Brassinosteroid Signaling In Arabidopsis PP2A-C Mutants

Akeela Lewis
Tiffany Oliver
Biology Department

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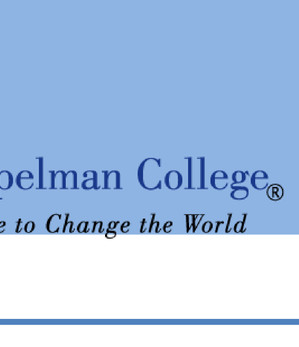
Introduction

In protein phosphorylation a phosphate group is attached to a protein. It is used in living organisms as a switch to transmit information using cell signaling circuits. The protein phosphorylation switch consists of a target protein that intrinsic characteristics are dependent on its phosphorylation status, a protein phosphatase, and a protein kinase. Protein kinase and phosphatase are responsible for the strength of the signal.

This experiment focuses on protein phosphatase 2A (PP2A), which are enzymes that are mostly responsible for the serine/threonine phosphatase activity within eukaryotic cells. PP2A is a heterotrimeric enzyme and has a subunit that acts as a catalyst (PP2A-C) and is regulated by the binding of two regulatory subunits (B and A).The B subunit is responsible for confirming the target before it bonds to the core dimer that was created by the catalytic subunit PP2A-C bound to PP2A-A.

Brassinosteroids are steroid hormones that promote growth and regulate physiological responses essential to plant development. They encourage cell division and expansion, regulate senescence, male fertility, pollen development, and fruit ripening and influence the plant's response to various environmental signals. As shown in Figure 1, brassinosteroids regulate transcription by activating a signal transduction after binding to the extracellular domain of a receptor kinase BRI1 on the cell surface. This experiment focused on mutations in the pp2a-c genes in Arabidopsis thaliana because this mutation alters the brassinosteroid related phenotypes of the plants. They are smaller in size compared to wild type Arabidopsis, produce less seeds, and have abnormal stomata patterns. The mutation in the PP2A-C genes alters the expression of BZR1pro:BRZR1-CFP or BES1pro:BES1-GFP. Arabidopsis' genome codes for five PP2A-C genes. PP2A-C1, -C2, and -C5 make up subfamily I. PP2A-C3, and -C4 make up subfamily II. It also codes for three A scaffolding subunits and 17 regulatory B, B', and B" subunits. It has been suggested that the B subunits confer substrate specificity and cellular localization to the PP2A. Due to the variety of subfamilies and subunits, there are plenty heterotrimer combinations that have the potential to function in different ways. This is believed to be the reasoning for genetic redundancy in plants. Genetic redundancy makes it hard to determine the physiological role of phosphatases in plants. PP2A-C subunits share 95% of sequence identity in a single subfamily and 80% between two subfamilies. Mutations in PP2A-A and B' subunits, when studied have resulted in apoptosis, change in phenotype, and a change in responses to brassinosteroids. A theory behind this is that the regulatory subunits form holoenzymes with the previous subfamily I in order to control regulatory responses.



Brassinosteroid Signaling in Arabidopsis

Objective

The goal of the research presented was to obtain a Arabidopsis plant with a mutation in the PP2A-C2, -C4, and b'B subunits. This was accomplished by introducing mutations in B' regulatory subunit into the pp2a-c2 pp2a-c4 mutant already available. Result analysis showed that the triple mutation had little effect on the BR-related phenotype of the plant.

Materials

Arabidopsis thaliana seedlings containing mutations in the PP2A C2-and C4 subunits and mutation in the b'B were used in this experiment to visually analyze the effects of mutations in regulatory protein phosphatases. The Arabidopsis grew in a green house that provided optimum conditions for growth of the plants. A forward and reverse primer was used to amplify the region of the DNA that was being examined. A Thermocycler machine was used to carry out the polymerase chain reactions (PCR).

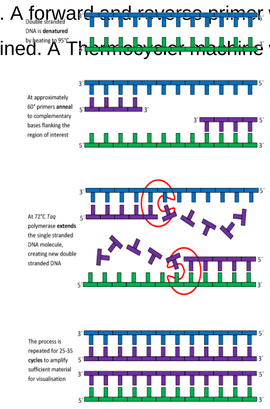
Methods

1.Sterilization of Seeds

2.Fertilization

3. DNA extraction

4. Polymerase Chain Reaction



Results

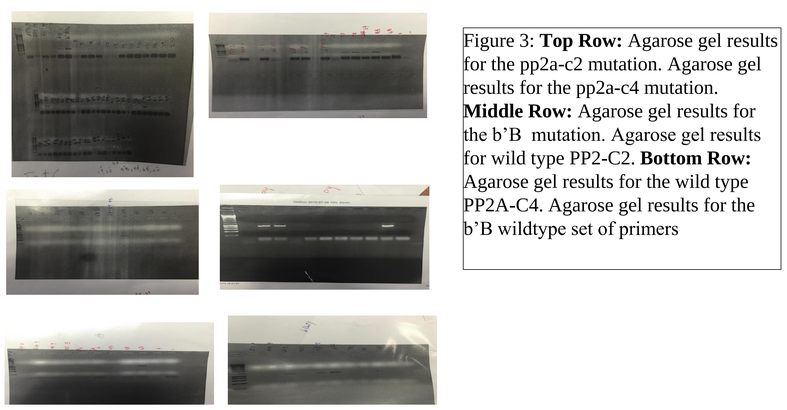


Figure 3: **Top Row:** Agarose gel results for the pp2a-c2 mutation. Agarose gel results for the pp2a-c4 mutation. **Middle Row:** Agarose gel results for the b'B mutation. Agarose gel results for wild type PP2-C2. **Bottom Row:** Agarose gel results for the wild type PP2A-C4. Agarose gel results for the b'B wildtype set of primers

Left: Image of wild type Arabidopsis plant. **Right:** Image of sample number 48 triple mutant Arabidopsis plant.

Discussion

The Arabidopsis plants with the double mutations in the experiment exemplified a dwarf appearance. This data confirms that PP2A-C2 and C4 affect brassinosteroid signaling. As stated before brassinosteroids promote plant growth. The dwarf phenotype of the mutants provide a visual evidence of the brassinosteroid being altered due to the mutation in the protein phosphatases, PP2A-C2 and C4. PP2A works against kinases to determine the strength of the transduction signal cascade. The transactional signal followed by the binding of brassinosteroids activates transcription. Because PP2A is believed to be involved in BR-induced BZR1/BZR2 dephosphorylation which results the expression of genes activated by BR (Zhang et. al, 2014) the mutation in the PP2A catalytic subunits are thought to be responsible for the altered phenotype in the dwarf.

The triple mutant had yet to be examined before this experiment. Two Arabidopsis plants exemplified the triple mutation. The small number of triple mutants that survived soil transfer could suggest that the mutation puts a strain on cell growth. the triple mutant Arabidopsis looked similar to the wild type Arabidopsis. is an example of a wild type Arabidopsis plant. The triple mutant stomata is slightly shorter than the wild type Arabidopsis thaliana's. The stem is taller than a dwarf plant but the leaves are similar to dwarf leaves, being that they are small in comparison to a wild type Arabidopsis.

Conclusion

It is possible that the mutation in the b'B subunit slightly restored the function of PP2A or activated a different enzyme to dephosphorylate the BZR1/BZR2. Allowing for signal transduction to occur as it would in a wild type Arabidopsis thaliana, resulting in transcription and the brassinosteroid induced genes to be expressed. It is hard to visually determine the exact cellular process that occurred as a result of the triple mutation.. The B subunits have a great deal of genetic redundancy, completely inactivating the B subunits would allow for a better analysis of the role, B subunits play in Brassinosteroid signaling in Arabidopsis plants.

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