Evaluation of a Peanut cDNA Library Enriched for Root-knot Nematode-Specific Transcripts by Suppression Subtractive Hybridization

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INTRODUCTION

Annual economic losses in cultivated peanut (Arachis hypogaea L.) due to nematodes are estimated at $102 million. Root-knot nematodes (Meloidogyne spp.) are the most important nematode pathogens of peanut, and the predominant pathogenic species in the southern U.S. is M. arenaria. In Alabama, Georgia, Florida, and Texas, two host races of M. arenaria have been defined based on the ability (race 1) or inability (race 2) to reproduce on the peanut cultivar Florunner. Resistance to root-knot nematodes from wild peanut species has been introgressed into cultivated peanut. Following a backcrossing program using Florunner, the nematode resistant, nearly isogenic cultivar, NemaTAM, was released by Texas A&M University. We hypothesized that subtractive hybridization of cDNAs from NemaTAM and Florunner challenged with root-knot nematodes would allow the identification of peanut genes involved in defense against M. arenaria.

MATERIALS AND METHODS

Individual plants of NemaTAM and Florunner were grown in earthen pots in a 1:1 mixture of sterile soil and sand. Ten days after planting, each pot was inoculated with a suspension of 5000 eggs of M. arenaria race 1. Root samples for RNA extraction were collected at five time points: 0, 12, 24, 48, and 72 hrs after inoculation. Equal amounts of RNA (0.25 mg) from each time point were pooled separately for each cultivar, and used to synthesize double-stranded cDNA. The suppression subtractive hybridization method was performed. NemaTAM as tester and Florunner as driver and vice versa were used during forward and reverse subtractions. The cDNA fragments obtained after subtraction were cloned into the pAL vector (Evrogen) and subsequently transformed into Escherichia coli.

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Differential screening of potential clones that were highly specific to NemaTAM and Florunner was done by probing nylon membranes dotted by pin replication (Bio-Rad) of ordered clones of each 96-well micro-titration plate with 32P-labeled subtracted cDNA of NemaTAM and Florunner. Based upon NemaTAM- and Florunner-specific signals, clones were selected and sequenced.

RESULTS

Differential screening yielded 140 NemaTAM-specific clones and 123 Florunner-specific clones.

A majority of the identified NemaTAM-specific sequences had pathogenesis-related and stress-related functions.

Analysis of Florunner-specific sequences revealed genes involved in signal transduction and cellular communication.