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VALIDATION OF SIGNALING AND DEFENSE GENES IN MYCORRHIZED YELLOW PASSION FRUIT AND UNDER FUSARIOSIS INDUCTION

Roberta Lane de Oliveira Silva¹; Brunara Evely de Araujo Lima¹; Natoniel Franklin de Melo²; Adriana Mayumi Yano-melo¹

¹. Rodovia BR-407, KM 12 Lote 543 S/n Projeto de Irrigação Nilo Coelho. Petrolina - Pernambuco. Universidade Federal do Vale do São Francisco (Campus Ciências Agrárias); ². Rodovia BR-428, Km 152, s/n - Zona Rural, Petrolina

- Pernambuco. Embrapa Semiárido

Abstract:

Inoculation with arbuscular mycorrhizal fungi (AMF) has been reported to be beneficial in several plant species, and in Passiflora edulis mycorrhizal colonization has provided greater tolerance to biotic and abiotic stresses in addition to the development of seedlings. The use of AMF in the biocontrol of soil pathogens has the potential to mitigate the negative effects of diseases in crops of economic interest, but the mechanisms involved are still little explored, e.g. fusariosis in yellow passion fruit. In this scope, this work aimed to select candidate genes related to signaling and defense in P. edulis and validate its expression using quantitative realtime PCR (qPCR). The experiment was carried out in a greenhouse under controlled conditions of temperature and humidity. The plants were inoculated with the suspension of Fusarium oxysporum f. sp. passiflorae - FOP (2.10⁶ conidia/mL) and fusaric acid - AF (400 mg/L) after 70 days of mycorrhizal inoculation (400 spores of Gigaspora albida and Entrophospora etunicata). Root tissues were collected at 24 hours and 7 days after exposure of plants to inducers (FOP and/or AF). RNA extraction was performed using a commercial kit, following the manufacturer's recommendations. The samples were evaluated for integrity, purity, and concentration, and used to synthesize complementary DNA (cDNA). The primers were designed and evaluated for their specificity in Primer3Plus and Primer-BLAST tools. The qPCR reactions were performed using the SYBR Green detection system and three reference genes to normalize the relative expression data. They were drawn in five pairs of primers for target genes (PeLecRK, PeSERK1, PePR-1, PePR-12 and PeWRKY) previously selected and characterized in the draft genome of the species with bioinformatics tools. The efficiency values of primers ranged from 98.69 to 109.81%, corroborating with what is recommended in the specialized literature. The results of the qPCR analyses demonstrated that all candidate genes were differentially expressed as a function of the tested treatments. Most targets had their expression repressed 24 hours after AF application or FOP inoculation, showing values of fold change - FC less than 0.66. In contrast, the PePR-1 had its constitutive expression in almost all treatments. In turn, when assessing the most prolonged period of stress (7 days) it was found that, except for PePR-1, the other candidates showed induction in almost all treatments. PeWRKY stood out among the others, since it presented high induction in all treatments, in addition, it was unique in presenting induction (FC - 15.08) in the treatment with AF. The results suggest that the time of stress application was crucial to change the expression profile of these targets and show promising candidates for application in biotechnological inferences or in the genetic improvement of P. edulis. Palavras-chave: Passiflora edulis; AMF; Fusarium oxysporum; qPCR;

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