

Efforts to Propagate Sorghum In Vitro to Support Local Food

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Abstract: Indonesia is a country known for its abundant biodiversity. However, the majority of its people still make rice as a daily staple food, making the price of rice continue to increase until it is difficult to reach by low-income people. Therefore, it is necessary to develop alternative local food to replace rice to avoid a food crisis. One of the alternative plants is sorghum. Because sorghum has many benefits besides as food as well as bioenergy. That way it is necessary to make efforts to develop sorghum, one of which is by means of embryogenic callus tissue culture techniques by adding a concentration of 2,4-D. This research was conducted in the Laboratory of Nutraceutical and Pharmaceutical Division of Center for Development of Advanced Science and Technologies (CDAST) UPA Waste Management and Integrated Laboratory, University of Muhammadiyah Jember by using Completely Randomized Design (CRD) with a single factor namely A0 (control), A1 1 ppm, A2 2ppm, A3 3ppm, A4 4 ppm which was repeated 3 times. The variables under observation are the callus's color, texture, percentage, and time of formation. According to the study's findings, the application of 4 ppm 2,4-D produced the best results when compared to other 2,4-D treatments. These treatments were able to produce callus at a rate of 4.11 his, 100% of the total number of callus, crumbly texture, and pale yellowish color.

Keywords: Sorghum, Callus Induction, 2,4-D

INTRODUCTION

Indonesia is a country rich in biodiversity. One of them is food crops in the form of cereal crops. Although Indonesia is famous for its abundant biodiversity, the majority of Indonesian people still focus on rice as their daily staple food. With this condition, the price of rice continues to increase until it is difficult to reach by some low-income people. [1] To anticipate a food crisis due to the price and quantity of rice, it is necessary to develop local food as an effort to improve food security. One of the alternative local food crops as a substitute for rice is sorghum [2]

Sorghum (*Sorghum bicolor* L.) is one of the cereal plants that has a variety of benefits that can be taken from parts of the plant such as seeds, leaves and stems which can be used as industrial raw materials, fuel and animal feed [3] With the various benefits it has, sorghum has a high economic value that has the potential to be developed for food diversification in Indonesia. Besides being prospective in various economic and food sectors, sorghum can function as a bioenergy material because the carbohydrates contained in sorghum seeds and sap stems can be converted into bioethanol [4]

Various sorghum development techniques have been carried out to support its productivity and quality. One technique that can support this development is the tissue culture technique. Tissue culture technique is one of the modern plant development techniques to produce large numbers of virus-free plants in a relatively faster time than conventional techniques [5]. Tissue culture techniques are performed by planting explants on

a growing medium. The media used depends on the type of plant and explant used [6]. Tissue culture propagation through embryogenic callus is one of the efforts to propagate plants to produce bipolar plants, resembling zygotic embryos and somatic embryos. This callus culture can genetically engineer plants and can also reproduce plants by regenerating callus into plantlets. In this tissue culture method, growth regulators are needed to stimulate explant growth. The growth regulator given to induce callus is using auxin in the form of 2,4-D. according to [7] Among the auxins used for callus induction 2,4-D is most efficient for inducing somatic embryogenesis of sorghum.

METHOD

This research was conducted at the Laboratory of Nutraceutical and Pharmaceutical Division of Center for Development of Advanced Science and Technologies (CDAST) UPA Waste Management and Integrated Laboratory, University of Jember, Jember, East Java. The study used a complete randomized design (CRD) consisting of 1 factor, namely 2,4-D with treatment combinations A0 (control), A1 (1 ppm), A2 (2 ppm), A3 (3 ppm), A4 (4 ppm). The implementation of this research consists of several stages, including;

Preparation of planting media consisting of Murashige & Skoog (MS) 4.43 gr/L, sucrose 30 gr/L, proline 1 gr/L, agar 8 gr/L, distilled water, hormone 2,4-D 10,000 ppm. Preparation for planting, local sorghum seeds were sterilized with soap until clean then soaked with alcohol for 10 minutes then rinse sterile distilled water three times then soak clorox 2.5% for 15 minutes then rinse then soak again with 70% alcohol for 2 minutes then rinse three times using sterile distilled water then plant. After that, it is incubated in a dark room with a room temperature of 23-25 C. Observations made include; the time of callus appearance (hsi) percentage of callus appearance, color and texture of callus.

RESULTS AND DISCUSSION

In sorghum seeds planted in 2,4-D media, callus began to form in the first week to the second week after planting. The speed of callus formation depends on the ability of the seeds to absorb nutrients in the planting medium.

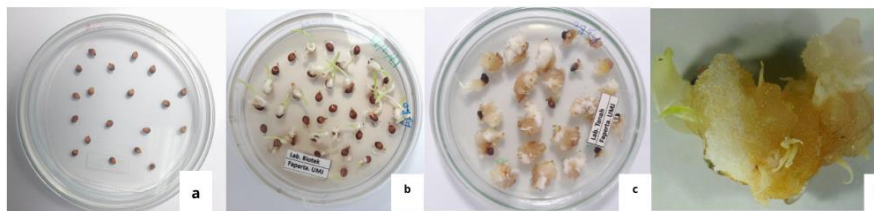


Figure1. Seed germination (a), callus development in 2,4-D medium after 7 days (b), sorghum callus 14 hsi (c), embryogenic callus

Based figure 1 The process of callus formation starts from seeds that experience germination and then accompanied by the appearance of callus characterized by swelling of the plant. After the second week of cutting the shoots, this is done to stop the growth of the shoots so that the growth focuses on the callus. When callus is induced, two types of callus will develop: non-embryogenic callus and embryogenic callus. A callus with the capacity to grow back into a perfect plant is known as an embryogenic callus [8]. Embryogenic callus is characterized by crumbly textured callus and has a yellowish white color and is not slimy. While non-embryogenic callus has a compact texture with a blackish brown color and slimy. Dark callus has a relatively slow growth and less optimal results [9].

Table 1. Average callus formation time and percentage of callus formation to the addition of 2,4-D with various concentrations.

treatment	When callus formation (hsi)	Percentage of callus formation (%)
A0 (Kontrol)	0 c	0 b
A1 (2,4-D 1 ppm)	4,89 a	88,89 a
A2 (2,4-D 2 ppm)	6,56 a	55,56 a
A3 (2,4-D 3 ppm)	5,00 a	77,78 a
A4 (2,4-D 4 ppm)	4,11 ab	100 a

Note: numbers accompanied by the same letter indicate that they are not significantly different in the Duncan test at the 5% level.

Based on table 1. The average callus emergence based on 5% Duncan's further test shows that treatment A4 or 2,4-D concentration of 4 ppm gives the fastest callus formation results at 4.11 hsi. While the slowest callus formation is in the A2 treatment which is 6.56 hsi. In line with research [10] which states that the provision of 2,4-D with a concentration of 2 ppm callus formed at 7.6 hst or about 1 week. The difference in callus growth time is not only influenced by an increase in the speed of cell division due to the effect of 2,4-D administration but also influenced by the conditions of genesis. In the percentage of callus formation, the A1, A2, A3, and A4 treatments are significantly different from the A0 treatment, this is because the provision of 2,4-D in the planting media can trigger the formation of callus while in the A1 (control) media or without 2,4-D there is no visible callus formation only produces buds. This is reinforced by the statement [11] which states that the use of auxin in the form of 2,4-d is able to encourage callus formation because this ZPT has the ability to increase osmotic pressure, cell wall pressure, and protein synthesis.

Table 2. average value of sorghum callus color

treatment	textures	Measurement Results				
		L*	a*	b*	C*	H*
A0	0	0	0	0	0	0
A1	Kompak	41,71 ± 2,82	± 8,04 ± 0,96	22,54 ± 2,18	± 23,95 ± 2,00	± 70,24 ± 3,19
A2	Remah	40,13 ± 3,49	± 10,25 ± 3,04	± 23,63 ± 2,99	± 25,79 ± 3,89	± 66,87 ± 3,81
A3	Kompak	36,92 ± 4,13	± 8,06 ± 1,19	19,65 ± 5,34	± 21,27 ± 5,28	± 67,09 ± 4,22
A4	remah	47,20 ± 4,13	± 6,73 ± 1,71	18,27 ± 2,86	± 19,52 ± 2,80	± 69,65 ± 5,22

Notes: L*= lightness, a*= red/green, b*= blue/yellow, C*= saturation, h0 = angle

Based on table 2. The average value of callus color is done using a colorimeter tool. the results of observations on the L* value show that the treatment has the highest level of brightness, namely in the A4 treatment with an L* value of 47.20, almost the same as in the A1 and A2 treatments which have an average value of 41.71 and 40.13 while in A3 has a low L* value of 36.92. the L* value of color using a colorimeter shows the level of brightness, the higher the L* value, the brightness level will increase and vice versa if the L* value is low, the brightness level will be lower [12].

The a* value indicates the degree of redness. If the a* value is (+) then the color tends to be reddish while if the a* value is (-) then it will tend to be greenish. In various treatments, the value of A1 which is 8.04 is almost the same as the A3 treatment which is 8.06. The a* value in the A2 treatment is 10.25 and in A4 is 6.73. The results of the a* value as a whole are positive, indicating that the color of the treatment tends to be reddish.

The b* value indicates the yellowish level, if the b* value is (+) then the color will tend to be yellowish while if the b* value is (-) then the color will tend to be bluish. The a* value in A1 has a value of 22.54 and in

A2 has a value of 23.63 while in A3 has a value of 19.65 and A4 has a value of 18.27. The overall b* value shows a positive value so that it can be mentioned that the color of the callus tends to be yellowish.

Other components in colorimeter color measurement are c-value and hue angle. The c value (chroma) indicates the saturation of a color. If the c value is low, the saturation is dull and not bright. Among several treatments, the lowest c* value on A4 is 19.52. While h* (hue) or hue angle shows the perceived color of the stimulus angle in the color spectrum. At angles 00 and 1800 refer to red and green colors, while at 900 and 2700 refer to yellow and blue colors.

In the treatment of A2 2,4-D concentration of 2 ppm and A4 2,4-D concentration of 4 ppm produces a crumbly texture characterized by nodular callus, has a small cell size, and easily brittle. While in the treatment of A1 2,4-D concentration of 1 ppm and A3 2,4-D concentration of 3 ppm has a compact texture. The characteristics of compact callus texture is the arrangement of cells tight and dense and watery which indicates that the callus is difficult or even unable to experience further development [13].

The color and texture of callus is one indicator of callus quality. Callus with high quality is characterized by a crumbly texture and yellowish white indicates that the callus cells are mature to go to the active division phase. the color formed in the callus produces different colors this is influenced by the composition of the media used. According to a change in the color of the callus is also influenced by the declining nutrient content of the hormones given in the media can also be caused by the age of the callus that is getting older[6].

CONCLUSION

The addition of 4 ppm 2,4-D affects the time of callus formation of sorghum plants faster, namely 4.11 hsi compared to other 2,4-D concentration treatments. In the treatment of 4 ppm 2,4-D, the percentage of the best callus is 100%, and the callus has a crumbly texture with a light yellowish color.

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