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RESEARCH ARTICLE

Response of Selected Kenyan Rice Cultivars to Infection by Root Knot Nematode (*Meloidogyne incognita*)

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Abstract

Meloidogyne incognita causes huge yield losses in rice which is the third most important cereal crop in Kenya. The aim of this study was to identify *M. incognita*-resistant rice cultivars from Kenya and relate the responses to known resistance pathways with *OsPR1a, OsPAL1,* and *OsJAMYB* as marker genes in rice. Five rice cultivars BW 196, Basmati 217 (Pishori), Sindano, IR 2793-80-1 (grown in lowland irrigated fields), and NERICA 4 (grown in upland rainfed fields) were evaluated for resistance to *M. incognita* under greenhouse conditions in two separate trials. The number of nematode eggs, reproduction factor (RF), and the level of galling were determined. The RF was used to select resistant cultivars. There was a significant difference ($P < 0.001$) in the number of eggs, galling index, and RF among the cultivars. NERICA 4, BW 196, and Sindano were classified as resistant with an RF <1. There was differential expression of the three marker genes between susceptible and resistant cultivars. *OsJAMYB* gene was up-regulated in leaves of all rice cultivars after 1 and 3 days post inoculation (dpi). *OsPAL1* was up-regulated in leaves of all varieties at 3 dpi while *OsPR1a* was down-regulated in leaves of resistant plants at 1 dpi and 3 dpi. These results provide an insight on sources of *M. incognita* resistance in Kenyan rice and it also forms an interesting starting point for further studies on defense responses of common rice varieties to root knot nematode infection.

Key words : Defense response, *Oryza sativa*, root knot nematode, resistance[1]

[1]dpi-days post inoculation; GI-Galling index; J2s-Second stage juveniles; KALRO-Kenya Agricultural and Livestock Research Organization; Pf-Final population; Pi-Initial population; RF-Reproduction factor; RKN-Root knot nematodes

Introduction

Rice is the third most important grain crop in Kenya and is mostly cultivated by smallholder farmers under irrigated and rainfed conditions. Consumption of rice in Kenya has increased (12%) compared to that of wheat (4%) and maize (1%). However, production does not meet demand and the deficit is covered by importing rice (MOALF 2016). Low production is attributed to different abiotic and biotic constraints that include water shortage, high cost of production, pests, and diseases (MOA 2008). *Meloidogyne* spp. are among the most damaging nematode pests of rice. Control of these nematodes is mainly through the use of nematicides that pose a risk to the environment and human health while at the same time increases the cost of production. Continuous flooding has been previously used to control *Meloidogyne* spp. although with low efficacy (Tandingan et al. 1996). *Meloidogyne* spp. causes high (80%) yield losses (De Waele and Elsen 2007) and susceptible varieties show a 50% reduction in root dry weight (Shrestha et al. 2007). These nematodes also reduce grain weight and affect nutrient

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quality (Patil and Gaur 2014). *Meloidogyne* spp. can withstand flooded conditions and they are also damaging to aerobic rice (De Waele and Elsen 2007; Luc et al. 2005). Win et al. (2011) observed 289 infective second-stage juveniles (J2)/g of roots and a galling index (GI) of 4.1 in rice grown in lowland fields and 4.2 $J2g^{-1}$ of roots and a galling index (GI) of 1.2 in upland varieties. In Myanmar, six rice varieties grown in upland and lowland fields showed a similar reduction in yield and growth traits (Win et al. 2015). The ability of *Meloidogyne* spp. to damage rice in different water regimes (Prot and Matias 1995) poses a threat to rice production.

At optimal conditions *Meloidogyne* spp. reaches adult stage within 11 days in flooded conditions and 13 days in non-flooded fields (Fernandez et al. 2014). This short lifecycle results in high population densities of *Meloidogyne* spp. during one crop cycle. The nematodes get into plants through the root tip and form feeding sites which turn into hook-like galls three days after infection (Luc et al. 2005). Yield reduction occurs as a result of reduced nutrient and water uptake that is caused by formation of galls (Williamson 1998). These nematodes also cause expression of different genes that are involved in plant defense responses (Kyndt et al. 2012b).

Rice accessions of *Oryza longistaminata* and *Oryza glaberrima* show resistance to *Meloidogyne* spp. (Cabasan et al. 2012; Petitot et al. 2017; Soriano et al. 1999). Resistance to *Meloidogyne* spp. from African rice *Oryza glaberrima* has been incorporated into the New Rice for Africa (NERICA) through the rice variety TOG5681. NERICA rice varieties are grown in Kenya under upland rainfed conditions. Despite its moderate resistance to *Meloidogyne* spp. this rice variety is affected by other diseases and pests (Adeyemo et al. 2015; Mogga et al. 2012). It is therefore imperative that other sources of resistance to *Meloidogyne* spp. are identified in common rice cultivars that are adapted to Kenyan environmental conditions.

Upon attack, plants exhibit resistance to pathogens by triggering defense response mechanisms which are mediated by various signaling pathways such as the salicylic acid (SA), jasmonic acid (JA) pathogenesis related proteins (PR), and ethylene (ET) biosynthesis pathways. These pathways, compounded by expression of various plant transcription factors result in defense responses that ultimately guard the plant against further damage (Pieterse et al. 2009). Rice varieties react differently after root knot nematode (RKN) attack and these reactions are controlled by numerous genetic loci (Bimpong et al. 2010). It is therefore important to study gene expression for different crop species and more importantly specific crop cultivars so as to better understand the resistance mechanism to RKN infection. In the current study, we sought to determine the response of five economically important rice cultivars to the most injurious RKN *M. incognita.* Since RKN infection has been shown to elicit local suppression of defense genes in rice roots to enhance their infection (Gheysen et al. 2011), we hypothesized that the genes regulating the defense pathways are likely to be differently expressed in infected and non-infected tissues.

Materials and Methods

RKN inoculum

The RKN, *Meloidogyne incognita* was collected from rice fields in Kenya and multiplied in the greenhouse (Pokharel et al. 2007). Nematode eggs were extracted by agitating 10 g of root sections in 0.6% NaOCl for 4 min and rinsing the homogenate with distilled water through an 80 and 500-mesh sieve (Hussey and Barker 1973).

Nematode resistance screening

The rice cultivars BW 196, Basmati 217 (Pishori), Sindano, IR 2793-80-1 which are grown in lowland irrigated fields, and NERICA 4 which is grown in upland rainfed conditions were used in the resistance screening experiments and were obtained from the Kenya Agricultural and Livestock Research Organization (KALRO, Mwea, Kenya). Ten seeds of each rice cultivar were planted in 20 × 25 cm pots containing mineral clay loam soil under greenhouse conditions. Each treatment was replicated five times in a completely randomized block design and uninoculated rice cultivars acted as controls. Greenhouse temperature was maintained at 25 ± 3°C and plants were watered as required. Five days after planting, an initial population (Ni) of 5000 eggs was used to inoculate each pot. The plants were harvested after 60 days and the roots were washed before evaluation of galling severity using 1-9 scale where; 1 = no galls observed (healthy roots), 2 = ≤ 5%, 3 = 6-10%, 4 = 11-18%, 5 = 19-25%, 6 = 26-50%, 7 = 51-65%, 8 = 66-75%, and 9 = 76-100% of the roots galled (Mullin et al., 1991). The final nematode egg population (Nf) was determined by extracting eggs from 10 g of root samples as previously described and counting them using a stereomicroscope. Roots (20 g) were dried at 80°C before weighing.

The reproduction factor RF, (RF = Nf / Ni, where Nf = final number of eggs per root system and Ni = initial number of eggs inoculated), was estimated for each rice cultivar. Cultivars with Rf < 1.00 were considered resistant while those with Rf ≥ 1.00 were considered as susceptible (Oostenbrink 1966). The experiment was carried out twice in different years, using the same set of cultivars to determine the consistency of differences in nematode resistance. Data were tested for normality and log (x+1) transformed where required. Data from trials 1 and 2 were combined and treated as a single trial during subsequent analyses. The mean number of eggs, galling index, and RF for each rice cultivar was compared using ANOVA with subsequent means separation using Fisher's Least Significant Difference test. The correlation between GI and number of eggs was determined using Pearson's correlation coefficient in SPSS (Norusis 1995).