Genomic Isolation and Sequence Comparison of Sweet Potato and Geranium GAPDH gene

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ABSTRACT

Gene cloning and sequencing are challenging technique frequently used in the creation of an exact genetic copy of an organism or plant and determining the nucleotide order of their given DNA. The main goal for this research is to extract genomic DNA then isolate, sequence and characterize the *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) gene from the plant species of sweet potato (Ipomoea) and Geranium (pelargonium) to find out if *GAPDH* gene evolved with these plants in their evolutionary development. *GAPDH* gene is known as a housekeeping gene which serves as a vital enzyme for plants and a

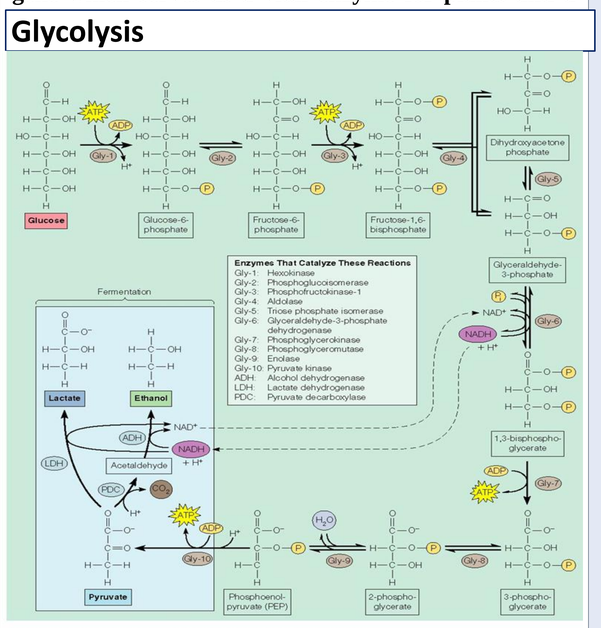

Figure 1: *GAPDH* phosphorylates glyceraldehyde-3-phosphate into 1,3-Bisphosphoglycerate in the sixth step of glycolysis

INTRODUCTION

Sweet potato is a dicotyledonous plant that belongs to the family of Convolvulaceae. It originated from South America, and is mostly grown in warm weather conditions (Adam, K.L). Geranium is also a dicotyledonous plant made up of 422 species of annual, biennial, and perennial plants. This plant originated from South Africa and is mostly grown in the temperate regions of the world and the mountains of the tropic (Reeves, Sonja L.S). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was extracted from sweet potato and geranium with a nucleic acid extraction module. The GAPDH gene codes for the enzyme that catalyzes the sixth step of glycolysis (Fig 1). This gene can be used for evolutionary comparison since it is widely conserved in the genome of plants.

OBJECTIVE

Our objectives for this experiment were to gain experience in biotechnology and techniques used in DNA recombination as well as to extract genomic DNA from two plant species for an evolutionary comparison. Our attention was on the *GAPDH* gene and its amplification, looking for either the similarities or differences between the band sizes of the DNA isolated from the two plants.

MATERIALS AND METHODS

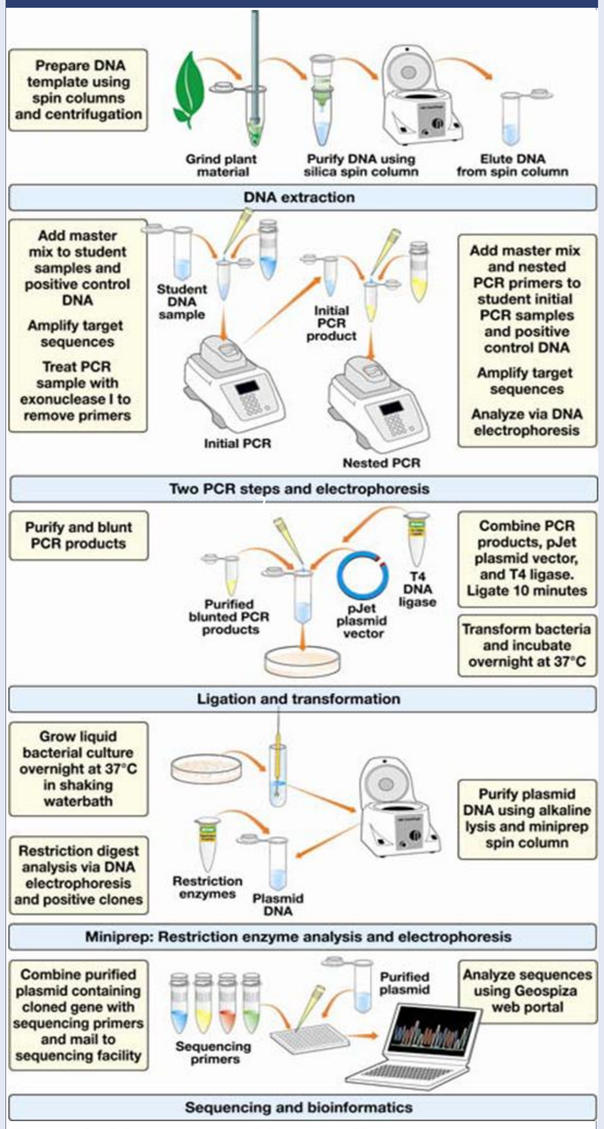

Figure 2: Materials and Methods: This figure gives a brief description of the techniques used to purify our genomic DNA as well as techniques used to isolate and purify the *GAPDH* gene.

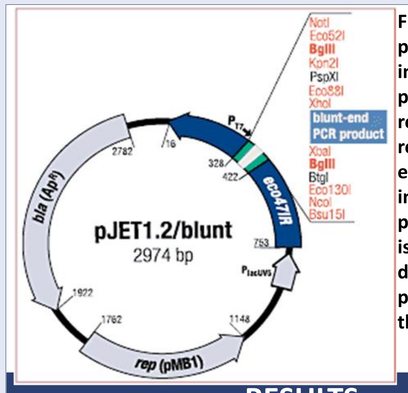

Fig 3: The blunted PCR product was inserted into the vector. The pJet1.2 contains a BglII restriction enzyme recognition site on either site of the insertion site. Once the plasmid DNA was isolated , a restriction digestion reaction was performed to determine the size of the insert

RESULTS

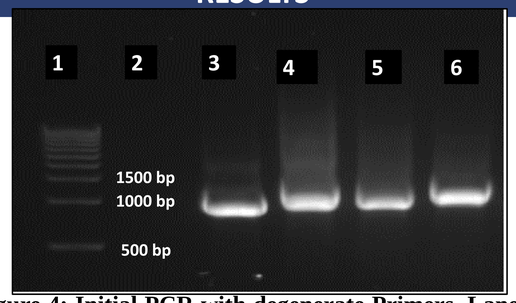

Figure 4: Initial PCR with degenerate Primers. Lane 1: 500bp ruler. Lane 2: Negative control. Lane 3: Positive control. Lane 4: Arabidopsis gDNA. Lane 5: Sweet potato. Lane 6: Geranium.

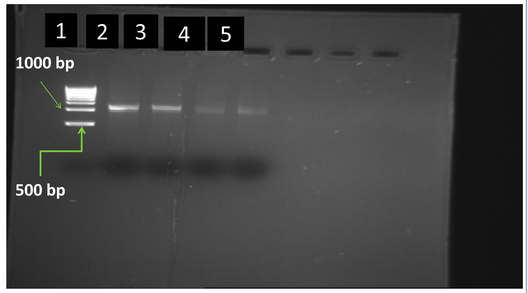

Figure 5: Purified PCR product from Nested PCR. Lane 1: 500bp ruler. Lane 2: Sweet potato DNA 1µl. Lane 3: Geranium DNA 1µl. Lane 4: Sweet potato DNA 2µl. Lane 5: Geranium DNA 2µl.

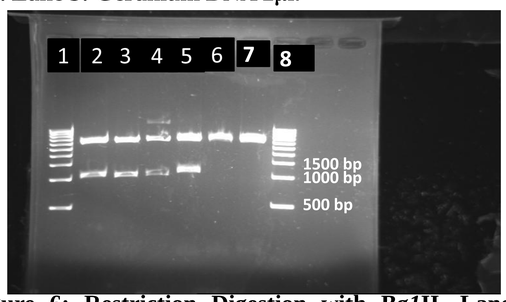

Figure 6: Restriction Digestion with Bg1II. Lane 1: 500bp ruler. Lane 2-4: Sweet potato colony. Lane 5-7: Geranium colony. Lane 8: 500bp ruler

DISCUSSION

We harvested fresh plant tissue and physically disrupt the tissues by grinding to isolate genomic DNA from the plants chloroplast. We lyse the cell with lysis buffer containing chemicals and enzymes to disrupt the cell membranes. Insoluble cell components such as cell walls and membranes were removed by centrifugation. The DNA collected in the supernatant was used to run an initial PCR containing degenerate primers to amplify the gene coding for the enzyme GAPDH. Results of DNA isolation could be seen after running a 1% agarose gel (Fig 4). The band size of geranium matched that of Arabidopsis and sweet potato matched the positive control. The next step was to get rid of primers that didn't get attached to target DNA. Before a second PCR was ran, exonulease 1 was inactivated by heat so it doesn't affect the nested PCR primers. We used the enzyme exonuclease 1 because it catalyzes the hydrolysis of single stranded DNA. The second PCR was performed with nested primers to ensure only targeted DNA were amplified. Our next step was to purify the PCR sample using a BIO-RAD kit to remove DNA polymerase, nucleotides, and extra primers. A 1% agarose gel electrophoresis was ran to check the DNA content (Fig 5). We used a cloning vector pJET 1.2 for blunting and ligation. Blunting was done to convert our DNA fragment with protruding ends to DNA fragment with blunt ends. Heat shock was done to enable the cells to become more competent to ease transformation. After the cells had been transformed, we grew them on LB plates containing ampicillin to selected the bacteria that contained our recombinant DNA. We inoculated three of the transformed colonies from each plant overnight to perform a plasmid purification. Digestion restriction with Bg1II enzyme was done overnight to cut the DNA fragment from the plasmid. A 1% agarose gel electrophoresis was ran to test the final DNA content (Fig 6).

CONCLUSION

A close examination was made in the section of the GAPDH gene amplified. The band sizes seem to indicate close evolutionary similarities between sweet potato and geranium. Gene sequencing is the next step to expand our research to get a better knowledge of how similar these two plants evolve.

REFRENCES

1. Adam, K.L. 2005. Sweet potato Organic Production. NCAT Agriculture Specialist.

2 .Reeves, Sonja L. 2007. Geranium bicknellii. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: http://www.fs.fed.us/database/feis/ [2011, April 10].

Research Opportunity

Research opportunity for Biology, Chemistry and Health Sciences undergraduate students are available in Dr. Muatasem Ubeidat's Lab. If you want more Information, come by Dr. Ubeidat's office (SCI 213). Call 580-774-3298 or E-mail muatasem.ubeidat@swosu.edu