Resistance of rice cultivars and lines to the white tip nematode *Aphelenchoides besseyi*

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Abstract
Aphelenchoides besseyi Christie is a major nematode pathogen in rice known as the causal agent of white tip disease. Application of resistant cultivars requires the identification of resistance sources, which can be included in breeding programs. Nine accessions of rice were evaluated for resistance to *A. besseyi* under field conditions. The experiment was conducted as split plots in a randomized complete block design with three replications. The results showed that the cultivars and lines differed in their response to infection. After the artificial inoculation with the white tip nematode, the highest percentages of plants showing symptoms were cultivars Osmanchik 97 and Cameo. There are no immune genotypes among the studied ones. Only cultivar HG 1 was highly resistance to *A. besseyi.*

Key words: rice; resistance; white tip nematode; *Aphelenchoides besseyi*; symptom expression

INTRODUCTION

Rice (*Oryza sativa*) is one of the major food crops for more than a third of the world's population (Xie et al., 2019). In order to meet the high requirements of production, modern rice varieties should have a set of valuable features and qualities. Thus, along with high productivity, the new cultivars must be characterized by good quality indicators and resistance to abiotic and biotic stress factors (Muhammad et al., 2013).

Rice is an essential food crop hosting various pests and diseases including plant-parasitic nematodes which pose a threat to production. Among over 200 plant-parasitic nematodes associated with rice, the rice white tip nematode, *Aphelenchoides besseyi*, is considered to be a major contributor to the seed-borne pathogens of rice (Duncan & Moens, 2013; Prot, 1994). It is widely distributed throughout almost all the rice-growing regions of the world, including in Bulgaria (Bridge et al., 2005; Samaliev & Stoyanov, 2008). The white tip nematode is included as a quarantine pest in the list of European and Mediterranean Plant Protection Organization (EPPO, 2017). The yield losses caused by this nematode in rice showed differences according to rice cultivar, growing year, temperature, cultural practices, and other variable factors. In infested fields, the average yield losses range from 10% to 30%, in fields where all plants have been attacked, yield losses of up to 70% for susceptible cultivars and 20% for resistant cultivars have been reported (Prot, 1992).

As a seed-borne nematode, *A. besseyi* survives anhydrobiotically in stored grain for several years (Tiwari & Khare, 2003). When infected seed is planted, the nematodes become active, emerge from the seed and feed on the above-ground tissues of rice, parasitising the leaves and other young tissues as a foliar nematode. During the early growth stages of rice, *A. besseyi* is found in low numbers in all green tissues, but is more abundant in the stem. A rapid increase in nematode numbers takes place at the flowering stage, when nematodes are mainly found in the florets of the panicles (Xie et al., 2019).

Affected plant tillers the tip of the leaves whiten for a distance of 3 to 5 cm. This causes typical symptoms of "white-tip" in leaves, which later become necrotic, then die off and shred. The panicles are shorter and often atrophied at the tips. The fertile flowers sometimes produce misshapen grains with a low and delayed germination potential (Tülek et al., 2014).

In recent years, monoculture growing of rice has become increasingly common. This way of production, the multiplication of some weed species and fungi of the genus *Fusarium*, which are also a good host of the white tip nematode, created favorable conditions for increasing the population of *A. besseyi* (Karov, 2001).

Due to increasing concern about environmental contamination by pesticides, plant resistance is considered to be the most promising component in parasitic nematode management programmes. Resistance to nematodes has been defined as the ability of plants to restrict or prevent nematode reproduction (Jamali & Mousanejad, 2011; Trudgill, 1991).

The aim of this study is to evaluate the resistance to white tip nematode of introduced rice cultivars and selected by us Bulgarian lines.

MATERIAL AND METHODS

Plant materials and growth conditions

The study was conducted during the period 2017-2019 at Maritsa Vegetable Crops Research Institute, Plovdiv under field conditions. The included accessions were selected randomly without prior information about their reaction to the white tip nematode. Osmanchik 97 and Gala with origin - Turkey, the Italian cultivars Cameo, Luna, CRLB 1 and Fenomino, HG 1 from Japan and the Bulgarian lines №19 and №77 were evaluated. The experiment was conducted as split plots in a randomized complete block design with three replications (artificially infected plants and uninfected control). Each experimental plot was 2 m^2. Rice plants were inoculated at tillering stages with 500 nematodes/plant.

Nematode preparation

Aphelenchoides besseyi was initially isolated from infected seeds collected from rice fields of Southern Bulgaria (Pazardzhik and Plovdiv). The nematodes were extracted by the modified Baermann funnel technique both before the sowing and after the harvest (Hooper, 1986). Seeds were separated from hulls (lemma and palea). Each sample was kept in water for 48 hours. The nematodes in two aliquant parts of 1 ml water suspension from each extract were counted in counting dishes. Using a stereomicroscope, the average value of both aliquant parts was calculated referring to 100 seeds.

Method for resistance assessment

White tip symptoms were recorded 3 to 6 weeks after inoculation and the percentage of infected plants were determined. Nematode population density was measured at the end of the growing season by counting the number of nematodes in 100 seeds. Resistance of the rice cultivars/lines to the *A. besseyi* was assessed by counting the numbers of nematodes from the plants and the development of white tip symptoms, using a diseases index scale (Popova et al., 1989; Popova, 1991; Popova et al., 1994).

The resistance rated as follows:

0- White tip symptoms and nematodes absent.

1- White tip symptoms absent and nematode numbers 1-10 per plant.

3- White tip symptoms absent and nematode numbers >10 per plant.

5- White tip symptoms present and many nematodes present.

The average index of infection of each cultivar was estimated using the formula:

$$P = \frac{\Sigma(B \times n)}{N}$$

Σ (B x n) - Sum of the number of plants (n) and corresponding index of infection (B). N - total number of the infected plants.

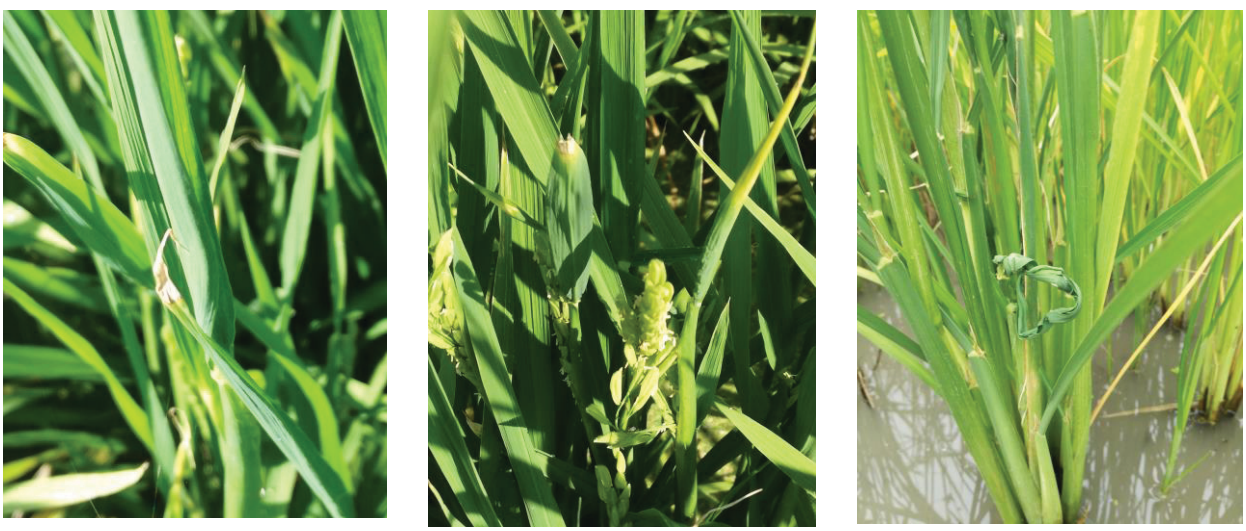
Each tested cultivar/line was classified on basis of the average index of infection: 0 – immune, 0.1-1.0 – highly resistant, 1.1-3.0 – moderately resistant, 3.1- 4.0 – moderately susceptible, 4.1-5.0 – highly susceptible.

RESULTS AND DISCUSSION

In field conditions, *Aphelenchoides besseyi* infects and multiplies in all tested cultivars and lines. During the flowering, plants of each cultivar were examined and evaluated for presence of symptoms. In nematode-infected plants we reported the pres-

ence of the typical whitening of the leaf tip of the central stem and the tillers, the panicles are half out of the flag leaf or are shorter with immature underdeveloped grains. Also we founded the presence of deformed and twisted leaves (Fig. 1), as well as plants without symptoms.

The results of the observations and the determined percentage of plants with and without symptoms are presented in Table 1. With regard to the response to white tip nematode, the tested cultivars/lines varied. Plants with the symptom of "white tip" disease, partially emerged panicle, underdeveloped and sterile grains, deformed and twisted leaves were reported in the cultivars Osmanchik 97 and Cameo. The cultivar Gala and lines 19 and 77 showed the symptom of "white tip" disease. In cultivars HG 1, Luna, Fenomino and CRLB 1 (except 2017), no symptoms of the disease were established. Feng et al. (2014) also assessed the effect of *A. besseyi* on 27 cultivars of rice (23 japonica and 4 indica) in the field. They established that most of cultivars were lack of the characteristic symptom of white tip,



A B C

Fig. 1. Typical symptoms on rice

White tip on leaf; B. Damaged panicle; C. Twisted leaf

Table 1. Response of the studied rice cultivars and lines to *Aphelenchoides besseyi*

Cultivar/Line	Symptoms of disease	Infected plants, %		
		2017	2018	2019
Osmanchik 97	White tip; partially emerged panicle; immature and empty grains	40	40	35
Cameo	White tip; partially emerged panicle; immature and empty grains; deformed and twisted leaves	60	55	75
HG 1	No symptoms	0	0	0
Line №19	White tip; partially emerged panicle; immature and empty grains	25	25	10
CRLB 1	White tip and no symptoms	10	0	0
Line №77	White tip	25	20	10
Luna	No symptoms	0	0	0
Gala	White tip	35	25	20
Fenomino	No symptoms	0	0	0

which was seen less frequently than the other two symptoms, namely small grains and erect panicles. Different reaction of rice plants infested by the nematode was observed by Jamali & Mousanejad (2011). Susceptible plants express also other typical symptoms such as shortening of flag leaf which twisted at apical portion and hinders the emergence of panicle, reduction of panicle length and the grain number, spikelets with distorted glumes and deformed kernels, production of tillers from the uppers nodes.

The highest percentage of plants with symptoms in all three experimental years was reported in the Italian cultivar Cameo from 55% to 75%, respectively. No infested plants were observed in cultivars HG 1, Luna and Fenomino (Table 1).

According to assessment method, the results showed that no accession was immune. Three cultivars, Osmanchik 97, Cameo and Gala were moderately susceptible. Moderate resistance was identified with lines №19, №77, and cultivars CRLB 1, Luna and Fenomino. Only one cultivar HG 1 was highly resistant and the results obtained correspond to the assessment during flowering when no identified plants with symptoms (Table 2).

All cultivars/lines were infected successfully by *A. besseyi* and varied in the nematode population density, with Osmanchik 97 showing the highest number of nematodes per 100 seeds and HG 1 showing the lowest (Table 2).

Resistant cultivars are effective in controlling *A. besseyi* (Tülek & Cobanoglu, 2010). Many resistant cultivars have been developed, which have greatly limited economic losses (Jamali & Mousanejad, 2011; Peng & Moens, 2003). Resistance to *A. besseyi* was reported in the USA in three cultivars - Arkansas Fortuna, Nira 43, and Bluebonnet, which subsequently became the sources of many more resistant cultivars (Bridge et al., 1990). In Russia, an assessment of the resistance to *A.besseyi* of 1003 rice cultivars from different regions was made. Three North American cultivars Bluebonnet, Bluebonnet 50 and Starbonnet were immune, ten were highly resistance, 164 were moderately resistant and 826 were susceptible or highly susceptible to *A. besseyi* (Popova et al., 1994).

CONCLUSIONS

No immune accessions were found in the study of the varietal response to the white tip nematode *Aphelenchoides besseyi* Christie.

In view of the purposes of breeding in the direction of resistance to the white tip nematode, cultivar HG 1 can be included in the breeding programs as a source of resistance.

Bulgarian lines are moderately resistant, which is a promising start for working with this nematode.

Table 2. Results of assessment of rice cultivars for resistance to white tip nematode (*Aphelenchoides besseyi*)

Cultivar/Line	Origin	Resistance category*	Average index of infection	Nematode/Plant Average (min- max)
Osmanchik 97	Turkey	MS	3.6	97.34 (0-220)
Cameo	Italy	MS	3.2	71.31 (0-200)
HG 1	Japan	HR	0.9	6.41 (0-35)
Line №19	Bulgaria	MR	2.4	56.29 (0-180)
CRLB 1	Italy	MR	2.0	45.26 (0-160)
Line №77	Bulgaria	MR	1.6	35.25 (0-120)
Luna	Italy	MR	1.6	34.90 (0-120)
Gala	Turkey	MS	3.3	86.30 (0-210)
Fenomino	Italy	MR	1.8	43.50 (0-140)

*HR: Highly Resistant, MR: Moderately Resistant, MS: Moderately Susceptible, HS: Highly Susceptible.

The Italian cultivars CRLB 1, Luna and Fenomino are tolerant to *Aphelenchoides besseyi* and can be recommended for growing.

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