

**Effect of Stress on Quantity and Quality of Plant Leaf RNA**

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## **Abstract**

The purpose of this project is to find out the effects stress had on plant leaf RNA quality and quantity. Plants undergo changes when they are under biotic or abiotic stress. Stress may lead to changes in gene expression to give rise to different RNA transcripts. Stress may also lead to degradation of RNA molecules. In this project the stress of different plants under different light intensities were tested to see how the stress affects leaf RNA. It was hypothesized that the stress will compromise the RNA quantity and/or quality. All of the samples were processed under a nanodrop-spectrophotometer for the quantification and the Agilent 2100 bioanalyzer for the quality assessment of the RNA. The testing showed that there was less RNA in the stressed plants but the quality of that RNA remained the same as compared to a healthy plant.

## **Executive Summary**

Ribonucleic acid (RNA) is essential to all of life. RNA, specifically messenger RNA (mRNA) relays messages from plant DNA to the cell so the cell can make the necessary proteins an organism to function. If an organism, in this case a plant, were to undergo any changes then the plant needs to adapt to these changes. For the plant to do this it needs to make different proteins than it would have made if it were in its normal environment. These factors that change the plants genome may stress the plant causing it to break off or decrease the plants RNA, thus not allowing the DNA to send the necessary signals to the cell and eventually causing death to the plant.

This purpose of this project was to find out what would happen to the quality and quantity of a plants RNA if a plant was stressed. Abiotic stress in the form of high light intensity was used. Samples of plant leaves were taken and then tested to see how the stress affected the RNA

composition. The results from the testing showed that when the light intensity is higher, then the quality of the RNA in a plant leaf remains the same, but the quantity of the plant decreases.

## 1. Introduction

Plants endure different changes in nature whether it is too much or too little of any of their necessary resources for survival. The plants need to adapt to these changes. For the plant to adapt to different environmental conditions it must change what it produces. For example, in an instance of drought, the plant would have to adapt in conditions of less water and prevent water loss from the plant. How would a plant adjust to these conditions for its survival? In the plant genome, the expression of the genes changes in different environmental conditions, which eventually changes the type and amount of transcripts or mRNA, a type of ribonucleic acid (RNA), which is formed. The survival of the plant depends on how well the plant adapts to the changing condition.

In natural environments, the plant is exposed to several biotic and abiotic stresses. Factors like high or low temperature, drought, alkalinity, salinity, UV stress, pathogen infection, etc. are potentially harmful to the plant. The plant responds to these stresses in several ways like leaf curling, hypersensitive response, defense response, etc. A plant can resist these stresses either by tolerance or escaping the stressful condition. Plants, being immobile, have to resist these changes. Tolerance of plants occurs at the cellular level, which consequently manifests as physiological change that causes the plant to tolerate the stress (Parvaiz and Satyawati, 2008). If the stress is present for very long periods of time like several years, the plant adapts to the change.

The aim of this project is to determine the effect of stress on the quantity and quality of RNA (ribonucleic acid) in the plant leaves. The stress inflicted on the plants will be an increase in light intensity. The plant used in the study is *Arabidopsis thaliana*, a model plant to study plant

biology and genetics. It is well documented that the plant leaves are healthy only in certain intensities of light. If the plant were to be put in any other light intensity then the plant will be stressed. To study how the quantity and quality of RNA will be affected by light intensity, the plant RNA of different plants, kept in different conditions, will be observed to see the differences between them.

Extraction of high quality of RNA is important for all downstream applications like cDNA library construction, reverse transcription polymerase chain reaction (RT-PCR), gene expression studies, etc. (Tattersall et al. 2005). In the eukaryotic cell, there are several pathways for degradation of RNA molecules. However, there is an equilibrium maintained in the cell between the synthesis and degradation of RNA in normal conditions. When there is a deviation from a normal condition, the equilibrium can shift more towards degradation leading to production of several smaller oligonucleotide fragments instead of intact RNA molecules. Also, the concentration of RNA molecules in the cell would decrease. Using such a data can lead to incorrect conclusions. It can thus be hypothesized that under stress, the quantity and/or quality of RNA in leaves will be compromised as compared to healthy leaves.

## **2. Materials and Methods**

### **2.1 Materials:**

*Arabidopsis thaliana* plants

Growth Chambers where different growth conditions are maintained

Liquid Nitrogen

Electric driller and pestle

Qiagen plant RNeasy mini kit

Nanodrop-spectrophotometer

Bioanalyzer

## **2.2 Methods:**

All protocols were performed under the guidance of a graduate student in the laboratory.

Treatment of the plant:

There were three groups of plants; the control group (C) was under a normal light intensity of 85  $\mu\text{E}/\text{m}^2/\text{s}$ , experimental group 1 (E1) which was stressed due to it being under a high light intensity between 189-192  $\mu\text{E}/\text{m}^2/\text{s}$  for twenty-four hours, and experimental group 2 (E2) which appears stressed due to undetermined environmental factors for more than a week. Part of this study was also to see if the reason for stress in E2 is high light intensity; although they were grown in normal light, it was seen that the growth chambers were not functioning properly and this could have resulted in high light intensity for some time in the chamber. Stressed was defined as purpling of leaves. The growth conditions are 22<sup>0</sup>C and 65% relative humidity.

Collection of the leaf tissue:

Three leaves were collected from all three groups from the second rosette layer and then frozen in liquid nitrogen. This causes the plant tissues to freeze in the same state as they were when collected. Multiple leaves were collected so the results could be reproducible. The leaf tissue was stored at -80<sup>0</sup>C until used for RNA extraction.

RNA extraction:

To extract the RNA, Qiagen plant RNeasy mini kit was used. In this kit, the collected leaf tissue was grinded to a fine powder using a mechanical driller. Lysis solution was added which will basically lyse (to induce lysis, or to cause dissolution or destruction of a cell membrane with lysis) the plant cells. Then that solution was passed through a filter which will separate the RNA from the other cell components in the solution. The solution is then passed through a membrane which specifically binds to RNA. In the last step, RNA is removed from the membrane using clean water and used for subsequent protocols.

RNA quantification:

For this step the nanodrop-spectrophotometer was used. It will give us the yield of RNA extracted. The instrument is provided by the Core Facility of Biology department.

Assessment of RNA quality:

In this step an instrument called the Agilent 2100 bioanalyzer was used. It will show us the quality of the RNA isolated. This instrument is provided by the Core Facility of Biology department.

### **3. Results:**

#### **3.1 Observation of the plant leaves:**

The leaves of both of the stressed plants in the experimental groups were purple. The leaves of the plant in E2 appeared to be more purple than others as seen in Figure 1. This could

be because E2 was stressed for more than a week while E1 was stressed for only twenty-four hours.



Picture 1: Control Plant



Picture 2: Experimental group 1 (high light intensity)



Picture 3: Experimental group 2 (stressed for undetermined environmental factors): 2 pictures – before and after

### 3.2 RNA assessment

**Table 1. Quantity and quality of RNA in control and experimental groups**

Condition	RNA quantity (Nanodrop spectrophotometer)			Quality of RNA (Bioanalyzer)
	Concentration (ng/ $\mu$ L)	260/280 nm	260/230 nm	
<b>Normal light intensity (Control)</b>	268.73	2.16	2.30	Good
<b>High light intensity (E1)</b>	219.61	2.17	2.22	Good
<b>Plant stressed for unknown reasons (E2)</b>	34.57	1.99	1.65	Good

In the Nanodrop-spectrophotometer RNA is measured with UV light. A beam is shot through the sample, which is 2  $\mu$ L and is measured at different wavelengths. The RNA molecules are absorb at the wavelength of 260nm and this absorption is directly related to the amount of RNA that is present in a given sample. The first column in table 1 shows how much RNA is present per microliter of the sample. The columns 260/280 and 260/230 addresses the quality of the RNA. 260/280 ratio shows presence of protein impurity and 260/230 ratio shows the presence of other impurities like phenol, EDTA, etc. that absorb at 230nm..

The first column shows the amount of RNA that is present in the sample. The first two samples have a good amount of RNA and the third one is significantly lower. This means that something happened to this plant causing the amount of RNA to decrease. The column, 260/280, determines the purity of the RNA. The values in this column must fall in between 1.8-2.0 or more for the

sample to be considered pure. The first two samples in the table are above 2.0 which are good. Therefore, the samples are very pure. If the sample were to fall below 1.8 then that would mean that there is some sort of contamination in the sample. So, all of the three samples are pure. The last column, 260/230 also determines the purity of the RNA in a sample. The range of a pure sample would be in between 2.0-2.2. The first two samples fall in this range. Like in the 260/280 ratio, if the value is above the range then it is a good sample. The third sample does not fall into the range of 2.0-2.2. This means that there is some sort of contaminant that was absorbed at this wave length, making this sample impure.

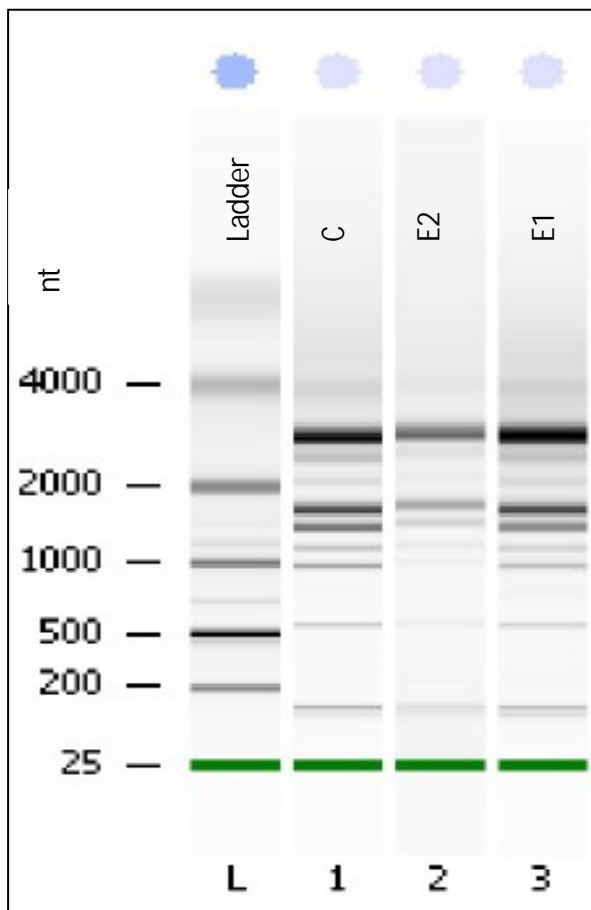


Figure 2. Total RNA seen on a gel obtained from Bioanalyzer. C- Control group; E1&E2- experimental groups 1 and 2.

The Agilent 2100 bioanalyzer is a machine that determines the quality of RNA in a sample. As seen in figure 2, the thick bands seen in each sample are of bigger size (between 2000-4000 nucleotides). This means that most of the RNA in the sample (as seen by thickness of the band) is long and intact and not degraded. The results of the bioanalyzer conclude that the RNA of each sample was good. Also, the band intensity in lane 2 (E2) is lighter than the other two, thus agreeing with the result of Nanodrop in it having least RNA concentration. The results from the bioanalyzer show that most of the RNA is intact.

#### **4. Discussion and conclusion:**

##### **4.1 Discussion**

In the testing of the RNA, the results were intriguing. It was hypothesized that the amount and the quality of the RNA in the stressed plants would be less than the amount in the control group. But, as the showed in the results of the bioanalyzer the amount and quality of RNA of the plant that was stressed under a high light intensity was similar to the healthy plant and the RNA of the plant that was stressed due to unknown factors had a significantly less amount of RNA than the other two plants. This can be on account of the amount of time the plant was stressed. E1 was only in that light intensity for twenty-four hours while E2 was stressed for over a week. The difference in time was quite significant which matches with the results of the Nanodrop. The time factor took a big role in this experiment. If E1 stayed under that light intensity for the same amount of time that E2 was then the results would have been different. E1 could not have been stressed that long in that light intensity because it would have died. This is known from previous experiments. From the results, it is also possible for E1 to recover and become healthy, but that this is only an inference not a conclusion. It can be also inferred that the factor responsible for stress in the plant in E2 is not light at all, as seen by equally good yield of

RNA in E1 and C and less yield in E2. A plant can be stressed due to several factors like lack of nutrients, less watering, high/low pH, etc. The reason still remains to be determined.

This study was a preliminary study for further experiments in the lab involving gene expression studies in plants infected with disease causing bacterium. In such experiments, the changes in transcripts in the plant leaf due to infection with a bacterium will be monitored. In such studies, it is very important to have good quality RNA. This study gives us an idea that having a plant at high light intensity will not necessarily cause RNA degradation, although changes in gene expression should be expected. However, it needs to be determined whether the stress of both high light intensity and bacteria together will lead to RNA degradation. This kind of study is very important when the conditions required for an experiment are to be determined. Performing an experiment in one set of conditions, without knowing the effect of that condition on the outcome, can give rise to misleading results.

## **4.2 Conclusion**

It can be concluded that having a stress of high light intensity for 24 hours does not cause a decrease in the quantity and quality of RNA extracted from plant leaves. This study could also conclude that the stress-responsible factor for the plant that appeared stressed due to undetermined environmental factors is probably not high light intensity. If the aim of this research had not been focused on the effects of high light intensity stress on a plant, but on the effects of stress on a plant in general, then the plant that was stressed due to unknown factors could have been of better use.

If time and money had permitted then sequencing of the RNA of the healthy and the plant in high light intensity could have been the next step in this project. Sequencing of RNA is now possible

with next generation sequencing technologies and it can give more information about which transcripts were produced in high light stress as compared to healthy plants. By knowing the sequence of the RNA in the plants then the differences in their RNA will be known and can be used for future research. If the plant that was stressed due to unknown factors were to be sequenced then we will know what was missing in the RNA. This research showed that E2 had less RNA than the healthy plant, by sequencing it could show us bases were lacking in the RNA of the plant.

**References:**

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2. Tattersall, E. A.R, Ergul, A, AlKayal, F, DeLuc, L, Cushman, J. C., Cramer, G. R. (2005). Comparison of methods for isolating high-quality RNA from leaves of Grapevine. *American Society for Ecology and Viticulture*, 56:4.