Infestation of the rice white tip nematode, *Aphelenchoides besseyi* (Christie, 1942), in Myanmar

Lin Zar Ni[1], Yu Yu Min[2] and Koki Toyota[3]

[1]Department of Agricultural Research, Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar
[2]Department of Agricultural Microbiology, Yezin Agricultural University, 15013, Yezin, Nay Pyi Taw, Myanmar
[3]Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, 184-8588, Koganei, Tokyo, Japan
e-mail: dr.yuyumin@yau.edu.mm

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Summary. The investigation of the rice white tip nematode *Aphelenchoides besseyi* infestations was carried out on rice seeds. One hundred and eleven samples of rice seeds were collected from Ayeyarwaddy, Bago, Shan State, Mandalay and Nay Pyi Taw regions, Myanmar, in which various local varieties (rainfed-lowland, irrigated-lowland, quality, upland, drought tolerance, submergence, salt tolerance, aerobic and hybrid rice) were grown. Nematodes were extracted from 100 discoloured rice seeds with three replications. *Aphelenchoides besseyi* was detected in rice seed samples from all collected regions, in which 53 out of 111 rice seed samples were infested with *A. besseyi*. The maximum population was observed in upland, quality and rainfed-lowland rice varieties, ranging from 193-459 indiv. (100 rice seeds)$^{-1}$. Four rainfed-lowland, one irrigated-lowland, five quality and five upland rice varieties were infested with the economic damage level of > 30 indiv. (100 rice seed)$^{-1}$. The densities of *A. besseyi* in relation to rice varieties are discussed.

Key words: *Oryza sativa*, rice varieties.

Rice (*Oryza sativa* L.) is widely cultivated in different regions of Myanmar in both rainfed and irrigated production systems. Among the rice producing countries in the world, Myanmar is the sixth in sown area and seventh in total production of rice (FAORAP, 2014). The total sown area was 7.26 million ha and the yield was 3,920 kg ha^{-1} in 2018. Rice production is sufficient to fulfill the local consumption needs and the surplus is being exported. Since export of rice contributes to Myanmar economy, an effective strategy for Myanmar's economic development is to increase the rice production and improve the quality of rice seeds for quarantine purposes.

The white tip nematode (*Aphelenchoides besseyi*) is a seed borne nematode and remain a quarantine pest in rice growing countries because of the potential danger of the emergence of more virulent pathotypes (CABI, 2019). After sowing rice seeds, anabiotic *A. besseyi* become activated in water and then they first feed on the tender primordium of sprouting seeds. It feeds endoparasitically in the coleoptile for 7-10 days and ectoparasitically within the innermost leaf sheath during other plant growth stage. The nematode number increases at the later tillering stage and is associated with the reproductive phase of plant growth (Tsay *et al.*, 1998).

According to a survey result of the Rice Research Section in 2017, 66% of total rice cultivated areas grow different rice varieties of which 36.5% are high yielding varieties, 12.5% are local rice varieties, 1.6% are upland rice varieties, 14% are quality rice varieties that have good aroma and eating quality, 0.6% are others and 1.4% are hybrid rice varieties in Myanmar (DAR, 2017). These rice varieties are cultivated depending on the climatic and environmental conditions of different growing regions. White tip disease has been recorded in rice fields of Myanmar (Aung *et al.*, 1993) but research on *A. besseyi* is very limited. There must be potential for infestation of *A. besseyi* in different rice varieties and areas because *A. besseyi* can survive on the seeds for several years and, thus, can be distributed by seeds (Tulek & Çobanoğlu, 2012). Therefore, we aimed to analyse

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the occurrence of *A. besseyi* in rice seed samples to generate information on the extent of its distribution in the five major different rice growing areas of Myanmar, and on the levels of infestation on different rice varieties.

MATERIAL AND METHODS

Collection sites of rice seeds samples. Rice seed samples were collected in the 2017 monsoon rice growing season from the regions of Ayeyarwaddy (16°N, 94°E), Bago (east) (17°N, 95°E), Mandalay (south) (21°N, 96°E), Nay Pyi Taw (19°N, 96°E) and Shan State (north) (22°N, 97°E) (Fig. 1). All seed samples were stored at room temperature (27-30°C) and names of rice varieties from where the samples were obtained were recorded. A total of 111 rice seed samples were sampled and 100 g of each rice seed sample were taken for analysis.

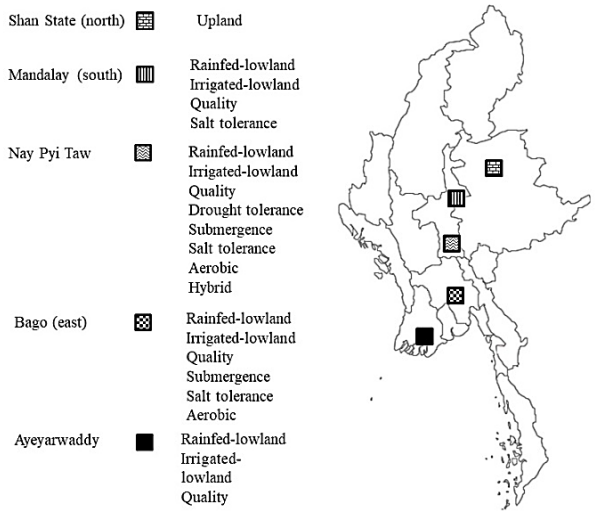

Fig. 1. Rice seed samples collection sites across the major rice growing regions of Myanmar and its cultivated rice varieties.

Nematode extraction and counting. From each seed sample, 100 discoloured seeds (deformed seeds with distorted glumes) were picked up and pounded with a motor and pestle. The crushed grains were placed on a piece of muslin cloth fitted over a sieve (1 mm mesh size) and then put in a Petri dish. The water level in the Petri dish was adjusted to touch the bottom of the sieve and muslin cloth. Another Petri dish was used to cover on the top of the assembly to prevent water evaporation. The Petri dishes were left for 24 h (12 h in the light and 12 h in the dark) at room temperature to allow the nematodes to move out of the seeds into the water. The sample solution from each Petri dish was collected and the presence of nematodes was examined in counting dishes, under a compound microscope (OLYMPUS CX22 LED RFS1). For the identification, nematodes recovered from rice seeds were killed by hot water (100°C for 1 min). The nematode was identified as having body shape slender, straight to slightly arcuate when relaxed; annules fine, stylet slightly offset; medium pharyngeal bulb oval; vulva transverse with slightly raised lips, tail conoid, with terminal mucrons with 2-4 pointed processes, spicule present (male) (CABI, 2019). The number of *A. besseyi* was counted and expressed per 100 rice seeds. Based on the number of nematodes present, seed samples were indexed at different levels of infestation according to Shahabi *et al.* (2016): low level of population (< 50 nematodes), moderate population (50-100 nematodes) and very high population density (> 100 nematodes).

RESULTS

Aphelenchoides besseyi was detected in rice seed samples from all regions, with 47.8% (53 out of 111) of seed samples being infested. The highest infestation was 91% in Ayeyarwaddy, followed by 58% in Bago (east), 46% in Shan State (north), 23% in Nay Pyi Taw and 7.7% in Mandalay (south) regions. The estimation of nematode population per 100 grains was used to determine the severity of *A. besseyi* infestation and among the infested samples, 83.0% had a low population level (< 50 nematodes (100 seeds)$^{-1}$), 7.6% had moderate population (50-100 nematodes (100 seeds)$^{-1}$) and 9.4% had very high population density (> 100 nematodes (100 seeds)$^{-1}$) (Table 1).

Among the rice varieties, 58% infestation in rainfed-lowland, 48% in irrigated-lowland, 46% in upland, 52% in quality, 0% in drought tolerance, 25% in salt tolerance, 50% in aerobic rice, 33% in submergence and 0% in hybrid rice were recorded. The maximum population ranged from 1 to 459 indiv. (100 seeds)$^{-1}$ in upland seed sample while the minimum population ranged from 1 to 3 indiv. (100 seeds)$^{-1}$ in aerobic rice variety (Table 2).

Four rainfed-lowland, one irrigated-lowland, five quality and five upland rice seed samples were infested with *A. besseyi* ranging from 31 to 459 indiv. (100 seeds)$^{-1}$. Yamaguchi (1977) reported that 30 or more live *A. besseyi* (100 seeds)$^{-1}$ can cause economic losses in a susceptible variety on rice. Of nine types of rice varieties, the maximum populations ranging from 193-459 indiv. (100 seeds)$^{-1}$ were observed in the upland rice variety followed by quality and rainfed-lowland rice varieties.

Table 1. Distribution and incidence of the white tip nematode, *Aphelenchoides besseyi*, in five different rice growing regions.

Rice growing region	Major type of rice varieties grown	No. of samples collected	No. of samples infested	Nematode population (100 seeds)$^{-1}$ (min-max)	Percentage of sample infested
Ayeyarwaddy	Rainfed-lowland	8	8	2-181	100
	Irrigated-lowland	6	5	0-51	83
	Quality	8	7	0-150	88
		22	20	2-181	91
Bago (east)	Rainfed-lowland	2	2	4-31	100
	Irrigated-lowland	5	3	2-11	60
	Quality	7	5	0-31	71
	Submergence	2	1	0-2	50
	Salt tolerance	2	0	0	0
	Aerobic	1	0	0	0
		19	11	2-31	58
Shan (north)	Upland	35	16	0-459	46
Nay Pyi Taw	Rainfed-lowland	6	1	0-5	17
	Irrigated-lowland	5	1	0-9	20
	Quality	6	1	0-13	17
	Drought tolerance	1	0	0	0
	Submergence	1	0	0	0
	Salt tolerance	1	1	2-13	100
	Aerobic	1	1	0-2	100
	Hybrid	1	0	0	0
		22	5	2-13	23
Mandalay (south)	Rainfed-lowland	3	0	0	0
	Irrigated-lowland	4	0	0	0
	Quality	5	1	0-2	20
	Salt tolerance	1	0	0	0
		13	1	2	8
Total		111	53	0-459	47.8

Data are means of three replications.

Table 2. White tip nematode, *Aphelenchoides besseyi*, infestation in collected rice varieties, nematode population and percentage of infested seed samples.

Type of rice variety	No. of samples collected	No. of samples infested	Nematode population (100 seeds)$^{-1}$ (min-max)	Percentage of samples infested	Mean nematode population (100 seeds)$^{-1}$	No. of rice varieties infested with over 30 *A. besseyi* (100 seeds)$^{-1}$ (min-max)
Rainfed-lowland	19	11	2-193	58	58	4 (52-193)
Irrigated-lowland	21	10	1-77	48	26	1 (33-77)
Upland	35	16	1-459	46	50	5 (32-459)
Quality	25	13	1-240	52	115	5 (31-240)
Drought tolerance	1	0	0	0	0	0
Salt tolerance	4	1	2-21	25	7	
Aerobic rice	2	1	1-3	50	2	0
Submergence	3	1	1-4	33	2	0
Hybrid	1	0	0	0	0	0
Total	111	53	1-459	48		

Data are means of three replications.

DISCUSSION

The infested rice seed samples were identified as having the potential to spread and cause severe infection, which can be damaging to rice plants and adversely affect yield. It was noticed that the cultivation period of rice depends on the length of the vegetative growth stage of each variety, and the reproduction of *A. besseyi* reached a peak during the plant reproductive stage (Tsay *et al.*, 1998). Generally, the reproductive stages of rice varieties lasts for 65 days; therefore, it can be assumed that the difference duration of rice varieties is not related to the population level of *A. besseyi* because the peak population of *A. besseyi* was detected only at the milky stage of rice plants (unpublished data), which is earlier than the reproductive stage.

The white tip nematode is very much widespread in almost all the rice growing areas of the world because of its dissemination in seed, but its importance varies between regions, countries and localities (Jamali *et al.*, 2006). This nematode resulted in average yield losses of up to 70% in susceptible varieties (Rahman & Miah, 1989). Aung (1997) suggested that *A. besseyi* is widely distributed throughout the entire country in Myanmar and yield losses can reach up to 50% depending on the nematode population density and the susceptibility of the crop.

In this study, *A. besseyi* was detected in rice seed samples of different rice varieties in all collected regions. The level of infestation differed among the varieties and some varieties were infested with high numbers of *A. besseyi*. The farmers can overlook the presence of *A. besseyi* in rice seeds and are faced with the constant risk of introducing foliar nematode-infected asymptomatic rice seeds into their production, thus causing rapid spread of the nematodes to adjoining healthy fields and to other rice growing regions. The data indicate that infested rice seeds should be treated before sowing to control its damage and to prevent further spreading of the nematode disease. Garcia *et al.* (2000) eradicated *A. besseyi* by heat treatment at 60°C for 10 min and 57°C for 15 min, suggesting that hot water treatment is useful to control the disease. However, further studies are needed to investigate crop loss assessment, and effective management methods for the control of rice white tip nematode disease.

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Lin Zar Ni, Yu Yu Min and Koki Toyota. Рисовая нематода *Aphelenchoides besseyi* (Christie, 1942) в Мьянме.

Резюме. Проведено обследование посадочного материала риса с целью выявления его пораженности рисовой нематодой *Aphelenchoides besseyi*. В нескольких районах Мьянмы: штате Шан, провинциях Пегу, Мандалай и Иравади, а также столичном регионе Нейпьидо было собрано 111 проб местных сортов семян риса. Изученные сорта различались по своим особенностям и включали суходольные и поливные низинные сорта, высококачественные сорта, горные сорта, устойчивые к засухе, затоплению, повышенному содержанию соли, аэробные и гибридные сорта. Нематод извлекали из 100 обесцвеченных семян с тремя повторностями. *Aphelenchoides besseyi* был отмечен в образцах семян риса из всех изученных регионов страны. Всего зараженными *A. besseyi* оказались 53 образца из 111 обследованных. Максимальная численность нематод была отмечена в горных районах страны, на высококачественных и суходольных сортах при численности в 193-459 особей на 100 семян риса. Четыре суходольных сорта, один поливной низинный, пять высококачественных и пять горных сортов были поражены рисовой нематодой с численностями выше уровня экономического ущерба – > 30 особей на 100 семян риса. Обсуждается связь численности особей *A. besseyi* с сортом пораженного риса.