

THE EFFECTS OF PHOSPHATE ON DESIREE POTATO CULTIVARS AND *UF3GT* ON ANTHOCYANIN PRODUCTION IN NORLAND POTATO CULTIVARS (*SOLANUM TUBEROSUM L. CV.*)

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Introduction

Red potatoes for fresh produce markets are a significant part of the potato industry. Farmers have noticed that red potatoes fade in storage. When faded potatoes are marketed, they are less appealing to customers (1). More vivid potatoes have an accumulation of anthocyanin. Anthocyanins are the pigments responsible for coloration in the skins of red potatoes (2).

In previous research that led up to this project, genes in the anthocyanin biosynthetic pathway (seen in Figure 1) for chalcone synthase (CHS), chalcone isomerase (CHI), dihydroflavonol 4-reductase (DFR), and leucoanthocyanidin dioxygenase (LDOX) were cloned. These are the genes that come before the last gene in the anthocyanin process, which is UDP-glucose:flavonoid 3-o-glucosyltransferase (*UF3GT*). This research showed those genes that come before *UF3GT* in the anthocyanin pathway were not responsible for the red coloration in potato skins (2).

Figure 1: Anthocyanin Biosynthetic Pathway

CHS -> CHI -> FLS -> DFR -> LDOX -> UF3GT

Ding et al. (2001) suggested that the *UF3GT* gene is crucial in the anthocyanin biosynthesis in grape skins. Their study examined seven anthocyanin biosynthetic genes, including the *UF3GT* gene, and compared the coding sequences of *UF3GT*. They found that *UF3GT* expression was detected only in red-skinned grapes but not in white grape skins. Their research concluded that the change from white to red in grape skins was the result of a possible mutation in a regulatory gene controlling the expression of *UF3GT* (2).

Another study showed that the lack of phosphorus in the form of potassium phosphate (K_3PO_4) increases anthocyanin production. The results suggest that by decreasing K_3PO_4 , anthocyanin production may increase in red potatoes (3).

There were two goals to the project presented here. The first goal was to determine if K_3PO_4 affected the color of 'Desiree' (*Solanum Tuberosum L. cv.*) potato tubers. The first hypothesis was that with less K_3PO_4 , more anthocyanin would be produced in 'Desiree' potatoes. It was thought that as the potato tubers grew, anthocyanins become thinly spread, causing fading in potatoes, so decreasing concentration of K_3PO_4 would result in redder potatoes. The second goal was to clone the *UF3GT* gene from 'Norland' (*S. tuberosum L. cv.*) callus to use in future work to determine its role in the anthocyanin process.

Materials and Methods

K₃PO₄ Experiments:

Two-week-old 'Desiree' potato plants were placed into each of 24 magenta boxes with 80 mL of tissue-culture media. The tissue-culture media was made with 60 g of sucrose, 4.3 g of Murashige & Skoog basal salts, 1 mL of Nitch and Nitch vitamins, and made to 1000 mL with dH₂O. The pH of the liquid media was kept between 5.6 and 5.7 by adding 1.0M HCl or 1.0M KOH. This was then autoclaved. Two plants were placed into each box to increase the sample size and ensure more accurate results. The two-week-old plants were transferred into magenta boxes under the hood, using forceps that were flamed between each transfer. Each box was sealed immediately to prevent contamination.

Four different concentrations of K₃PO₄ were used: 0x, 1/2x, 1x, and 2x. The 1x K₃PO₄ was made by using the typical Murashige and Skoog basal salt mix with 170 mg/L of K₃PO₄.

Six replicate magenta boxes were used for 24 treatments. Six of these were dedicated to one of the four K₃PO₄ concentrations. Three replicate boxes were wrapped in aluminum foil, to keep the plants in the dark. The other three replicate boxes were kept under continuous fluorescent light. All of the boxes were labeled and left to grow for three weeks. The same procedure was replicated. Data were collected on whether plants produced callus and/or tubers. In addition the colors of the tubers were recorded as "red", "pink", "green", or "white".

Cloning UF3GT:

The second part of the project was to amplify and clone the gene *UF3GT*. First, RNA was extracted, using a Qiagen RNeasy kit. 'Norland' callus were ground into a fine powder and kept cold by adding liquid nitrogen every 30 seconds for 15 minutes while grinding. The frozen tissue was scraped into a microfuge tube. A volume of 450 µL of buffer RLT was added to the tissue and vortexed vigorously. This mixture was transferred to a shredding column and centrifuged for two minutes at 14000 x g.

The mixture was then transferred to a microfuge tube without disturbing the pellet. A volume of 250 µL of ethanol was added. This mixture was mixed by pipetting. The mixture was transferred to a spin column and centrifuged for 15 seconds at 10000 x g. The flow-through was discarded, and 700 µL of RW1 buffer were added to the column. The column was centrifuged for 15 seconds at 10000 x g, and the flow-through discarded. The mixture was transferred to a collection tube, and 500 µL of RPE buffer were added. This was centrifuged for 15 seconds at 10000 x g, and the flow-through was discarded. A volume of 500 µL of the RPE buffer were added to the collection tube and centrifuged for two minutes at 14000 x g. This mixture was transferred to a microfuge tube and centrifuged again for one minute at 14000 x g. This was transferred to a 1.5-mL microfuge tube, and 50 µL of RNase-free water were added. This was centrifuged for one minute at 10000 x g.

The total RNA was transcribed into cDNA using Superscript III reverse transcriptase, or mRNA was first purified from total RNA and then transcribed into cDNA. First-strand cDNA synthesis was used to obtain products for the polymerase chain reaction.

The two primers of the *UF3GT* gene were diluted with 1 mL of RNase-free water and then used in PCR along with oligo, random, and diluted gene-specific primers. The products were electrophoresed on a 1.5% agarose gel. A Clontech Advantage 2 kit was used for the PCR. PCR was run using different annealing temperatures ranging from 55°C to 68°C and elongation times ranging from one minute to three minutes. The concentration of the diluted mRNA solutions was analyzed using spectrophotometry. PCR products were visualized by running them on a 1.5% agarose gel stained with ethidium bromide.

Results

Table 1: Trial 1 of K₃PO₄ Treatments in Light and Dark

	Treatment	Callus	Number of Tubers	Tuber Color	Tuber Size
Light	0x	white callus black stems	1	pink	small
	1/2x	red leaf tips white callus	1	green	large
	1x	white callus thick stems	8	green/red	6 large 2 small
	2x	white callus thick stems	8	green/red	large
Dark	0x	black stems thin stems	0		
	1/2x	thin stems white callus	0		
	1x	white callus red leaf tips	0		
	2x	white callus red leaf tips	3	white	large

Table 1 shows that in the light the 1x and 2x K₃PO₄ boxes grew the most tubers with eight each. Of the three out of the four boxes of plants grown in dark conditions, the 0x, 1/2x, and 1x K₃PO₄ produced no tubers. All boxes except 0x K₃PO₄ in the dark grew white callus.

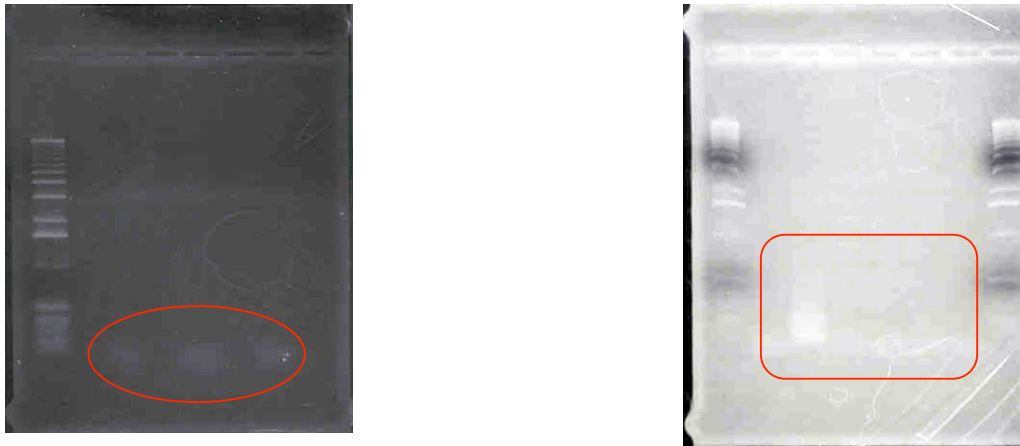
Table 2. Treatments of Trial 2 in Light and Dark

	Treatment	Tubers/Callus	Number of Tubers	Tuber Color	Tuber Size
Light	0x	white callus thin stems	4	pink/green	small
	1/2x	white callus red leaf tips	9	3 pink 3 red 1 white	large small large
	1x	white callus pink leaf tips	7	green/red	large
	2x	white callus thick stems	4	red/green	large

Dark	0x	thin stems white callus	0		
	1/2x	thin stems white callus	2	white	large
	1x	white callus pink leaf tips	2	red/pink pink	large large
	2x	foggy white/ peach liquid	1	white	large

Table 2 displays the results from the second trial of K_3PO_4 experiments. The colors of tubers varied from pink, red, white, pink/green, and green/red. The 0x K_3PO_4 treatment for the dark experiment produced no tubers. The 1/2x K_3PO_4 light treatment produced nine tubers, and the 1x K_3PO_4 light treatment produced seven tubers.

Figure 2: Smears of UF3GT and Nested Primers



In Figure 2, the areas circled in red are the smears of *UF3GT* and the nested primers from PCR. The bands should be about an inch higher in order for the amplification to have been successfully completed (1).

Table 3: Annealing and Elongation Times

Denaturing	Annealing	Elongation
94° C for 15 seconds	68° C for 1 minute	68° C for 1 minute
94° C for 15 seconds	65° C for 1 minute	68° C for 1 minute
94° C for 15 seconds	65° C for 1 minute	68° C for 3 minutes
94° C for 15 seconds	55° C for 1 minute	68° C for 3 minutes
94° C for 15 seconds	60° C for 1 minute	68° C for 3 minutes

Table 3 displays the annealing and elongation times and temperatures used for PCR. The denaturation times remained constant throughout. The conditions that successfully produced the smear results, seen in Figure 2, were an annealing temperature of 60° C for one minute and an elongation temperature of 68° C for three minutes.

Conclusion

The data from this project showed that the K_3PO_4 treatments had no significant effect on the color of either small or large potatoes. It was thought that decreasing K_3PO_4 concentrations would

increase anthocyanin production, but this seems not to be the case. This indicates that concentration levels of K_3PO_4 in soil will not affect the coloration of potatoes.

Although this project did not successfully amplify the *UF3GT* gene, the smears on the gels showed enough success that the amplification process described here could be used in future projects to clone sufficient amounts of *UF3GT*. Because the smears of *UF3GT* and other nested primers did not show up on the first five gels, there was speculation about the PCR conditions.

This project determined more successful PCR conditions for annealing and elongation times and temperatures, as seen in Table 3.

Problems that arose during the K_3PO_4 treatments were that plants of 'Norland' were mistakenly used instead of 'Desiree'. In addition there was some contamination in the magenta boxes. A tray that contained both 'Norland' and 'Desiree' plants, which were mislabeled as 'Desiree' plants, resulted in 'Norland' plants being mistakenly used. This was discovered six weeks into the K_3PO_4 treatments. To replace the plants, the magenta boxes had to be opened under the hood, possibly resulting in contamination.

In addition to the contamination that may have come from the magenta boxes being re-opened, it was found that 'Desiree' plants did not grow in the 0x K_3PO_4 dark treatments. So no results could be drawn from the 0x K_3PO_4 dark boxes.

The next step of this project is to clone the *UF3GT* gene and grow it in bacteria to determine its role in the anthocyanin process, using PCR conditions determined in this project. The next step for the K_3PO_4 treatments is to test concentrations less than 1/2x K_3PO_4 in order to determine the effects of low levels of K_3PO_4 .

Sources cited

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