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Analysis of the reducing sugar and carbohydrates total of cassava (*Manihot esculenta* Crantz) resistant to drought stress

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Abstract

Cassava (*Manihot esculenta* Crantz) is the third most important crop in the world and a staple food source throughout the tropics. Cassava land centers in Indonesia are controlled by Lampung Province. This situation makes Lampung a supplier of one-third of the national cassava production of the national production. However, there are still many production constraints in cassava cultivation, including drought stress. Drought stress is one of the main factors causing cassava plant death. So far, there is no drought-resistant cassava cultivar. The use of cassava seeds which are resistant to drought stress with high yields is expected to be an important alternative for drought stress control by using *polyethylene glycol* 6000 (PEG 6000). The purpose of this study was to analyze the reducing sugar content and the total carbohydrate content of cassava plants which were effective and resistant to drought stress. This study used a completely randomized design with one factor, namely the concentration of PEG 6000 consisting of 5 levels 0%, 10%, 20%, 30% and 40%. The results showed that the higher the concentration of PEG 6000, the total dissolved carbohydrate content and reducing sugar content in cassava plants which were resistant to drought stress increased. The highest reducing sugar content and carbohydrate content were found in the treatment of 40% PEG 6000 concentration.

Keywords: Carbohydrates; Cassava; Drought Stress; PEG 6000; Reducing Sugar

1. Introduction

Cassava (*Manihot esculenta* Crantz) is the third most important crop in the world and a staple food and income source throughout the tropics. Cassava cultivation can be a livelihood for more than 500 million farmers [1,2]. Cassava is an important food commodity in Indonesia and in the future this commodity will play an increasingly strategic role in people's lives and the country's economy. Based on the harvest area of food commodities, cassava ranks third after rice and corn, all three of which are the main sources of carbohydrates for the community [3]. The nutritional content of cassava leaves is omega-3, protein and minerals which are no less than other green vegetables [4, 5]. Cassava leaves are consumed as vegetables in several Asian and African countries such as Indonesia, Malaysia, Nigeria and Tanzania [5]. Manihot belongs to the Euphorbiaceae family which consists of 300 genera and 8000 species [6].

Cassava has the potential as a source of food, feed, and industrial raw materials that are resistant to drought conditions. However, in conditions of water shortage, cassava productivity decreases and can result in plant death; therefore, cassava tolerance to drought needs to be improved in order to increase its productivity. Cassava breeding needs to be done to maintain its productivity, especially on dry land. The most effective breeding technique for cassava is to use molecular techniques and provide compounds that are tolerant to water shortages. The flowering time of cassava is quite long, around 8-10 months, which is influenced by genotype and environmental conditions [7]. The use of superior

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varieties that are resistant to drought stress is one alternative that can be used to overcome climate conditions that are always changing. One of the most popular approaches to inducing drought stress is to use high molecular weight osmotic substances such as Polyethylene glycol (PEG) [8].

Polyethylene glycol is the best material to control water potential and cannot be absorbed by plants [9]. PEG that is completely soluble in water has the ability to reduce water potential and is expected as a selective condition to determine the response of planted tissue to drought stress [10], as well as to isolate cells or variant tissues that have tolerance to stress so that they can be used to stimulate the magnitude of soil water potential [11]. Polyethylene glycol compounds are compounds that are able to reduce the osmotic potential of solutions through the activity of ethylene oxide sub-units which can bind water molecules with hydrogen bonds [12]. The addition of Polyethylene glycol solution to the planting medium is expected to stimulate drought stress conditions because of its nature which can absorb water so that the water supply for plants is reduced. Polyethylene glycol is a stable compound even in environmental conditions with high temperatures, acids, bases, and high oxidation. In addition, the advantages of using Polyethylene glycol are that it is non-halogenated, low toxicity and the costs required are small [13]. The occurrence of drought stress causes photosynthesis results to decrease. If the results of photosynthesis are no longer sufficient, then the breakdown of dissolved carbohydrate molecules is used to maintain the metabolic process. [14]. Plants experiencing drought stress conditions have increased total soluble carbohydrate content [15] and reducing sugar content. Plant breeding and productivity are greatly influenced by plant resistance to drought. Plants that are tolerant to drought stress can respond to drought through morphological, physiological and molecular responses.

The use of PEG 6000 in tolerant concentrations has never been reported with certainty and accuracy in the induction of cassava resistance to drought stress, therefore, this study is interesting to do. The purpose of this study was to determine the optimum reducing sugar content and total soluble carbohydrate content in cassava plants treated with PEG 6000.

2. Materials and Methods

The materials used in this study were one-month-old cassava (*Manihot esculenta* Crantz), glucose, polyethylene glycol 6000 (PEG 6000), distilled water, nelson reagent A & B, arsenomolybdate, 96% phenol, H_2SO_4, water bath, analytical balance, and spectrophotometer. The administration of PEG 6000 consisted of five concentration levels, namely 0%, 10%, 20%, 30%, and 40% with each treatment repeated five times.

2.1. Reducing Sugar Content

Materials for the analysis of reducing sugar content using cassava leaves that have been given PEG 6000 and without treatment (control). Analysis of reducing sugar content using the Somogyi-Nelson method with the following steps.

2.1.1. Making Reducing Sugar Calibration Curve

Preparation of standard glucose solution (10 mg glucose/100 mL), dilution of glucose solution with concentrations of 2, 4, 6, 8, and 10 mg/100 mL, each concentration was put into each test tube and 1 tube containing distilled water as a blank. Then 1 mL of Nelson Reagent (Nelson a 25 parts and Nelson b 1 part) was added to each tube. The solution that had been added Nelson was then heated in a boiling water bath for 20 minutes. The solution was then cooled in a beaker containing cold water until the tube temperature was 25 °C, then 1 mL of Arsenomolybdate Reagent was added, shaken until homogeneous. The solution was measured with a Vis spectrophotometer at a wavelength of 540 nm, then a calibration curve was made of the relationship between glucose concentration and absorbance.

2.1.2. Determination of Reducing Sugar Content

Fresh cassava leaf extract aged 40 days (the extract solution must be clear) each with a concentration of 1 mL was taken and put into each test tube and 1 ml of Nelson's Regensia was added to each tube. The solution that had been added with Nelson's Regensia was then heated in a boiling water bath for 20 minutes. The solution was then cooled in a beaker containing cold water until the tube temperature was 25 °C. After that, 1 mL of Arsenomolybdate Regensia was added to the solution and then shaken until all the precipitate was dissolved again. After the solution was mixed homogeneously, 7 mL of aquadest was added to the solution and shaken again until homogeneous. The solution was measured with a Vis spectrophotometer at a wavelength of 540 nm. The next step is to make a calibration curve of the relationship between glucose concentration and absorbance. The reducing sugar content is calculated using the following formula:

$$\text{Reducing Sugar Content (\%)} = \frac{X \text{ x } Df}{SW} \text{ x } 100\%$$

Description:
X : Sample x value
Df : Dilution factor
SW : Sample weight

2.2. Total Soluble Carbohydrate Content

Analysis of total dissolved carbohydrate content was carried out using the Phenol-Sulfur method. Cassava leaves were taken and weighed as much as 0.1 grams from each ~~plantlet~~ plant leaves. The leaves were pounded with a mortar and then given 10 ml of aquadest, then filtered with Whatman No. 1 filter paper, after which they were put into a test tube. Furthermore, 1 ml of filtrate was taken and 1 ml of H_2SO_4 was added and 2 ml of phenol was added. The total dissolved carbohydrate content was calculated by making a standard glucose solution consisting of several concentrations and then measured on a spectrophotometer with a wavelength of 490 nm. The results of the absorbance of the standard solution were made into a linear regression equation so that the equation was obtained:

$$y = ax + b$$

The absorbance result of the cassava sample is known as y and is substituted into the linear regression equation. So that the total dissolved carbohydrate content is obtained.

The data were analyzed using Analysis of Variance or Anova at a 5% significance level and further tested with the Tukey test at a 5% significance level.

3. Results and Discussion

3.1. Reducing Sugar Content

Measurement of reducing sugar content in cassava plants given PEG 6000 with various concentrations in soil media was carried out using the Somogyi-Nelson method. The results of determining the standard curve of reducing sugar are in and presented in Table 1.

Table 1 Comparison of Reducing Sugar Concentration and Absorbance

Reducing Sugar Concentration (ppm)	Absorbance
20	0,215
40	0,279
60	0,328
80	0,423
100	0,463

Based on the standard curve of reducing sugar in Table 1, it can be used to find the reducing sugar content of each treatment by substituting the absorbance value (y) of the cassava leaf extract solution sample at the maximum wavelength into the linear regression equation y = ax + b obtained from the reducing sugar calibration curve to obtain the concentration (x). The value of x is then substituted into the formula for calculating the reducing sugar content. The results of the characterization of the reducing sugar content of cassava plants in soil medium added with PEG 6000 with various concentrations obtained from the calculation results in and are presented in Table 2.

Based on Table 2, the results of the characterization of the reducing sugar content of cassava plants added with PEG 6000 at various concentrations showed that the increasing reducing sugar content was in line with the increasing concentration of PEG 6000 added to the soil medium. The increase in the reducing sugar content of cassava plants at PEG 6000 concentration was significantly different from the control treatment (0%), The addition of PEG 6000 concentration to the soil medium was linearly related to the reducing sugar content of cassava plants. Based on Table 2, regarding the graph of reducing sugar content in cassava plants, the results showed that the greater the concentration of PEG 6000 added to cassava plants, the higher the reducing sugar content of cassava plants. The highest increase in reducing sugar content was in the addition of PEG 6000 with a concentration of 40%. Based on Table 2, it shows that

the addition of PEG 6000 with increasing concentrations resulted in cassava plants experiencing an increase in reducing sugar.

Table 2 Average Reducing Sugar Content of Cassava at Several PEG 6000 Concentrations

PEG concentration (%)	Average Reducing Sugar Content (%) (Mean ± Stdev)
0	0,453 ± 0,331[a]
10	0,569 ± 0,111[ab]
20	0,8837 ± 0,0650[b]
30	1,1616 ± 0,0766[b]
40	2,292 ± 0,480[b]

Note: Numbers followed by the same letter indicate no significant difference between treatments at a 95% confidence level.

Plants increase the concentration of soluble sugars to maintain cell turgor under drought stress. This increases the ability of cells to retain and absorb water [16]. Soluble sugars play an important role in maintaining the osmotic balance of plants under drought stress [17]. Reducing sugars can be an indicator to identify water-stressed plants. Glucose and sucrose serve as substrates for osmolytes and cellular respiration in plants, helping to maintain cellular homeostasis [18].

3.2. Total Soluble Carbohydrate Content

Total soluble carbohydrate content is one of the parameters used to indicate cassava resistance to drought stress by administering PEG 6000. Total soluble carbohydrate content in cassava that has been treated with PEG 6000, with 5 concentration levels is presented in Table 3.

Table 3 Average Total Soluble Carbohydrate Content of Cassava

PEG concentration (%)	Average Total Soluble Carbohydrate Content (Mean ± Stdev)
0	0,175 ± 0,036[c]
10	0,465 ± 0,096[c]
20	0,703 ± 0,055[c]
30	1,471 ± 0,332[b]
40	2,189 ± 0,074[a]

Note: Numbers followed by the same letter indicate no significant difference between treatments at a 95% confidence level.

The results of the cassava total soluble carbohydrate content test on drought stress with PEG 6000 administration are presented in Table 2, showing an increase in total soluble carbohydrate content along with the increase in the concentration of PEG 6000 given. The total soluble carbohydrate content increased in cassava given PEG 6000 treatment compared to no treatment (0%). The highest carbohydrate content occurred in cassava given PEG 6000 treatment with a concentration of 40%, this shows that the higher the concentration of PEG 6000, the higher the total soluble carbohydrate content obtained. This is due to the influence of PEG 6000 which is applied directly to the planting medium so that it is absorbed by cassava resulting in an increase in carbohydrate content in plants.

The results of this study are in line with previous studies that in drought stress conditions plants are able to maximize the increase in carbohydrates so that they can withstand decreased osmotic potential [19]. The results of high total soluble carbohydrates are in line with the increasing accumulation of proline in a plant. According to ~~Murningsih et al.~~ [20] the increasing drought stress causes the accumulation of proline and total sugar content in corn to increase. The results of research on bean plantlets (*Phaseolus vulgaris* L.) inoculated with Rhizoctonia solanii mycorrhiza did not affect the total soluble carbohydrate content under drought stress conditions [21]. In addition, research on orchid plantlets [*Phalaenopsis amabilis* (L.) BI.] stated that the addition of fusaric acid to the Vacin and Went (VW) medium increased the total soluble carbohydrate content compared to the control [22].

Cavendish banana plantlets have an effect on increasing carbohydrate content under salinity stress conditions in vitro, as seen from the higher NaCl concentration, the higher the carbohydrate content produced [23]. The plant's efforts to increase carbohydrate content are a plant defense response in responding to extreme environmental conditions [21].

4. Conclusion

The optimum reducing sugar content and total soluble carbohydrate content were produced in PEG 6000 treatment with a concentration of 40%. The higher the concentration of PEG 6000, the higher the reducing sugar and total soluble carbohydrate content.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest

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