Evaluation of Chinese rice varieties resistant to the root-knot nematode *Meloidogyne graminicola*

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**Abstract**

The root-knot nematode *Meloidogyne graminicola*, which is distributed worldwide, is considered a major constraint on rice production in Asia. The present study used the root gall index and number of nematodes inside the roots to evaluate resistance/susceptibility to *M. graminicola* in different subpopulations of *Oryza sativa* (aus, hybrid aus, indica, hybrid indica, temperate japonica, tropical japonica). Nematode development in highly resistant varieties was also evaluated. Analyses of randomly selected 35 varieties showed the number of *M. graminicola* nematodes inside the roots correlated very strongly \((r=0.87, P≤0.05)\) with the nematode gall index, and the results from pot and field experiments revealed similar rankings of the varieties for resistance/susceptibility. Among the 136 tested varieties, temperate *japonica* displayed the highest gall index, followed by tropical *japonica*, hybrid *indica*, and hybrid *aus*. Zhonghua 11 (aus) and Shenliangyou 1 (hybrid aus) and Cliangyou 4418 (hybrid *indica*) were highly resistant to *M. graminicola* under both pot and field conditions. Further examination of nematode development suggested that compared to susceptible rice, *M. graminicola* penetrated less often into highly resistant varieties and more frequently failed to develop into females. The promising varieties found in the present research might be useful for the breeding of hybrid rice in China and for the further development of practical nematode management measures.

**Keywords:** *Meloidogyne graminicola*, rice varieties, resistant, susceptible, resistance evaluation

1. **Introduction**

Cultivated rice (*Oryza sativa*) is the predominant staple food for most countries in Asia and provides 20% of the dietary energy supply worldwide (Schatz *et al*. 2014). Rice varieties in Asia are traditionally classified into two major groups: *indica* and *japonica* (Chang 2003). *Indica* varieties are widely cultivated in lowland tropical areas, whereas *japonica* varieties are cultivated in both lowland and high-elevation upland areas. Based on differences in
isozymes and simple sequence repeats (SSR) markers, the indica group of Asian rice can be further subdivided into two subpopulations (indica and aus) and the japonica group into three subpopulations, temperate japonica, tropical japonica and aromatic (Glaszmann 1987; Garris et al. 2005; Zhao et al. 2010). These subpopulations exhibit broad differences in geographical distribution, morphological traits, physiological differentiation and genetic divergence (Chang 1976).

The root-knot nematode Meloidogyne graminicola is widely distributed in every rice-producing country in South and Southeast Asia and is considered one of the most important pests affecting Asian rice (Kynadt et al. 2012; Ji et al. 2013). After second-stage juveniles (J2s) hatch from eggs, they invade at the root tips and inject pharyngeal secretions into vascular cells to induce a specialized nematode feeding site called a giant cell (Kynadt et al. 2013). Root-knot nematodes keep these giant cells, which are embedded in galls after J2 root penetration (Gheysen and Mitchum 2011), alive as a food resource throughout their life cycle. These root galls are typically direct reflection of infection level of nematode and always correlate with susceptibility of plants to nematode (Wang et al. 2012). M. graminicola infection causes substantial damage to rice root systems in nurseries and significant yield loss in the field (Patil and Gaur 2014; Dimkpa et al. 2016). In Bangladesh and Thailand, nematicide application in M. graminicola-infested rice fields resulted in yield increases of 16 to 33% (Arayarungsarit 1987; Sharma-Poudyal et al. 2004). In the Philippines, rice yields following two crops of cowpea and treatment with carbofuran resulted in a 34% increase, and nematicide application in upland rice fields in Indonesia resulted in yield increases of 28 to 87% (Dutta et al. 2012). Although M. graminicola has been identified in most rice-growing areas in southern China, systematic investigation of rice yield loss after nematode infection is lacking.

Many economical practices have been used alone or in combination to manage M. graminicola population densities below the damage threshold. For example, crop rotation with non-host plants, flooding and fallowing for several months can effectively decrease populations of M. graminicola and reduce yield losses (Ventura et al. 1981; Rahman 1990). However, these management practices have a number of drawbacks. The wide host range of M. graminicola limits the use of crop rotation, and flooding cannot be applied in water-limited areas. Furthermore, areas left fallow for several months or a crop season can significantly reduce overall output (De Waele et al. 2013). Popular in many rice-planting areas, soil sterilization with chemical nematicides is the most effective management method. Nonetheless, chemical nematicides are expensive, environmentally harmful and pose potential risks to beneficial organisms. Accordingly, searching for resistant or tolerant rice varieties may be an eco-friendly management strategy to manage this nematode.

To date, resistance to M. graminicola has been evaluated in a number of Asian rice accessions, the majority of which are susceptible to M. graminicola. Indeed, only a few are truly resistant (Bridge et al. 2005). One accession (WL02) of Oryza longistaminata and three accessions of Oryza glaberrima (TOG7235, TOG5674, TOG5675) in the Philippines were found to be resistant to M. graminicola (Soriano et al. 1999), and two commonly grown rice cultivars, Masuli and Chaite-6, were moderately resistant to M. graminicola in Nepal (Sharma-Poudyal et al. 2004). The recurrent parent Teqing and the donors Type 3, Zihui 100 and Shwe Thwe Yin Hyv were resistant to the nematode in India (Prasad et al. 2006). Regardless, in China, limited information is available on the resistance of rice accessions, and very little effort has been devoted to identifying and breeding resistant rice cultivars. Thus, the aim of this study was to evaluate the response of the most commonly grown commercial rice varieties to M. graminicola in China.

2. Materials and methods

2.1. Rice seed collection

In total, 136 rice varieties were used to characterize the response of plants to M. graminicola, as well as one highly resistant (Rongyou 368, a hybrid indica rice variety) and one susceptible (Boyoyu 998, a hybrid indica rice variety) control. Of the 138 rice varieties, 16 are aus, 33 are hybrid aus, 10 are indica, 45 are hybrid indica, 10 are temperate japonica, and 24 are tropical japonica (Appendix A). Most hybrid varieties were collected from the China National Hybrid Rice R&D Center; others were obtained from seed markets in different provinces. All the information of rice varieties can be find at China Rice Data Center (http://www.ricedata.cn).

2.2. Nematode culture and inoculation

M. graminicola was originally collected from an infested field in Hanshou County, Hunan Province, China, and maintained on O. sativa cv. Nipponbare in a greenhouse at 26°C. Eggs of nematodes were collected from root galls and hatched in a 200-µm sieve. J2 nematodes were extracted with a modified flotation-sieving method and collected using a 25-µm sieve, as described in Huang et al. (2015). Nematode number was estimated under a
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