BIOTECHNOLOGY STRATEGIES TO NEMATODE CONTROL IN CROP PLANTS: AN OVERVIEW

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Abstract
Plant parasitic nematodes constitute an important group of plant pathogens for important crops, such as potato, soybean and wheat. The biotechnological approaches to nematode control aims to exploit natural resistance present in gene pools of crop species and their relatives to be introgressed in adapted cultivar in genetic breeding programs or to employ synthetic alternatives of resistance based on transgenic approaches. The transgenic tools explores either the molecular mechanisms of the resistance response in the host side or the essential and parasitism machinery from the pathogen side. These alternatives can based on disruption of feeding cells, expression of specific proteins/ toxic compounds to the nematodes or on host induced gene silencing based on RNAi technologies.
Key words: Effectors, Genetic resistance, Host Induced Gene Silencing, Transgenic

Theme Development

Nematodes are widely distributed and occur in almost all ecosystems. They can be free living, feeding on bacteria or fungi or lives as parasites of animal and plants. According to their life style they can divide into migratory and sedentary parasites. Cyst nematodes and root knot nematodes are the main groups of the plant sedentary parasites. In Brazil, migratories species, like Pratylenchus brachyurus has been increasing importance, with significative yield losses on soybean production regions, such as in the central region of Brazil.

So far, the nematode control in crops of agronomic importance has been done mainly by adoption of management practices, use of resistant cultivars or by chemical control. The natural resistance to plant nematodes is the most common alternative explored to nematode control and involves deploying of natural resistance sources associated with marker assisted selection. Many plant resistance (R) genes have been characterized and exploited to develop of resistant varieties in genetic breeding programs. Plant resistance to nematodes is often governed by specific resistance genes that interact with avirulence genes of the pathogen, leading to a gene-for-gene interaction (FLOR, 1971). This interaction implies that pathogen develop races that differ in avirulence genes for a certain plant resistance gene. To exploit natural variation for resistance, large-scale screening of germplasm is often employed, together with molecular markers and/or positional cloning to identify R genes or metabolites that confer resistance to particular nematodes in a wide range of germplasm of crop plants and their wild relatives. Additionally, the new generation sequencing plataforms have been improving the capacity to access genetic variability at genomic level, reveling important polymorphisms among individuals with differences in infection responses to the pathogen.

The marker-assisted selection to improve the resistance to cyst nematode has been the most significative in genetic breeding programs to important crops as potato, soybean and wheat. Recently, the genetic resistance governed by the major genes Rhg1 and Rgh4 in soybean against *H. glycines* was successfully elucidated. Resistance at the Rhg4 locus involves a serine hydroxymethyltransferase (LIU et al., 2012), while the resistance at the Rhg1 locus involves copy number variation of a stretch of three different genes in a 31-kilobase segment, one of them coding for an amino acid transporter (COOK et al., 2012). The resequencing of different

soybean lines reveled that susceptible lines contain one copy of the 31-kilobase segment per haploid genome while resistant can have 10 copies. These results emphasize the delicate balance between cyst nematodes and their host plants to keep the syncytium functional, and reveled that the resistance can be dependent of copy number variation, genetic polymorphism in the resistance gene and epigenetic control (COOK et al, 2014). These knowledge have been explored as a important tool to marker assisted selection based on single polymorphisms (SNPs) and/or copy number variation in the region containing R genes to screening germoplasm and/or breeding lines to selection.

The resequencing of the whole genome of Brazilian cultivars mainly developed by Embrapa and the parental resistant germoplasm have revealed important SNPs that can be explored to marker assisted selection in tropical adapted germoplasm. A total of 2,149 SNPs related to resistance against SCN were identified in Rhg1/Rhg4 loci and some QTLs spread on chromosome 7, 10, 11, 17, and 18. The most meaningful SNPs associated to resistance against SCN were detected in Rhg1 loci. Moreover, we identified a non-synonymous triallelic variation in an exon of the major gene of Rhg1 loci responsible for the increase of the resistance against SCN. This SNP could differentiate susceptible and resistant accessions, and also the two major source of resistance against SCN: Peking or PI 088788. Additionally, a total of 4,461 SNPs associated to resistance against RKN were identified in a QTL region of chromosome 10, being most of them upstream 5 k of genes inside this interval.

The transcriptome studies in plant root tissues after nematode infection has been also contributing for the understanding of molecular mechanisms of plant-nematodes interaction. These studies has been revealing that the success in the establishment of cyst and root-knot nematode parasitism in host plants is associated with dynamic suppression of plant defense mechanisms (BARCALA et al., 2010; KLINK et al., 2007) and upregulation of genes involved in the cell cycle control, cell wall modification and metabolism, which are crucial for nematode feeding site formation and development of giant cells and syncytia (DE ALMEIDA ENGLER et al., 2012). Biotechnology approaches based on host genes are focused on strategies which can disrupt feeding site formation and function, and basically direct the expression of the target genes specifically or highly upregulated in feeding cells, using tissue specific plant promoters.

Alternatively, many studies have been focusing on pathogen genes whose products are vital for different processes of nematode parasitism, such as root location, invasion, host defense evasion, general metabolic and developmental processes, and feeding or feeding site formation. In particular such research is leading to identifying effectors secreted by nematodes that allowed them avoid or neutralize host plant defenses, migrate within roots and, depending on the species, induce the formation of long-term feeding sites. These knowledge has supporting the development of biotechnology alternatives, such as RNA interference (RNAi) to target nematode essential and parasitism genes.

The RNAi-based approach to silence nematode effector and/or essential genes relies on the production of stable transgenic plants expressing a dsRNA corresponding to targeted nematode genes or by ectopic administration of dsRNA or siRNA molecules similar to the target genes. The dsRNA is recognized by the RNA silencing machinery and results in depletion of the targeted transcripts and its translated protein, causing loss-of-function phenotypes and consequently impaired infection or development of the pathogen. Many important genes for nematodes parasitism were characterized by this strategy (STEEVES et al., 2006; YOUSSEF et al., 2013; XUE et al., 2013; JAOUANNET et al., 2013). Nowadays, RNAi has emerged as a potent and the one most promising alternative for nematodes and pest control in crop plants, and can be obtained by transgenic or by innovative strategies to ectopic delivery stable dsRNA molecules to the plants.

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