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# Phenylalanine Ammonia-lyase 1 (PAL1) Expression During Cold Stress in Two Japanese Tomato Varieties

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## Abstract

Cold stress is one of the most important and deleterious stresses for *Solanum lycopersicum* (tomatoes) during their growth, causing enzymatic browning, stunted growth or, in severe cases, death. *Phenylalanine Ammonia-lyase 1* (PAL1) is known to be induced during cold stress in several species of plants, but the specific role of PAL1 is still unknown. Some tomato varieties are more cold tolerant than the others, which may be in part due to PAL1 expression. In this study, our goal is to observe whether PAL1 expression varies between tomato cultivars available in Japan. Using semi-quantitative RT-PCR, PAL1 expression in both Fruitica and Momotaro cultivars was compared. The percent change between the control and treatment of both varieties was surprisingly not statistically different. The results show that both varieties are not significantly different in terms of PAL1 expression. Better understanding of cold stress tolerance genes in tomatoes can help farmers decide which varieties to buy in an ever-changing climate.

*Keywords: Cold stress in Tomatoes, Fruitica, Momotaro, Phenylalanine Ammonia-lyase 1*

## Introduction

In Japan, more than 720,000 tons of tomatoes are grown annually (Japan CROPs). Tomatoes have the best growth rate in places with direct sunlight throughout the day and prefer temperatures between 20 and 24°C. Cold stress in tomatoes is the state where there are substantial cellular and physical changes as a result of exposure to cold temperatures. Because tomatoes are sensitive to cold temperatures, when and where tomatoes can be grown is limited. Temperatures below 15°C are too cold for tomato plants resulting in damage (Foolad and Lin, 2001). However, when temperature reach 0°C and below, the cold will kill the plant and severely damage the fruit. In January 2021, temperatures in Tokyo, Japan averaged 5.4°C, which is too low for tomatoes to grow (Japan Meteorological Agency, 2021). Even in March, temperatures in Tokyo can fall as low as 5°C.

Small modifications such as expression of various transcription factors, post-translational proteins, metabolic enzymes, and photosynthesis-related genes may play vital roles in endurance of cold stress in tomatoes (Liu et al,. 2012). The taste of the tomatoes is determined by the amount of sugar, the organic acid content, and the volatile compound composition, all of which will be abnormal if the environmental temperatures are low (Mikkelsen, 2005). The cold first affects the plant at a cellular level and the problem eventually elevates when other cells die as a result. Damage to the exocarp, the outer layer of the tomato fruit, as well as discoloration of the fruit, leaves and stem tissues are consequences of cold stress, which can make the plant inedible. Both physical and cellular effects damage the plant and should be avoided at all costs.

One of the important genes for cold tolerance in tomatoes is *Phenylalanine Ammonia-lyase 1* (PAL1). PAL1 has been shown to be upregulated during cold stress in plants (Rivero et al. 2000). PAL1 synthesizes trans-cinnamic acid from L-phenylalanine; trans-cinnamic acid can become phenolic compounds which help protect against cold stress (Janas et al., 2000). In Ortega-García and Peragón (2009), PAL1 has shown to participate in the recovery process from cold stress by creating phenylpropanoids and protecting the plant from cold injury. PAL1, DPPH, flavonoid contents and total phenolic content help preserve the tomato plant at low temperatures for a long period of time (Rezaie et al., 2020). Jian-ye Chen et al. (2008) discovered that PAL1 may also play a role in heat pretreatment-induced chilling tolerance in bananas. PAL1 has many effects and supports other enzymes that maintain the health during the cold throughout several plant species.
Tomatoes are a common ingredient used in many dishes throughout all seasons, but at the same time fragile and prone to bruising and from chilling injury. In order to prevent this, people have come up with solutions to grow them in winter. Many farmers often use greenhouses to make a fitting environment and grow tomatoes in them. Breeders have created tomato species that could withstand certain temperatures. The new hybrid tomatoes are stronger to cold and can withstand below 12°C while tasting the same. However, the change in expression of PAL1 in different varieties available to farmers in Japan is still unknown. The goal of this study is to compare the expression of *Phenylalanine Ammonia-lyase* during cold stress in different tomato varieties available in Japan. I hypothesize that the different tomato varieties will show different tolerance levels depending on the amount of PAL1.

## Methods

*Plant materials and growth conditions*

The seeds of Frutica tomatoes (**フルティカ**) and Momotaro Fight (**桃太郎 ファイト**) cultivars were obtained from Amazon.com in early July 2021. They were grown for 23 days with 16 hours of sunlight at 24°C. They received 100 ml of water per pot each week. On the final day of growth, half of the plants were kept in a cold chamber for 24 hours at 4°C. Approximately 80 mg of tissue were used per biological replicate (n = 3 for each variety and treatment).

TABLE 1: RNA quality for each sample. Variety 1 was Fruitica and Variety 2 was Momotaro. “C” indicates a control sample and “T” indicates a cold-stressed sample.

| Sample | A260 | 260/280 | Concentration (ng/uL) |
|---|---|---|---|
| V1T1 | 0.128 | 1.52 | 512 |
| V1T2 | 0.141 | 2.47 | 564 |
| V1T3 | 0.193 | 2.10 | 772 |
| V1C1 | 0.221 | 2.17 | 884 |
| V1C2 | 0.218 | 1.90 | 872 |
| V1C3 | 0.202 | 2.20 | 808 |
| V2T1 | 0.187 | 2.46 | 748 |
| V2T2 | 0.147 | 2.58 | 588 |
| V2T3 | 0.205 | 2.5 | 820 |
| V2C1 | 0.093 | 2.52 | 372 |
| V2C2 | 0.152 | 2.38 | 608 |
| V2C3 | 0.180 | 2.4 | 720 |

TABLE 3: Primer sequences and amplicon sizes. Primers Tubulin-1F and Tubulin-1R are from Løvdal and Lillo (2009).

| Name | Sequence | Sequence ID | Amplicon size (base pairs) |
|---|---|---|---|
| PAL-1F | TGCGTTAAGGCTCAACAACA | SGN-U239712 | 200 |
| PAL-1R | AGTTGCACAGTCACGTCTTT | SGN-U239712 | 200 |
| PAL-2F | GGTAAAGACGTGACTGTGCA | SGN-U239712 | 204 |
| PAL-2R | GCCAAAGACTCCAGCATTCA | SGN-U239712 | 204 |
| Tubulin-1F | AACCTCCATTCAGGAGATGTTT | DQ205342 | 180 |
| Tubulin-1R | TCTGCTGTAGCATCCTGGTATT | DQ205342 | 180 |

*RNA isolation and cDNA synthesis*

The RNA isolation process was done using the "NucleoSpin® RNA Plant" kit made by Macherey-Nagel (Catalog Takara U0120B) without liquid nitrogen for homogenization. The first strand of cDNA was made using PrimeScript 1st strand cDNA synthesis kit as described (Catalog Takara 6110A). 3.5 µg of RNA was used for cDNA synthesis. No reverse-transcriptase (no-RT) controls were also made for each of the biological samples.

*Semi-quantitative PCR and gel electrophoresis*

The PCR were as follows: 10 µL of EmeraldAmp MAX PCR Master Mix (Catalog #RR320A), 0.5 µL of cDNA, 0.4 µL (2µM) of both forward and reverse primers, and finally, 8.7 µL of ddH2O. Table 2 shows the given primer sequences. Primers Tubulin-1F and Tubulin-1R are taken from Løvdal and Lillo (2009). All PCR conditions were as follows: 98°C for 2 minutes; 98°C for 15 seconds, 58°C for 30 seconds, and 72°C for 1 minute which are repeated for 30 cycles; 72°C extension for 10 minutes. PCR was done by using a miniPCR® mini16 thermal cycler (SKU: QP-1016-01). Gel electrophoresis was done by using blueGelTM electrophoresis with a built-in transilluminator (SKU: QP-1500-01).

*Data Analysis*

After the gel electrophoresis, the band size was quantified using Fiji (Fiji is just ImageJ) (Schindelin et al., 2012). The BioFormats plugin within Fiji was used to measure band size and peak area (Linkert et al., 2010). Finally, Microsoft Excel was used for statistical analysis and generating figures. Statistical significance was determined via one-way ANOVA. Unless otherwise indicated, "n = 3" indicates that a total of 12 samples were included in analysis (for both varieties, 3 samples each from control and cold-stressed samples).

## Results

Figure 1 compares the PAL1 expression under control and cold-stressed conditions in both cultivars. As expected, our housekeeping gene, Tubulin, had a visible band in every sample, and the difference between samples was not statistically significant ($p = 0.74$, $n = 3$). PAL1 expression was only visible in cold-stressed samples, with an exception of one Frutica control sample. There was no statistically significant difference of PAL1 expression between the Fruitica and Momotaro varieties. Furthermore, PAL1 expression was not significantly different between control and treated Fruitica samples ($p = 0.07$, $n = 3$), nor between control and treated Momotaro samples ($p = 0.14$, $n = 3$).

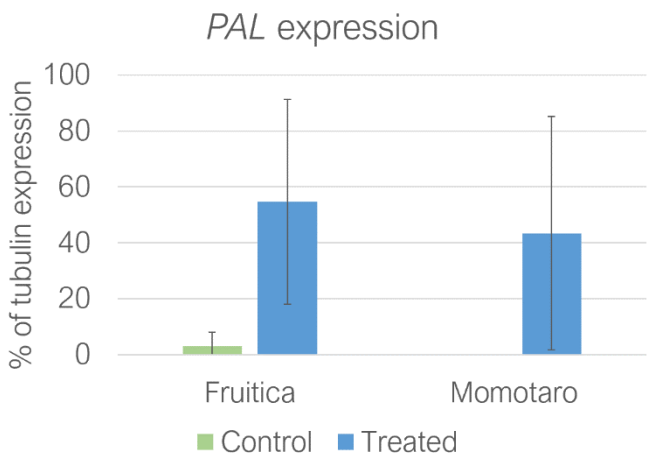


FIGURE 1: PAL1 expression in both control and treated samples of the varieties Fruitica and Momotaro.

## Discussion

Based on the semi-quantitative PCR, there were no statistically significant differences of PAL1 expression between the treated and control samples of both Fruitica and Momotatro varieties. One-way ANOVA showed that our housekeeping gene, Tubulin, also was not statically different between samples. As shown in Figure 1, the standard deviations are tremendously large, so it is not surprising that the difference between control and cold-stressed samples is not significant.

One explanation is that the duration of the cold stress was not sufficient to induce PAL1. It is also possible that the temperature was either too severe or too mild for PAL1 to be expressed. Although unlikely, the plants may not have been mature enough to express PAL1. Slight variation of the growth condition and environment, like amounts of light and water, may also affect the amount of PAL1 expression. One possible explanation for the treated sample with no PAL1 expression is that the plant died before the experiment.

There were some limitations and challenges throughout our experiment. We were unable to use liquid nitrogen for RNA isolation; however, all samples were kept on ice and lysed as quickly as possible to avoid RNA degradation. In our PCR tests, the first set of PAL1 primers were a failure. The second set successfully amplified PAL1, but there was a secondary band likely due to genomic DNA contamination. Future work should focus on optimizing primer efficiency. Furthermore, the experiment could be more accurate with the power of qPCR.

Ultimately, we hope that this work contributes to scientists' understanding of cold stress tolerant genes, including PAL1, in Japanese tomato varieties. This information could benefit future farmers because they will be able to decide which varieties to plant depending on their climate and may give farmers peace of mind. Furthermore, better understanding of which local varieties are cold tolerant could allow farmers to extend their growing season and could even increase the rate of tomato production.

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