Transformation of *Arabidopsis thaliana* for Tandem Affinity Purification of Chloroplast Proteins

by

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Abstract

Matrix attachment region-binding filament-like protein 1 (MFP1) is part of a chloroplast protein complex that interacts with the thylakoid membrane and chloroplast DNA. The proteins in this complex need to be identified in order to determine the complex's function. Mechanisms such as tandem affinity purification can be used to identify the proteins that interact with MFP1. The purpose of this project is to use *Agrobacterium tumefaciens* to transform an MFP1-TAP expression construct into MFP1-knockout mutant *Arabidopsis thaliana* plants via floral dipping. The genotype of the mutants was confirmed via polymerase chain reactions (PCR). The success of the transformation was also confirmed via PCR. The goal of this project is to generate MFP1-TAP plants to purify protein complexes and identify the proteins that interact with MFP1.

Acknowledgements

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Introduction

MFP1 Protein

Matrix attachment region-binding filament-like protein 1, or MFP1, is a plant-specific protein that binds double stranded DNA. This protein was identified first in the tomato, *Solanum lycopersicum*, second in tobacco, *Nicotiana tabacum*, and then third in the subject of this project, *Arabidopsis thaliana* (Samaniego *et al.*, 2000). MFP1 can be found in two different organelles of the plant cell, the chloroplast thylakoid membrane, and the nuclear matrix. It is a filamentous protein that is relatively conserved in plants and has been found to share many characteristics with filament-like proteins from animals and yeast (Samaniego *et al*., 2000). MFP1 contains an N-terminal domain and a C-terminal domain. The N-terminal domain is a transmembrane domain that anchors the protein to the thylakoid membrane, while the C-terminal domain is a DNA-binding domain that is exposed to the stroma (Jeong *et al.*, 2003). The secondary structure of MFP1 contains α-helices that fold together to form a coiled-coil structure that is stabilized through tight interactions between hydrophobic side chains (Rose *et al*., 2004).

As its name suggests, MFP1 binds to segments of DNA known as matrix attachment regions, or MARs. MARs are segments of DNA that are rich in adenine and guanine and are often several hundred base pairs long (Meier *et al*., 1996 & Gindullis and Meier 1999). MARs interact with the nuclear matrix, which is why MFP1 is often localized in the nucleus and nuclear matrix (Gindullis *et al.*, 1999). MFP1 can also be found on the stroma side of thylakoid membranes within mature chloroplasts (Samaniego *et al.*, 2005). It has been suggested that a possible function of MFP1 is the anchoring of nucleoids to the thylakoid membranes in chloroplasts as well as binding chloroplast DNA (Jeong *et al.*, 2003).

MFP1, like other coiled-coil proteins, is a subunit of a larger protein complex. This project will test the hypothesis that these protein complexes are involved in photosynthesis by using mutant *Arabidopsis thaliana* plants that have had the MFP1 protein knocked out and determining their photosynthetic efficiency compared to wildtype plants. The mutant plants will be used instead of the wildtype because they lack the MFP1 protein so there would be no competition for binding partners from the untagged wildtype protein. In this project, *Arabidopsis thaliana* mutant plants that lack the MFP1 protein will have a gene coding for a tagged MFP1 inserted into their genomes. This will be done by cloning the coding sequence for the MFP1 protein into a cTAPi vector (Rohila *et al*., 2004). The cloned expression construct for the tagged MFP1 protein will then be inserted into the *Arabidopsis thaliana* genome using *Agrobacterium*-mediated transformation and confirmed via PCR and future studies will confirm the presence of the tagged protein using Western blot.

Mutant versus Wildtype

The subject of this project is a K-8-5 mutant and wildtype (MS ecotype) of *Arabidopsis thaliana*. These plants contain an inserted T-DNA that carries a gene for kanamycin resistance inserted into the MFP1 gene. The insertion of the T-DNA has the potential to alter the plant's genotype and phenotype (Kryson *et al*., 1999). As a result, the gene is knocked out and no protein is produced in the mutant plants (Jeong *et* al., 2003). This project will use two different methods that assess the genotype of the plants, including PCR analysis and testing of antibiotic resistance. To test the hypothesis that MFP1 might be involved in photosynthesis complexes, photosynthetic efficiency will be measured.

Tandem Affinity Purification

To better understand the function of MFP1, it will be necessary to identify its interaction products. This project will prepare plants that contain TAP-tagged MFP1 protein to purify complexes via TAP from chloroplasts. Tandem affinity purification, or TAP, is a method of purification of proteins and protein complexes under native conditions. The TAP method originated as a method to purify protein-protein complexes and to identify protein-protein interactions in yeast, but has since been modified for use in other organisms including mammals, bacteria, and plants. (Xu *et al.*, 2010). This method of purification requires the fusion of a TAP tag to a targeted protein at either the N-terminus or the C-terminus. The tag is composed of a calmodulin-binding domain and an IgG-binding domain that are separated by a tobacco etch virus protease cleavage site (Rohila *et al*., 2010). Once the tag is fused, the tagged protein is then expressed in a host organism and subject to a two-step purification process in which additional proteins in the complex may be identified (Puig *et al*., 2001).

Materials and Methods

Plants and Growth Conditions

The plants used in this project are K-8-5 mutant and WS ecotype wildtype *Arabidopsis thaliana*. The mutant plants have a T-DNA inserted into the genome (Jeong *et* al., 2003) and lack MFP1 gene expression. The plants were grown in round 2.5 inch diameter pots in MetroMix 360 potting soil (Sungro, Agawam, MA, USA) with 1.15 g of Osmocote 14-14-14 slow release fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH). Pots were allowed to saturate with deionized water and seeds were planted. Trays were incubated at 4 ºC for three days then transferred to a growth chamber (Percival Environmental Chamber E-30B, Percival Scientific Inc., Boone, IA, USA) under long-day conditions (16 hours light, 8 hours dark, 23 ºC day temperature, 22 ºC night temperature).

Photosynthesis Measurements

For photosynthesis measurements, two week old *Arabidopsis thaliana* plants were transferred to Ray Leach Cone-tainers (model SC-7, Stuewe and Sons Inc., Tangent, OR, USA) and the soil surface sealed with soft modeling clay. Plants were allowed to rest for 24 hours and then fitted into the Whole Plant Arabidopsis Chamber in the LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, NE, USA). Conditions in the chamber were set to match those of the growth chamber (temperature = 23 ºC, [CO_2] = 400 ppm, relative humidity = 50-75%). Measurements were taken based on CO_2 uptake per time and leaf area. Leaf area was determined using a CanoScan 9000F Mark II scanner (Canon, Melville, NY, USA) and the Black Spot Leaf Area Calculator (http://www.ncbs.res.in/blackspot.html; Varma and Osuri, 2013). Once the plants were placed in the chamber, they were exposed to 350 µM of light for 10 minutes, 1500 µM of light for 30 minutes, and then back down to 350

µM until measurements were stable, approximately ten minutes. Two measurements were made at each light interval, roughly one minute apart to confirm consistency within each interval.

Seed Sterilization and Plating for Selection

Seeds were sterilized by soaking in a sterilization solution consisting of 2 mL bleach (6% sodium hypochlorite), 10 µL Tween, and 10 mL sterile, deionized water. The seeds were soaked in the solution for ten minutes, centrifuged for 8 seconds at top speed in Eppendorf centrifuge, and the supernatant removed. The seeds were then rinsed twice with sterile, deionized water.

To determine the limit of kanamycin resistance of non-transformed *Arabidopsis thaliana* plants, 0.5x MS agar plates (pH 5.7) were created containing five varying concentrations of kanamycin: 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 300 µg/mL. MFP1-knockout mutant and wildtype seeds were sterilized, plated, and placed in the growth chamber under long day conditions. The plants were observed for 3 weeks to determine the lethal concentration of kanamycin.

Agrobacterium *and Binary Vector cTAPi*

Agrobacterium tumefaciens strain GV3101 cells were previously transformed with a cTAPi vector which contained the expression construct for the tagged MFP1 shown in Figure 1 below.

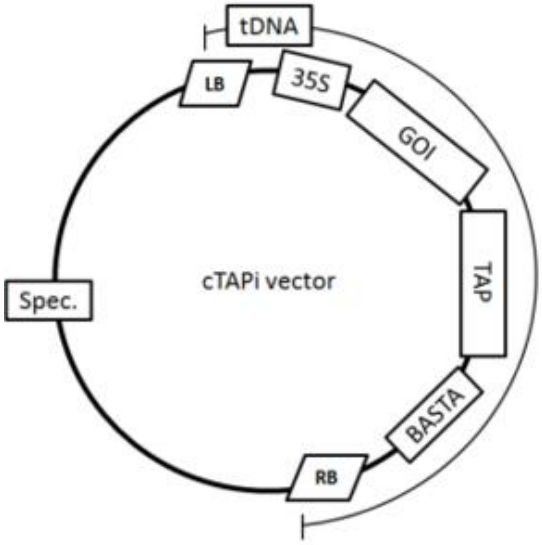


Figure 1. cTAPi vector depicting left and right borders (LB, RB), spectinomycin resistance gene (Spec.), gene of interest (GOI) which will be MFP1, promoter (35S), TAP-tag gene (TAP), and BASTA herbicide resistance gene (BASTA). The half circle represents the T-DNA that will be inserted into the plant genome.

The presence of the MFP1/cTAPi construct was confirmed using colony PCR with primers designed to amplify cTAPi and MFP1 displayed in Table 1 below.

Primer	**Sequence**	**T_m**
MFP1 Forward	5'-ACTTCAACGATCACTAGGAGAGGCA-3'	64.6 ºC
MFP1 Reverse	5'-ATTGCCTCTTTCATCAGCCAAAGCG-3'	64.6 ºC
cTAPi Forward	5'-CCTCGGATTCCATTGCCCAGC-3'	66.5 ºC
cTAPi Reverse	5'-TCGCTTCGGCGAGCAGGTTG-3'	66.6 ºC

Table 1. Primers and sequences used for PCR with melting temperatures (T_m).

Figure 2a and Figure 2b show where the MFP1-forward, MFP1-reverse, and JL-202 primers will anneal on the MFP1 gene.

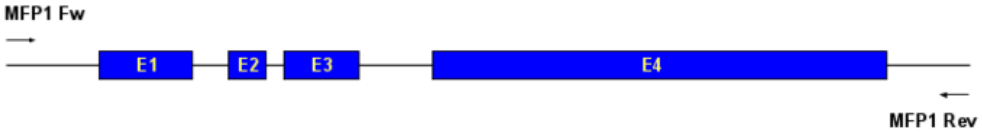


Figure 2a. MFP1 gene depicting where the forward and reverse primers anneal.

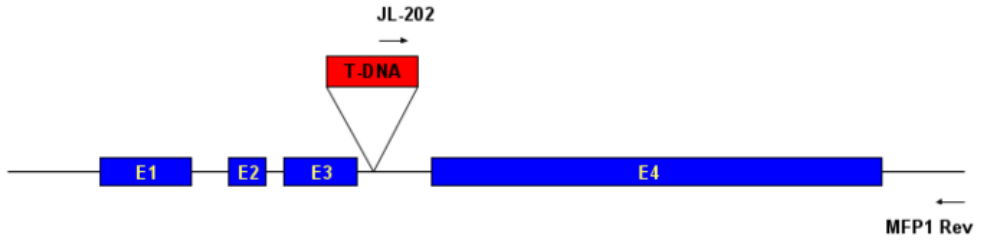


Figure 2b. MFP1 gene with T-DNA insertion depicting where the JL-202 and MFP1-reverse primers anneal.

Each of the four PCR reactions contained 1 µL of a forward primer and 1 µL of a reverse primer. Two reactions contained cTAPi forward and MFP1 reverse while the other two reactions contained cTAPi reverse and MFP1 forward. Within the two reactions that used cTAPi forward and MFP1 reverse, one of the reactions contained a GV3101 colony that had been streaked from glycerol stocks onto an LB agar plate containing rifampicin (50 mg/mL), gentamycin (50 mg/mL), spectinomycin (100 mg/mL), and streptomycin (50 mg/mL), and the other reaction contained 0.5 µL MFP1/cTAPi DNA (1:5 dilution) and served as a positive control. As for the two reactions that used the cTAPi reverse and MFP1 forward primers, the first reaction also contained a GV3101 colony, and the second contained MFP1/cTAPi and served as a second positive control. Each of the four reactions also contained 0.4 µL 25 mM dNTPs, 0.5 µL Taq polymerase, 5 µL 10x PCR buffer, and deionized water to bring the final volume to 50 µL. The cycle program for the colony PCR for the amplification of the *Agrobacterium tumefaciens* strain GV3101 is displayed in Table 2 below. The machine used was an Applied Biosystems 9700 PCR thermal cycler system.

The PCR reactions were then subject to 1% agarose gel electrophoresis at 90 volts for one hour.

Stage	Temperature	Time
First Cycle	95 ºC	5 minutes
Denaturing	95 ºC	1 minute
Annealing	60 ºC	1 minute
Extending	72 ºC	3 minutes
Last Cycle	72 ºC	5 minutes
Number of Cycles	25	

Table 2. PCR protocol for the amplification of *Agrobacterium tumefaciens* strain GV3101

Transformation of Arabidopsis thaliana

LB media was inoculated with a GV3101 colony that contained the cTAPi vector from the antibiotic-containing agar plates grown at 28 ºC for five days. The *Agrobacterium* solution was then spun down and resuspended to OD_{600}=0.742 in 5% sucrose solution. Silwet-77 (Helena Chemical Company, USA) was added to the solution to a concentration of 0.05% and mixed well. Silwet acts as a surfactant to aid in the attachment of bacteria to the plant cells.

Seeds collected from the MFP1-knockout and wildtype *Arabidopsis thaliana* were planted and allowed to grow under long day conditions (16 hour days at 23 ºC, 8 hour nights at 20 ºC). Once the plants began to flower, the first bolts were clipped to encourage the growth of secondary bolts. Six days after the first bolts of the plants were clipped, the above-ground parts of the flowering plants were dipped in the *Agrobacterium* solution for 2-3

seconds with gentle agitation. The dipped plants were placed under a plastic cover for 24 hours to maintain high humidity and then watered and grown normally. The plants were then dried and the dry seeds collected.

DNA Extraction and PCR Analysis

DNA was extracted from leaf tissue by grinding the tissue, adding extraction buffer (0.2 M Tris-HCl, pH 9.0; 0.4 LiCl; 25 mM EDTA; 1% SDS), centrifuging, and then centrifuging the resulting supernatant at top speed in a microcentrifuge for five minutes. Isopropanol was added to the centrifuged supernatant in a ratio of 1:1, mixed by inversion, and centrifuged again for five minutes. The resulting pellet was dried and resuspended in TE buffer (10 mM Tris pH 8, 1 mM EDTA). Each reaction contained 2 µL DNA, 5 µL 10x Taq buffer, 1 µL dNTPs, 1 µL of each primer, 0.5 µL Taq polymerase, and deionized water to bring the total volume to 50 µL. Eight samples of DNA were collected from pooled leaves of four transformed mutant and wildtype *Arabidopsis thaliana.* Each sample of DNA was subjected to four different PCR reactions. The first reaction tested the presence of the wildtype allele using the primers MFP-RP and MFP-LP. The second reaction tested the presence of the mutant allele using the primers MFP-RP and JL-202. The third and fourth reactions tested the presence of the MFP1 gene using the primers cTAPi-forward and MFP1-reverse as well as cTAPi-reverse and MFP1-forward. The sequences and melting temperatures for these primers can be found in Table 1 and Table 3. The reactions were run on a 1% agarose gel (1.34 g agarose, 134 mL 1x TAE, 6.7 µL EtBr) at a constant 90 volts for one and a half hours.

Primer	Sequence	T_m
MFP-LP	5’-GGGCTTCTGTGTTCGAGTAATGTCG-3’	66.2 ºC
MFP-RP	5’-TTCTTATGAGTTCTTCCTTCTGCTGTTTG-3’	63.2 ºC
JL-202	5’-CATTTTATAATAACGCTGCGGACATCTAC-3’	63.2 ºC

Table 3. Primers and sequences with melting temperatures used for PCR of *Arabidopsis thaliana* to confirm T-DNA insert.

Results and Discussion

Mutant and wildtype plants do not differ in photosynthetic efficiency

The first test to identify a difference between mutant and wildtype *Arabidopsis thaliana* involved measuring the photosynthetic efficiency of each line of plants to determine if the MFP1 complex is involved in photosynthesis. The carbon dioxide uptake data collected from this experiment is displayed in Table 4 below. Each data collection was normalized for the surface area of the leaves of each plant that was measured.

	Wildtype 1 (SA=5.2)	Wildtype 2 (SA=15.8)	Wildtype 3 (SA=16.8)	Mutant 1 (SA=3.6)	Mutant 2 (SA=14.1)	Mutant 3 (SA=11.3)
350-1	N/A	4.70	1.56	1.90	4.24	1.15
350-2	N/A	4.79	1.61	2.09	4.29	1.23
1500-1	5.38	8.59	5.45	6.14	9.30	4.41
1500-2	5.31	8.60	5.46	5.98	9.41	N/A
350-3	2.41	5.23	1.51	2.83	4.57	0.98
350-4	2.49	5.26	1.51	N/A	4.63	1.00

Table 4. Measurements of photosynthesis of mutant and wildtype *Arabidopsis thaliana* under normal and high light conditions. Surface area measured in cm^2. Light level is in the leftmost column and is measured in µM. Measurements indicate carbon dioxide uptake in µmol CO_2 m^2/s^2.

The amount of carbon dioxide taken in by the mutant and wildtype plants suggest that there is not a difference in photosynthetic efficiency between the two. The size of each plant's leaves affects the amount of photosynthesis that can occur; larger plants can exchange more carbon dioxide, however the data in Table 4 have been normalized to account for this difference in leaf size. Wildtype 2 (SA=15.8 cm^2) and Mutant 2 (SA=14.1 cm^2) plants used

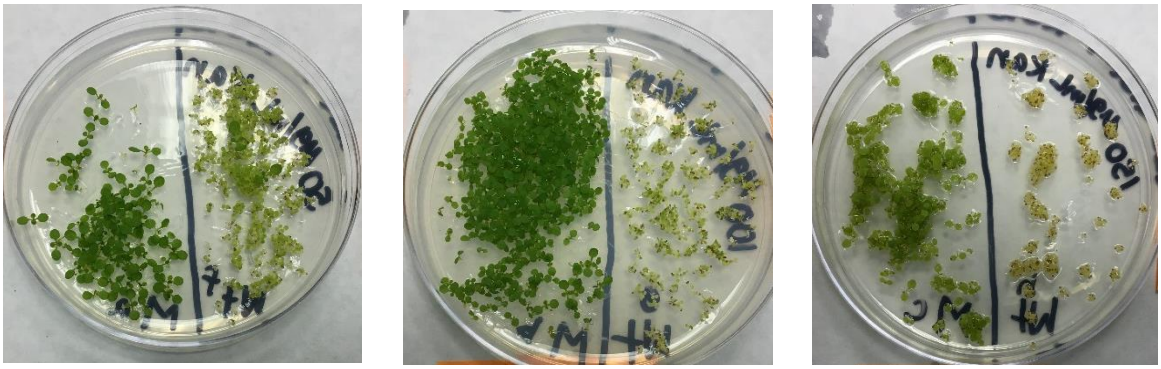
similar amounts of carbon dioxide, suggesting that there was no difference in the amount of photosynthesis that occurred. Both plants had a constant photosynthetic rate around 4.5 for normal light, around 9 for high light, and around 4.8 when returned to normal light, suggesting that there is no difference in photosynthetic efficiency between mutant and wildtype plants.

The photosynthesis experiment also aimed to determine if the MFP1 protein complex is a photosynthetic complex. It was hypothesized that when the plants were subjected to high light, it would damage the mechanism responsible for successful photosynthesis. When the plants were returned to normal light after being shocked by high light, it was predicted that the photosynthetic rate would drop much lower in the mutant than what it originally demonstrated. This result was not seen. After exposure to high light, the plants resumed similar photosynthetic rates as demonstrated before the increase in light intensity. This could have several explanations. It is possible that the intensity of the light was not high enough to shock the plants. It is also possible that the MFP1 protein complex is not involved in photosynthesis, so there would be no phenotypic difference between the mutant and wildtype plants when it comes to photosynthesis.

Mutant plants are resistant to kanamycin

In order to determine if the mutant *Arabidopsis thaliana* plants contained the T-DNA insertion, seeds from both the mutant and wildtype plants were planted on plates containing kanamycin to test the plants' resistance to the antibiotic. Plants that successfully grow on the kanamycin plates are suspected to contain the inserted T-DNA because it contains a kanamycin resistance gene as a genetic marker. If the plants do not contain the T-DNA, then they are expected to die because the levels of kanamycin in the plates are too high for the

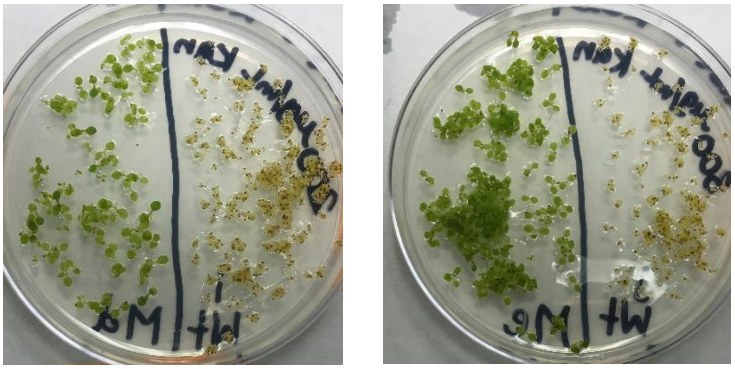
wildtype plants to survive. The images in Figure 3 and Figure 4 show the progression of the growth of the mutant and wildtype plants in varying concentrations of kanamycin. The effects of the kanamycin on the plants were not immediate, which is why the images were taken ten days apart. This gave the plants plenty of time to absorb and react to the high levels of kanamycin. The mutant plants were more resistant to the kanamycin than the wildtype plants. After 7 days, the wildtype plants began looking withered at the lowest concentration of kanamycin, 50 µg/mL. As the concentration increased, the plants looked less and less healthy until they were completely dead in the 200 µg/mL and 300 µg/mL concentrations. After 17 days, the wildtype plants in all five concentrations of kanamycin were dead. As for the mutant plants, the green color of the leaves was less vibrant beginning at 150 µg/mL than at 50 µg/mL and 100 µg/mL. After 17 days, there was a significant number of plants that were killed by 150 µg/mL kanamycin, however a few plants managed to survive, which is shown in Figure 4. This observation was also seen in the 200 µg/mL and 300 µg/mL concentrations. It is expected that the plants that are successfully growing on the kanamycin plates contain the T-DNA.



Mutant a Wildtype f
50 µg/mL kan.

Mutant b Wildtype g
100 µg/mL kan.

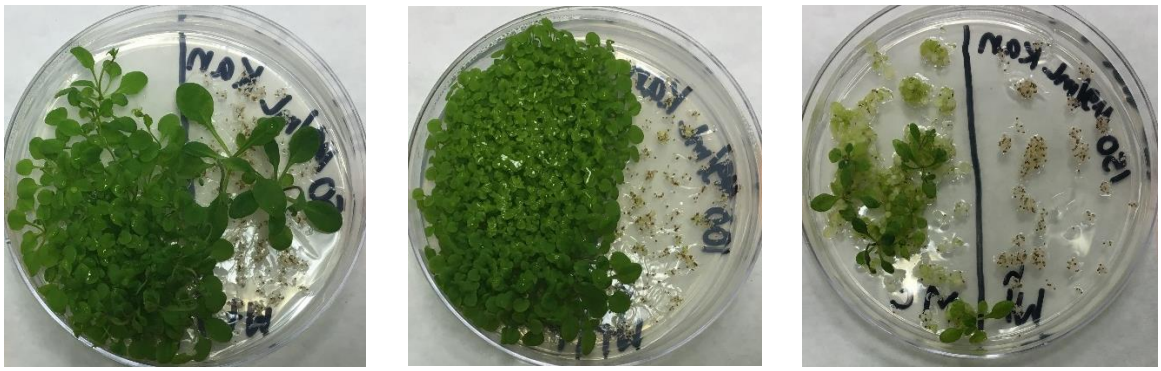
Mutant c Wildtype h
150 µg/mL kan.



Mutant d Wildtype i
200 µg/mL kan.

Mutant e Wildtype j
300 µg/mL kan.

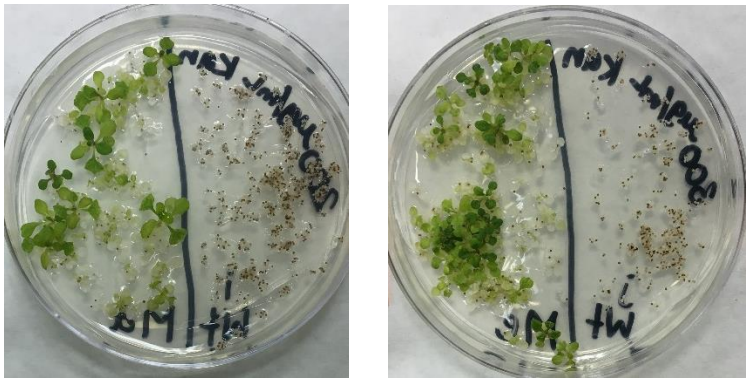
Figure 3. Non-transformed *Arabidopsis thaliana* grown on 0.5x MS agar plates with varying concentrations of kanamycin 7 days after planting.



Mutant a Wildtype f
50 µg/mL kan.

Mutant b Wildtype g
100 µg/mL kan.

Mutant c Wildtype h
150 µg/mL kan.



Mutant d Wildtype i
200 µg/mL kan.

Mutant e Wildtype j
300 µg/mL kan.

Figure 4. Non-transformed *Arabidopsis thaliana* grown on 0.5x MS agar plates with varying concentrations of kanamycin 17 days after planting

Mutant plants carry a T-DNA insertion in the MFP1 gene

To confirm that T-DNA-containing plants had the insertion in the MFP1 gene, plants were genotyped using PCR. DNA was extracted from the leaf tissue of mutant *Arabidopsis thaliana* plants, that were selected for kanamycin resistance. The concentration and purity of the DNA in each reaction was determined by the Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and is displayed in Table 5.

	Concentration (ng/µL)	260/280	260/230
Mutant 1	66.5	2.24	0.53
Mutant 2	86.9	2.28	0.62
Mutant 3	147.6	2.23	1.58
Mutant 4	325	2.16	1.14
Mutant 5	162	2.24	1.32
Mutant 6	341.9	2.18	1.52
Mutant 7	160	2.23	0.99
Mutant 8	132.3	2.22	1.22

Table 5. Concentration and purity of extracted DNA from mutant *Arabidopsis thaliana*.

The concentration data represents how much DNA was collected from each sample of leaf tissue. The 260/280 and 260/230 measurements represent the purity of the isolated DNA sample. The ideal value for 260/280 is approximately 1.8 for pure DNA and 2.0 for pure RNA. Each of the 260/280 values for the collected samples were higher than the ideal for DNA, suggesting that the DNA was mixed with RNA and other contaminants. The 260/230 value represents contamination from protein, phenolics, and carbohydrates and is ideally higher than the 260/280 value for pure DNA. Because the 260/230 values from the isolated DNA are lower, it suggests that the DNA is mixed with contaminants, likely carbohydrates common in plant cells. This was expected because the protocol used was for a crude isolation of DNA. Although the DNA samples contained RNA, protein, and carbohydrates, these contaminants are not expected to interfere with the PCR reaction.

The samples were then subjected to four PCR reactions. The reactions were then subjected to agarose gel electrophoresis. Because leaf tissue was only collected from the plants that successfully grew on the kanamycin plates, it is expected that the PCR will show bands representing the mutant allele and no bands representing the wildtype allele, suggesting that the mutant *Arabidopsis thaliana* does contain the T-DNA and is homozygous mutant as opposed to heterozygous mutant. The resulting gel was imaged and is displayed in Figure 5 below.

Reactions 1 through 8 in the PCR results from Figure 5 below have no bands present. This is expected because the primers were designed to amplify the wildtype allele. Because there are no bands representing the wildtype allele, it suggests that the mutants are homozygous mutant as opposed to heterozygous mutant. There are five faint bands present in reactions 9 through 16. These bands represent the mutant allele and were expected to be present. Because these plants only contain the mutant allele and not the wildtype allele, it confirms that the mutant plants are homozygous mutant. The DNA fragment that represents the wildtype allele is expected to be approximately 1.3 kb, while the mutant allele is expected to be slightly smaller. Some of the reactions that produced no fragments could be a result of the very crude DNA extraction protocol that was used. This experiment could be repeated using higher concentrations of DNA or with better purified DNA. It would also be beneficial to test a control reaction with wildtype DNA to confirm that the wildtype primers are working effectively.

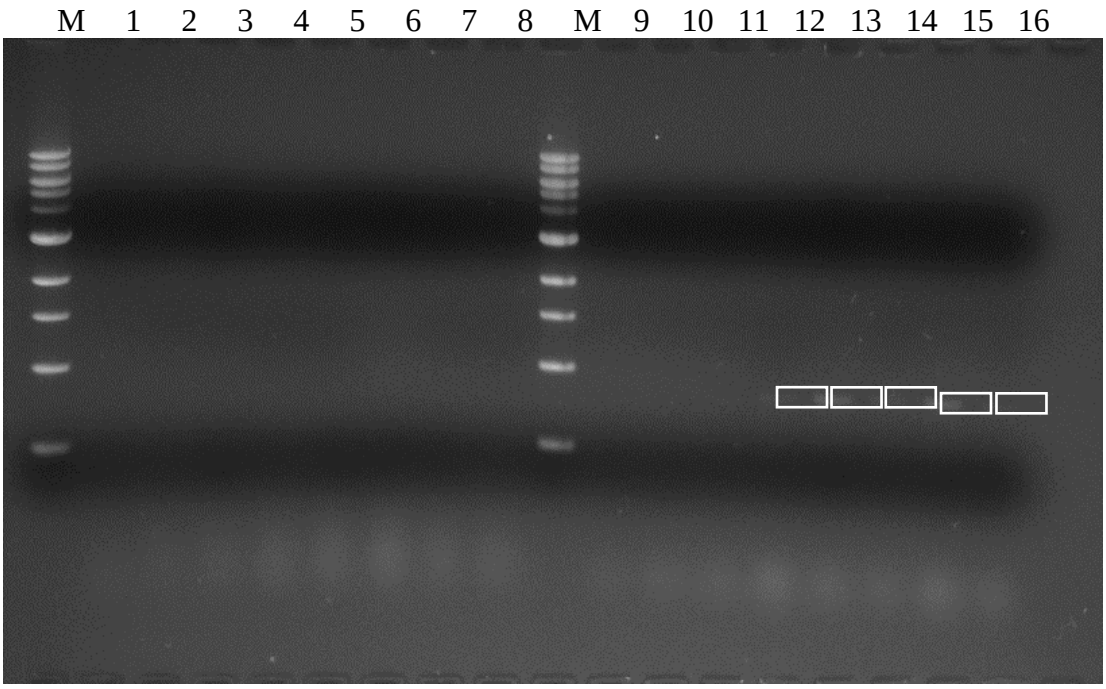


Figure 5. PCR reactions of mutant *Arabidopsis thaliana* selected for via kanamycin resistance run on a 1% agarose gel. For reactions 1-8, primers MFP-LP and MFP-RP were used. For reactions 9-16, primers MFP-RP and JL-202 were used. The size marker is a 1 kb ladder.

Confirmation of transgenic Agrobacterium

The *Agrobacterium tumefaciens* that was used in this project was subjected to a colony PCR reaction to ensure that the tagged the construct coding for MFP1 that was to be inserted into the *Arabidopsis thaliana* genome was in fact present in the GV3101 strain. Four reactions were performed to test for the presence of the target gene. Two reactions tested a colony of GV3101 and the other two reactions were controls and tested plasmid MFP1/cTAPi. The products of the reactions were then run on an agarose gel; an image of the gel is displayed in Figure 6.

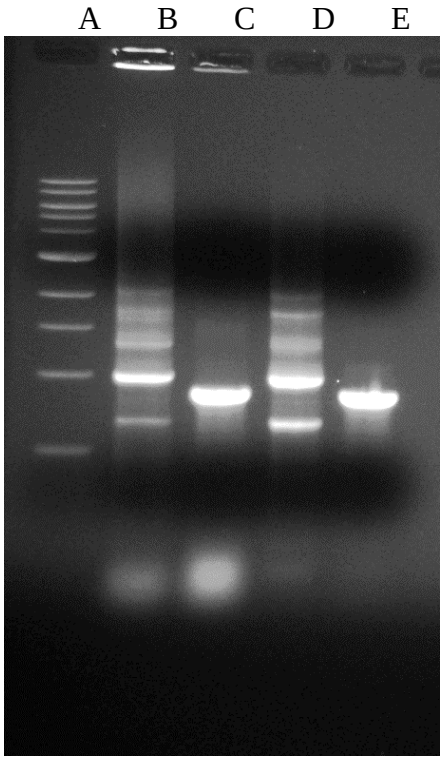


Figure 6. Colony PCR reactions of *Agrobacterium tumefaciens* strain GV3101 run on an agarose gel. Lane A: size marker, 1 kb ladder. Lane B: cTAPi-For, MFP1-Rev, GV3101 colony. Lane C: cTAPi-Rev, MFP1-For, GV3101 colony. Lane D: cTAPi-For, MFP1-Rev, control. Lane E: cTAPi-Rev, MFP1-For, control.

The reaction from Lane B in Figure 6 above used the same primers as the control reaction from Lane D, while the reaction from Lane C used the same primers as the control reaction from Lane E. The bands that represent the 5' border of the cloned MFP1 construct are expected to have sizes of approximately 1.5 kb, while the bands that represent the 3' border of the cloned MFP1 construct are expected to have sizes of approximately 1.3 kb. There are additional bands present in the lane representing the 5' border of the cTAPi vector. This could be a result of the primers binding to multiple regions of the T-DNA, which could potentially be avoided with a higher dilution of the primers or altering the annealing temperature. Because the banding patterns are the same for Lanes B and D and the same for

Lanes C and E, it suggests that the construct for the MFP1 protein is present in the *Agrobacterium tumefaciens* strain.

Confirmation the success of plant transformation after floral dipping

The cloned MFP1-TAP construct was transformed into mutant plants using floral dipping. DNA was extracted from the transformed *Arabidopsis thaliana* and was subjected to two PCR reactions with the primers cTAPi-reverse and MFP1-forward as well as cTAPi-forward and MFP1-reverse. Each PCR reaction that is displayed in Figure 7 below contained DNA that was combined from four different plants. All of those collected samples except for Lane 6 show distinct bands. These banding patters are similar to those in Figure 6 which represents the MFP1/cTAPi plasmid. This suggests that the bands in Figure 7 represent the inserted MFP1/cTAPi. The presence of these bands indicates that the transformation of the plants was successful. Future studies should repeat these PCR reactions and include a control with wildtype and untransformed mutant DNA to confirm that this is not unspecific binding of primers. A cTAPi control DNA should also be run on a gel for a comparison of fragment sizes.

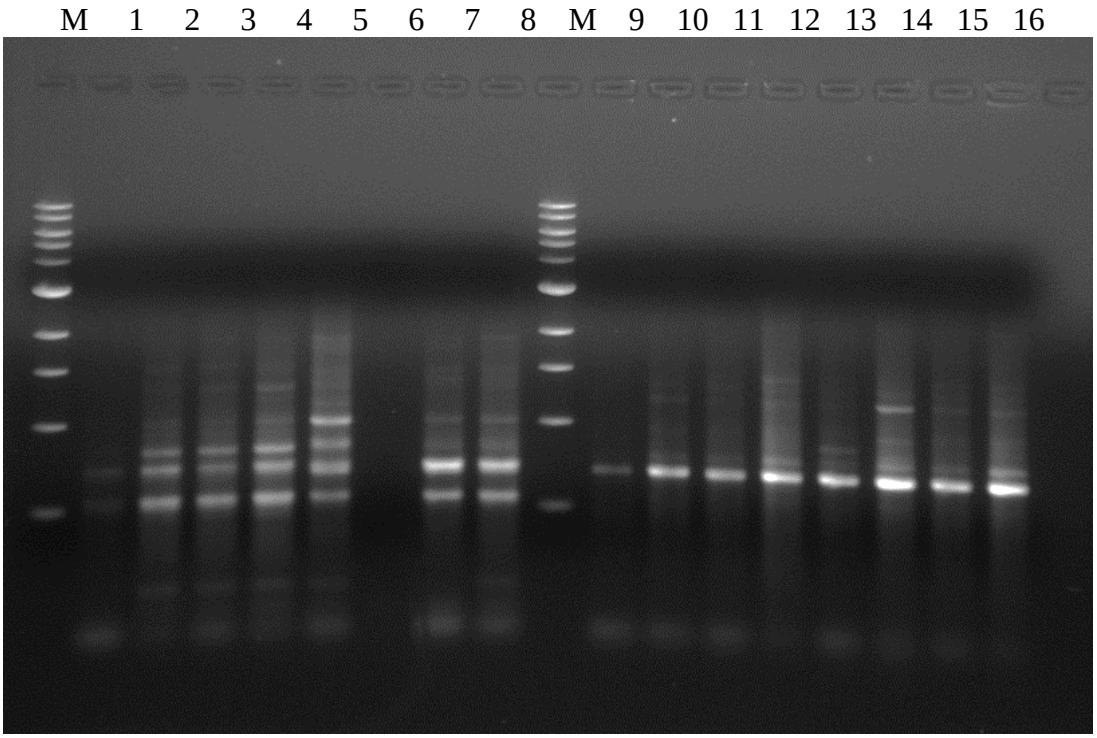


Figure 7. Image of PCR reactions of transformed mutant *Arabidopsis thaliana* run on a 1% agarose gel. For reactions 1-8, primers cTAPi-reverse and MFP1-forward were used. For reactions 9-16, primers cTAPi-forward and MFP1-reverse were used. The size marker is a 1 kb ladder. Each reaction contained DNA from leaves pooled from four plants.

Conclusion and Future Studies

The purpose of this experiment was to create a line of transgenic *Arabidopsis thaliana* plants containing a tagged MFP1 protein to purify complexes and identify interaction partners. The transformation was done via floral dipping in a solution containing *Agrobacterium tumefaciens* strain GV3101. Once the transformation was complete, seeds were collected from the transformed plants and subjected to PCR to confirm the presence of the tagged MFP1 construct. The results indicate that plants were indeed successfully transformed.

The success of this project will allow future studies on this subject to be performed. Protein can be isolated from the transformed plants and Western blotted to confirm the presence of the tagged MFP1 protein. These successfully transformed *Arabidopsis thaliana* plants can also have protein complexes isolated from chloroplasts and used in tandem affinity purification for identification of MFP1 binding partners. The protein complex that contains MFP1 can be isolated and the remaining proteins can be identified by mass spectrometry. Because this project found that the MFP1 protein is not functionally involved in the photosynthetic complex, the identification of MFP1's actual binding partners can provide clues about the function of this complex. The methods used in this project can also be adapted to insert different tagged proteins and use different strains of *Agrobacterium tumefaciens*. Overall, this project serves as a basis for countless other studies involving the MFP1 protein.

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