Detection and Analysis of Delayed-Ripening Genes in the Growth of Strawberries (*Fragaria x ananassa*) Treated 1-Naphtaleneacetic Acid

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**Abstract.** Strawberries (*Fragaria* spp.) are non-climatic fruits that are popular due to its good taste, fragrance and attractive colors. It contains anthocyanins and other phenolic compounds capable of preventing such diseases as cancers and heart diseases. Rapid decay of fruits is among farmers’ post-harvest problems. 1-Naphtaleneacetic acid (NAA) is a hormone known to be capable of delaying fruit ripening. Molecular studies of growth and development control genes have been carried out, including color pigment control in strawberry fruits. *FaCHS* and *FaPYR1* are genes that play a role in fruit development, especially in ripening strawberry fruits. The purpose of the present study was to detect fruit ripeness-delaying genes in *Fragaria x ananassa* treated with 1-Naphtaleneacetic acid (NAA). It was conducted by inducing strawberry plants from Banyuroto, Magelang of Central Java, with 10 ppm, 30 ppm and 60 ppm of NAA. DNA was isolated from fruit using the Nucleon Phytopure Kit and then tested qualitatively and quantitatively using electrophoresis and spectrophotometry. Genes were detected using the MyTaqTM-Bioline PCR method. The phenotypic characteristics showed that there was an increase in the leaves area and stem diameter in the cultivar ‘KP Brite’ and the fruit size in the cultivar ‘Crystal’ after 10-ppm NAA treatment. Results of amplification of *FaCHS* DNA fragments showed *FaCHS* was not detected in the cultivar ‘KP Brite’ after 60-ppm NAA treatment, while *FaPYR1* could only be detected in the red stage of the cultivar ‘Crystal’ after 10-ppm NAA treatment and the pink and red stages of the cultivar ‘KP Brite’ after 30-ppm NAA treatment.

**Keywords:** delayed-ripening genes, *FaCHS*, *FaPYR1*, *Fragaria x ananassa*, 1-Naphtaleneacetic acid

1. **Introduction**

Strawberries (*Fragaria* spp.) belong to herbaceous plants firstly discovered in Chile, America. Strawberry fruits are non-climatic fruits that are popular due to their good taste, fragrance and attractive colors, making them potential for development of fruit-picking agro-tourism in various places. Strawberry fruits contain anthocyanins and other phenolics capable of preventing such diseases as cancers and heart diseases. Ellagic acid in their seeds is capable of beautifying the skin, whitening...
teeth, eliminating bad breath, and increasing strength [1]. Among the widely consumed strawberry species is *Fragaria x ananassa*. This species is quite large and easily found during the summer. Its unique flavor make this fruit much sought after and liked [2].

Relatively fast decay of strawberry fruits is one of the obstacles that should be overcome by farmers in the post-harvest fruit handling. 1-Naphtalenecetic acid, or NAA, is one of auxin synthetic hormones. In addition to its known function in the induction of cell expansion and root elongation, this hormone is known to be capable of delaying fruit ripening [3, 4], making it effective in delaying decay. This hormone can prevent fruit ripening since it works to inhibit ABA [5].

Molecular studies of growth and development control genes have been carried out, including color pigment control in strawberry fruits. *FaCHS* and *FaPYR1* are genes that play a role in fruit development, especially in ripening strawberry fruits.

The purpose of the present study was to screen and detect fruit-ripeness delaying genes in *F. x ananassa* of cultivars ‘Crystal’ and ‘KP Brite’ induced with NAA at concentrations of 10 ppm, 30 ppm and 60 ppm.

2. Materials and Methods
2.1. Time and place
Strawberry planting and germination were carried out in the Agro-tourism areas of Desa Banyoroto, Magelang, Central Java. Strawberries (*Fragaria x ananassa*) of cultivars ‘Crystal’ and ‘KP Brite’ were induced with 10 ppm, 30 ppm and 60 ppm of NAA after they were ±3 months old. The present study was conducted from January to May 2019 at Genetic and Breeding Laboratory, Faculty of Biology, Gadjah Mada University.

2.2. Observation of NAA induction results
Such physiological characters as leaf length, leaf width, leaf number, plant height, stem diameter, fruit diameter, and fruit weight were measured and observed.

2.3. DNA isolation
DNA was isolated from fruits using plant genome isolation methods from Illustra™ Nucleon Phytopure Kit. Those fruits were taken from the four stages of fruit ripeness, namely the green, white, pink and red stages.

2.4. Detection of *FaCHS* and *FaPYR1*
*FaCHS* and *FaPYR1* were amplified using the MyTaq™-Bioline PCR method. PCR used the target *FaCHS*, 26S-18S RNA housekeeping gene, *FaPYR1* gene, and Actin primers (Table 1).

| Name of Primer          | Oligonucleotide Sequence       |
|-------------------------|--------------------------------|
| *FaCHS*                | F 5'-GCCTTTTGAGCTGGTCT-3'    |
|                         | R 5'-CCCCAGAACATCTTGGAGG-3'  |
| 26S-18S RNA housekeeping gene |    |
|                        | F 5'-ACCGTTGATTCGACAATTGGTCATCG-3' |
|                         | R 5'-TACTGCGGGTGGCAATCGGACG-3' |
| *FaPYR1*               | F 5'-ATGGAGAAACCATCATCGGC-3' |
|                         | R 5'-TCAGACCTGGGAGTTCG-3'   |
| *Actin*                | F 5'-TGCGTTGTGCTGGAGATGAT-3' |
|                         | R 5'-CAGTAGAAGACTGGGTCG-3'  |

Table 1. Primers used in PCR [6, 7].
2.5. **ANOVA analysis**

Physiological characters observed were analyzed using the analysis of variance (ANOVA) at a significance level of 0.05 or a confidence level of 95% in order to determine the significance of the effect of various concentrations on physiological characters of plant.

2.6. **Qualitative analysis**

DNA isolates were qualitatively tested by electrophoresis using 0.8% agarose gel. PCR amplification products were qualitatively tested by electrophoresis using 2% agarose gel. Electrophoresis used a 100-bp marker or ladder to determine the successful amplification of *FaCHS* (118 bp), *26S-18S RNA housekeeping gene* (146 bp), *FaPYR1* (627 bp), and *Actin* (262 bp).

2.7. **Quantitative analysis**

Isolates were analyzed using an Ultraviolet-Visible (UV-Vis) spectrophotometer by observing the ratio of purity of A260/A280 nm absorbance values in the range 1.8 to 2.0 [17, 18]. The DNA concentration calculation formula is:

\[
\text{DNA (µl/ml)} = A_{260} \times fp \times 40 \text{ (µl/ml)}
\]

Notes:

- \( A_{260} \) = absorbance value at λ 260 nm
- \( fp \) = dilution factor

3. **Results and Discussion**

3.1. **Physiological Characteristics of NAA-Induced Strawberries**

Results of ANOVA of the effects of NAA induction on the growth of cultivars ‘Crystal’ and ‘KP Brite’ showed that NAA concentration had effects on leaf length, leaf width, leaf number, plant height, stem diameter, fruit diameter, and fruit wet weight (Table 2). Table 2 shows that NAA treatment at concentrations of 10 ppm, 30 ppm, and 60 ppm did not indicate any interaction with vegetative growth of plants, such as leaf length, leaf width, leaf number, plant height, and stem diameter of cultivar ‘Crystal’. The role of NAA in the cultivar “Crystal” was not shown in the vegetative phase, but it did in the generative phase of plants. NAA showed a significant effect on fruit development in terms of fruit diameter and wet weight of the cultivar ‘Crystal’.

The 10-ppm NAA-induced cultivar ‘Crystal’ showed a significant difference from the control plants. The 10-ppm NAA treatment had an effect on increased fruit diameter of 2.365 ± 0.092 cm relative to the control fruits of 1.952 ± 0.037 cm. Similarly, the 10-ppm NAA treatment significantly affected the fruit weight of cultivar ‘Crystal’. The concentration can increase the wet weight of the fruits to 6.006 ± 0.964 grams, or nearly twice the weight of the control fruits of 3.748 ± 0.251 grams. This shows that the addition of exogenous NAA can increase fruit diameter and weight. This is in consistent with Satriowibowo *et al.* (2014) [8] saying that NAA can increase fruit weight. However, at concentrations of 30 and 60 ppm, NAA did not show a significant difference in fruit diameter or wet fruit weight of the control. Wilkins (1992) [9] argued that healthy plants have optimal levels of substances that regulate growth, including auxin (NAA). NAA treatment at a higher concentration will show a different reaction from plants treated with low concentrations of NAA. Responses of plants to NAA may depend on the concentration given. In the present study, high concentrations of NAA (30 and 60 ppm) led to different results from low concentrations of NAA (10 ppm), where the concentration of 10 ppm was the optimum concentration to increase the size and weight of cultivar ‘Crystal’.

Different results were shown by the cultivar ‘KP Brite’ (Table 3), in which leaf length and leaf width of the controls were not significantly different at NAA concentrations of 10 ppm and 30 ppm, but it was significantly different at NAA concentration of 60 ppm. In addition, observation of leaf length and leaf width did not show a significant difference between NAA concentrations of 30 ppm and 60 ppm. The 30-ppm and 60-ppm NAA treatments had an effect on decreasing the leaf length and leaf width relative to those of controls. The leaf length and leaf width of cultivar ‘KP Brite’ induced with 60 ppm of NAA were 4.875 ± 1.217 cm, smaller than those of controls with a leaf length and leaf...
width of $6.500 \pm 1.134$ cm. NAA induction at various concentration also affected the leaf number of cultivar ‘KP Brite’. The leaf number of controls did not show a significant difference at NAA treatments of 10 ppm and 30 ppm, but it was significantly different from those at the NAA treatment of 60 ppm. The 10-ppm, 30-ppm and 60-ppm NAA treatments were not significantly different. The three concentrations showed a decrease in leaf number relative to that of controls, but the effect of 60-ppm NAA treatment showed a significance, which was $21.625 \pm 13.016$, far lower than those of controls of $41.625 \pm 20.170$. The plant height of controls was not significantly different at the 10-ppm NAA treatment, but it was significantly different at 30-ppm and 60-ppm NAA treatments. The 10-ppm NAA treatment was not significantly different from the NAA 30 ppm treatment, while the NAA 30 ppm treatment was not significantly different from the 60-ppm NAA treatment. The stem diameter of cultivar ‘KP Brite’ of controls at 10-ppm and 30-ppm NAA treatments was not significantly different but it was significantly different from that of the 60-ppm NAA treatment (Table 3).

### Table 2. Physiological characteristics of the NAA-induced cultivar ‘Crystal’.

| NAA Treatment (ppm) | Leaf Length (cm) | Leaf Width (cm) | Number of Leaf | Plant Height (cm) | Stem Diameter (cm) | Fruit Diameter (cm) | Fruit Weight (g) |
|---------------------|-----------------|----------------|---------------|------------------|-------------------|-------------------|-----------------|
| Control             | $6.143 \pm 1.376$ | $5.357 \pm 1.281$ | $36.143 \pm 19.608$ | $17.714 \pm 4.261$ | $2.643 \pm 0.027$ | $1.952 \pm 0.037$ | $3.748 \pm 0.251$ |
| 10 ppm              | $6.143 \pm 1.069$ | $5.286 \pm 1.185$ | $50.000 \pm 31.091$ | $17.857 \pm 2.410$ | $2.714 \pm 0.567$ | $2.365 \pm 0.092$ | $6.006 \pm 0.964$ |
| 30 ppm              | $5.286 \pm 1.524$ | $4.929 \pm 1.427$ | $62.429 \pm 37.398$ | $16.929 \pm 3.220$ | $2.643 \pm 0.090$ | $1.962 \pm 0.049$ | $2.626 \pm 0.394$ |
| 60 ppm              | $5.786 \pm 0.756$ | $4.929 \pm 0.535$ | $42.714 \pm 19.164$ | $17.857 \pm 2.545$ | $2.500 \pm 0.408$ | $1.941 \pm 0.115$ | $3.188 \pm 1.626$ |

Means followed by similar letters show no significant difference based on the Duncan’s multiple range test at a significance level of 5%.

### Table 3. Physiological characteristics of the NAA-induced cultivar ‘KP Brite’.

| NAA Treatment (ppm) | Leaf Length (cm) | Leaf Width (cm) | Number of Leaf | Plant Height (cm) | Stem Diameter (cm) | Fruit Diameter (cm) | Fruit Weight (g) |
|---------------------|-----------------|----------------|---------------|------------------|-------------------|-------------------|-----------------|
| Control             | $6.500 \pm 1.134$ | $6.500 \pm 1.134$ | $41.625 \pm 20.170$ | $19.313 \pm 1.907$ | $3.938 \pm 0.622$ | $2.132 \pm 0.064$ | $5.350 \pm 0.644$ |
| 10 ppm              | $6.625 \pm 1.061$ | $6.625 \pm 1.061$ | $37.875 \pm 16.120$ | $16.938 \pm 2.245$ | $4.000 \pm 0.802$ | $2.227 \pm 0.032$ | $5.350 \pm 0.649$ |
| 30 ppm              | $5.313 \pm 1.193$ | $5.313 \pm 1.193$ | $35.250 \pm 15.755$ | $15.375 \pm 3.335$ | $3.250 \pm 0.169$ | $2.323 \pm 0.035$ | $6.460 \pm 0.943$ |
| 60 ppm              | $4.875 \pm 1.217$ | $4.875 \pm 1.217$ | $21.625 \pm 13.016$ | $12.438 \pm 2.821$ | $2.125 \pm 1.157$ | $2.524 \pm 0.192$ | $8.007 \pm 2.637$ |

Means followed by similar letters show no significant difference based on the Duncan’s multiple range test at a significance level of 5%.

Induction with 10 ppm of NAA can increase the leaf length, leaf width, and stem diameter of the plants. This is in line with the fact that NAA is among the auxin hormones serving to stimulate cell elongation and expansion, especially in vegetative cells [10]. NAA is capable of increasing the plasticity of cell walls. NAA can stimulate certain proteins in the plasma membrane of plant cells to pump H+ ions onto the cell walls. The H+ ions located on the cell walls lead to the activation of certain enzymes that are able to break some of the hydrogen cross-links of cellulose molecular chains that make up the cell walls. This leads to the plasticity of cell walls to increase and the cells absorb more water through osmosis, causing the plant cells to increasingly elongate [11]. Table 3 shows that an increase in NAA concentrations can lead to a decrease in the leaf number. This is because an increase in auxin (NAA) concentration can trigger ethylene production and stimulation. This excess ethylene may result in leaf falls and decrease in leaf number [12, 13].

Observation of the physiological character of the cultivar ‘KP Brite’ showed that NAA induction of 60 ppm had a significant effect. The cultivar ‘KP Brite’ treated with NAA of 60 ppm experienced a decrease in plant growth in terms of leaves, stems and height. An increase in auxin concentration did not necessarily lead to an increase in plant growth. An increase in auxin at certain concentrations...
could reduce plant growth. NAA at high concentrations can inhibit plant growth due to an excessive high NAA. At a high dose of auxin, growth inhibition can also be caused by excessive ethylene production. In addition to leaf falls, this excess ethylene may cause the plants to die through leaf damage and aging [14].

3.2. Detection of FaCHS and FaPYR1

In gene analysis and detection, strawberry fruit samples were collected to obtain DNA isolates. Samples were collected from the 4 stages of fruit development, including the Green (8 days), White (18 days), Pink (22 days), and Red (25 days) stages. The collected fruits were subsequently extracted using Nucleon Phytopure Plant DNA Extraction Kit. The purpose of the DNA extraction was to separate genomic DNA from such other components as protein, carbohydrates, phenols, etc. This kit is designed with the ability to prevent contamination of polysaccharides commonly contaminating the plant DNA. Those polysaccharides need to be removed to prevent further inhibition of enzymatic analysis of DNA [15]. Genomic DNA was quantitatively tested by spectrophotometry using a spectrophotometer to determine the concentration and purity of genomic DNA isolated from cultivars ‘Crystal’ and ‘KP Brite’ (Table 4).

Nucleic acids, both in the form of DNA and RNA, can absorb maximum UV lights at a wavelength (λ) of 260 nm. Contaminants in the form of proteins and phenols can absorb maximum lights at a wavelength (λ) of 280 nm, while contaminants in the form of polysaccharides will absorb maximum lights at a wavelength (λ) of 230 nm. Thus, the absorbance ratio of $A_{260/280}$ indicates the degree of genomic DNA purity relative to protein/phenolic contaminants, and the absorbance ratio of $A_{260/230}$ indicates the degree of genomic DNA purity relative to polysaccharide contaminants [16]. Genomic DNA purity can be considered good if the value of the $A_{260/280}$ ratio is 1.8 to 2.0 [17; 18]. The value of the $A_{260/280}$ ratio above 1.8 indicates the presence of contamination with RNA, while the value of the $A_{260/280}$ ratio of less than 1.8 indicates the presence of contamination with proteins [19].

Table 4 shows that the value of the $A_{260/280}$ ratio for cultivar ‘Crystal’ induced with NAA of 60 ppm at the green and white stage and that of cultivar ‘KP Brite’ induced with NAA of 30 ppm at the red stage were 1.8 to 2.0. According to Singh and Singh (2015) [18], the DNA purity can be considered quite good and can be used for PCR analysis. A large amount of DNA isolates showed values below 1.8, indicating contamination with proteins or phenols or polysaccharides [20].

The isolated genomic DNA was subsequently amplified by the polymerase chain reaction (PCR) method using primers specific for the target genes FaCHS and FaPYR1 (Table 1). PCR amplification of DNA is a method to amplify a sequence of nucleotides exponentially by means of in vitro enzymatic processes [21].

FaCHS plays a role in fruit ripening [22]. Fruit ripening occurs simultaneously with discoloration due to the activation of anthocyanins that provide fruit colors. Meanwhile, FaPYR1 serves as an ABA receptor coding gene. NAA has the role of inhibiting ABA, in which ABA is capable of initiating the synthesis of ethylene that plays a role in decay [23]. The present study used 28S-16S RNA housekeeping gene as the control gene for FaCHS and Actin as the control gene for FaPYR1.

The annealing temperature in the present study for FaCHS and 28S-16S RNA housekeeping gene was 54°C. The annealing temperature for FaPYR1 and Actin was 60°C. Annealing temperature is an important part of the amplification process. An excessively high and low annealing temperature may cause the primers cannot attach to a specific site and, therefore, target gene amplification products cannot be obtained [24].

The PCR products were then electrophoresed using 2% agarose gel. Electrophoretic visualization showed that FaCHS was amplified at 118 bp, FaPYR1 at 627 bp, and 28S-16S RNA housekeeping gene at 146 bp, while Actin was amplified at 262 bp. Therefore, the present study used 100-bp markers to visualize PCR products.

Amplification of FaCHS DNA fragments showed that FaCHS was detected in both cultivars at each stage of development except at a concentration of 10 ppm in the green stage and strawberries induced with 30 ppm of NAA at the white stage. Amplification of FaPYR1 DNA fragments in the
Table 4. Spectrometric data of DNA isolates.

| Crystal | Treatment | Fruit | ng/µl | 260/230 Ratio | 260/280 Ratio |
|---------|-----------|-------|-------|---------------|---------------|
|         | Control   | Green | 210.28| 0.34          | 1.44          |
|         |           | White | 144.65| 0.25          | 1.37          |
|         |           | Pink  | 105.84| 0.21          | 1.53          |
|         |           | Red   | 89.10 | 0.25          | 1.35          |
|         | 10 ppm    | Green | 261.85| 0.29          | 1.31          |
|         |           | White | 72.10 | 0.33          | 1.65          |
|         |           | Pink  | 238.34| 0.27          | 1.38          |
|         |           | Red   | 198.35| 0.23          | 1.20          |
|         | 30 ppm    | Green | 156.43| 0.29          | 1.67          |
|         |           | White | 18.16 | 1.64         | 1.30          |
|         |           | Pink  | 65.23 | 0.30          | 1.45          |
|         |           | Red   | 122.38| 0.14         | 1.17          |
|         | 60 ppm    | Green | 323.75| 0.41          | 1.80          |
|         |           | White | 280.41| 0.40          | 1.82          |
|         |           | Pink  | 201.65| 0.23          | 1.08          |
|         |           | Red   | 1.82  | -0.15         | 1.77          |

Table 5. PCR Amplification of FaCHS and FaPYR1.

| Treatment | Crystal          | FaCHS | Housekeeping | FaPYR1 | Actin | KP Brite | FaCHS | Housekeeping | FaPYR1 | Actin |
|-----------|------------------|-------|--------------|--------|-------|----------|-------|--------------|--------|-------|
| Control   | Green v*         | v     | v            | v      | v     | v        | v     | v            | v      | v     |
|           | White v          | v     | -            | v      | v     | v        | v     | -            | v      | v     |
|           | Pink v           | v     | v            | v      | v     | v        | v     | -            | v      | v     |
|           | Red v            | v     | -            | v      | v     | v        | v     | -            | v      | v     |
| 10 ppm    | Green -          | -     | -            | -      | v     | -        | v     | -            | -      | v     |
|           | White v          | -     | -            | -      | v     | v        | v     | v            | v      | v     |
|           | Pink v           | v     | v            | v      | v     | v        | v     | -            | -      | -     |
|           | Red v            | v     | v            | v      | v     | v        | v     | -            | v      | v     |
| 30 ppm    | Green v          | v     | v            | v      | v     | v        | v     | v            | v      | v     |
|           | White -          | -     | -            | -      | v     | v        | v     | v            | v      | v     |
|           | Pink v           | v     | v            | v      | v     | v        | v     | v            | v      | v     |
|           | Red v            | v     | v            | -      | v     | v        | v     | v            | v      | v     |
| 60 ppm    | Green v          | v     | v            | -      | -     | -        | -     | -            | -      | -     |
|           | White v          | -     | -            | -      | -     | -        | -     | -            | -      | -     |
|           | Pink v           | v     | v            | v      | v     | v        | v     | v            | v      | v     |
|           | Red v            | v     | v            | -      | -     | v        | -     | -            | -      | -     |

* v = amplified
Figure 1. Detection results of FaCHS, 26S-18S housekeeping gene, FaPYR1, and Actin. 100-bp Marker
Notes: a. Control cultivar ‘KP Brite’ (1C-4C = target FaCHS; 1H-4H = 26S-18S housekeeping gene; 1P-4P = target FaPYR1; 1A-4A = Actin) 1 = green, 2 = white, 3 = pink, 4 = red.
b. Cultivar ‘KP Brite’ (5C-8C = target FaCHS, 10 ppm; 13C = target FaCHS; 60 ppm; 5H-8H = 26S-18S housekeeping gene, 10 ppm; 13H = 26S-18S housekeeping gene, 60 ppm) 5 = green, 6 = white, 8 = red, 13 = green.
c. Cultivar ‘Crystal’ (EC = target FaCHS, 10 ppm; EH-HH = 26S-18S housekeeping gene, 10 ppm; IC-LC = target FaCHS, 30 ppm; IH-LH = 26S-18S housekeeping gene, 30 ppm) E, I = green, H, L = red.
d. Cultivar ‘KP Brite’ (9C-12C = target FaCHS; 9H-12H = 26S-18S housekeeping gene) 9 = green, 10 = white, 11 = pink, 12 = red.
e. Cultivar ‘Crystal’ (EP-HP = target FaPYR1, 10 ppm; EA-HA = Actin, 10 ppm; IP-LP = target FaYR1, 30 ppm; IA-PA = Actin, 30 ppm) E, I = green, H, L = red.
f. Cultivar ‘KP Brite’ (5P-8P = target FaPYR1, 10 ppm; 5A-8A = Actin, 10 ppm) 5 = green, 6 = white, 8 = red, 13 = green.
   Cultivar ‘Crystal’ (FP-GP = target FaPYR1, 10 ppm; FA-GA = Actin, 10 ppm) F = white, G = pink.
g. Cultivar ‘Crystal’ (FC-HC = target FaCHS, 10 ppm; FH-GH = 26S-18S housekeeping gene, 10 ppm) F = white, G = pink, H = red.
h. Cultivar ‘Crystal’ (JC-KC = target FaCHS, 30 ppm; JH-KH = 26S-18S housekeeping gene, 30 ppm; MC-OC = target FaCHS, 60 ppm; MH-OH = 26S-18S housekeeping gene, 60 ppm) M = green, J, N = white, O, K = pink.
i. Cultivar ‘Crystal’ (JA-KA = Actin, 30 ppm; MA-OA = Actin, 60 ppm) M = green, J, N = white, O, K = pink.
   Cultivar ‘KP Brite’ (13P = target FaPYR1, 60 ppm; 13A = Actin, 60 ppm) 13 = green.

Controls showed that FaPYR1 could only be detected in the green and red stages. Treatment results showed that FaPYR1 was only amplified in strawberries treated with 10 ppm of NAA (Table 5). This indicates that NAA is able to inhibit ABA by inhibiting FaPYR1 formation in strawberries induced with 30 or 60 ppm of NAA. Inhibition of ABA will prevent the synthesis of ethylene which plays a role in strawberry fruit decay. NAA concentrations of 10, 30 and 60 ppm cannot inhibit the formation of FaCHS which plays an active role to provide colors via activation of anthocyanins. At the NAA concentration of 30 ppm at the white stage FaCHS could not be amplified since the DNA isolates obtained had a fairly low concentration of below 20 ng/µl.

FaCHS could not be amplified in cultivar ‘KP Brite’ induced with 30 ppm of NAA at the white stage. In addition, FaCHS was not amplified in the isolates of strawberry fruits treated with 60 ppm of NAA at the green, white, and pink stages.

FaPYR1 fragments of cultivar ‘KP Brite’ could only be detected in DNA isolates of strawberry fruits treated with 30 ppm of NAA and 10 ppm of NAA at white stage (Table 5). These results indicate that the 60-ppm NAA induction treatment can inhibit the formation of FaCHS in the accelerated decay of fruits. FaCHS could be detected in the strawberry fruits of cultivar “KP Brite” treated with 60 ppm of NAA at the red stage due to the formation of anthocyanins that provide strawberry fruits with the red color.

4. Conclusion
Treatment of NAA induction in Crystal cultivar of strawberry plants has no effect on physiological characters (such as leaf length and width, number of leaves, plant height, and stem diameter) of plants in the vegetative stage. The treatment of 10 ppm has increasing the fruit diameter and weight for the Crystal cultivar strawberry plants. The treatment of NAA 30 and 60 ppm induction affected the FaPYR1 gene profile in Crystal cultivars. While the 60 ppm NAA induction treatment was able to influence the FaCHS gene profile in Kp-Brite cultivars.
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