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World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

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Analysis of reducing sugar levels of *Cattleya* sp. orchid plantlet after induction fusaric acid *in vitro*

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World Journal of Advanced Research and Reviews, 2022, 14(02), 095–099

Publication history: Received on 22 March 2022; revised on 02 May 2022; accepted on 04 May 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.14.2.0369

Abstract

Cattleya sp. orchid is one type of orchid plant that is widely known in Indonesia. The queen of orchids is the nickname given to the *Cattleya* sp. orchid because of the elegant shape and color of the flowers of the *Cattelya* sp. orchid. This research was conducted to determine the level of reducing sugar produced by plantlets of *Cattleya* sp. after the addition of fusaric acid in *in vitro* media. Determination of reducing sugar content was carried out using the Nelson – Somogyi method. This study used a completely randomized design (CRD), with five levels of treatment and five replications for each level. The treatment consisted of adding fusaric acid with concentrations of fusaric acid in the medium: 0 ppm, 10 ppm, 20 ppm, 30 ppm and 40 ppm. Data analysis used Anova analysis, and continued with Tukey's test at 5% significance level. Based on the analysis, there was an increase in reducing sugar levels in the plantlets of *Cattleya* sp. orchids after adding fusaric acid to the growing media.

Keywords: Orchid; Fusaric Acid; *Cattleya* sp.; Sugar Reduction; *in vitro*

1. Introduction

Orchid plants are included in the Orchidaceae family, are one of the most popular commercial ornamental plants in Indonesia and the world, because they have very beautiful and unique types, variations in shape, color, and flower characters, and have high economic and aesthetic value. *Cattleya, Vanda, Phalaenopsis, Dendrobium, Oncidium,* and *Aranthera* are some of the orchid species that are in great demand [1]. The *Cattleya* orchid itself is part of the Kingdom Plantae, Spermatophyta Division, Class Monocotyledoneae, Order Asparagales, Family Orchidaceae, and Genus *Cattleya* [2]. The beauty and beauty of the flowers make this plant called the queen of flowers. In Indonesia, *Cattleya* sp orchid is a plant that has high economic value. Conventional propagation of orchids takes a long time and gets a limited number of seeds, because it is done by separating the tillers. By means of *in vitro* seeds will be obtained in large quantities in a relatively short time and uniform [3].

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Reducing sugar is the ability of sugar to reduce, which is caused by the presence of free aldehyde or ketone groups [4]. Compounds that oxidize or are reducing agents are oxidizing metals such as Cu (II). Examples of sugars including reducing sugars are glucose, fructose, lactose, maltose, and others. Monosaccharides have the ability to reduce a compound. The reducing property of a sugar is determined by the presence or absence of a reactive free hydroxyl group. The principle of analysis is based on monosaccharides which have the ability to reduce a compound, the presence of monosaccharide polymerization affects its reducing properties [5].

The analysis that can be used to determine the reducing sugar group is the Nelson-Somogyi method [6]. In this analysis, the sample will be reacted with arsenomolybdate which will produce blue molybdin, this blue material will then be measured its absorbance value. The intensity of the blue color formed is equivalent to the amount of reducing sugar in the sample. The higher the absorbance value, the more reducing sugar content in the sample. This study aims to determine the level of reducing sugar produced after the addition of fusaric acid in plantlet media of *Cattleya* sp. orchids *in vitro*

2. Material and methods

The research was conducted from October 2021 to January 2022 in the *in vitro* culture room of the Botany and tissue culture laboratory, Department of Biology, FMIPA, and University of Lampung. This study used a completely randomized design with 5 treatments of fusaric acid administration on Vacint & Went medium as much as 0 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm with each treatment 5 repetitions.

2.1. Planting *Cattleya* sp plantlets in Vacin and Went medium that has been given Fusaric Acid

Vacint & Went medium in sterilized culture bottles was added with fusaric acid with concentrations of 0 ppm (control), 10 ppm, 20 ppm, 30 ppm, and 40 ppm. Prior to use, fusaric acid was dissolved with distilled water until the specified concentration was obtained, then filtered. Filtering using a 0.45 m diameter filter twice and with a 0.22 m diameter filter once. Filtration is carried out in a sterile room in Laminar Air Flow in sterilized culture bottles. Prior to use, the medium was incubated for 7 days at room temperature of 25 ^{0}C to ensure that the fusaric acid was well filtered. If within 7 days there is no contamination in the medium, then the medium can be used.

Plantlets of *Cattleya* sp. were carried out in a sterile room in Laminar Air Flow (LAF) Cabinet plantlets from culture bottles were removed with a sterile scalpel and one by one placed on a 10 cm diameter petri dish, then the plantlets were sorted one by one, after that they were planted in each Each culture bottle containing 3 plantlets of medium. Plantlet selection was carried out for 30 days. On day 30, an evaluation was carried out to determine the tolerant concentration of AF for the selection of *Cattleya* sp plantlets *in vitro.*

2.2. Analysis of Reducing Sugar Levels

2.2.1. Preparation of Standard Sugar Solution

Analysis of reducing sugar was carried out by making a standard glucose solution [7] (10 mg glucose/100mL), carried out 5 dilutions of glucose solution with a concentration of 2ml, 4ml, 6ml, 8ml, and 10 mg/100 ml, each of which was added to each concentration. Each test tube and 1 tube containing distilled water as a blank. Add 1 mL of Nelson's reagent (Nelson A 25 parts Nelson B 1 part) into each tube. The solution to which Nelson was added was then heated for 20 minutes. The solution was cooled in a beaker until the tube temperature was 25 ^{0}C, then 1 ml of arsenomolybdate reagent was added, shaken until all the precipitate dissolved again. After homogenizing again, 7 ml of distilled water was added and shaken until homogeneous. The solution was measured with a Vis spectrophotometer at a wavelength of 695 nm, then a calibration curve was made for the relationship between glucose concentration and absorbance.

2.2.2. Determination of Reducing Sugar Content

Determination of reducing sugar content using the Nelson – Somogyi method [8]. Fresh *Cattleya* sp. orchid leaf extract (extract solution must be clear), each concentration of 1 ml is taken. put into each test tube and add 1 ml of Nelson's reagent into each tube, the solution to which Nelson has been added is then heated for 20 minutes. The solution was cooled in a beaker until the tube temperature was 25 ^{0}C, then 1 ml of arsenomolybdate reagent was added, shaken until all the precipitate dissolved again. After homogenizing again, 7 ml of distilled water was added and shaken until homogeneous. The solution was measured with a Vis spectrophotometer at a wavelength of 695 nm, then a calibration curve was made for the relationship between glucose concentration and absorbance.

The reducing sugar content is calculated by the formula 1:

$$\text{Reducing Sugar Content (\%)} = (X.FP)/BS \times 100\% \quad (1)$$

Information:
X= Concentration (ppm)
FP= Dilution factor of sample solution
BS = Sample weight (g)

3. Results and discussion

Measurement of reducing sugar content on the standard curve of reducing sugar. The standard curve is obtained from the ratio of the concentration of reducing sugar to the absorbance value. The results of determining the standard curve of reducing sugar can be seen in Table 1.

Table 1 Comparison of Reducing Sugar Concentration and Absorbance

Reducing Sugar Concentration (ppm)	Absorbance
20	0.318
40	0.347
60	0.367
80	0.387
100	0.405
120	0.432

Based on the data in Table 1, the standard curve of reducing sugar is obtained. So that the linear equation y = 0.0011x + 0.2996 is obtained and has a correlation value of R2 = 0.9946 which shows the diversity between the concentration of reducing sugars and the absorbance value. The standard curve of reducing sugar and absorbance is show in Figure 1

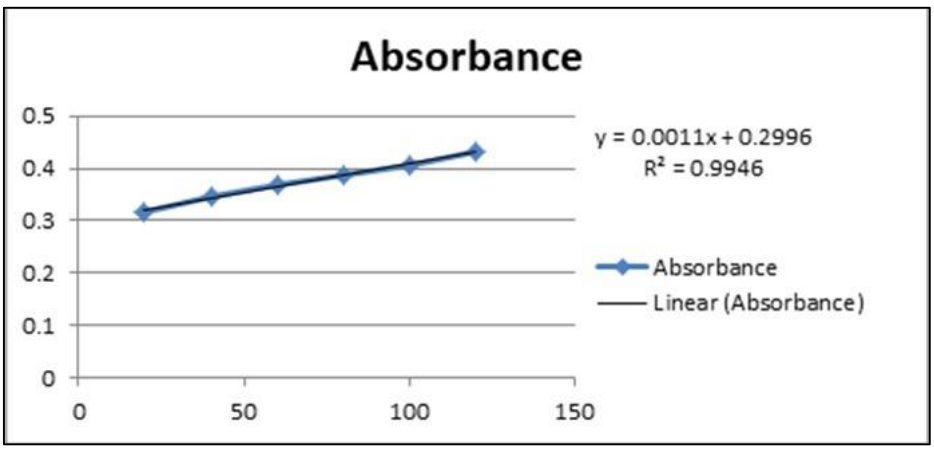

Figure 1 Reducing Sugar Standard Curve

Based on Figure 1, it can be calculated the reducing sugar content of each treatment by substituting the absorbance value from the spectrophotometer results of the plantlet leaf extract of *Cattleya* sp. The absorbance value is entered into the linear equation y= ax – b from the standard curve where y is the absorbance value. The calculation results obtained the value of x as concentration, then substituted in the reducing sugar calculation formula. The results of the calculation of reducing sugars of *Cattleya* sp. orchid plantlets with the addition of fusaric acid at several concentrations are presented in Table 2.

The results of observations based on Table 2 increased reducing sugar occurred from the control 23.476 to 23.506 at a concentration of 10 ppm fusaric acid. Then at a concentration of 20 ppm it became 27.402 followed by 28.405 at a concentration of 30 ppm and 30.142 at a concentration of 40 ppm. These results indicate that there is an effect on the administration of fusaric acid, where the higher the concentration of fusaric acid given, the higher the content of reducing sugar contained in the plantlets of *Cattleya* sp.

The increased reducing sugar came from starch and sucrose as a result of carbohydrate conversion. Some carbohydrates are also used as respiration materials and form other compounds, this causes reducing sugar levels to increase but carbohydrates to decrease [9].

Table 2 Reducing Sugar Levels of *Cattleya* sp. Orchid Plantlets Induced by Fusaric Acid

Treatment/ 30 Days	Orchid *Cattleya* sp reducing sugar levels (%)
0 ppm (Kontrol)	23. 476 ± 1.069
10 ppm	23. 506 ± 2.712
20 ppm	27. 402 ± 2.408
30 ppm	28. 405 ± 2.023
40 ppm	30. 142 ± 2.629

Changes in reducing sugar content are influenced by several factors, one of which is the heating process. Heating during the test for reducing sugar content causes the chemical structure to change. As a result of heating the glycosidic bond breaks causing non-reducing sugars (sucrose) to break down into reducing sugars such as glucose and fructose [5]. In addition, the content of reducing sugar is also influenced by the administration of fusaric acid. The high content of fusaric acid can reduce reducing sugar levels, as in previous studies conducted on cassava [10].

4. Conclusion

The results showed an increase in reducing sugar levels in plantlets of *Cattleya* sp. This shows that there is an effect on the administration of fusaric acid, where the higher the concentration of fusaric acid given, the higher the reducing sugar content contained in the plantlets of *Cattleya* sp.

Compliance with ethical standards

Acknowledgments

The researcher would like to thank the Botanical Laboratory, especially the In Vitro Culture Room, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Lampung, Indonesia, which has provided a place to conduct and process research data.

Disclosure of conflict of interest

All authors have no conflicts of interest

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