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Circadian rhythm: effect of sodium in tobacco plant circadian regulation

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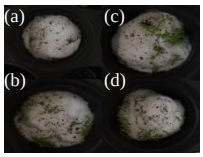
GRAPHICAL ABSTRACT



(a) Tobacco seed without application of bio-plastic fertilizer after 7 days and (b, c, d) Tobacco seed with application of bio-plastic fertilizer 10g, 30g and 50g of sodium respectively after 7 days.

ABSTRACT

The rising demand for agriculture production due to growing number of global population required advance technology in crop development thus genetically modified plants was introduced to increase the crops' productivity. However genetically modified product recently had arise a few concerns that may related to possible danger or risk in human body and environment. In this research, a new approach to increase crops' productivity was studied by modifying the plants circadian rhythm. Tobacco was chosen as plant sample since it's the first plant that was genetically modified and due to its distinctive qualities in physiology related to day length rhythm. A demonstration of the circadian clocks controlling the elongation of tobacco upon germination was done by using sodium as the controlled variable. The goal was to provide an overview how external cue may affect the biological clock of plants. Bio-plastic fertilizer was synthesized as sodium supplier and expected to release sodium in a slow rate manner. The rate of sodium release by bio-plastic fertilizer was evaluated by using flame photometer. The findings revealed that, bio-plastic fertilizer synthesize in this study was failed to act as a controlled-released fertilizer while sodium does contribute in modifying the tobacco circadian rhythm; however the different concentration of sodium applied to the tobacco seed does not gave significant changes in the germination rate. Additionally, this study produced significant and relevant information for future studies regarding plants' biological clock.

Keywords: circadian rhythm, biological clock, tobacco, controlled-released fertilizer.

1. INTRODUCTION

The global populace was increased from 7.2 billion in 2014 to 7.5 billion in March 2017; and predicted to continue to rise until 12.3 billion by 2100 thus increasing the demand on worldwide production of food [1]. It is estimated that extreme environmental changes such as drought and flood may cause the crop loss by 50% in 2050 [2].

Since the number of crops production does not concomitant with the populace, an urgent need for crops' development to improve the plants tolerance towards abiotic stress was introduced. Previously, genetically modified technique was introduced in order to boost the basic efficiency of plants thus increase plants productivity. In the field trials, tobacco was used as plant sample due to its properties which rich with flavoring and a perfect plant for nutrient and physiology studies. Professor Stephen Long, who led the work at the University of Illinois said that this modification are targeting a universal process that is the same in all crops, thus trial in tobacco plants might gave the same result to the other crops including plants that produce foods. However recent studies suggested that biological clock might contributes in maintaining plant fitness by enhancing the abiotic stress tolerance and might be much safer that GMOs. This suggestion was supported by a convincing evidence which plants whose its circadian clock are in rhythm to the environment rhythmic are performed best in competition experiments [3].

Basically, the biological clock work at the individual cell level which consist of other three basic components. These basic components are input pathway, central oscillator and output pathway [4]. Ability to modify plants circadian clock may result in a favorable position to the world agriculture sector since biological clock may contribute in the plant cold tolerance by modifying its cold-response pathway. Besides that, circadian rhythm can help increase the rate of plants growth and crops might be able to grow in season or places that are now currently not possible [5].

The main focus of this research is to study does sodium mineral as an external cue affect the tobacco plant circadian oscillation or biological rhythm through the observation of the cell elongation during the germination period. The synthesizing of bio-plastic fertilizer is included in this research as the sodium supplier. The rate of sodium intake by the tobacco seed sample is determined by using flame photometer.

2. EXPERIMENTAL

The experiment was divided into three main stages. The first stage was focused on the synthesized of controlled-released fertilizer which starch-based bio-plastic polymer as medium to deliver sodium to the tobacco seed sample was used since it is cheap, renewable and completely bio-degradable [7]. In addition, the second

stage was germination of tobacco seed with three different concentration of sodium was applied as controlled variable. Cotton ball was used to replace soil during the germination period to control the element uptake by the seed and temperature was depended by the room temperature. The cell elongation of the seed sample was observed. The last stage was the degradation determination of bio-plastic fertilizer using flame photometer. The concentration of sodium intake by the tobacco seed was determined. The flame photometer used in this study is from JENWAY, model PFP7, single channel emission flame photometers designed for determination of sodium (Na) and the limit of detection (LOD) for sodium is 0.20 ppm.

3. RESULTS AND DISCUSSION

3.1. Effect of Sodium to the Tobacco Seed Circadian Rhythm

In this study, four sample of bio-plastic fertilizer contained different concentration of sodium was applied to the seed in order to observe the cell elongation during its germination time. In normal condition, tobacco seed required about 10 days for it to start germinate. However, upon application of controlled-released fertilizer that supplied sodium nutrient, the seed was observed to start produced leaves as early as day five.

Figure 1 Tobacco seed 7 days after application of sample 1

Figure 2 Tobacco seed 7 days after application of sample 2

Figure 3 Tobacco seed 7 days after application of sample 3

Figure 4 Tobacco seed 7 days after application of sample 4

From the observation, it is shown that sodium does act as an external cue that affect the biological clock of the tobacco seed by accelerated the germination processes. However the concentration of sodium supplied by the bio-plastic fertilizer does not gave any significant difference towards the seed. Thus it is shown that different concentration hold by the fertilizer does not affect the tobacco germination rate.

3.2. Determination of Sodium Released by the Bio-Plastic Fertilizer

The controlled-released fertilizer was analyzed by using flame photometer to determine the final concentration of sodium. The solution of known concentration sodium standard solution and its intensities from flame photometer was used to make a calibration curve by which the sodium concentration in the bio-plastic fertilizer sample was determined.

Table 1 Intensity reading of standard calibration solution by flame photometer

ppm / Rep	2	4	6	8	10	12	14	16	18	20
1	0.03	0.07	0.09	0.14	0.16	0.21	0.23	0.28	0.32	0.37
2	0.03	0.08	0.11	0.14	0.18	0.22	0.24	0.29	0.34	0.38
3	0.03	0.06	0.10	0.14	0.17	0.20	0.24	0.29	0.33	0.37

The value of intensities was substituted in to the formula to calculate the concentration of sample. From the equation, the sodium concentration of sample 2, sample 3 and sample 4 was 9.06 *ppm*, 12.30 *ppm* and 15.55 *ppm* respectively.

Table 2 Average intensity of standard calibration solution

Intensity	Concentration, *ppm*
0	0
0.03	2
0.07	4
0.10	6
0.14	8
0.17	10
0.21	12
0.24	14
0.29	16
0.33	18
0.37	20

These results were used in plotting a calibration curve; intensity versus sodium concentration

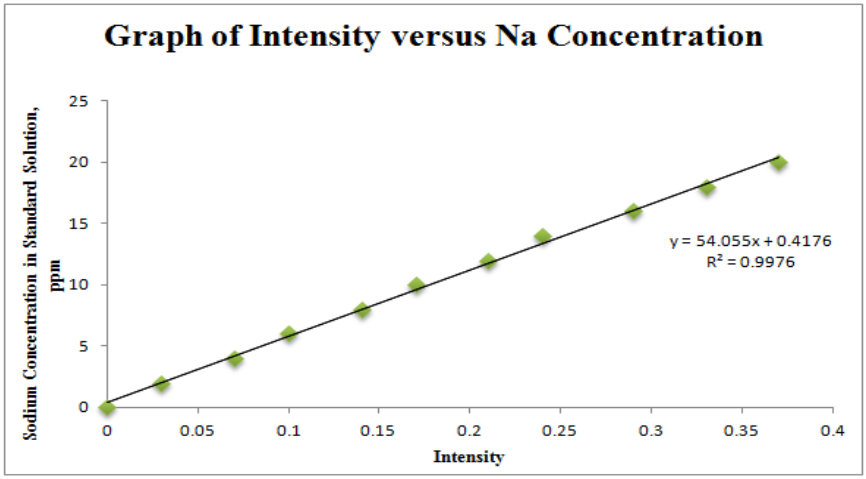


Figure 5 Graph of intensity versus sodium, na concentration

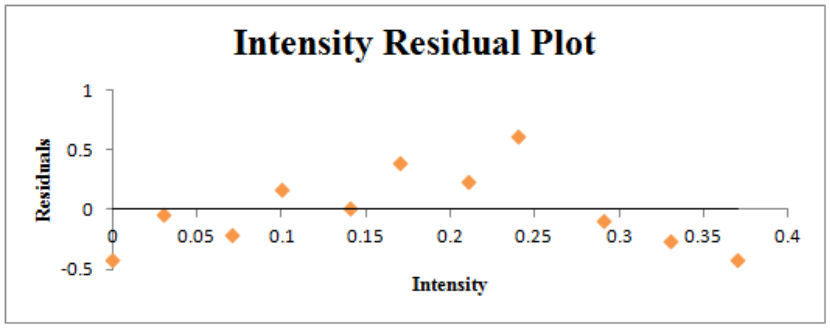


Figure 6 Residual plot of intensity

The residual plot in figure 4.5 shows the residuals are scattered randomly around zero and no trend in the spread of residuals with concentration.

3.3. Degradation of Sodium Concentration in Bio-plastic Fertilizer

Table 3 Concentration of Sodium, Na in bio-plastic fertilizer

Sample	Concentration of Sodium in Bio-plastic Fertilizer in (W/V) %		
	Initial	Final	Loss
1	16.670	0.009	16.660
2	50.000	0.012	49.990
3	83.330	0.016	83.310

Table 4.10 show the initial concentration, final concentration and concentration of sodium loss through the entire experiment in weight per volume. From the objective, it was expected that bio-plastic fertilizer synthesized in this study to acts as a controlled-released fertilizer by releasing sodium in a very slow rate manner. However, from the analysis, the results obtained showed that the bio-plastic fertilizer released the sodium in uncontrollable manner thus proved that synthesizing of controlled-release fertilizer were a failure.

4. CONCLUSION

Based on the current findings of the research, the following conclusions were proposed; (i) Bio-plastic fertilizer was prepared using starch soluble reagent; does not successful in releasing sodium in slow rate manner since most of the sodium was release 7 days after applied to the media. (ii) Sodium does acting as an external cue that affects the plants' circadian clock since it's accelerates the germination process (*i.e.* faster cell elongation observed) of the tobacco seed sample. (iii) The concentration of sodium content in the controlled-released fertilizer does not affect the tobacco seed germination rate.

There are several limitations that occur thus interrupting the process of study. Theoretical and conceptual problems occur since circadian clock is a study with limited information. A limitation in research strategy by limited information was obtained in order to modify the circadian rhythm of tobacco plants or any other plants. A longer period of time to observe the changes of cell elongation of tobacco are required in order to gain more information about the study

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