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Description of *Aphelenchoides besseyi* from Abnormal Rice with 'Small Grains and Erect Panicles' Symptom in China

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Abstract: The abnormal rice with small grains and erect panicles were found on a large scale in China, which showed shortend rice panicle and decreased number of grains in comparison with normal rice, and the grain was small and black-brown, and some of them were distorted, while the flag leaf was normal. A kind of nematode of *Aphelenchoides* was isolated from the grains of rice variety Wuyujing 3 which performed 'small grains and erect panicles' symptom. There were 2014 nematodes in one hundred grains infected, and up to 74 in single grain, 92 percent of the grains tested had nematodes in the infested panicles. The diagnosis characters of nematode include lateral fields about one-fourth as wide as body, with 4 incisures. The terminus bears a mucro of diverse shape with 3-4 pointed processes. The female post-vulval uterine sac extends less than 50% of distance from vulva to anus, no sperm in it. Oocytes usually arrange in 2-4 rows. The male spicules have a moderately developed rostrum. Morphological measurements showed it to be conspecific with *Aphelenchoides besseyi* Christie, 1942.

Key words: *Aphelenchoides besseyi*; abnormal rice with small grains and erect panicles; identification; nematode; nematodiasis

In recent ten years, the symptom 'small grains and erect panicles' in rice was widespread in most rice growing areas of China, which generally caused yield loss ranging from 10% to 30%, even as high as 50% in heavily infected regions [1]. It is a serious problem in the middle of Jiangsu Province and some regions of southern Jiangsu Province. The occurrence of the phenomenon 'small grains and erect panicles' in rice totaled 3.3×10^5 ha during 2001-2003 in Jiangsu Province, resulting in at least 500 million kilogram of yield loss [2].

Some studies suggested that the 'small grains and erect panicles (SGP)' symptom was directly caused by the nematode *Aphelenchoides besseyi* [1, 2] or by fungal disease, herbicide, fertilizer or other environmental factors. The typical symptoms caused by *Aphelenchoides besseyi* included whitening of the top several centimeters of the leaves, necrosis, distortion and crinkling of the flag leaf, reduction in panicle development and a brown stripe at the boundary of ill and healthy parts. The aim of this research was to ascertain the nematodes attacking the improved varieties grown widely in Jiangsu Province were *A. besseyi* or other nematodes (the root-lesion nematode *Hirschmanniella oryzae* also attacks rice [3]), and evaluate the morphological characters of the nematode isolated from the unhealthy rice with SGP symptom in Jiangsu Province.

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MATERIALS AND METHODS

Distribution of *A. besseyi* on the unhealthy grains

The field experiments were conducted on Sanhe farm, Xuyi County, Jiangsu Province, China, with rice cultivars Wuyunjing 7 and Wuyunjing 3. The panicles with SGP symptom were collected and stored at 4℃ until being used. The seeds from each sample were split open, and soaked in water in Petri dish at 25℃ for 5 h. The distribution of *A. besseyi* on a panicle was determined by counting the nematode number per seed under a microscope.

Extraction, killing, fixing and measurement of nematode

The nematodes isolated from the rice with SGP symptom were collected by using Baermann funnel techniques, killed and fixed with fixatives FP4:1. Then the morphological characters were measured with Zeiss Axiovision 4 software and calculated with de Man formulae.

Preparation of nematode sample for scanning electron microscopy (SEM)

The killed nematodes were fixed with 2% glutaraldehyde coated by the qualitative filter paper for 2-4 h. After primary fixation, the nematodes were rinsed with double-distilled water for three times and each lasted for 20 min, and then were fixed with 1% osmium tetroxide for 3 h. After post-fixation, the nematodes were washed with phosphate buffer for 20 min three times. A five-graded series of acetone solution including 30%, 50%, 70%, 90% and 100% was employed to dehydrate the specimens and each step was 20-min long. The last step of 100% acetone should be repeated for three times. After immersion in the mixture of epoxy resin and acetone (1:3) for 1 h, the specimens were embedded with pure epoxy resin for 5 h and transferred directly onto the sieve, polymerized at 70℃ for 3-5 h and rinsed with epoxy propane after refrigeration. The nematodes were sputter-coated with gold and observed on the sample table [6].

RESULTS

Typical symptoms and distribution of *A. besseyi* on the panicles with SGP symptom

As shown in Fig. 1, the typical symptoms of small grains and erect panicles (SGP) in rice cultivar, Wuyunjing 7 included high sterility, small panicles, distorted glumes, small and chaffy grains, declined grain numbers, and stunted plant but without white tip on flag leaf. Compared with apparently healthy panicles, the abnormal panicles was shortened by 15.0-28.8%; The number of grains was decreased about 30%, sometimes even reaching 50%; Percentage of filled grains was reduced by 5-25% and 1000-grain weight was lightened by 10-30%. As listed in Table 1, the nematodes in filled grains were more than those in unfilled grains. The average number of nematodes per grain was 20.14 and the highest value was 74. The percentage of grain infested by the nematode was 92% in the abnormal panicle.

Fig. 1. The symptom of small grains and erect panicles of rice.

A, B, Abnormal panicles with small grains; C, D, E, Normal panicles.

Table 1. Distribution of *Aphelenchoides besseyi* in the rice panicle with SGP symptom.

Grains in the primary branches (from bottom to upside)	Primary branches in the panicles (from bottom to upside)											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
1st	35	14	13	10	4	5	2	23	9	6	5	3
2nd	35	6	59	7	43	53	15	34	45	5	11	13
3rd	31	24	21	8	25	13	35	9	17	4	39	
4th	10	2	9	1	16	23	16	17	12	47	8	
5th	10	13	40	3	12	37	12	0	5	0	6	
6th	0	36	5	0	45	14	25	16	6	18	0	
7th		67	39	8	3	17	2	0	24	5		
8th		39	58	4	8	50	2	52	9	2		
9th		32	20	32	46	3	15	63	0	8		
10th		35	46	18	48	25	30	37	12			
11th		21	21	5	55	51	40	38				
12th		15	3	1	0	32	74	3				
13th				1	35	0	26	22				
14th				7	0	51	16					
15th				17	17		25					
16th				38								

Rice cultivar, Wuyunjing 7. Samples were collected from Sanhe farm, Xuyi county, Jiangsu Province in China in October 2004.

SEM morphological characters and measurements of *A. besseyi*

The morphological characters of *A. besseyi* viewed with SEM (Fig. 2) were as follows: lateral fields were about one-fourth as wide as body, with 4 incisures, lip region was round, slightly offset and wider than body at lip base, about half as wide as mid-body, height of head was less than width, labial framework was hexaradiate, with 4-5 annules fine, indistinct. Terminus bore a mucro of diverse shape with 3-4 pointed processes. Comparison of measurements of *A. besseyi* from abnormal panicles in Jiangsu, China with one of the Senegal by Fortuner (1970) showed that they were same except for longer spicules, stylet and male testis in our results (Table 2).

Structural characters of nematode body by light microscope

Female: arcuate ventrally when relaxed, labial framework lightly sclerotized, stylet slender with slight basal swelling, anterior part of stylet sharply

Table 2. Comparison between measurements of *A. besseyi* from abnormal rice panicles with SGP symptom in Jiangsu, China and one of the Senegal by Fortuner (1970).

Character	*Aphelenchoides besseyi* (Senegal; Fortuner, 1970)		*Aphelenchoides besseyi* (Jiangsu, China; 2004)	
	♂	♀	♂	♀
L(mm)	0.57-0.84	0.53-0.61	0.68-0.83	0.55-0.64
a	39.00-53.00	40.70-46.90	32.38-41.19	29.39-41.52
b	9.20-13.10	8.87-10.70	9.84-10.66	8.55-9.89
b'	4.06-5.77	3.57-4.91	4.20-5.08	3.03-4.83
c	13.80-20.40	16.00-20.00	14.91-21.20	14.10-19.07
V	68.70-73.60	-	70.23-75.43	-
Stylet (μL)	10.00-12.50	10.00-12.50	11.51-13.10	11.44-13.08
T	-	28.00-52.00	-	34.51-77.69
Spicules(μL)	-	18.00-21.00	-	19.40-22.33

L, Total body length in mm; a, Body length/greatest body width; b, Body length/distance from anterior end to junction of oesophagus and intestine; b', Body length/distance from anterior end to posterior end of oesophageal glands; c, Body length/tail length; V, Distance of vulva from anterior×100/body length; Stylet, Stylet length in μL; T, Distance from cloaca to most anterior part of testis ×100/ length; Spicules, Spicules length in μL.

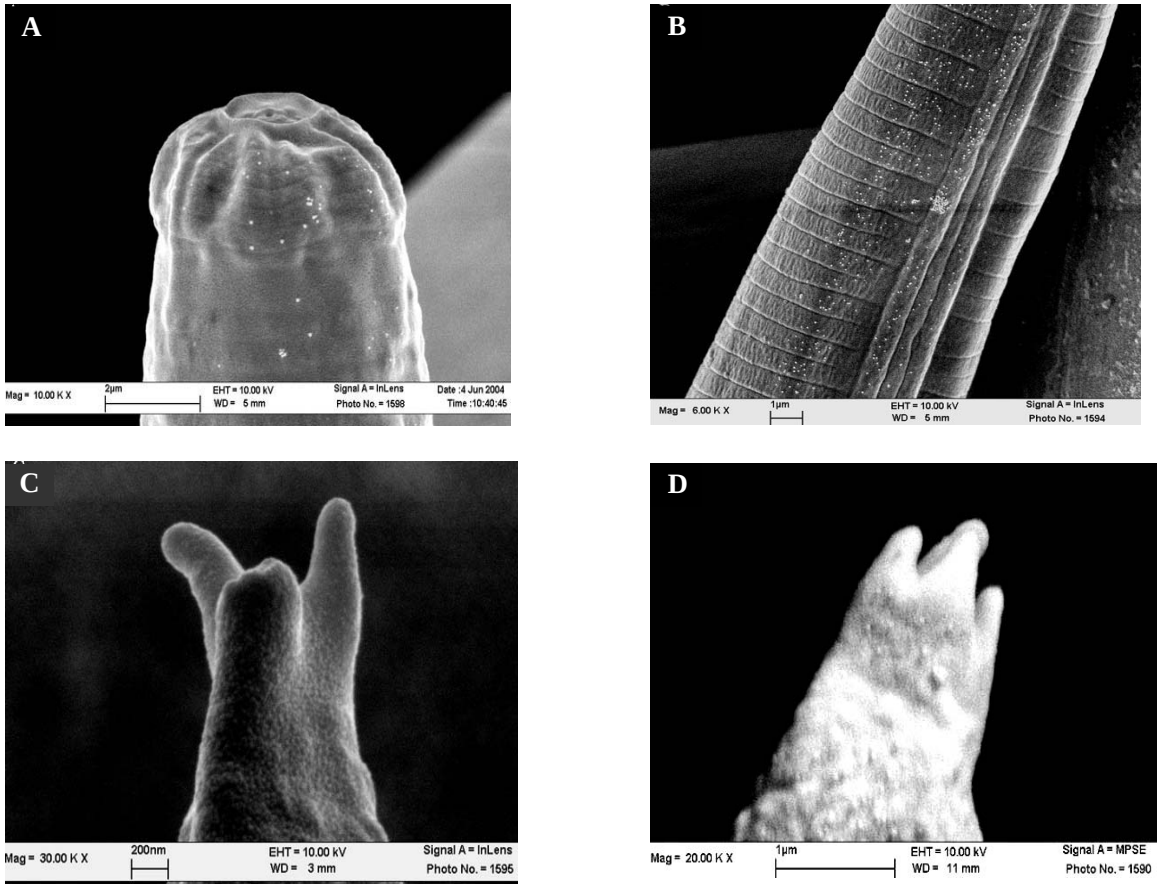

Fig. 2. SEM morphological characters of *A. besseyi* from abnormal panicle with SGP symptom in rice.

A, Female head; B, Lateral field; C, D, Shape of mucro.

Table 3. Comparison of morphological character on *A. besseyi*, *A. ritzemabosi* and *A. fragariae*.

Character	*A. besseyi*	*A. ritzemabosi*	*A. fragariae*
Incisure	4	4	2
Mucro	Diverse shape with 3-4 pointed processes	Terminal peg which has 2-4 minute processes	Simple blunt spike
Oocytes	Multiple rows	Multiple rows	Single file
Post-vulval distance	< half of the vulva-anus distance	> half of the vulva-anus distance	> half of the vulva-anus distance
Apex	Lack	Lack	Moderately developed apex
Rostrum	Moderately developed rostrum	Lack	Moderately developed rostrum

poited, about 45% of total stylet length. Median oesophageal bulb oval, with a distinct valvular apparatus slightly behind its centre. Oesophageal glands extending dorsally and subdorsally for 4 to 8 body-widths over intestine, Vulva transverse, with slightly raised lips. Ovary relatively short and not extending to oesophageal glands, with oocytes in 2-4 rows, spermatheca elongate oval, usually packed with sperms. Post-vulval uterine sac narrow, not containing sperms, 2.5-3.5 times anal body width long but less than one-third distance from vulva to anus. Tail conoid 3.5 to 5 anal body widths long.

Male: About as numerous as females, posterior end of body curved to about 180 degrees in relaxed specimens. Lip region, stylet and oesophagus as described for females; tail conoid, with terminal mucro with 2-4 pointed processes. Spicules typical of the genus except that the proximal end lacks a dorsal process (apex) and only has a moderately developed ventral one (rostrum) (Fig. 3).

Diagnosis and relationships of the nematode

The nematode from the abnormal small panicles in rice cultivar Wuyunjing 3 was identified as *Aphelenchoides besseyi* Christie, 1942 by morphological characters and measurements.

It is characterized by its lateral fields about one-fourth as wide as body, with 4 incisures; the terminus bearing a mucro of diverse shape with 3-4 pointed processes; the female post-vulval uterine sac extends less than 50% of distance from vulva to anus, no sperm in it; oocytes usually have in 2-4 rows; the

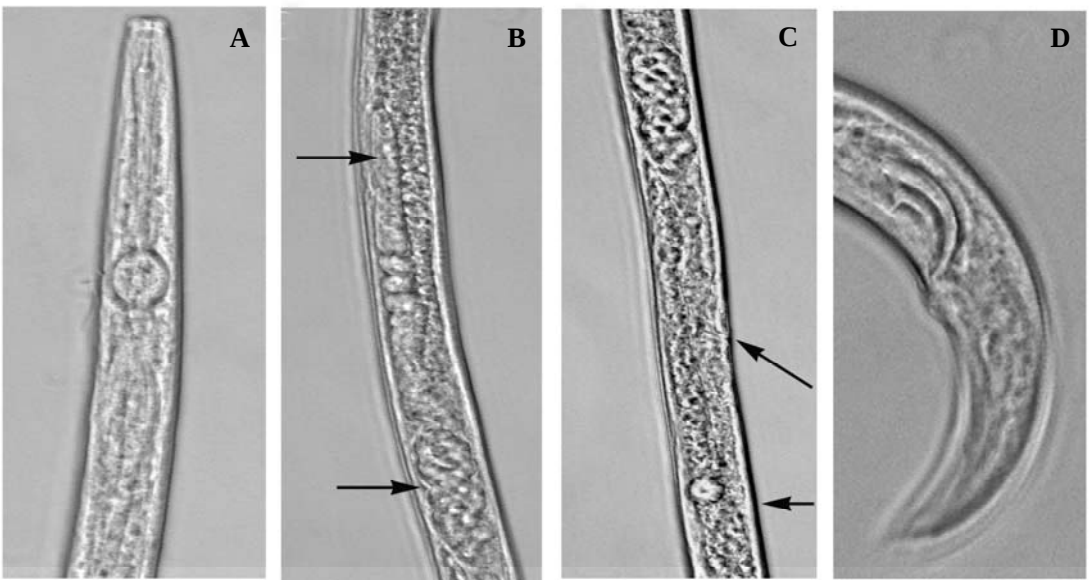

Fig. 3. Light micrographs of *A. besseyi* from abnormal panicles with SGP symptom in rice.

A, Median oesophageal bulb; B, Ovary anterior portion of the metacarpus showing theoocytes and spermatheca; C, Vulva and post-vulvaluterine sac; D, Spicules.

male spicules has a moderately developed rostrum.

Aphelenchoides besseyi Christie, 1942 is most similar to *A. ritzemabos*i Steiner & Buhrer 1932 from chrysanthemum with 4 incisures and oocytes usually arrange in multiple rows, but can be distinguished by the female post-vulval uterine sac extending for more than half the vulva-anus distance, and often containing sperms; tail bearing a terminal peg which has 2-4 minute processes pointing posteriorly and giving it a paint-brush-like appearance; lacking a dorsal or ventral process at proximal end. *A. ritzemabos*i is an obligate plant parasite, inhabiting leaves and buds in chrysanthemum, and it is one of the species popularly called bud and leaf nematodes. This nematode is a major pest of chrysanthemum in Europe, North America and Japan. Another important host is the strawberry on which it is usually found to be associated with another related species *A. fragariae.* However it was not found in association with rice.

DISCUSSION

Aphelenchodes besseyi was a migrating ectoparasitic nematode, feeding on the growing points of rice. It moved upward as the plant grows up. *A. besseyi* was capable of withstanding desiccation and found in a quiescent state beneath the hulls of rice grains, and transmitted through the seeds [8, 9]. It could also cause 'summer dwarf' on the strawberry in Jiangsu Province [10]. In Taipei, China, this nematode led to losses on the *Dendrobium nobile* at a certain extent [11]. It endangered the leaves and the tubers of *Dioscorea trifda* through blacking and drying the leaves, putrefying the tubers and chapping the surface in Latin America [11].

The rice attacked by *A. besseyi* showed various symptoms in different rice cultivars and different environments. The disease caused by *A. besseyi* on rice was known as 'white tip'. It was ectoparasitic feeding on the young tissue resulting in whitening of 1-8 cm of leaf tips, which later developed into necrotic, crinkled and distorted leaf enclosing the panicle. In the study, we found much more rice cultivars showed small grains and erect panicles but not the white tip, including Wuyujing 3 and Wuyunjing 7. The typical symptoms were as follows: whole rice plant was dwarfed, i.e. the lengths of rice panicles and flag leaf were shorter than normal, the number of grains was decreased, the grains were small and some of them were distorted, and yields were reduced. In our research, we also found that infested rice showed not only white tip but also small grains and erect panicles in Zhendao 2. These might be indicated that whether the small grains and erect panicles or not was connected with the heredity of rice. In addition, we observed that the environment could influence the symptoms of the desease. When the diseased seeds of Wuyujing 3, which showed small grains and erect panicles symptom at the farm of Baima Lake in Jiangsu Province, were planted in Hainan, the plants did not show typical symptoms and no nematode was detected within the harvested grains. It required more direct evidence to confirm the relationship between the rice and nematodes. The critical result of the nematode isolated from the rice with SPG symptoms manifested that the nematode was *A. besseyi*. All above-mentioned demonstrated that small grains and erect panicles of rice were caused by *A. besseyi*.

Masking of symptom means that original symptom was weak or disappeared when changing the environment or using the pesticides. But the pathogen has been existed in the plant yet, when the environment recovered or the effect of pesticide disappeared, symptom reappeared on the plant. Through investigation, we found that some other genotypes of rice such as Zhengdao 2 showed white tip and small grains and erect panicles on the plant. The results of this study showed that abnormal rice with small grains and erect panicles happening on the rice cultivars of Wuyujing 3 and Wuyunjing 7 was the new symptom caused by *A. besseyi*.

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