Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 410-413
Received: 15-11-2017
Accepted: 17-12-2017

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Comparison of Abscisic Acid hormone on the leaves of palm oil seeds with watered and not watered

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Abstract
The dryness had the effect to increase the level of abscisic acid in oil palm. The increasing level of abscisic acid in the leaves will induce and regulate stomatal closure so the dryness can be prevented. Dryness can be prevented by watered regularly. The average abscission rate was 2.2361 (µg/g) and watered leaves was 2,3832 (µg/g). Significance different test was at 95% confidence interval and obtained Pvalue equal to 0,005 and value $\alpha = 0,05$. The average abscisic of oil palm which not watered was higher than the average abscission acid level with watered.

Keywords: abscisic acid, hormone, oil palm, watered

1. Introduction
Abscisic acid was one of the growth hormone which related to environmental stress conditions. An increasing in the concentration of abscisic acid in plant roots was a chemical signal that will be transported to the leaves when the plant has lack water from the soil. When dryness stress, the increasing of the synthesis abscisic acid in plant roots as a response to deficit groundwater will happened. This increasing in abscisic acid was related to the status of water in plants [1]. The next process of abscisic acid will be transported from root to leaves through xylem. Besides in roots, the plant also synthesizes abscisic acid in the leaves in case there is an increasing abscisic acid level.
The plant growth and productivity are strongly influenced by natural condition in the form of various biotic and abiotic factors. The dryness is the most important abiotic factors in agriculture. Prolonged dryness stress can inhibit the growth of oil palm which affect the increasing of abscisic acid hormone. The increasing level of abscisic acid in the leaves will induce and regulate stomatal closure, whereas increased levels of abscisic acid in the roots increase hydraulic conductivity that increases water retrieval and transport. Dryness can be prevented by watering regularly. To determine the influence of watering the oil palm plant, then the comparison of abscisic acid in oil palm's seed which watered and not watered has been done [2].
This research was aimed to determine the effect of dryness against abscisic acid in oil palm seed by comparing the content of the sample with watered and with not watered. The result then processed statistically using minitab 17 software.

2. Material and methods
2.1 Plant Material
Seed of oil palms aged 10-11 months.

2.2 Sample Preparation
Oil palm leaves were put into plastic bag and stored temporarily in cool boxes and then cleaned of dirt and dust that was attached and then cut into small pieces and mashed with liquid nitrogen.

The Conditioning UPLC Tool
The mobile phase used were aquadest with pH 4, methanol, acetonitrile, and aquadest with flow rate 0.2 mL/ minute, 3 minutes retention time, 1 µL injection volume, and at 208 nm wavelength.

Qualitative Test
The sample was injected 1 µL into the mobile phase and will enter the column. And then

compound coming out of the column will be detected in the detector and then recorded in chromatogram form.

Determination of Abscisic Acid Level

The crushing of oil palm leaves weighed 5 g, put into erlenmeyer 100 mL then added 30 mL methanol 80%. The sample was shaked in an incubating shaker at 4°C for 24 hours at a speed of 80 rpm. The sample was filtered using whatman filter paper No.41. The filtrate was concentrated using a Rotary Evaporator at 50 ° C to about 10 mL. The filtrate was dissolved with 30 mL buffer phosphate pH 8.5. The solution was extracted using ethyl acetate (2x20 mL), the ethyl acetate phase was removed and the water phase adjusted at pH 2.5 using HCl 1 N. After pH 2.5 was reached, the solution was extracted with diethyl ether (2x20 mL), the water phase was removed and the diethyl ether phase was added CaCO3 to precipitate the remaining water. The diethyl ether phase was filtered and then evaporated with a rotary evaporator at 50^0C until dry, then dissolved in 2 mL of methanol (p). The solution was then filtered and inserted into the UPLC vial with a Syringe filter. The sample is injected into the UPLC [3].

As a standard, 100 ppm abscisic acid standard was dissolved with methanol (p) in 2 mL microtube. The standard was a mixture content of giberellic acid, indole asetic acid and abscisic acid. The abscisic acid level in the sample can be calculated using the following formula:

$$\text{Abscisic acid level } (\mu g/g) = \frac{(\text{LA sample}/\text{LA standard}) \times \text{C standard (ppm)} \times 0{,}002}{\text{Sample weight} \times 1000}$$

LA sample = Sample Area
LA standard = Standard Area
C standard = Standard Concentration (ppm)
0,002 = Volume of methanol in vial (mL)
1000 = Conversion Factor (µg /g)

T paired test [4]

Hypothesis:

H0 : $\mu_1 = \mu_2$

H1 : $\mu_1 \neq \mu_2$

Test Statistic

$$t_{hit} = \frac{\bar{x}_d}{s_d / \sqrt{n}}$$

$$t_{tabel}(\alpha, db = n-1)$$

$\bar{x}_d$ = average difference of level abscisic with watered and not watered (µg /g)

s_d = standard deviation of the difference of level abscisic with watered and not watered (µg / g)

N = the number of sample

Conclusion

If $|t_{hit}| \leq t_{tabel}$ then accept H0

If $|t_{hit}| > t_{tabel}$ then reject H0

The data was processing using minitab, conclusion was obtained by comparing P Value and α.

If $P_{value} < \alpha$ then reject H0 and if $P_{value} > \alpha$ then accept H0

3. Result and discussion

Qualitative Test

Chromatographic qualitative assays were performed to determine the presence of abscisic acid compound in standard and sample through chromatogram results [5]. The result of standard chromatogram can be seen in Figure 2.

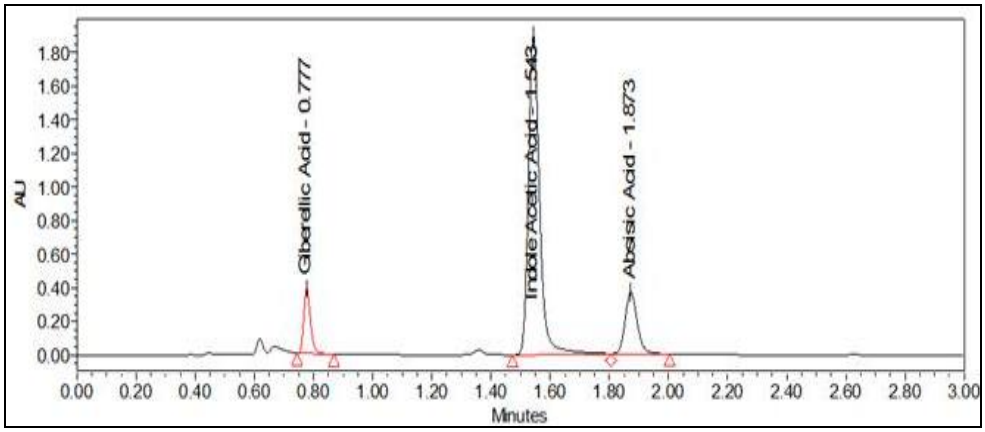

Fig 2: Standard Chromatogram

Based on the standard chromatogram (Figure 2), it was showed three types of hormones namely gibberelic acid, indole asitic acid and abscisic acid. Three hormones are the growth hormone in plants but have different function. Giberelic acid was a hormone that serves to encourage the process of cell lengthening, indole asetic acid was a hormone that works against the development and germination of embryo, and abscisic acid was a hormone that maintain the plant from dryness by dormancy (fallen leaves) [6].

In the standard chromatogram, it can be seen that peak of the giberellic acid hormone was at 0.777 minutes retention time, indole asetic acid peak was at 1.543 minutes retention time and abscisic acid hormone peak was at 1,873 minutes. The total area of the abscisic acid sample can be determined from the resulting sample chromatogram by adjusting the peak area of the sample which has same retention to the standard. Determination of abscisic acid content of sample was done by using UPLC tool [7]. The result obtained in the form of chromatogram can be seen in Figure 3.

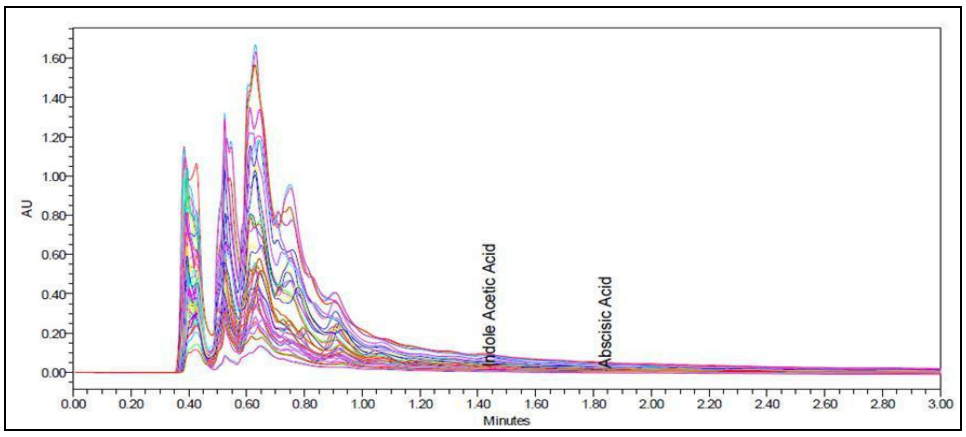

Fig 3: Sample Chromatogram

Figure 3 showed that 30 chromatograms of sample (15 samples were watered and 15 samples were not watered). The result showed that chromatogram have several peaks, according to the retention time of each hormone [8]. It showed that the sample contain the indole asetic acid and abscisic acid hormone. The abscisic acid hormone in the sample was read at the time of retention close to the retention time of standard abscisic acid, ie 1,873 minutes. The measurement of the retention time of abscisic acid hormone can be seen in Table 1.

Table 1: Sampling Retention Time (Minutes)

Sample	Retention Time (Minutes)	
(Seed the-)	Watered	Not watered
1	1,851	1,899
2	1,923	1,844
3	1,834	1,862
4	1,856	1,853
5	1,946	1,988
6	1,907	1,817
7	1,889	1,906
8	1,885	1,837
9	1,919	1,809
10	1,867	1,821
11	1,917	1,901
12	1,802	1,804
13	1,889	1,879
14	1,992	1,926
15	1,862	1,812

Based on Table 1, it can be seen from 15 samples of watered palm leaves seed had retention time in the range (1,802 - 1,992) minutes and not watered had retention time in the range (1,804 - 1,988) minutes.

Quantitative Test and Significant Different Test

The result of quantitative test of abscisic acid of oil palm seed leaves can be seen in Table 2.

Table 2: Level of the abscicid acid with watered and not watered.

Sample	Level of the abscicid acid (µg/g)		
	Watered		Not watered
1	2.2394		2.5901
2	2.2705		2.4137
3	2.3390		2.2258
4	2.2022		2.4300
5	2.3451		2.3367
6	2.2919		2.2724
7	2.2647		2.3569
8	2.3480		2.3125
9	2.0499		2.4835
10	2.1723		2.2563
11	2.1517		2.4305
12	2.2274		2.3890
13	2.2358		2.4767
14	2.2858		2.2442
15	2.1170		2.5291
P_{value}		0,005	
A		0,05	

Based on the results of the determination of abscisic acid level in Table 2 showed the sample with watered had abscisic acid level (2.0499 - 2.3480) µg / g, while the sample with no watered contained abscisic acid (2,2258 - 2,5901) µg / g. This result indicate that without watered can be increased the level of abscisic [9]. The dryness condition the abscisic acid can be increased immediately in a role of stimulating stomatal closure to maintain water balancing with aging or abort leaves [10].

From the t-paired test in Table 2, it showed that Pvalue 0,005 and α = 0,05. Pvalue was smaller than α value, so it can be concluded that the average of the measurement of abscisic acid level with watered is significantly different to the abscisic acid level without watered.

4. Conclusion

The average abscisic acid level was 2.2361 (µg/g) and no watered was 2.3832 (µg / g). Significant difference test at 95% confidence interval, of Pvalue equal to 0,005 and value α = 0,05. The result of data processing shows that the value of Pvalue was smaller than α, so it can be concluded that the level of abscisic acid on the oil palm leaves seed with watered was significantly different from the level of abscisic acid with no watered. The rate with watered was higher than the average of no watered. This means that the dryness had the effect to increase the level of abscisic acid in oil palm leaves.

5. Acknowledgement

The authors acknowledge the kindness and co-operation of the informants and local administrators in the study area, PT. Smart, Tbk Indonesia and the support of the Polytechnic AKA Bogor.

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