Asia-Pacific Journal of Science and Technology :Volume :3 Issue :05 Article ID: APST-30-05-12 Research Article

APST

Asia-Pacific Journal of Science and Technology

https//:www.tci-thaijo.org/index.php/APST/index

Published by Research and Innovation Department,
Khon Kaen University, Thailand

Detection and analysis of strawberry (*Fragaria* × *ananassa*) fruit ripening Genes *FaPYR1* and *FaCHS* treated by Indole-3-acetic acid induction

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Received 5 February 2024
Revised 12 February 2024
Accepted 29 April 2025

Abstract

Strawberries (*Fragaria* × *ananassa*) are economically significant commodities in Indonesia, where ripeness influences their quality and marketability. This study investigates the role of the *FaPYR1* and *FaCHS* genes, involved in fruit ripening, in strawberries treated with varying concentrations of indole-3-acetic acid (IAA). A key research gap lies in understanding how IAA affects these gene expressions and strawberry morphology. Strawberry plants were treated with 10 ppm, 30 ppm, and 60 ppm of IAA, and their genomic deoxyribonucleic acid (DNA) was analyzed using polymerase chain reaction (PCR), alongside morphological evaluations. Results revealed that 30 ppm of IAA significantly enhanced leaf dimensions and fruit genomic DNA concentration during the red stage. Conversely, the highest leaf count was observed at 60 ppm, while control plants exhibited the lowest morphological and genomic outcomes. PCR analysis confirmed *FaCHS* gene expression across all treatments, but *FaPYR1* expression was inconsistent. These findings highlight the potential of IAA, particularly at 30 ppm, to optimize strawberry growth and ripening, offering valuable insights for improving cultivation practices and fruit quality management.

Keywords: *FaCHS* gene, *FaPYR1* gene, *Fragaria* × *ananassa*, Fruit ripening, indole-3-acetic acid

1. Introduction

Strawberries (*Fragaria* × *ananassa*), members of the Rosaceae family, are non-climatic and subtropical fruits widely appreciated for their sweet taste, distinctive aroma, and high nutritional value [1–4]. Their global significance extends beyond Indonesia, contributing to substantial economic value in agricultural industries worldwide. However, strawberry production faces challenges such as declining yields and increasing consumer demand, which has led to a growing reliance on imports in regions like Indonesia. From 2012 to 2014, national production plummeted from 169,796 tons to 58,882 tons annually, highlighting an urgent need to boost local production with superior-quality berries [5,6].

Fruit ripeness is a critical quality indicator for strawberries, influencing both market value and consumer satisfaction [7,8]. Among the strategies to enhance fruit ripening and quality, the role of plant hormones, particularly indole-3-acetic acid (IAA), has drawn considerable attention [9,10]. IAA is a naturally occurring auxin that regulates multiple physiological processes, including growth, development, cell division, and ripening [10–12]. Additionally, IAA can stimulate abscisic acid (ABA) synthesis, a hormone known to regulate strawberry ripening through its intracellular receptor *FaPYR1*, which plays a key role in ABA signaling [13,14]. Moreover, secondary metabolites such as anthocyanins and flavonoids further contribute to strawberry ripening by

influencing color development, an essential indicator of fruit maturity [14,15]. *FaCHS*, a key gene in flavonoid biosynthesis, also acts as a reporter for signaling mechanisms that control pigment changes during ripening [16]. While previous studies have explored the individual roles of IAA and ABA in strawberry ripening, the specific interactions between these hormones and ripening-associated genes like *FaPYR1* and *FaCHS* remain underexplored. Understanding these molecular mechanisms could pave the way for strategies to improve fruit quality and local strawberry cultivation. The objectives of this study were to investigate the effects of IAA on strawberry growth and development, analyze its role in ripening processes, and detect the expression of ripening-related genes, *FaPYR1* and *FaCHS*. This research aims to provide insights into the hormone-gene interactions that underlie strawberry ripening, with potential applications in improving cultivation practices and enhancing the competitiveness of locally grown strawberries in domestic and global markets.

2. Materials and methods

The study was conducted at the Laboratory of Genetics and Breedings, Faculty of Biology, Universitas Gadjah Mada, from August to December 2018. Strawberry fruits were obtained from the Banyuroto Agro-tourism, Banyuroto Village, Sawangan District, Magelang Regency, Central Java.

2.1 Planting and Treatment with IAA

The study was performed for three months using two cultivars of *F.* × *ananassa* 'Crystal' and 'KP Brite'. Both seed seedlings were taken from Banyuroto Agro-tourism, Banyuroto Village, Sawangan District, Magelang Regency, Central Java. The design was made with eight repetitions of each treatment using Randomized Complete Block Design. The experiment was designed as follow: control, 10 ppm, 30 ppm, and 60 ppm [17] , and applied for thirty plants per treatment, respectively. Spraying was conducted twice a week, applying approximately 1 mL each time, over a period of three months, excluding the control plants. The spray was applied to the stems and leaves, which are key growth regions. The fruits were harvested at four stages of ripeness: green, white, pink, and red, after which deoxyribonucleic acid **(**DNA**)** isolation was performed. Environmental conditions were not measured, as the study was carried out under natural conditions typical of mountainous regions at an altitude of 1,500 meters above sea level. Standard plantation growing media were used, supplemented with animal and plant-based fertilizers from local farming sources. Strawberry seeds are planted in polybags and placed on the prepared land, then watered once a day. The growth period of strawberry plants until the fruit appears is around 5-6 months, in this study strawberry plants that have grown for about 2 months were used, then treated with IAA spraying. Two-month-old strawberries were treated by spraying twice a week for three months. Furthermore, plant phenotypes consisting of plant height, leaf width, leaf length, number of leaves, and stem diameter were observed. The analysis was made using the ANOVA test with a confidence level of 95% and continued with DMRT (Duncan's Multiple Range Test at a significance level of 5% ($\alpha = 0.05$) using the SPSS version 16 software application to determine the significant difference between control and treatment.

2.2 DNA Extraction

The genomic DNA was extracted using the Illustra Nucleon-Phytopure DNA Extraction Kit TM RPN 1851, which consists of reagents 1, 2, and resin. Four stages of fruit ripening were categorized. Each treatment categorized fruits into green, white, pink, and red. Fruits were weighed at 0.5 gr, ground to fineness, and put into a sterile 1.5 µL microtube. A total of 400-500 µL of the reagent Phytopure I was added, then 100 µL of the reagent Phytopure II was put and stirred slowly. Samples were incubated at 65ºC for 10 minutes. It was subsequently put into a freezer for 20 minutes at 5ºC. The sample was removed and then added with cold chloroform and 50-70 µL of Phytopure resin, which was centrifuged for 10 minutes at 3,000 rpm. The supernatant formed at the top was taken, added with isopropanol at the same volume, and then transferred into a new 1.5 µL microtube. Afterward, samples were centrifuged for 10 minutes at 10,000 rpm, and the DNA pellets formed at the bottom of the tube were then washed with 100 µl of 70 % ethanol before re-centrifuged for 10 minutes at 10,000 rpm. DNA pellets were eluted with TE buffer 1x and stored in a freezer. The genomic DNA was analyzed quantitatively using a spectrophotometer.

2.3 Amplification of FaPYR1, FaCHS, and Housekeeping Gene

The extracted DNA was amplified using the polymerase chain reaction (PCR) method. The target, reference Actin, and 26S-18S RNA housekeeping gene primers (Table 1) were used. Amplification was executed using the MyTaxTM HS Red Mix BIOLINE as the master mix. The amplification protocol consisted of the following steps: predenaturation at 95 °C for 1 minute; denaturation at 95°C for 15 seconds; annealing at 50-55 °C for 15 seconds; and extension at 72°C for 10 seconds, was performed. The *FaPYR1* and *FaCHS* amplification products were

analyzed qualitatively by electrophoresis. Successfully amplified genes produced the bands parallel to the marker according to the size of each gene. *FaPYR1* is 627 bp, *Actin* 262 bp, *FaCHS* 127 bp, *26S-18S RNA Housekeeping* 146 bp.

Table 1 Primer for amplification.

Primers	Sequence	Reference
FaPYR1 Forward	'5-ATGGAGAAACCATCATCGGC-3'	[18]
FaPYR1 Reverse	'5-TCAGACCTGGGGAGTTAGCG-3'	[18]
Housekeeping for *FaPYR1* (Actin Forward)	'5-TGGGTTTGCTGGAGATGAT-3'	[13]
Housekeeping for *FaPYR1* (Actin Reverse)	'5-CAGTAGGAGAACTGGGTGC-3'	[13]
FaCHS Forward	'5-GCCTTTGTTTGAGCTGGTCT-3'	[19]
FaCHS Reverse	'5-CCCAGGAACATCTTTGAGGA-3'	[19]
Housekeeping for *FaCHS* (26S-18S Forward)	'5-ACCGTTGATTCGCACAATTGGTCATCG-3'	[19]
Housekeeping for *FaCHS* (26S-18S Reverse)	'5-TACTGCGGGTCGGCAATCGGACG-3'	[19]

3. Results

3.1 Morphological Responses to IAA Treatment

Figure 1 shows the leaf morphology of strawberry cultivars (A) 'Crystal' and (B) 'KP Brite', which exhibit differences after IAA induction. Leaves are a vital part of plants for photosynthesis. IAA induction affected the two strawberry cultivars differently. Table 2 indicates that, for the cultivar 'Crystal,' there is no significant difference between the control and treatments regarding the number of leaves. The highest value of 59.250 ± 26.964 was found at the 60-ppm treatment.

Table 3 indicates that for the cultivar 'KP Brite', there is a significant difference between the control and treatments in terms of the number of leaves. The highest value of 28.875 ± 0.991 was found at the 60-ppm treatment. In addition to the number of leaves, the length and width were measured using the Duncan range tests. The results revealed that for the cultivar 'Crystal', there was a significant difference between the control and treatments in terms of leaf length (Figure 2). The highest value of 5.313 ± 1.319 was found at 60 ppm. The cultivar 'KP Brite' showed no significant difference between control and treatments in terms of leaf length and the highest value of 7.375 ± 0.916 was found at the 60-ppm treatment.

3.1.1 Leaf Characteristics

The morphological responses of strawberry cultivars to IAA treatment showed distinct patterns in leaf characteristics between 'Crystal' and 'KP Brite'. For leaf count, 'Crystal' exhibited no significant changes, but the 60-ppm concentration resulted in the highest average of 59.250 ± 26.964 leaves. Conversely, 'KP Brite' displayed significant differences, with the highest leaf number recorded at 60 ppm with an average value of 28.875 ± 0.991 leaves. Leaf length in 'Crystal' varied significantly, with the most extended found at the 60-ppm concentration with an average value of 5.313 ± 1.319 cm, whereas 'KP Brite' showed no significant differences. However, its maximum leaf length reached 7.375 ± 0.916 cm at the same concentration. Leaf width was broadest in 'Crystal' at 30 ppm, measuring 5.687 ± 0.798 cm, while 'KP Brite' achieved its widest leaves with an average value of 7.000 ± 0.886 cm at 60 ppm.

3.1.2 Plant Growth Parameters

Regarding plant height, 'Crystal' showed a solid response to IAA treatments, with the tallest plant height at 5.687 ± 0.798 cm observed at 30 ppm. In contrast, 'KP Brite' exhibited no significant differences across treatments, with its tallest plant value at 29.000 ± 5.130 cm in the control group. Stem diameter showed no significant differences in either cultivar. However, the highest values were recorded at 60 ppm for both 'Crystal' (2.687 ± 1.237 cm) and 'KP Brite' (2.500 ± 0.707 cm), with the latter also reaching the same diameter in the control group. These findings suggest that while 'Crystal' responded well to IAA for plant height, 'KP Brite' displayed limited responsiveness to the hormone.

3.1.3 Flower Development

The flower morphology differed significantly between the two cultivars (Figure 3). 'Crystal' exhibited higher flower production than 'KP Brite', contributing to its increased fruit yield. The higher flower production suggests that IAA may enhance the reproductive capacity of 'Crystal'. However, the extent to which this effect occurs in 'KP Brite' remains limited, potentially due to its lower responsiveness to IAA treatments.

3.1.4 Fruit Development Stages

Fruit development also varied between the cultivars. 'Crystal' fruits were larger, oval-shaped, and more abundant than the smaller and less numerous fruits of 'KP Brite' (Figure 4). Both cultivars underwent a color transition during fruit development, from green to red, indicating ripening. IAA effectively stimulated early-stage fruit development by promoting cell division and elongation during the green and white phases. As the fruits transitioned to the pink and red stages, IAA levels decreased, giving way to ABA and anthocyanins essential for the ripening process.

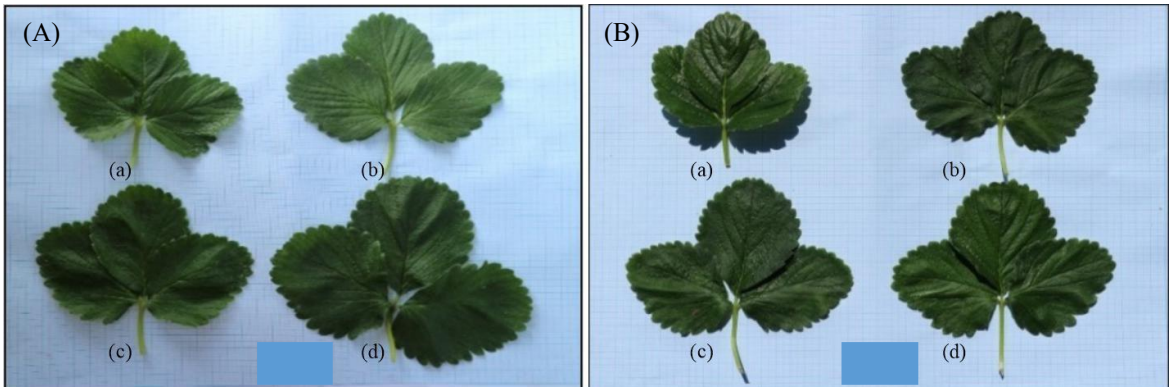


Figure 1 Leaf morphology of cultivars (A) Crystal, (B) KP Brite. (a) Control, (b) 10 ppm, (c) 30 ppm, (d) 60 ppm.

Table 2 Statistical analysis of morphological measurements of IAA-induced for cultivar Crystal.

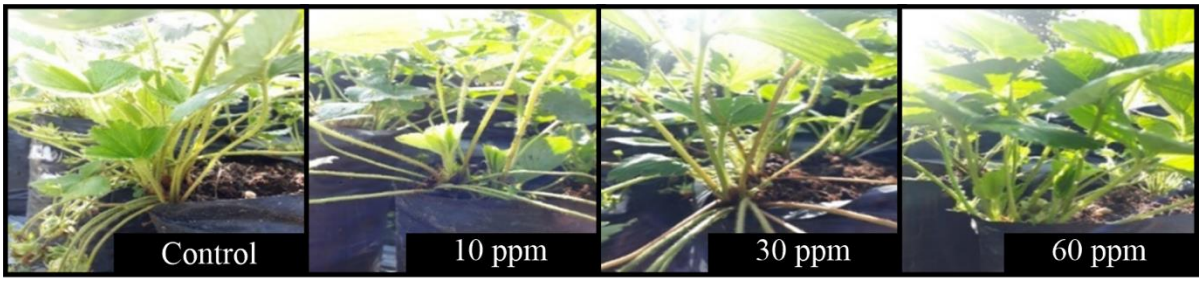
IAA Treatment (ppm)	Parameters				
	Leaf Length	Leaf width	Leaf Number	Plant Height	Stem Diameter
Control	6.375 ±1.903^{ab}	6.937 ± 1.568^{b}	43.500 ± 22.032^{b}	29.000 ± 5.130^{a}	2.500 ± 0.654^{a}
10	6.625 ±0.954^{ab}	6.062 ± 0.863^{ab}	41.125 ± 9.078^{ab}	19.687 ± 3.239^{a}	2.687 ± 0.703^{a}
30	5.625 ± 2.031^{a}	5.187 ± 1.771^{a}	27.625 ± 21.138^{a}	15.000 ± 3.693^{a}	2.375 ± 0.876^{a}
60	7.375 ± 0.916^{b}	7.000 ± 0.886^{b}	28.875 ± 0.991^{ab}	18.875 ± 2.216^{a}	2.500 ± 0.707^{a}

The means followed by similar letters shows non-significant difference based on the Duncan's multiple range test at 5% level of significance.

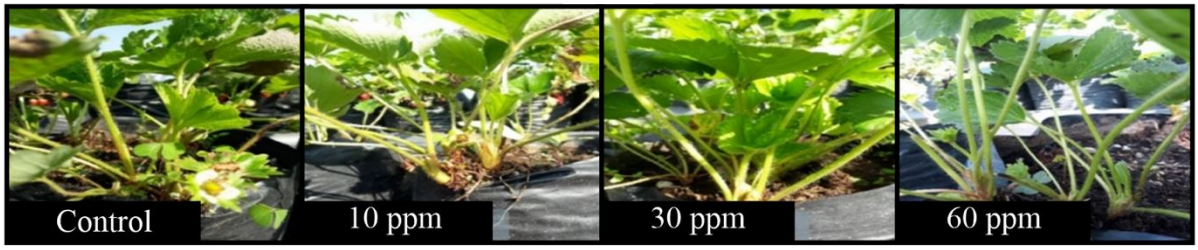
Table 3 Statistical analysis of morphological measurements of IAA-induced for cultivar KP Brite.

IAA Treatment (ppm)	Parameters				
	Leaf Length	Leaf width	Leaf Number	Plant Height	Stem Diameter
Control	4.500 ± 1.118^{a}	3.687 ± 1.163^{a}	31.750 ± 20.471^{a}	13.375 ± 4.373^{a}	1.750 ± 0.534^{a}
10	5.013 ± 0.036^{a}	4.250 ± 1.069^{ab}	48.625 ± 29.110^{a}	14.937± 4.373^{a}	2.312 ± 0.798^{a}
30	6.438 ± 0.863^{a}	5.687 ± 0.798^{c}	50.250 ± 24.376^{a}	20.312 ± 1.579^{b}	2.437 ± 0.886^{a}
60	5.313 ± 1.319^{b}	4.687 ± 1.032^{b}	59.250 ± 26.964^{a}	20.562 ± 4.386^{b}	2.687 ± 1.237^{a}

The means followed by similar letters shows non-significant difference based on the Duncan's multiple range test at 5% level of significance.



(A)



(B)

Figure 2 Morphology of cultivar (A) Crystal, (B) KP Brite.

3.2 Molecular Analysis

3.2.1 DNA quality and quantity

Molecular analysis of the cultivars 'Crystal' and 'KP Brite' of *F. ananassa* highlighted notable DNA purity (Figure 5) and concentration variations throughout different developmental stages and IAA treatments (Figure 6). For the cultivar 'Crystal', the highest DNA purity recorded was 1.93 during the green stage at a 30-ppm IAA treatment, which falls within the optimal purity range of 1.8 to 2.0. Conversely, the lowest purity level of 1.20 was noted at the 60-ppm treatment during the red stage, likely attributed to contamination from phenolic compounds, such as anthocyanins, prevalent in this stage. The other purity measurements for 'Crystal' varied between 1.28 and 1.83. Similarly, the 'KP Brite' cultivar exhibited a peak DNA purity of 1.95 during the green stage at a 10-ppm treatment, indicating lower contamination levels from phenolic compounds. The lowest purity observed for 'KP Brite' was 1.28 at the 30-ppm treatment during the pink stage, with its overall purity values ranging from 1.28 to 1.90. The analysis of DNA concentration revealed significant differences between the two cultivars studied. For the 'Crystal' cultivar, the highest recorded DNA concentration was 294.25 µg/µL at the 60-ppm treatment during the white stage, while the lowest concentration was observed at 41.77 µg/µL at the 30-ppm treatment during the same stage. The concentrations for other samples ranged from 59.72 µg/µL to 216.89 µg/µL.

In contrast, the 'KP Brite' cultivar exhibited its highest DNA concentration of 422.45 µg/µL at the 30-ppm treatment during the red stage. The lowest concentration for this cultivar was 61.39 µg/µL, recorded at the 60-ppm treatment during the white stage. Other samples within this cultivar showed a concentration range from 89.1 µg/µL to 208.79 µg/µL. These findings indicate the variability in DNA concentration between cultivars and across different treatment stages. Stage-specific observations highlighted that DNA purity was generally higher during the green stage for both cultivars, likely due to lower levels of phenolic compound contamination at this stage. Conversely, due to anthocyanin accumulation, DNA purity decreased during the red stage. DNA concentration peaked variably across treatments and developmental stages, indicating stage-specific responses to IAA treatment. These findings underscore the interplay between IAA treatment, developmental stages, and DNA quality, providing valuable insights into the molecular dynamics of the two strawberry cultivars.

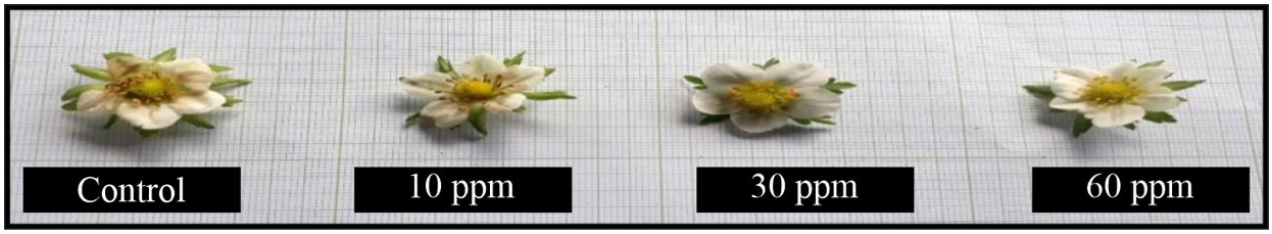
3.2.2 Gene expression patterns

The amplification results for *FaPYR1*, Actin, *FaCHS*, and 26S-18S RNA housekeeping genes revealed optimal extension sizes. Specifically, the *FaPYR1* product measured 627 bp, while the Actin product measured 261 bp. In contrast, the amplified *FaCHS* fragment was 118 bp, and the 26S-18S RNA housekeeping region was 146 bp. A concentration of 20 µM primers was utilized throughout this study.

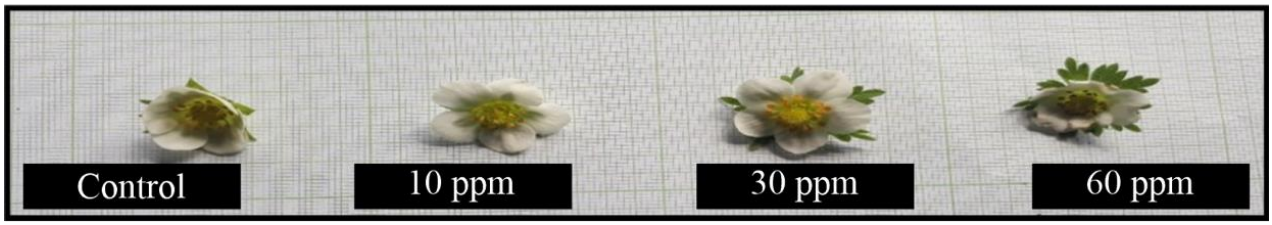
Figure 7 illustrates that both cultivars, 'Crystal' and 'KP Brite,' successfully amplified *FaCHS* and the 26S-18S RNA housekeeping gene. This was confirmed by comparing the bands on the electrophoresis gel against a 100-

bp marker. However, it is noteworthy that not all samples of *FaPYR1* and Actin from both cultivars yielded amplification, as shown in Figure 8.

The amplification of *FaPYR1* and Actin (Figure 8) in the cultivars 'Crystal' and 'KP Brite' across green, white, pink, and red stages indicates the active involvement of these genes in the regulation of ABA. The synthesis of ABA is influenced by auxin, as referenced in previous studies. Additionally, the amplified *FaCHS* gene demonstrates a significant role in the function of chalcone isomerase (CHI), which is essential for the stimulation of anthocyanin production during the ripening of strawberry fruits. Overall, the amplification of these genes, as evidenced by the electrophoregram bands, highlights the critical role of IAA induction in the process of fruit ripening.

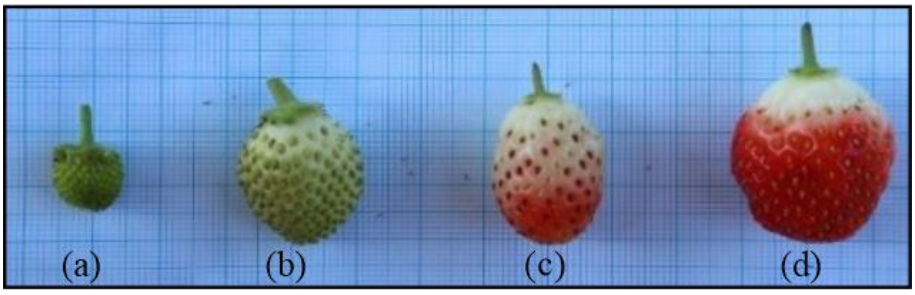


(A)

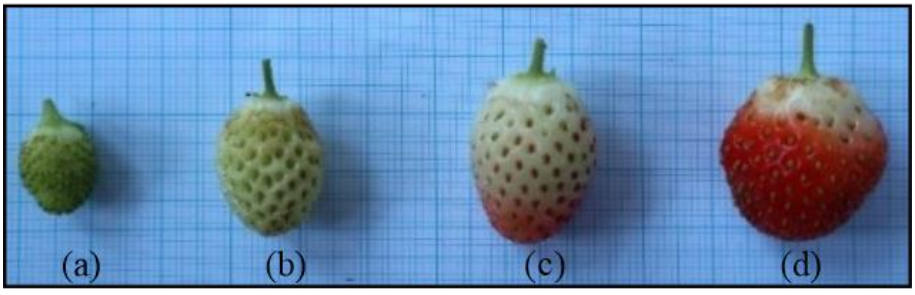


(B)

Figure 3 Flower morphology of cultivar (A) Crystal, (B) KP Brite.



(A)



(B)

Figure 4 Four stages of strawberries. (A) Crystal, (B) KP Brite (a) green, (b) white, (c) pink, and (d) red cultivar.

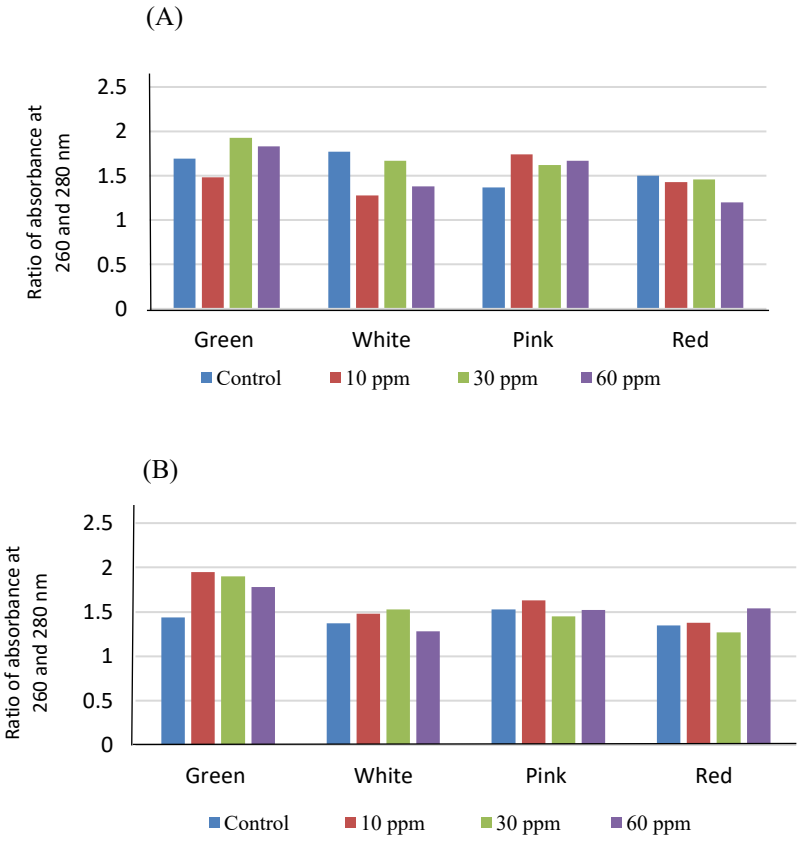


Figure 5 Quantitative DNA purity tests of (A) Crystal and (B) KP Brite.

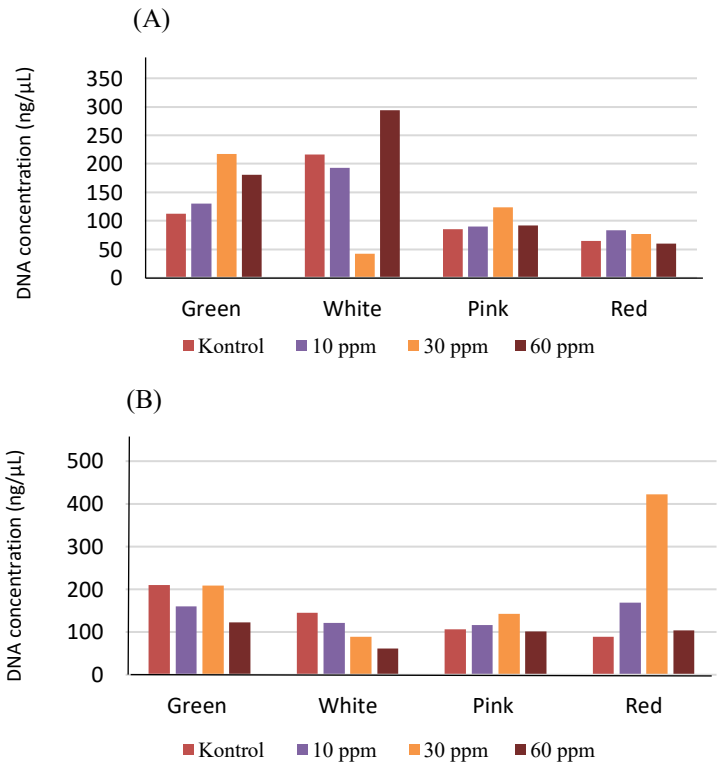


Figure 6 Quantitative DNA concentration tests of (A) Crystal and (B) KP Brite.

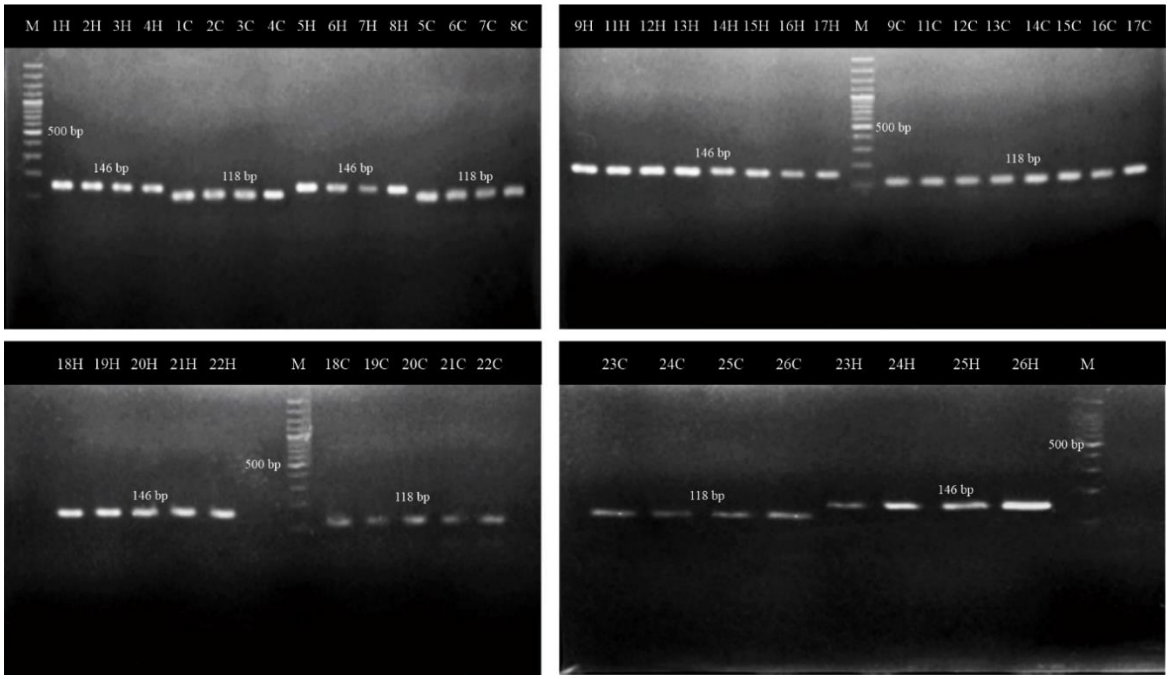


Figure 7 Electrophoregram of *FaCHS* and Housekeeping PCR products. C: *FaCHS* Gene, H: 26S-18S RNA Housekeeping Gene, M: (1) 'KP Brite', 10 ppm, green; (2) 'KP Brite', 10 ppm, white; (3) 'KP Brite', 10 ppm, pink; (4) 'KP Brite', 10 ppm, red; (5) 'Crystal', 10 ppm, green; (6) 'Crystal', 10 ppm, white; (7) 'Crystal', 10 ppm, pink; (8) 'Crystal', 10 ppm, red, (9) 'KP Brite', 30 ppm, green; (11) 'KP Brite', 30 ppm, pink; (12) 'KP Brite', 30 ppm, red; (13) 'Crystal', 30 ppm, green; (14) 'Crystal', 30 ppm, white; (15) 'Crystal', 30 ppm, pink; (16) 'Crystal', 30 ppm, 30 ppm, red; (17) 'KP Brite', 60 ppm, green; (18) 'KP Brite', 60 ppm, white; (19) 'KP Brite', 60 ppm, pink; (20) 'Crystal', 60 ppm, green; (21) 'Crystal', 60 ppm, 60 ppm, white; (22) 'Crystal', 60 ppm, pink; (23) 'Crystal', 60 ppm, red; (24) 'KP Brite', 60 ppm, white brite; (25) 'Crystal', 60 ppm, red; (26) 'Crystal'.

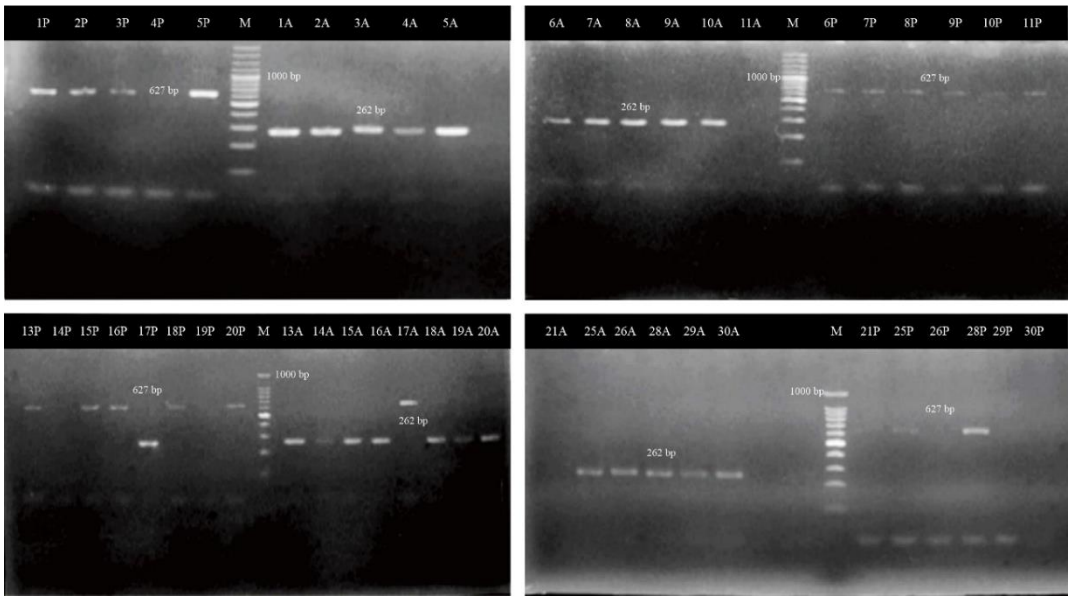


Figure 8 Electrophoregram of *FaPYR1* and *Actin* PCR products. P: *FaPYR1,* A: *Actin,* M: (1) 'KP Brite', 10 ppm, green; (2) 'KP Brite', 10 ppm, white; (3) 'KP Brite', 10 ppm, pink; (4) 'KP Brite', 10 ppm, red; (5) 'Crystal', 10 ppm, green; (6) 'Crystal', 10 ppm, white; (7) 'Crystal', 10 ppm, pink; (8) 'Crystal', 10 ppm, red; (9) 'KP Brite', 30 ppm, green; (10) 'KP Brite', 30 ppm, white; (11) 'KP Brite', 30 ppm, pink; (13) 'Crystal', 30 ppm, green; (14) 'Crystal', 30 ppm, white; (15) 'Crystal', 30 ppm, pink; (16) 'Crystal', 30 ppm, red; (17) 'KP Brite', 60 ppm, green; (18) 'KP Brite', 60 ppm, white; (19) 'KP Brite', 60 ppm, pink; (20) 'Crystal', 60 ppm, green; (21) 'Crystal', 60 ppm, white; (25) 'Crystal', 60 ppm, red; (26) 'Crystal', 60 ppm, white; (28) 'Crystal', 60 ppm, green; (29) 'Crystal', control, pink, (30) 'Crystal', control, red.

4. Discussions

4.1 Morphological Responses to IAA Treatment

IAA is plants' main auxin or growth hormone [20–22]. It is essential for plant growth and development [23,24]. IAA helps with cell division, growth, germination, and fruit ripening [25]. IAA is also involved in forming various plant organs, including leaves and adventitious roots, and it plays a significant role in the plant's response to directional growth, known as tropism [26,27].

Duncan's multiple range test results indicate that various concentrations of IAA hormone, precisely 10 ppm, 30 ppm, and 60 ppm, significantly influence strawberries' number of leaves, leaf length, and leaf width. Notably, the Crystal cultivar exhibited the lowest measurements for these three parameters in the control group. This is attributed to the absence of exogenous IAA application, which resulted in slower growth than plants receiving treatment [28,29]. In the KP Brite cultivar, the observed low results for the three parameters in the 30 ppm treatment indicate that this concentration is less effective in promoting optimal growth. However, the treatment's effectiveness relies on auxins at specific concentrations that enhance explant growth, especially leaf formation [30]. Research has indicated that the IAA hormone plays a significant role in developing meristem tissue in potential leaf growth [31,32]. Higher measurements of leaf length and width are indicative of increased leaf morphology.

Auxin plays a crucial role in the growth process by stimulating the extension of stem [33–35]. It is notably observed in the Cristal cultivar. Additional research also indicates that endogenous and exogenous auxins are essential in activating energy reserves, influencing cell elongation [36,37]. The KP Brite cultivar demonstrates high yields that do not align with conventional expectations, as there is no significant difference compared to the control group. This observation suggests that the impact of IAA may not be optimal for promoting cell division during treatment.

The findings from Duncan's multiple tests on the Crystal and KP Brite cultivars indicate that 60 ppm of IAA hormone concentration is the most effective for enhancing growth characteristics. This concentration significantly influenced vital parameters such as the number of leaves, leaf area, plant height, and stem diameter. It is believed that 60 ppm of IAA effectively complements endogenous auxin, playing a crucial role in cell differentiation, division, and overall cell development in plants [23,24]. In this study, the 'KP Brite' was observed to have the lowest results from Duncan's multiple distances. It was occurring with a treatment concentration of 30 ppm. This contrasts with the results seen in the 'Crystal' cultivar, leading to the hypothesis that the 30-ppm hormone concentration may not be optimal for triggering the growth process in 'KP Brite'. This aligns with existing research, suggesting that specific hormone concentrations can inhibit growth [26].

Previous research has shown that IAA is significant in various physiological processes. Studies indicate that it can significantly enhance root formation when IAA is applied in conjunction with other hormones, such as Gibberellic acid (IBA) and Thidiazuron (TDZ). This combination of hormones is often utilized to optimize plant growth and development [38]. Combining exogenous applications of indole-3-butyric acid IBA IAA has enhanced the proliferation of new shoots from vegetative tissue cultivated in vitro. This method can significantly optimize shoot formation [39,40]. IAA, ABA, jasmonic acid, and the expression of fruit development genes collectively contribute to fruit development. These factors regulate the growth and maturation of fruits [41]. Auxin and abscisic acid play crucial roles in the ripening and aging of strawberry fruit by influencing a signal transduction pathway. This pathway subsequently regulates structural genes that affect the physicochemical properties of the fruit. Notably, the application of exogenous IAA has been shown to delay the ripening process of strawberries after harvest. In contrast, ABA promotes ripening during the postharvest period [42].

4.2 Molecular Basis of IAA-Induced Ripening

The molecular mechanisms underlying IAA-induced ripening in strawberry cultivars 'Crystal' and 'KP Brite' underscore the hormone's essential function in influencing growth and development. Observations indicate that IAA application at a concentration of 60 ppm most significantly enhanced vegetative growth parameters, such as the number of leaves, leaf area, and stem diameter, particularly in the 'Crystal' cultivar. These findings support the notion that IAA works in tandem with endogenous auxins to facilitate processes like cell differentiation, division, and elongation [43]. While there was a notable improvement in certain vegetative growth metrics, the transition to flowering and fruit development suggested a redistribution of photosynthates toward generative processes, thereby constraining further vegetative growth.

Strawberry fruits develop through distinct stages which are green, white, pink, and red [44–46]. Each stage involves specific physiological and molecular changes significantly influenced by IAA [47]. During the green and white stages, high levels of IAA promote cell division and elongation, which are vital for the initial formation of the fruit [36]. As strawberries progress to the pink and red stages, IAA levels decrease and are replaced by abscisic ABA and anthocyanins [48–50]. These compounds play a crucial role in the maturation and coloration of the fruit. Molecular analyses have confirmed the expression of genes associated with ripening and the role for reinforcing of IAA in initiating and maintaining these developmental processes [12,51].

The IAA hormone significantly influences fruit development [52,53]. Aplication at later stages makes its application less impactful during the green stage. However, administering IAA at this stage can reduce the effectiveness of ABA and result in decreased expression of the *FaPYR1* gene, which functions as the receptor for ABA. Seed maturation is known to occur during the green stage, and gene expression analysis indicates that the *FaPYR1* gene plays a crucial role in ABA signaling [54]. The expression of the *FaPYR1* gene can be highest during the initial stages of fruit development, specifically at the beginning of the white stage, and declines during the pink and red stages [13]. During periods of elevated IAA hormone levels. It is expected that the synthesis of the ABA hormone does not increase. However, *FaPYR1* gene expression can rise under IAA treatment conditions [55]. As the fruit reaches the red stage, it is characterized by significant changes in aroma and color [56]. The expression of the *FaPYR1* gene can also increase. This stage is critical for accumulating the ABA hormone, which is essential in fruit ripening [55,57]. The higher ABA levels can enhance the synthesis process which leading to further elevated expression of the *FaPYR1* gene [13].

The *FaCHS* gene is crucial in initiating the phenylpropanoid biosynthesis pathway and essential for fruit coloring [58]. This process is regulated by ABA in conjunction with various metabolite compounds, hormones, enzymes, and transcription factors [57]. Collectively, these elements promote the expression of the *FaCHS* gene, leading to the catalysis of naringen compounds and their derivatives, which contribute to the fruit's coloration [59]. Furthermore, the mechanism will activate another gene in the fruit, namely CHI [60], and stimulate anthocyanin compounds that play a role in the red pigment coloring of the fruit. The *FaCHS* gene is induced by ABA and other compounds so that it can be expressed. IAA induction affects the *FaCHS* gene expression level at the white level, especially under conditions such as environmental stress [61]. The role of exogenous IAA in triggering the process of ABA synthesis will further induce the formation of the *FaCHS* gene and produce anthocyanin compounds [62]. Therefore, IAA treatment can trigger a higher expression level. The red pigment in strawberries results from anthocyanin expression triggered by *FaCHS* hormone. Compared to Crystal and KP Brite cultivars, the highest *FaCHS* gene expression level was the same at the red level.

5. Conclusions

The growth process of strawberry fruits in the 'Crystal' and 'KP Brite' cultivars comprises four phases: green, white, pink, and red. Notably, there was a significant difference in strawberry fruits induced by IAA at a concentration of 60 ppm compared to the control. Both 'Crystal' and 'KP Brite' cultivars exhibited the expression of *FaPYR1* and *FaCHS* as genes associated with fruit ripening.

6. Acknowledgements

Authors express gratitude to al farmers in Banyuroto Agro-tourism, Sawangan, Magelang, Central Java, and all parties for their involvement and assistance in completing the study. Author also appreciate to Head of Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada and the Dean of Faculty of Biologi Universitas Gadjah Mada for the support and faciltied for finishing this research.

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