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Opportunity for Undergraduate Research

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Abstract

There is possibly more than one PAL gene in soybeans. The PAL gene encodes part of the phenyl propanoid pathway. BLAST searches were done to find sequences that are similar to the already determined PAL gene. From there everything above .03% accuracy was keep for further study. Those sequences were place in Batch Entrez then consensus sequences were formed in Sequencher. This was done in order to try and produce primers for other possible PAL genes so that they can be seen when running a gel.

Introduction

The phenylpropanoid pathway is important in legumes and PAL is the first enzyme in the pathway which makes it really important. The pathway leads to many secondary metabolites in legumes as well as isoflavonoids. The genes that encode this pathway are useful for defense against pathogens, protection from cold, extended UV light and a lack of nutrients. Since PAL is a key enzyme in the pathway it is important to research.

Materials and Methods

A BLAST search was done on the NCBI website on the genetic code of the PAL gene that was already determined. From the results of the BLAST search everything above .03% accuracy was placed in Microsoft Excel and put them through a Batch Entrez search from the NCBI website. The results of this search were then put into Sequencher. Once they were in Sequencher, contigs were found by running the program and lining up the sequences based on genetic similaritly. Then the EST's in each contig were recorded in Microsoft Excel and transferred to Gene Tool for sequence alignment and evaluation. Once the consensus sequences were determined they the steps were repeated starting with the BLAST search. This allowed for new contigs to be made based on the new search results. The Excel files were then evaluated for repeated EST's so that the over lap between the first set of sequences and the second set of sequences wasn't based on repeated sequences instead of genetic similarity. Lastly, 4 micoL of 5X Green Go Taq Buffer, 4 micoL of 1:100 dilution dNTP, 2.5 microL of 1:20 dilution 010 Primer, 2.5 microL of 043 Primer 1 microL of 1:10 dilution of pP1053.3 and 5 microL of nuclease free water were put in a tube and PCR was run to amplify the DNA. Then a gel was run with 0.6g NuSieve 3:1 Agarose, 3 mL of 10X TBE, and 27 mL of dH2O. The stain used for the gel was 1:100 mL of ethidium bromide; the gel was stained for 10 minutes then destained for 10 minutes in distilled water.

Results and Discussion

The results of the first BLAST search followed by the Batch Entrez search produced five contigs in Sequencher. Each contig was then put into a BLAST search and

Batch Entrez search again by it's self to produce a varying number of contigs from the original ones. The contigs produced are still being evaluated for possible primers for other PAL related genes. The results from the gel showed a band of DNA in the appropriate place for the sequence we were looking for.

Nomenclature

A PCR is a polymerase chain reaction that amplifies specific sequences of DNA. Sequencher and Gene Tool are computer programs used to put together similar sequences. EST's are expressed sequence tags which is how each sequence is labeled.

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Soures

Genetic Analysis, 8th Edition, Griffiths et al.