

## **PROSPECÇÃO DE FONTES DE RESISTÊNCIA AO NEMATOIDE-DAS-GALHAS NA CULTURA DO ARROZ COMO ESTRATÉGIA DE CONTROLE**

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In this talk, I will summarize current and past attempts in breeding for rice resistance to root-knot nematodes. I will present the recent advances and new knowledge on rice innate immunity to nematodes gained through –omics technologies (genomics, transcriptomics...) and gene functional analysis. I will conclude by presenting some perspectives to control rice nematodes based on novel biotechnological strategies targeting essential nematode genes. Parts of the text below are extracted from my co-authored publications Nguyen *et al.* (2014), Kyndt *et al.* (2015) and Petitot *et al.* (2017). Asian rice (*Oryza sativa* L.) is the most-consumed cereal food in the world, particularly in tropical and subtropical regions. Rice is mostly grown in Southeast Asia, which accounts for 90% of the production, but some important rice-producing countries are also found in Africa (Egypt and sub-Saharan countries) and the Americas (Brazil and the United States). Global rice production was estimated at more than 740 million tons in 2016 (FAOStat, 2018). The crop is grown in a wide range of climatic conditions, from river deltas to mountainous regions, and cultivation is classified on the basis of hydrological characteristics: irrigated, rain fed–lowland, and rain fed–upland systems. Estimates of total annual yield losses due to plant-parasitic nematodes on the rice crop range from 10% to 25% worldwide. Fortuner and Merny (1979) made a list of more than 100 different species of parasitic nematodes that attack rice plants. The nematode species encountered largely depends on the rice cultivation system, with, for instance, the flooding-adapted *Hirschmanniella oryzae* dominating paddy fields, and root-knot nematodes (RKNs) *Meloidogyne* spp being widespread in upland and irrigated rice fields.

RKNs are obligate parasites that settle in roots and complete their life cycle by feeding from specially adapted host cells. These nematodes may significantly decrease rice production in Asia, West Africa and South America, where *Meloidogyne graminicola* (Golden & Birchfield, 1965), *M. incognita* (Kofoid & White, 1919; Chitwood, 1949), and *M. javanica* are prevalent (Fortuner and Merny, 1979; Soriano and Reversat, 2003). In southern Brazil, a recent survey identified that a *Meloidogyne* species complex is indeed parasitizing rice in irrigated fields (Negretti *et al.*, 2017).

*M. graminicola* may infect rice roots and complete its life-cycle in less than 3 weeks under favourable temperatures (Nguyen *et al.*, 2014). *Meloidogyne* spp. are highly adapted parasites able to escape from plant recognition, establish feeding sites and suppress host defense reactions. Nematode stage 2 juveniles (J2) enter roots close to the apex at the elongation zone, and then reach the root central cylinder where they initiate a feeding site from a set of parenchyma cells. This process may be observed in rice as soon as 2 days post infection (dpi) with *M. graminicola* (Nguyen *et al.*, 2014). In response to nematode signals, the parenchyma cells differentiate into multinucleate and metabolically active giant cells (GCs), enabling *M. graminicola* to withdraw nutrients from the plant's vascular system. During root invasion, *M. graminicola* juveniles express a high number of genes encoding cell wall degrading enzymes and virulence effectors to enter, migrate into roots and induce GC formation (Petitot *et al.*, 2015). In addition, transcriptome data from this compatible interaction showed that plant defence pathways are suppressed in *M. graminicola* –infected rice (*O. sativa* Nipponbare) roots (Petitot *et al.*, 2017).

Rice has been likely domesticated from wild rice (*Oryza rufipogon*) thousands of years ago in southern China, then subsequently crossed to local wild rice in South East Asia and South Asia, thus generating *japonica* and *indica* sub-types after many cross-differentiation-selection cycles (Huang *et al.*, 2012). Until recently, the lack of effective resistance in Asian rice germplasm had hampered an effective genetic breeding for *M. graminicola* resistance. Sources of specific resistance to *Meloidogyne* spp. were first identified in the related species *O. longistaminata* and *O. glaberrima* (Soriano *et al.*, 1999). *O. glaberrima* is a low-yielding species originating from Africa that has many interesting agricultural traits, such as resistance to biotic and abiotic stresses (Linares, 2002). In particular, the *M. graminicola*-resistant TOG5681 and CG14 varieties have been used as donor parent to develop some NERICA (New Rice for Africa) lines that provide good yield and are adapted to lowland ecosystems. Genetic analyses of the backcrossed TOG5681-introgression lines indicated that resistance to *M. graminicola* may be controlled by several genetic loci (Plowright *et al.*, 1999; Bimpong *et al.*, 2010). Penetration and development of *M. graminicola* in several *O. glaberrima* genotypes were compared by microscopic observation of infected roots and histological analysis of galls (Cabasan *et al.*, 2013; Petitot *et al.*, 2017). The low level of *M. graminicola* infection is mainly due to limited nematode penetration and to subsequent host resistance reactions that induce infection sites collapse and inhibit nematode female development. In incompatible plant-nematode interactions studied so far, three main resistance response types were described based on the timing, namely a rapid hypersensitive response (HR) that blocks GC initiation, or a resistance that restricts GC expansion, and a third type occurring later impairing GC to function as active transfer cells (Goverse and Smant, 2014). Localised necrosis close to the

nematodes, suggesting a HR-like reaction, was observed within a few days after nematode penetration in some resistant *O. glaberrima* genotypes (Cabasan et al., 2013).

Recently, two *M. graminicola*- resistant *O. sativa* accessions were identified from a large screening of a global collection of 332 diverse accessions including landraces and elite cultivars from 82 countries of the Rice Diversity Panel 1 (Dimpka et al., 2016). A genome-wide association study (GWAS) mapped 11 quantitative trait loci (QTL), associated with resistance to *M. graminicola*, and a set of 493 positional candidate genes within 200 kb of these QTL were described that may be associated with plant disease resistance (Dimpka et al., 2016). The identification of molecular markers that are closely associated with *M. graminicola* resistance QTL is currently underway (Cabasan et al., 2018).

Rice molecular responses to *M. graminicola* were assessed in *O. glaberrima* TOG5681 and the susceptible *O. sativa* genotype 'Nipponbare' by root transcriptome profiling at 2, 4 and 8 d post- infection (dpi) (Petitot et al., 2017). Gene expression changes in the African rice were observed as early as 2 days after infection, with genes predicted to be involved in defence responses, phenylpropanoid and hormone pathways strongly induced, in contrast to the susceptible accession of *Oryza sativa*. No specific hormonal pathway could be identified as the major determinant of resistance in the rice- nematode incompatible interaction. Candidate genes proposed as involved in resistance to *M. graminicola* in *O. glaberrima* TOG5681 were identified based on their expression pattern and quantitative trait locus (QTL) position, including chalcone synthase, isoflavone reductase, phenylalanine ammonia lyase, WRKY62 transcription factor, thionins, stripe rust resistance protein, thaumatins and ATPase3 (Petitot et al., 2017). Identification of such genes may be of high interest to produce rice varieties with enhanced resistance to RKN. As an example, thionins belong to the pathogenesis-related (PR) proteins family PR13, and are well known as antimicrobial peptides. When the gene *OsTHI7* was artificially overexpressed in susceptible *O. sativa* plants, the transgenic rice lines revealed decreased susceptibility to *M. graminicola* infection (Ji et al. 2015).

## Conclusions

Promising genotypes with resistance and/or tolerance to *M. graminicola* infection were identified that could be further developed into advanced breeding lines and ultimately resistant and/or tolerant cultivars (Dimpka et al., 2015; Cabasan et al., 2018). Transcriptomics study provided a novel set of candidate genes for *O. glaberrima* resistance to nematodes that could enhance the efficiency of the breeding programs that require several years to develop a new rice genotype with superior phenotypes for nematode resistance. Alternatively, other research programs are on-going to develop RKN control strategies using data obtained on genes essential for the nematode, either metabolism genes or those involved in parasitism.

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