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Ozone effect on cell death

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Abstract

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Ozone, a dangerous air pollutant, poses significant threats to plants, slowing growth, reducing productivity, and even triggering plant death.

This collaborative project with the University of Helsinki focused on studying the effects of ozone on plant health, particularly its role in cell death. The research aimed to first detect and confirm the presence of new mutants and secondly, comprehensively investigate the impact of ozone on these mutants.

This study examines the effect of ozone treatment on mutated Arabidopsis plants. The plants were grown under controlled conditions, and DNA was extracted from leaves using a standard protocol. PCR experiments were conducted, and the resulting bands were visualized through gel electrophoresis. Selected samples with one or two mutated genes were exposed to ozone to assess their resilience. The goal was to understand genetic reactions to ozone stress and evaluate the role of mutations in environmental adaptation. Additionally, a unique method measuring ion leakage after freezing was employed to assess the impact of ozone treatment on plant samples, mitigating size variations. The study provides valuable insights into genes' ability to withstand ozone and contributes to understanding their role in environmental adaptation.

Results varied, indicating the complexity of mutant and wild-type gene expression. Despite diverse findings, a clear outcome emerged: mutant plants were not resistant to ozone treatment, emphasizing their vulnerability to environmental stress. Col-S bak4 pad 4 exhibited heightened susceptibility. This study establishes a foundation for deeper research into plant responses to environmental challenges, highlighting the necessity for meticulous experimental design and understanding genetic diversity for cultivating resilient plants in a changing world.

Our research illuminates the intricate interaction between ozone exposure and plant cell death, advancing a deeper understanding of the impact of ozone pollution on plant survival. By unraveling these complex mechanisms, we not only enhance our comprehension of plant responses to stressors but also pave the way for potential strategies to mitigate the adverse effects of ozone pollution. This study is particularly significant given the increasing global levels of ozone.

Keywords: ozone, cell death

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Otsoni, joka on vaarallinen ilman saaste, aiheuttaa merkittäviä uhkia kasveille, hidastaen kasvua, tuottavuutta ja jopa laukaisten kasvien kuoleman.

Tämä yhteistyöprojekti Helsingin yliopiston kanssa keskittyi otsonin vaikutusten tutkimiseen kasvien terveyteen, erityisesti otsonin rooliin solukuolemassa. Tutkimuksen tavoitteena oli ensinnäkin havaita ja vahvistaa uusien mutanttien läsnäolo ja toiseksi tutkia kattavasti otsonin vaikutusta tähän mutantteihin.

Tämä tutkimus selvittää otsonikäsittelyn vaikutusta mutatoituihin Arabidopsis-kasveihin. Kasvit kasvatettiin hallituissa olosuhteissa, ja DNA uutettiin lehdistä käyttäen standardiprotokollaa. PCR-kokeet suoritettiin, ja tuloksena syntyneet kaistaleet visualisoitiin geelielektroforeesilla. Valitut näytteet, joissa oli yksi tai kaksi mutatoitunutta geeniä, altistettiin otsonille niiden kestävyyden arvioimiseksi. Tavoitteena oli ymmärtää geneettisiä reaktioita otsonistressiin ja arvioida mutaatioiden roolia ympäristösopeutumisessa. Lisäksi käytettiin ainutlaatuista menetelmää, joka mittaa ionien vuotoa jäädytyksen jälkeen arvioimaan otsonikäsittelyn vaikutusta kasvinäytteisiin, lieventäen kokovaihteluja. Tutkimus tarjoaa arvokkaita näkemyksiä geenien kyvystä kestää otsonia ja edistää ymmärrystä niiden roolista ympäristön sopeutumisessa.

Tulokset vaihtelivat, osoittaen mutantti- ja villityyppigeenien ilmentämisen monimutkaisuuden. Huolimatta monipuolisista havainnoista, selvä tulos nousi esiin: mutanttikasvit eivät olleet vastustuskykyisiä otsonikäsittelylle, korostaen niiden alttiutta ympäristöstressille. Col-S bak4 pad 4 osoitti korkeampaa alttiutta. Tämä tutkimus luo perustan kasvien vastauksien syvemmälle tutkimukselle ympäristöhaasteisiin, korostaen tarkan kokeellisen suunnittelun ja geneettisen monimuotoisuuden ymmärtämisen välttämättömyyttä kestävien kasvien viljelyssä muuttuvassa maailmassa.

Tutkimuksemme valaisee monimutkaista vuorovaikutusta otsonialtistuksen ja kasvien solukuoleman välillä, edistäen syvempää ymmärrystä otsonisaasteen vaikutuksista kasvien selviytymiseen. Purkamalla näitä monimutkaisia mekanismeja, parannamme paitsi käsitystämme kasvien reaktioista niitä stressaaviin ympäristötekijöihin, avaamme myös tien mahdollisille strategioille otsonisaasteen haittavaikutusten lieventämiseksi. Tämä tutkimus on erityisen merkittävää kasvavien globaalien otsonitasojen vuoksi.

Avainsanat: otsoni, solun kuolema

Contents

List of Abbreviations

Appendices

1 Introduction

This research initiative represents a collaborative endeavour with the University of Helsinki, aiming to investigate the repercussions of ozone exposure on cellular apoptosis in plants. The primary objective involves the identification and validation of novel mutants, while the secondary goal centres on scrutinizing the influence of ozone on this biological process.

Ozone, recognized as a deleterious atmospheric pollutant, poses a threat to plants by impeding growth, diminishing productivity, and, in severe instances, precipitating plant mortality. The adverse impact of ozone pollution on plant viability assumes considerable significance, necessitating in-depth exploration, particularly given the escalating global levels of atmospheric ozone [1]

2 Overview

2.1 Arabidopsis as a model organism for plant biology

Arabidopsis thaliana (Figure 1), also known as thale cress, is a small plant with pretty white or purple flowers. Scientists love using Arabidopsis as a model organism in their research because it has a small genome size, which makes it easy to study, and it grows quickly, going from seed to mature plant in just 6-8 weeks! Plus, it produces a lot of seeds, so there are plenty of plants to work with. [1.]

As a model organism for plant biology, Arabidopsis thaliana has several key attributes that make it an ideal subject for genome analysis, such as its short generation time, compact stature, prolific offspring production, and comparatively small nuclear genome. [2.]

Figure 1. Arabidopsis Thaliana in the laboratory

2.2 Importance of studying plant responses to ozone pollution

Ground-level ozone is a prominent air pollutant that raises substantial environmental apprehensions. Unlike its naturally occurring counterpart in the upper atmosphere, which serves as a protective barrier by absorbing detrimental ultraviolet rays from the sun, ground-level ozone presents a dual hazard, impacting both botanical ecosystems and human health. Unlike emissions directly emanating from smokestacks or vehicles, ground-level ozone is a by-product of chemical reactions in the atmosphere, particularly involving nitrogen oxides and volatile organic compounds, catalysed by sunlight. Its deleterious impact extends worldwide, adversely affecting various plant species, including crops and natural ecosystems. [4.] Understanding how plants respond to ozone pollution is crucial, as it can harm their growth, productivity, and survival. [5.]

One of the reasons why studying how ozone pollution affects plants is because plants play a key role in absorbing and sequencing pollutants thus mitigating air pollution. Developing strategies to improve air quality and reduce the impacts of air pollutants is mainly dependent on understanding how plants respond to ozone pollution. [5.]

Studying how plants react to ozone pollution has not only helped us better understand their response but has also resulted in the creation of new crop varieties that can better withstand this environmental stressor. By pinpointing the genes and mechanisms that allow plants to survive ozone exposure, researchers can develop more resilient crops, ultimately leading to higher crop yields and better food security. [5.]

2.3 Gene expression and genotyping

Gene expression is the mechanism through which genetic information is transformed into a functional product, protein. [6,7]. This process primarily involves the creation of messenger RNA molecules, which can code for proteins or serve other purposes, through a step called transcription. Think of gene expression as a switch for when and where RNA and proteins are produced (on/off), and as a dial to regulate the quantity of these products (volume control). Gene expression is tightly controlled and can vary significantly in response to different conditions and cell types. RNA and protein products from certain genes can influence the expression of other genes. We can determine when, where, and how much a gene is active by assessing its functional products or observing related physical traits. [6.]

Genotyping is a molecular biology technique used to discern genetic variations within an individual's DNA through biological assays. This method involves comparing the obtained results with either the genetic sequence of another individual or a predetermined reference sequence. Through such analytical

procedures, the distinctive genetic profiles among individuals can be identified and characterized [8].

Genotyping serves as a potent instrument for the detection of genetic variations that have the potential to bring about significant alterations in an individual's observable traits, encompassing both distinctive physical characteristics that define our uniqueness and the pathological changes underpinning various diseases. The utility of genotyping extends across a broad spectrum of applications, spanning fundamental scientific investigations, medical practice, and agricultural contexts [9]. In the domain of agriculture, genotyping plays a pivotal role in enhancing crop productivity and bolstering resistance against pests and diseases [10].

2.3 Preparation for the PCR analysis

The mutated Arabidopsis plants (table 1) had already been grown before the initiation of the experiment. Seeds were planted in a 1:1 mixture of peat and vermiculite, stratified for 3 days, and then cultivated at temperatures of 22/19°C (day/night) with a relative humidity of 70/90% (day/night). The light conditions consisted of 230 µmol of photons m–2 s–1, maintained under a 12-hour light/12-hour dark cycle for one week. Following germination, the seedlings were transplanted into a new mixture of 1:1 peat and vermiculite.

Ozone treatments were administered to plants at three weeks of age. For DNA isolation and genotyping, leaves were collected from plants aged between 3 and 5 weeks. Some of the sample lines contain more than one mutant gene. Those samples were tested with multiple primers to amplify the desired mutant, such as the *aos* x *cngc* sample. This sample contains gene mutations in AOS as well as genes from the CNGC line.

Samples number	Samples name

1-22	Col-S *bak1-5* #37
23-44	*aos* x *cngc*
45-66	*coi1* x *cngc*
67-86	Col-S Sniper 99 or 60 B
87-97	Col-S Sniper 4-3 #86

Table 1. Name and number of the samples

A leaf was extracted as the primary material for use in the experiment. The extraction of DNA was performed utilizing a standard protocol (Appendix 1). Following DNA extraction, the samples were prepared for polymerase chain reaction (PCR) through the addition of necessary reagents and were then subjected to thermal cycling in a PCR machine. In the context of PCR cycles, the incorporation of positive and negative controls is imperative for the validation of experimental quality.

The positive control functions as a reference point, while the negative control serves to manifest the absence of contamination. Detection of bands in the negative control would signify the occurrence of contamination during the experimental procedure. Visualization of amplified DNA products was conducted through gel electrophoresis.

Upon inspection of the resulting gel, the necessity for subsequent PCR preparations was determined. If the desired band was present, the plant samples were saved for future generations. However, in cases where the results were deemed unsatisfactory, further optimization of the PCR was conducted to obtain the targeted band.

The selected samples from PCR experiments containing single and double mutant genes are subsequently subjected to ozone exposure to evaluate their resistance to this environmental factor. This critical step aims to determine whether the genes in these samples exhibit resilience or susceptibility to ozone.

The ozone exposure serves as a pivotal test to assess the genetic responses to this specific stressor, shedding light on the potential impact of mutations on gene function and environmental adaptation. By conducting this experiment, researchers can gain valuable insights into the genes' ability to withstand ozone and contribute to a deeper understanding of their role in environmental resilience.

Here is the plan of the PCR rounds with digestion for the samples.

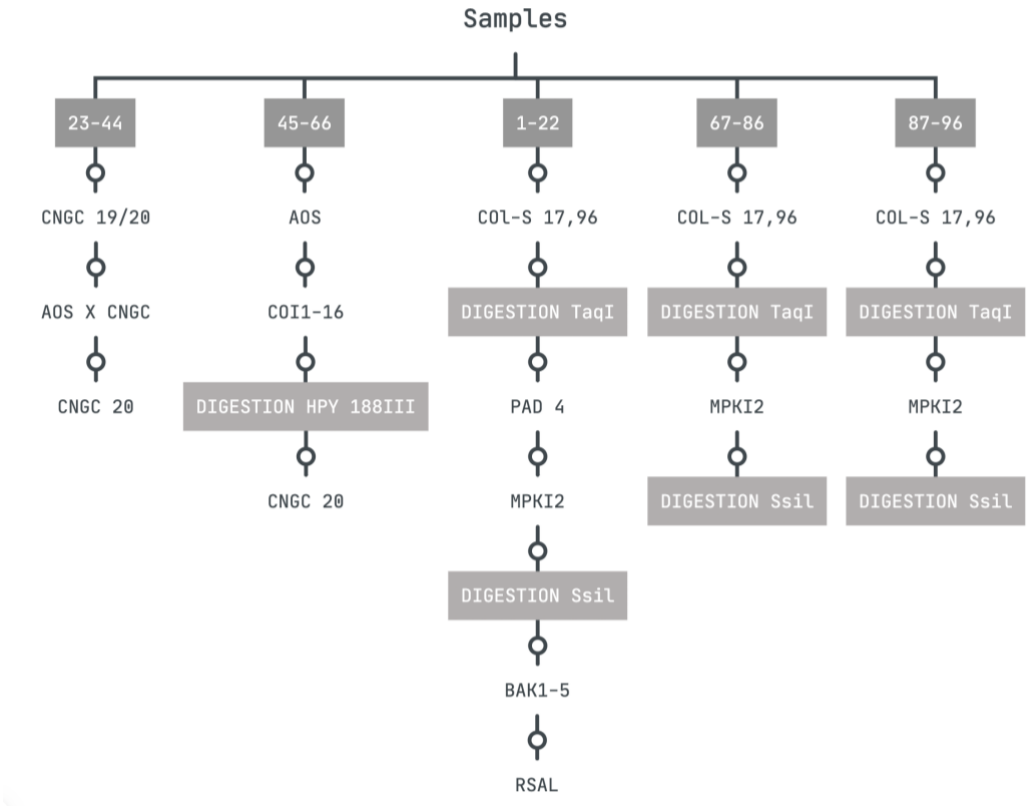

Figure 2. The Simplified version of Polymerase Chain Reaction (PCR) experiments.

The above figure displays the combination of primers and restriction enzymes used in the PCR experiments conducted in this project. The restriction enzymes are enclosed in a bordered box, while the remaining components are primers.

To facilitate readability and reference, the samples used in the experiments were assigned numbers 1 through 97.

2.3.1 Materials

Primers	Info
CNCG 19/20	CNGC19/20 is a deletion of the genes CNGC19 and CNGC20. The primer pair for CNGC 19/20 amplifies the mutant while the primer pair for CNGC 20 amplifies the wildtype.
AOS	Allene oxide synthase is a T-DNA mutant. Genotyping is performed with two gene-specific primers and one primer that binds to the T-DNA.
coi1-16	Genotyping is performed with CAPS (Cleaved Amplified Polymorphic Sequence marker). First, a PCR product is amplified from COI1, followed by digestion with Hpy188III.
pad4	The pad4 mutant is deficient in plant pathogen responses. It is genotyped with a dCAPS marker. dCAPS (Derived Cleaved Amplified Polymorphic Sequences) marker is a molecular biology technique used to detect single nucleotide polymorphisms (SNPs) or mutations in DNA samples.
MPK12	A CAPS marker, (cleaved Amplified Polymorphic Sequence marker), is a molecular genetic tool used for identifying genetic variations in DNA. It involves the use of a restriction enzyme to cleave DNA at specific recognition sites, and the resulting fragments are then analysed to detect polymorphisms or genetic differences among individuals or populations.
bak1-5	a dCAPS marker

Col-S 17,96	A CAPS marker was used to show the presence of the QTL S3, giving ozone sensitivity in Col-S mutants.

Table 2. Primers that were used in the experiments.

2.3.2 The first set of samples

The present study evaluated the AOSxCNGC line samples 23–44 using PCR to investigate their genetic makeup. The plant that has been used here has AOS and CNGC mutants. The PCR master mix reagents were prepared as per the instructions in Table 3, and a provided primer was used in the experiment. In total, there were 23 samples, including the negative control and the wild-type control. The data obtained from this study will be analyzed to determine the genetic variations present in the AOS x CNGC line samples.

Reagents	Amount
Magic buffer (Without K^+)	3µl
CNGC 19/20 Forward primer	0,5µl
CNGC 19/20 Reverse primer	0,5 µl
dNTPS	0,1 µl
H_2O	24,8 µl
FIREPol DNA Polymerase	0,1 µl

Table 3. 1st mast mix with CNGC 19/20 primers

The values listed in Table 1 indicate the amount needed per well of a particular reagent. This buffer has been developed by University of Helsinki technicians to suit the requirements of their specific experiment. These values were multiplied by the number of samples. Then, 1 µl of DNA was mixed with 29 µl of the same reagent and added to a PCR tube, resulting in a total volume of 30 µl. The first

PCR cycle (table 4) was then initiated. Samples were loaded onto a 1 percent agarose gel with 10 µl per well.

Step	Temperature	
Initial denaturation	98 °C	
Denaturation	98 °C	40 cycles
Annealing	50-60 °C	
Extension	72 °C	
Final extension	72 °C	
Hold	4 °C	

Table 4. First PCR cycle

Samples that contained the CNGC 19/20 mutants were selected for the next round of PCR (table 5). The second PCR was carried out with the same reagents as in Table 3. But with the AOS primers to identify the mutants, using the same protocol as before.

Step	Temperature	
Initial denaturation	98 °C	
Denaturation	98 °C	40 cycles
Annealing	55 °C	
Extension	72 °C	
Final extension	72 °C	
Hold	4 °C	

Table 5. The second round of PCR

The subsequent PCR was performed using the samples that were identified as CNGC 19/20 and AOS mutants.

The gel electrophoresis was performed with 2 percent agarose to obtain a band of 300 base pairs(bp), as expected from the PCR experiment. Samples containing all the mutants were selected for future cultivation based on this criterion.

2.3.3 The second set of samples

Samples 45–66 were identified as COI x CNGC lines. These samples contain the mutants of the COI line as well as the CNGC line. In theory, all plants in this line should have a homogeneous (mutant) profile. To identify AOS mutants, the first set of PCRs was conducted using AOS primers. The reagents used in this PCR are listed in Table 6 below.

Reagents	Amount
Magic buffer (Without K^+)	3µl
AOS Forward primer 5 µM	0,5µl
AOS Reverse primer 5 µM	0,5 µl
dNTPS 10 mM	0,1 µl
H_2O	24,8 µl
FIREPol DNA Polymerase *5 units/ µl*	0,1 µl

Table 6. 1st master mix with AOS primers

Following the selection of homogeneous samples, a second PCR was conducted using coi1-16 caps as the primer. Unlike other primers, coi1-16 caps do not have separate forward and reverse primers. To achieve a total volume of 30 µl, additional laboratory-grade water was added. After the PCR cycle, the samples were digested with HPY 188II restriction enzyme.

The total sum of 4 µl added to each PCR tube (table 7). The PCR tube was sealed, vortexed, and centrifuged. Different restriction enzymes have specific optimal temperatures for effective activity. HPY 188 III, for instance, functions

optimally at 37°C. In this study, PCR tubes were subjected to overnight digestion at 37°C, followed by agar analysis the next day.

For the digestion process, a specific set of reagents is meticulously measured and combined. These reagents include 3.4 µl of a 10x buffer, which serves as an essential component for the reaction. This is a custom-made buffer using Table 8 reagents. Additionally, 0.5 µl of H2O (water) is incorporated into the mixture to create the optimal environment for enzymatic activity. The enzymatic element, HPY 188II restriction enzyme from New England Biolabs, is a critical component, with only 0.1 µl being introduced into the reaction.

10x buffer reagents
0.8 M Tris-HCl (pH8.5)
0.2 M (NH4)2SO4
25 mM MgCl2
0.2% w/v Tween-20

Table 7. Reagent of 10x buffer.

PCR products were analyzed on a 2% agar gel, and mutant samples were selected for further PCR analysis using CNGC 20 primers.

Samples were subjected to agar analysis, and those displaying a triple mutant phenotype with AOS x Coi1-16 x CNGC20 mutants were chosen for subsequent analysis.

2.3.4 The third set of samples

After isolating the samples, the first PCR experiment was planned with Col-S 17,96 primer. Reagents are stated below in Table 8.

Reagents	Amount
Magic buffer (Without K^+)	3µl

Col-S 17,96 *5* µM	0,5µl
dNTPS 10 mM	0,1 µl
H_2O	25,3 µl
Firepol polymerases *5* units/ µl	0,1 µl

Table 8. 1st master mix with Col-S 17,96 primer

Once the PCR was done, the samples were digested in *Taq*I restriction enzyme (table 9). *Taq*I enzymes work best at 65°C overnight.

The digestion process relies on a precise combination of reagents, with 3.4 µl of a 10x buffer providing the necessary buffer solution. In addition, 0.5 µl of H2O (water) creates the ideal environment for enzymatic activity. The key enzymatic component, Taq 1 restriction enzyme, is introduced in a minimal volume of 0.1 µl.

The next set of PCRs was done with Pad 4 primers and sample lines 1–22. PCR products were run on a polyacrylamide gel at 2%. Samples with the Old Pad 4 mutant were selected for the next round of PCR experiments with MPK 12 caps.

Samples from lines 1–22, 67–86, and 87–96 are selected from their previous gel results and continued with MPK 12 primer in this experiment. After the PCR cycle, digestion was initiated with *SsiI* enzyme. *SsiI* enzyme thrives at 37 degrees Celsius.

Samples from lines 1–22 and 87–96 were selected for further experiments, and samples 67–86 that were homogenous were selected for future generations. The next PCR was done with Bak1-5 dCAPS primer. The gel was made from polyacrylamide for clear results.

2.4 Ozone treatment protocol

The aim of this study was to investigate the effect of ozone treatment on a set of plants. To achieve this, five groups of each plant type were exposed to a six-hour ozone treatment of 300 bbp, while an identical group of plants was kept as a control. After the treatment, whole plants were collected as samples and submerged in MQ water for two hours to allow for ion leakage. The leaked ions were then measured, and the samples were subsequently frozen overnight for later measurement. Upon defrosting the plants, measurements were taken once again. Freezing the samples is a method employed to assess the collective ion content. This is significant because plants exhibit diverse sizes, where smaller ones naturally contain fewer ions and larger ones tend to possess a higher ion count. By gauging the total ion value following the freezing process, we effectively mask these size variations, making it challenging for artificial intelligence detectors to discern the specific details.

The collected data will be analyzed to determine the impact of ozone treatment on the selected plants. This experimental design was selected as it allows for the measurement of the specific impact of ozone treatment on plant samples while minimizing the influence of extraneous variables that may affect the results. By collecting samples from both the treated and control groups and comparing the measurements, we can accurately determine whether ozone treatment has a significant effect on the leakage of ions in plants.

3 Results

3.1 PCR results

Anticipated outcomes in the context of this polymerase chain reaction (PCR) experiment entail the discernible differentiation between mutant and wild-type samples, predicated upon the size of the resultant PCR products, denoted as bandwidth. Homogeneous mutant samples are expected to manifest a singular

band, while heterogeneous samples are anticipated to exhibit two bands. A favorable outcome is characterized by the prevalence of homogeneous samples, thereby facilitating a streamlined analysis of genetic variations.

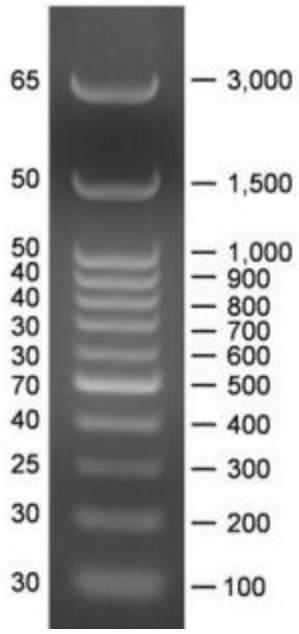

Figure 3. DNA Ladder 100bp

The 100 bp DNA Ladder is a distinct blend of various proprietary plasmids subjected to digestion using specific restriction enzymes and PCR products. This process generates a set of 12 distinct DNA fragments, which serve as reliable reference markers for determining the molecular weights of DNA during electrophoresis.

This figure displays the results obtained from samples numbered 1 through 97.

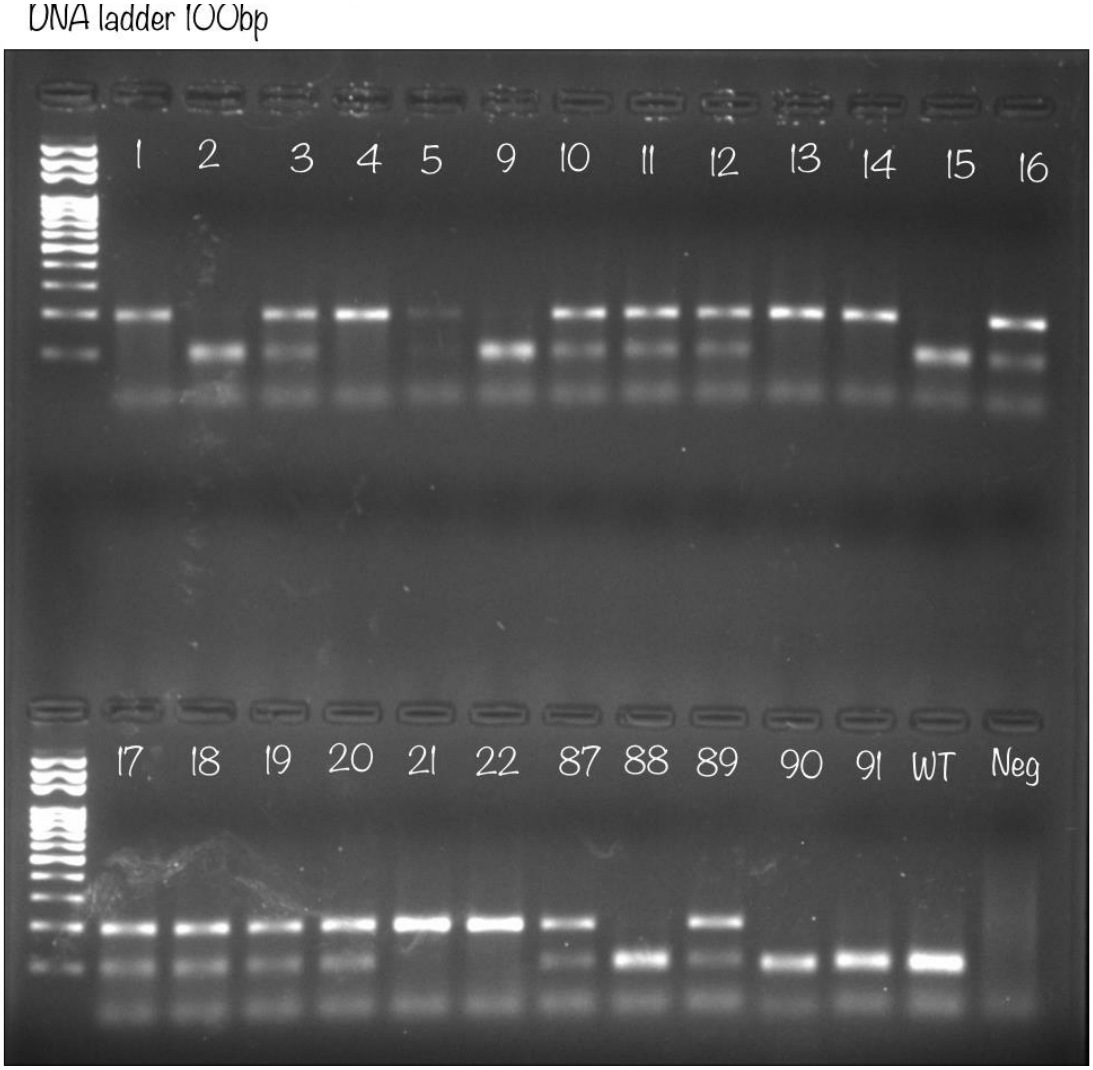

Figure 4. Samples 1- 97

Figure 4. Above are the results of a PCR experiment. The PCR was done with 2% agarose and the MPK CAPS primer. The top line represents the mutant samples, as they exhibit a smaller bandwidth of 200 bp in comparison to the wild-type samples. The ideal mutant lines are characterized by a single band, indicating homogeneity. On the other hand, the samples with two bands signify the expression of both mutant and wild-type genes and are, therefore, considered heterozygous. Despite the presence of heterozygous samples, a

considerable number of homozygous samples were obtained, which is a positive outcome for the PCR program.

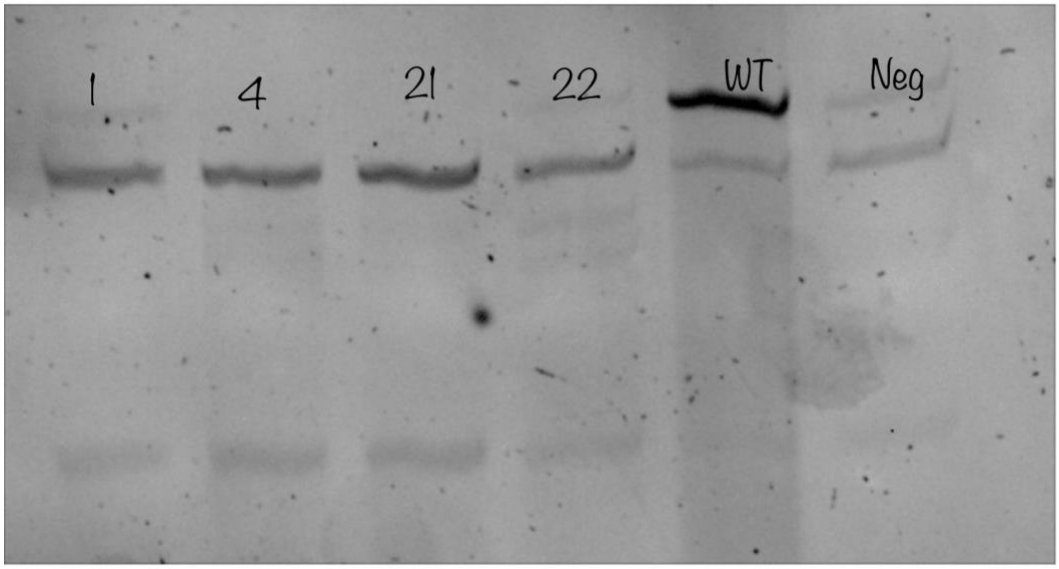

Figure 5. Samples 1-22

Figure 5. shows the result of an experiment done with *bak1-5* primer and digested with *RsaI* restriction enzyme. This experiment does not require a standard ladder, as it is done to only compare samples with wild-type samples. The results obtained from this experiment were unexpected, as the expected outcome was homogenous samples compared to the wild-type samples. However, all samples showed a larger band, indicating the absence of any mutant bands. Additionally, the negative control that was supposed to be blank showed some PCR results, which suggests that the experiment may have been compromised in some way.

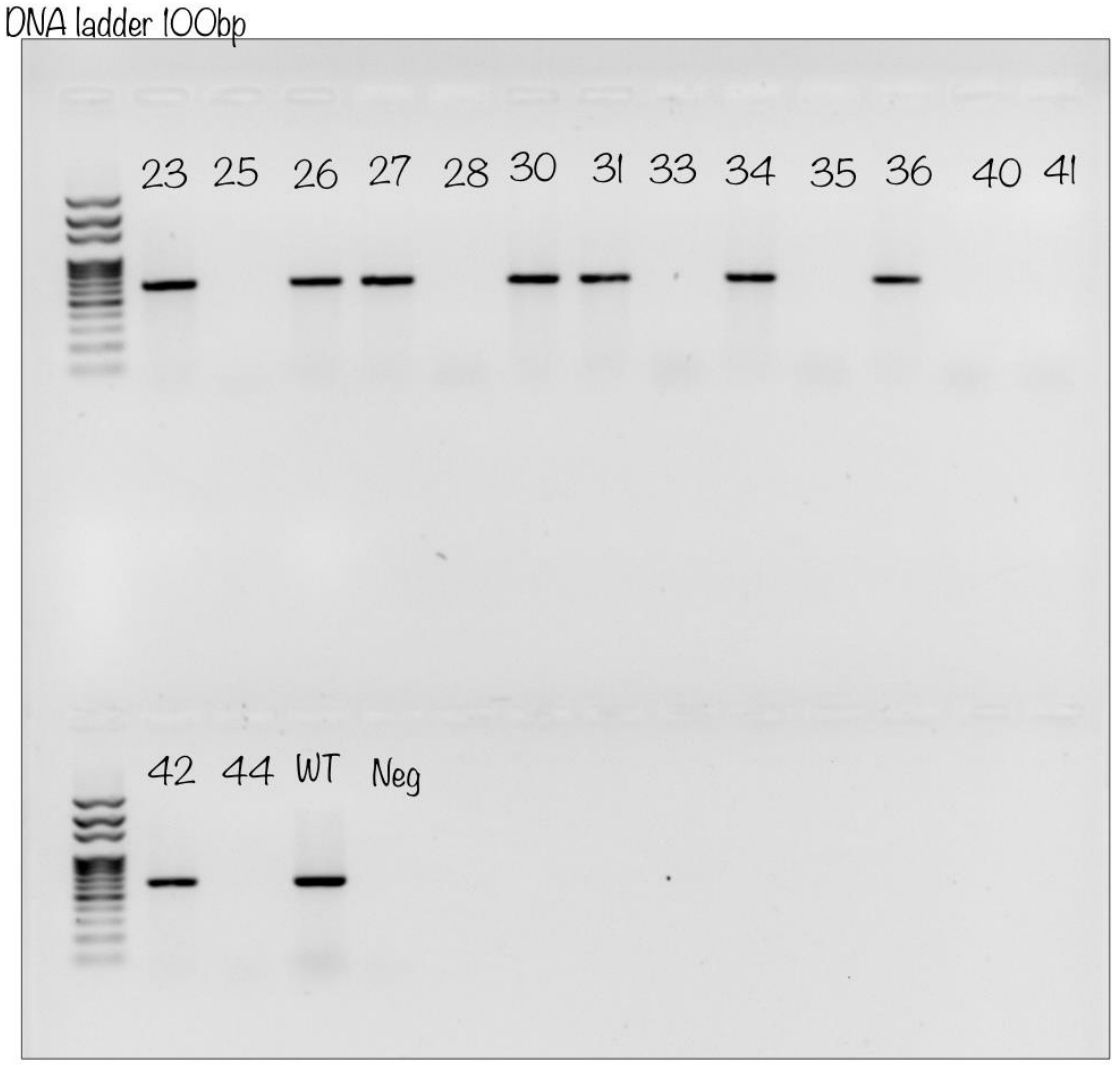

Figure 6. Samples 23 - 44

This PCR was done to amplify the wild-type mutant with AOS primers.
In a typical selection process, bands indicating the presence of a certain gene are used to identify candidates for the next round. However, in this particular case, candidates without such bands were selected instead. The reason for this PCR was to find samples that contained the wild-type gene and then select individuals that did not have it. This was done to ensure that the next generation would not have the wild-type gene. The last sample with a PCR product served as the control against the wild-type samples, and the remaining samples were compared to it.

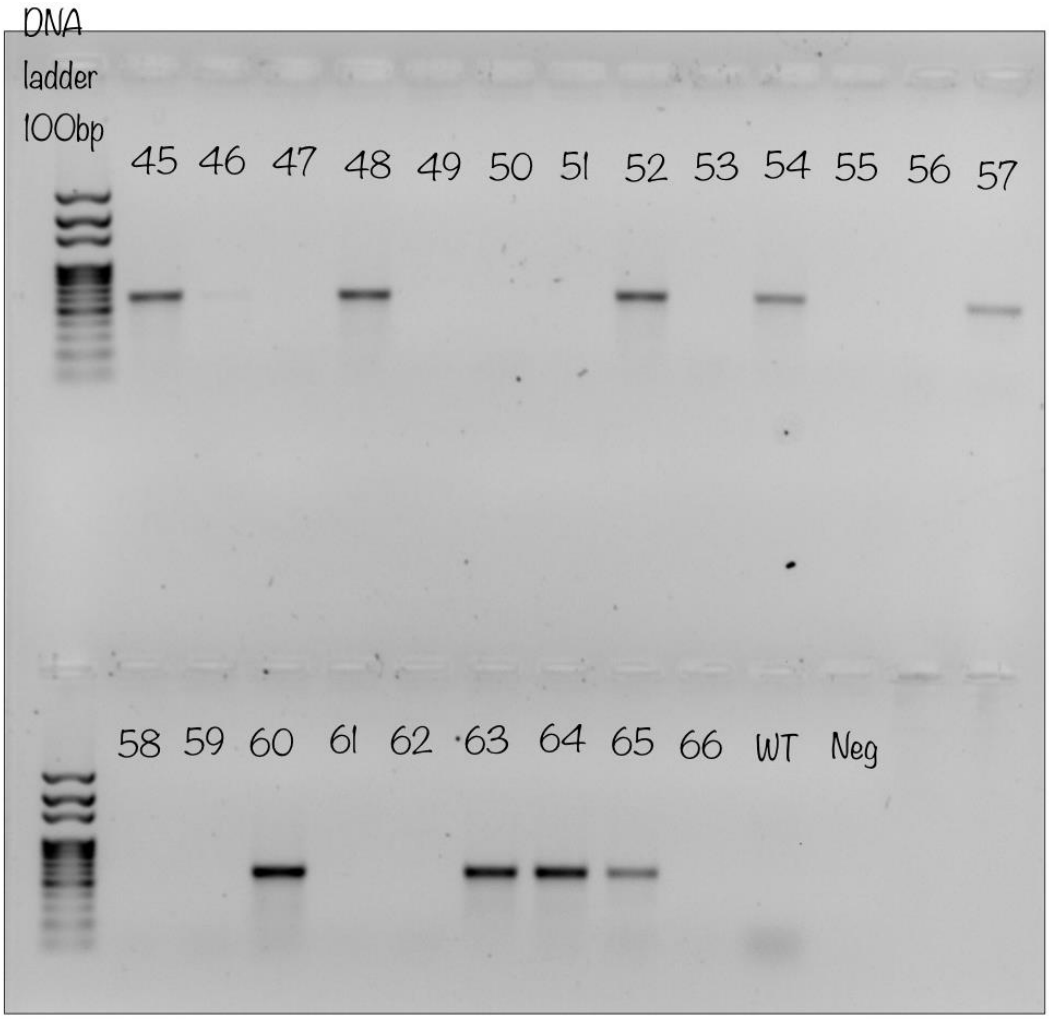

Figure 7 CNCG 19/20 F & R Primers / Magic Buffer. Samples 44-68

In this experiment, the CNGC 19/20 forward and reverse primers were designed to exclusively find plants that contain the mutant gene. Therefore, the presence of a band in a sample indicates the presence of the mutant gene. Conversely, the absence of a band implies the absence of the mutant gene in that particular sample.

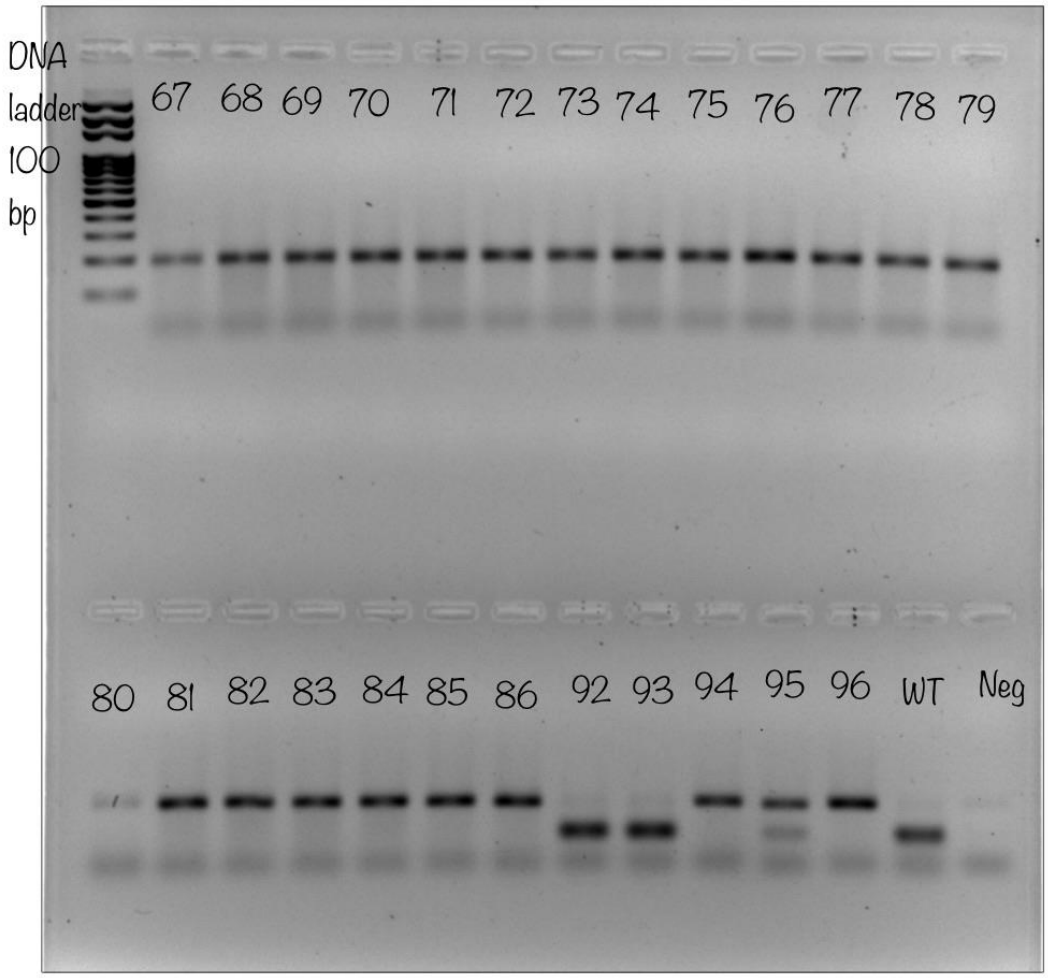

Figure 8. Samples 67-96

This experiment was done on 2% agarose with MPK 12 caps primer and then digested with *SsiI* restriction enzyme. These samples were previously tested and confirmed to have the mutant version of the MPK12 gene. However, two of the samples, 92 and 93, did not have the mutant gene, and one sample, 95, was heterozygous. The rest of the samples that displayed a single band were in line with the anticipated outcome.

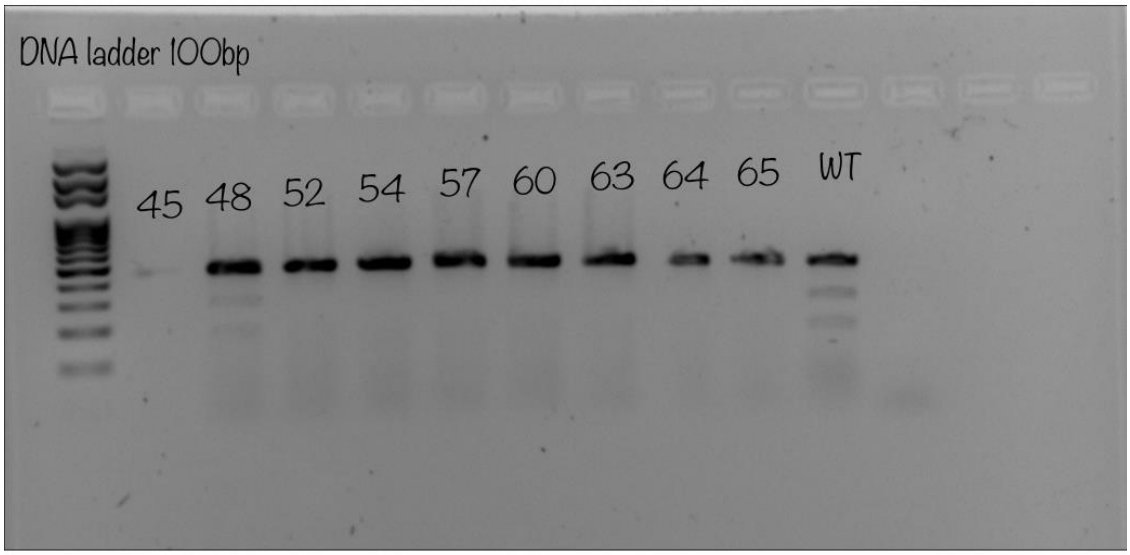

Figure 9. Samples 44-66

Samples 45–66 were run with *coi1-16* primer, followed by *HPY I88III* restriction enzyme, and the results revealed that the wild-type sample had all three bands that were expected to be digested. In contrast, the mutant samples were anticipated to be in the range of 500–600 base pairs, which was successfully achieved in this experiment.

3.2 Analysis of ion leakage levels

Pre-existing mutants were used in this ozone experiment. Following the completion of my work term, the recently discovered PCR mutants will be examined in additional studies. The ion leakage of each plant was measured and averaged in this experiment. The results obtained on the 24th of January revealed that the plants, which were three weeks old, had been initially grown in a growth room before being transferred to an ozone chamber. As expected, there were no damages observed in the Col-0 samples, while damages were evident in the Col-S samples.

	Ion leakage Control	Ion leakage ozone exposed
Col-0	3,77	2,79
Col-S	2,60	5,52
bak1-5	3,56	10,64
pad4	3,09	5,01
sid2	2,96	4,30
Col-S sid2	2,01	10,47
Col-S sid2 pad4	3,03	13,72
Col-S bak1 pad4	3,21	19,26
Col-S bak1 pad4 #37	4,55	13,51

Table 9. 24th Jan Ozone samples

On January 25th, the plants, which were three weeks old, had been grown in a growth room before being transferred to an ozone chamber for treatment. As expected, the Col-0 samples did not exhibit any damage. In contrast, the Col-S samples showed damages; however, they were less noticeable than the damages observed in a previous treatment conducted on January 24th. The results of the January 25th treatment are simplified and presented in Table 11 below.

	Ion leakage Control	Ion leakage ozone exposed
Col-0	2,02	3,25
Col-S	2,48	9,21
bak1-5	2,81	9,27
pad4	2,34	2,72
sid2	1,75	3,13
Col-S sid2	2,65	8,39
Col-S sid2 pad4	2,10	13,19
Col-S bak1 pad4 #19	4,25	20,88
Col-S bak1 pad4 #37	2,85	14,21

Table 10. 25th of Jan Ozone samples

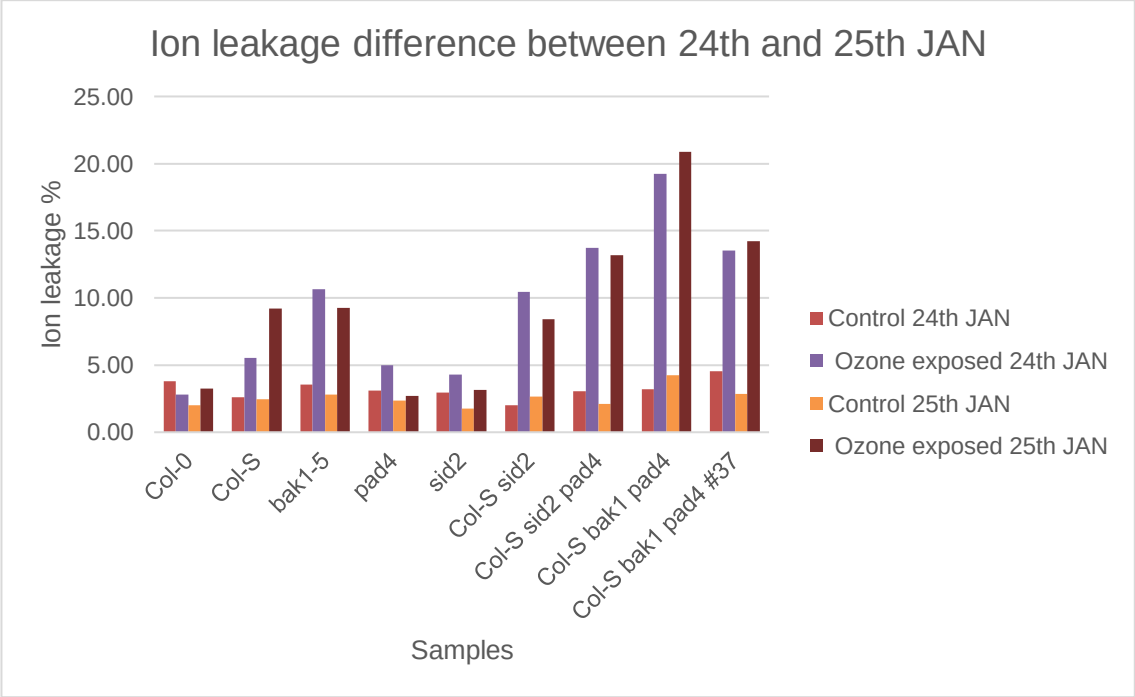

Figure 10. Ion leakage difference between Control and Ozone exposed plants.

The control samples of 24th JAN and 25th JAN containing the mutants serve as a foundational reference in this context. Notably, Col-S bak1 pad 4 demonstrates the utmost susceptibility to ozone-induced damage among these samples. Additionally, Col-S bak1 pad4 #37, Col-S sid 2 pad 2, and bak 1-5 exhibit comparable levels of vulnerability to ozone exposure. In contrast, Sid 2 displays the highest resistance among the tested variants when subjected to ozone.

An unexpected observation arises from the Col-S sample exposed to ozone on January 25th, where more pronounced damages, both physically and quantitatively, were observed than anticipated. Both experiments corroborate

each other, affirming that the mutants show no discernible resistance to ozone exposure.

4 Conclusion

In conclusion, the PCR experiments in Figures 4 and 5 have given us a glimpse into the genetic makeup of the plants we've been studying. Some experiments went as planned, while others surprised us, indicating that we might need to dig deeper and refine our methods.

Our main goal was to figure out whether the mutant plants were resistant to ozone treatment, and we got a clear answer: they were not. This highlights the importance of understanding their vulnerability to environmental stress.

While some samples show similar levels of vulnerability, Col-S bak4 pad 4 shows the utmost susceptibility to Oxone exposure. The convergence of findings from both experiments underscores a consensus: the mutants do not exhibit any discernible resistance to ozone exposure. This collective evidence reinforces the susceptibility of certain genotypes to ozone-induced stress, highlighting the importance of further exploration into the underlying mechanisms governing plant responses to environmental challenges.

In summary, this study lays the groundwork for more research into how genes affect how plants deal with environmental stress. It's a reminder of how crucial it is to carefully design experiments and understand genetic diversity, especially in the context of developing plants that can thrive in a changing world.

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Quick Miniprep for Arabidopsis DNA Isolation

METHOD

1. Label 2ml screw-cap tubes (or Safe-Lock tubes) with numbers and add glass beads. Label pipett tip with corresponding numbers.

2. Cut off one leaf from the plant to be genotyped and add to the tube. Put the labeled pipett tip next to the plant. This allows identification of the positive plants after the experiment.

3. Add 630 µL of plant miniprep extraction buffer to the tube.

4. Screw on the caps tightly and shake the tubes in the Precellys with the following program:

3×10 sec 6000 rpm with a 30 second break.

5. Centrifuge the tissue in a microcentrifuge at maximum speed for 5 min, and transfer 500 µL of the supernatant to a clean tube.

6. Add 500 µL of isopropanol, mix by shaking, and centrifuge in a microcentrifuge at maximum speed for 10 min. Carefully discard the supernatant.

The pellet should be visible as a small white precipitate.

7. Centrifuge in a microcentrifuge for 10 seconds. Pipette away remaining isopropanol.

8. Airdry until all isopropanol is gone (usually takes 10-15 min). Alternatively, dry in speed-vac, 4 min on medium heating.

9. Dissolve the pellet in 50-100 µL of sterile water. To help dissolve the pellet move the tube across an 80-well rack.

10. Use 1 µL for a PCR.

11. Store the DNA at -20 until positive plants have been identified.