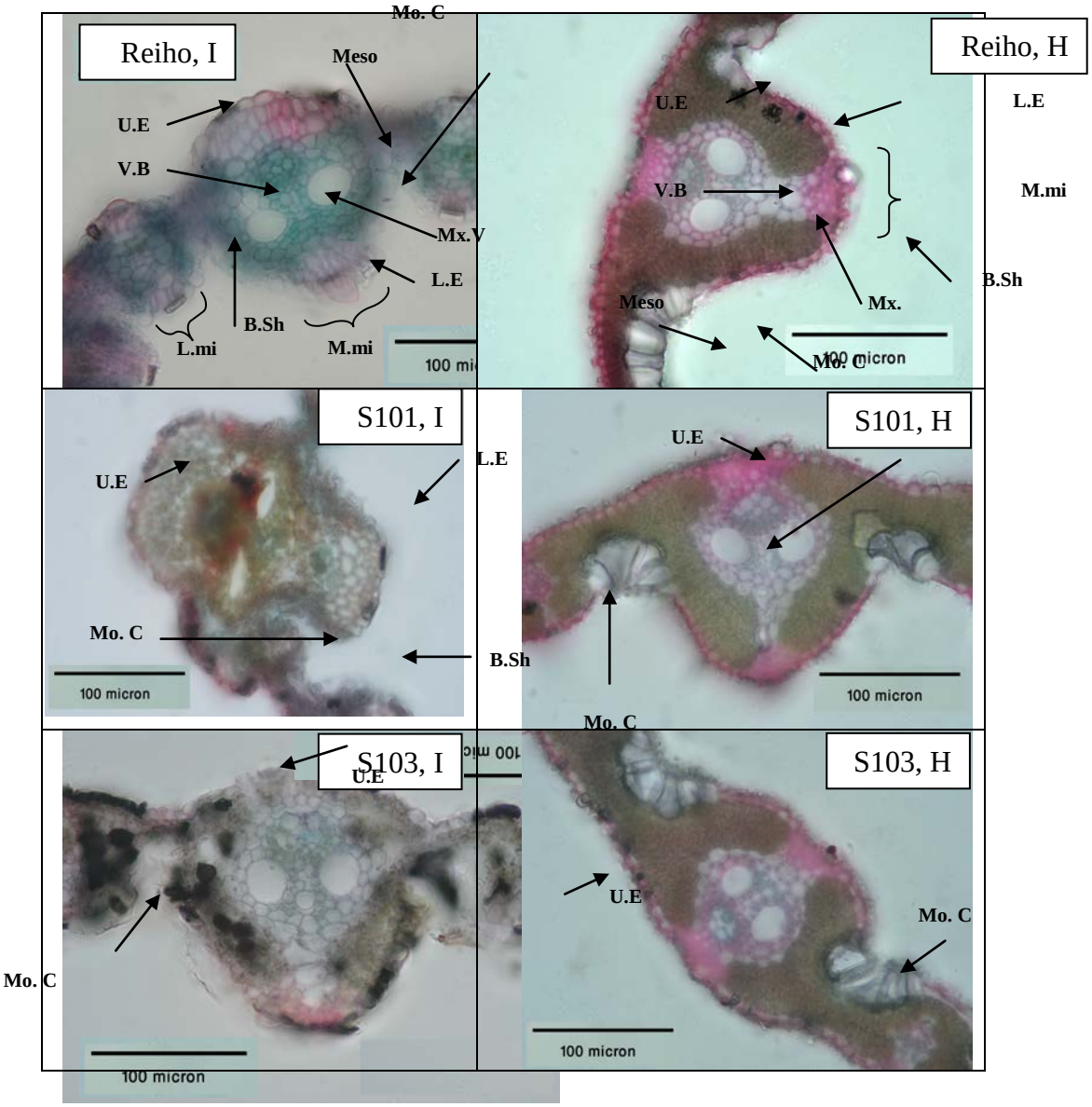

Fig. (2): Transfer sections in healthy (H) and infected (I) flag leaf of rice cultivars; Reiho, Sakha 101 and Sakha 103. (x40)

U.E. Upper epidermal cells L.E, Lower epidermal cells
Meso, Mesophyll layer V.B, Vascular bundle
Mx.V, Meta xylem vessel B.Sh, Bundle sheath
Mo. C, Motor cells M.mi, Main midrib, L.mi, Lateral midrib

For Mesophyll layer thickness, it was sharply decreased by 1.7-3.4 times compared with the healthy plants, whereas the lowest one was 17.97 µ of the infected leaves of Sakha 103 and the highest value 61.28 µ for healthy leaves of the same cultivar (Fig. 2) and (Table 2).

The reduction in a Mesophyll thickness was associated with sharp reduction in chlorophyll content, so it should significantly affect the photosynthesis process and subsequently reduces the grain yield of infected plants.

On the other hand, bundle diameter and midrib length was highly affected with the infection. Bundle diameter increased form 86.41 in the healthy flag leaves to 108.29 µ in the infected leaves for cultivar Sakha 103. For midrib length, the midrib length was significantly increased from 167.93 to 203.02 with Sakha 101 and from 127.38 to167.93 µ with Sakha 103.

For motor cells, the deformation of flag leaf as a result of white tip nematode infection was highly associated with death and damage of motor cells, subsequently the lamina be twisted, (Fig. 1) and (Fig. 2).

The xylem and vessel diameter differed in their response to infection with different cultivars. In Reiho and Sakha 103; the diameter increased due infection, while the diameter of xylem and vessel decreased with Sakha 101.

As a result of deformation of flag leaf structure due to nematode infection, some growth process and nutrients translocation were affected. The deformation of leaf structure was associated actually with yield reduction.

Anatomical structure of flag leaf of susceptible rice cultivars significantly changed in response to white tip nematode infection . These findings are in accordance with Artyukhova and Popova (1981), who recorded ultrastructural changes caused by *A. besseyi* to leaves of susceptible rice plants. They reported that nematode causes crimping and chlorosis, considerable changes in the lamina, the structure of the epidermis, and misalignment, underdevelopment and deformation of the motor cells. Also, these results are in harmony with findings of Jairajpuri and Baqri (1991), who reported that the injury due to the infection of *A. besseyi* caused by the stylet leads to the disintegration of phloem cells. It is clear that white tip nematode disease deforms and damages the anatomical structure of rice flag leaf which is the important organ for photosynthesis. *Aphelenchoides besseyi* caused a sharply decrease in the chlorophyll content of the cell, so the photosynthesis rate is severely and negatively affected. According to the deformation of flag leaf due to infection of white tip nematode all agronomic traits will be decreased. White tip nematode infection deformed the motor cells which controlled the rolling and expanding of leaf blade, so the leaf area which exposed to the light of sun sharply diminished as a result of this damage the photosynthesis rate reached to the lower level. Vascular bundle, xylem diameter were negatively affected with white tip nematode infection as a result of this damage the translocation of nutrients from source to the sink will be affected. As a result of all above-mentioned damages, the growth of

the plant will be affected, this findings in accordance with results of Ou, (1985) who reported that the diseased plants are stunted, lack vigor and produce small panicles and the affected panicles show high sterility, distorted glumes and small and distorted kernels.

3- Relationships between anatomical structure of flag leaf in some rice cultivars and their reactions of white tip nematode infection.

All japonica rices under study were susceptible or highly susceptible to *A. besseyi* except Giza 176 and Sakha 104 that were resistant (symptomless). Giza 178 as indica japonica and H1 hybrid as indica rice were resistant to *A. besseyi.* Although, Sakha 101 and Sakha 103 were infected with white tip nematode but these cultivars have wide levels of tolerance to the disease.

Data in Table (3) and Fig (3) indicated that the response of rice cultivars to white tip nematode infection significantly differed according to their differences in anatomical structure. H1 hybrid as a highly resistant cultivar was superior in almost studied characters compared with the other cultivars specially the susceptible ones. Also; Giza 178 and Sakha 104 surpassed in all studied characters compared with Reiho as a highly susceptible cultivar.

Table (3): Structure of flag leaf in resistant and susceptible rice cultivars as affected by white tip nematode infection.

Character (µ) / variety	Leaf thickness (µ)	Mesophyll (µ)	bundle sheath (µ)	bundle (Ø)	xylem (Ø)	vessel (Ø)	midrib length (µ)	midrib width (µ)	epidermal layer (Ø)	Plant type	Type of reaction
Reiho	50.96	41.53	20.52	82.19	33.46	24.24	138.64	148.73	9.43	J	HS
S101	76.72	57.93	36.19	95.81	36.71	27.96	167.93	156.63	18.78	J	S
S103	82.29	61.28	34.59	86.41	32.41	20.48	129.85	129.85	21.01	J	S
S104	64.51	54.97	37.48	92.93	30.83	21.97	172.29	151.00	9.54	J	HR
G176	51.77	32.84	27.77	92.76	30.69	23.49	121.66	145.13	18.93	J	HR
G178	83.88	59.84	36.53	97.62	35.85	25.59	169.08	143.99	24.04	I J	HR
H1	103.00	77.99	36.80	97.80	31.50	22.45	180.22	161.73	25.00	I	HR
L.S.D 5%	3.358	2.966	3.026	3.449	4.228	3.706	2.218	6.170			
1%	4.708	4.158	4.242	4.835	5.927	5.195	3.109	8.650			

HS, highly susceptible, S, susceptible, HR, Highly resistant, J, Japonica, I, Indica, IJ, Indica Japonica

Fig. (3): Transfer sections in flag leaf of some (resistant and susceptible) rice cultivars as affected by white tip nematode infection. (x40)

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التغيرات التشريحية الحادثة في ورقة العلم نتيجة الإصابة بنيماتودا القمة البيضاء في نباتات الأرز

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الملخص العربي

يؤدي إنتشار مرض القمة البيضاء التي تسببه نيماتودا ***Aphelenchoides*** **besseyi** إلي خسارة كبيرة لمحصول الأرز في معظم أنحاء العالم. أجرى هذا البحث لدراسة تأثير الإصابة بهذا المرض علي التغيرات التشريحية لورقة العلم لبعض أصناف الأرز الحساسة (ريهو، سخا ١٠١ ، سخا ١٠٣) والمقاومة (جيزة ١٧٦ ، جيزة ١٧٨ ، سخا ١٠٤ ، هجين ١) و اثر ذلك على إنتاجها لذا قد أقيمت تجربتين حقليتين في مزرعة مركز البحوث الزراعية والتدريب في الأرز بسخا في شمال الدلتا لموسمي ٢٠٠٨ و ٢٠٠٩ .حيث أوضحت النتائج المتحصل عليها الأتي: –

أ– حدثت تغيرات معنوية في التركيب التشريحي لورقة العلم لأصناف ريهو، سخا ١٠١ وسخا ١٠٣ كرد فعل للإصابة بمرض القمة البيضاء مما أدي إلي نقص سمك النصل وكذلك طبقة الميزوفيل وكذا سمك غلاف الحزمة وكذلك نقص سمك العرق الوسطي مقارنة بتلك الصفات في الأوراق السليمة. بينما إزداد قطر الحزمة الوعائية و إتسع طول العرق الوسطي بها معنوياً في هذه الأصناف الثلاثة. وقد أدي حدوث الإصابة بالمرض إلي إتلاف وموت الخلايا المحركة مما ترتب عليه إلتفاف نصل أوراقها.

ب– اختلفت الأصناف في رد فعلها للإصابة بالمرض إختلافات متباينة وذلك كنتيجة طبيعية للإختلاف فيما بينها في التركيب التشريحي حيث تفوق صنف الهجين ١ كصنف مقاوم في

جميع الصفات التشريحية المدروسة يليه جيزة ١٧٨ ثم سخا ١٠٤ مقارنة بالصنف ريهو شديد الحساسية.

ت- أدي حدوث التشوه في التركيب التشريحي لورقة العلم إلي حدوث نقص واضح في محتوي الأوراق من الكلوروفيل وكذلك المساحة الورقية وبالتالي نقص واضح في المحصول لأصناف الأرز الحساسة مقارنة بالأصناف المقاومة.

وأخيرا تؤكد النتائج أن هناك علاقة وثيقة بين تمتع أوراق بعض أصناف الأرز بخصائص تشريحية أفضل ومقدرتها على مقاومة المرض قد يكون هذا سبب تفوقها في الإنتاج عن غيرها و هي :-هجين ١ يليه جيزة ١٧٨ ثم سخا ١٠٤.