GENE ISOLATION AND VECTOR CONSTRUCTION FOR TOBACCO TRANSFORMATION AIMING GENE SILENCE OF PLANT PARASITIC NEMATODE *Meloidogyne incognita*

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Plant parasitic nematodes are responsible for huge economic losses in world agriculture that reaches US$ 125 billion yearly. The most important of these phytopatogens, *Meloidogyne incognita*, has six developmental stages during the life cycle (egg, four juveniles and female). The infective juvenile 2 (J2) enters the host root via mechanical force and enzymatic degradation to establish the host-pathogen interaction and differentiate in apomitic adult female, which deposits around 2,000 eggs. Current agronomic practices have usually been unsuccessful and expensive, so the cultivation of resistant varieties is actually the most efficient way to control nematodes. The phenomenon of gene expression interference mediated by RNA (RNAi) found in eukaryotic organism was recently discovered and our understanding has been constantly improved. Parallelly, its application as molecular biological tool has been continually improved too. Beyond RNAi had become a powerful strategy for gene function identification, this technique is a promising tool for gene therapy and viral, protozoal, fungal, nematodal, acaridal, arthropodal, insectidal control in different organisms. Ingestion of double strand RNA (dsRNA) by nematode leads to RNAi process that culminates in specific gene expression silencing, resulting in nematode physiology alterations. In this work, aiming transform *Nicotiana tabacum* with plasmidial constructions for double strand RNA expression for bioassays by infection with *Meloidogyne incognita*, the nematode target genes chosen were: Isocitrate Lyase, Arginine Kinase, 14-3-3 Protein, Heat Shock Protein 90, Aspartic, Cysteine and Serine proteinases, β-1,4-endoglucanase and an esophageal gland gene named Mi-2. One region of each target gene was selected, isolated and subcloned in the RNAi vector pK7GWIWG2(I) for tobacco transformation using *Agrobacterium tumefaciens*. In addition, it was done an expression analysis of Isocitrate Lyase, Arginine Kinase, 14-3-3 Protein and Heat Shock Protein 90 target genes, normalized with housekeeping genes β-actin and 18S rRNA, using quantitative real time PCR. This experiment showed that these genes are differentially expressed when compared during egg, J2 and female phases. The major gene expression difference observed was in Isocitrate Lyase pattern, which is a hundred times more expressed in J2 or female when compared with egg. The Arginine Quinase expression was five-fold higher in J2 and ten-fold shorter in female compared with egg. The 14-3-3 expression had insignificant variation in all three stages. Finally, the HSP90 expression level was the same in egg and J2, but was 32 times lesser in female than in egg.

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