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Meristem Culture of Strawberry Plants (*Fragaria x Ananassa* Duch.) Sachinoka Variety on Various Types of Basic Media In Vitro

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Abstract: The large number of in vitro media circulating in the market is a challenge to find the most effective media for sachinoka strawberry meristem culture, because the need for in vitro media depends on genetic intelligence. The purpose of this study was to find the best media from the various tested media to reproduce the Sachinoka variety strawberry seedlings in a relatively short time using the meristem culture method. This study used a completely randomized design (CRD) with 6 media treatments and repeated 7 times, namely: M1 (WPM without BA), M2 (WPM + 4 ppm BA), M3 (NP without BA), M3 (NP + 4 ppm BA), M5 (BMM without BA), M6 (BMM + 4 ppm BA). The variables observed were the time of emergence of shoots, the number of shoots and the number of leaves. The results of this study indicate that there is no significant difference for the emergence of shoots variable. Treatment M1 produced the highest number of shoots (12.29) but not significantly different from M5 (10.14), both were significantly different from both treatments. The highest number of leaves is in M1 (27.14). It can be concluded that the best medium for the growth of sachinoka strawberry meristem culture is WPM media with the addition of 4 ppm BA, while the second-best medium is BMM media with the addition of 4 ppm BA.

Keywords: Meristem culture, basic in vitro media, sachinoka, virus.

1. Introduction

Strawberry plants are sub-tropical fruit plants that have great potential to be developed in Indonesia, especially in highland areas. Strawberries (*Fragaria x ananassa* Duch.) Sachinoka variety is one of the varieties preferred by producers (farmers) and also consumers in the strawberry production center in Bali (Pancasari). This variety is a strawberry variety originating from Japan, which has the advantage of being sweet and aromatic due to its high volatile compound content.

Conventional strawberry breeding causes many disease infections and is not enough to meet the needs of commercial seeds (Mozafari & Gerdakaneh, 2012). The problem with strawberries in Indonesia is the lack of availability of quality and disease-free seeds. The use of ruuner seeds, namely strawberry saplings taken from the field, reduces strawberry yields due to virus attacks. It is possible that the runner seeds taken from the field were contaminated with viruses from the parent plant.

One solution to the problem regarding the availability of quality and virus-free seeds is to try to improve the

seeds. So far, strawberry farmers have been buying seeds from outside, the quality is decreasing, with the fruit becoming rarer and smaller. Therefore, tissue culture methods are needed for strawberry cultivation (Shohael, 2008).

Tissue culture is a method for isolating parts of plants such as protoplasm, cells, tissues, organs and growing them under aseptic conditions so that these parts can reproduce and regenerate into whole plants again (Gunawan, 1995). Plant cells, tissues and organs are grown in a controlled environment and in aseptic conditions or free of microorganisms (Santoso and Nursandi, 2003).

Tissue culture will have a greater percentage of success if it uses meristem tissue. Meristem tissue is young tissue, namely tissue consisting of cells that are always dividing, the walls are thin and do not have thickening from pectin, the plasma is full and the vacuoles are small (Hendaryono and Wijayanti, 1994). One part of the meristem tissue in plants is found in the shoots. Explants in the form of shoot shoots are the explants with the highest percentage of producing plantlets, especially if grown on media without auxin (Irawati, 2000).

One of the determining factors for the success of carrying out tissue culture work is providing nutrients in the correct quantities and ratios in the culture medium. There are various media used for in vitro plant culture. The choice of medium depends on the type of plant used, the desires, goals and calculations of each researcher (Sherrington, 1984 in Indrianto, 2002). Media is the main factor in propagation by in vitro culture and has a huge influence on the growth and development of explants and the seeds they produce (Tuhuteru *et al*., 2012).

The exact media to be used in tissue culture cannot be ascertained because there are still influencing factors, such as the type of plant being cultured, the age of the parent plant, the age of the explant, the type of explant used, the need for growth regulators, and the process carried out in tissue culture. (Wetherell, 1982).

The addition of growth regulators (ZPT) is also a determinant of the success of meristem culture besides the media. Because ZPT affects the growth and morphogenesis of cell, organ and tissue cultures. If the concentration of auxin is greater than cytokinin, callus will grow, and if the concentration of cytokinin is greater than auxin, shoots will grow (Gunawan, 1987 cit. Sudarmadji, 2003).

This research aims to find the right media and growth regulators for the meristem culture of sachinoka variety strawberry plants which will be cultivated by strawberry farmers in Indonesia and especially by farmers in Bali (Pancasari). Apart from that, to meet the need for strawberry seeds that are free from viruses in large quantities.

2. Methodology

This research was conducted at the Tissue Culture Laboratory, Experimental Garden, Faculty of Agriculture, Jalan Pulau Moyo No. 16 Pegok South Denpasar Bali. The research starts from May 2022 to September 2022. The research uses a complete experimental design (RAL) with 6 treatments. The treatments are:

M1 = WPM (Woody Plant Medium);
M2 = WPM + 4 ppm BA + 0.5 ppm NAA;
M3 = BMM (Banana Multiplication Medium);
M4 = BMM + 4 ppm BA + 0.5 ppm NAA;
M5 = NP (New Phalaenopsis Medium); and
M6 = NP + 4 ppm BA + 0.5 ppm NAA.

Each treatment was repeated 7 times. Observed variables: when shoots appear, number of shoots, number of leaves, number of roots. Observations were made a week after culture. The data obtained was then subjected to a BNT test at 5% level.

3. Results

3.1 Time of emergence of shoots, number of shoots and number of leaves on different media

The results of analysis of variance showed that three types of media treatment with the addition of 4 ppm BA had no significant effect on the time of shoot emergence. The results of the three media treatments with the addition of BA were not significantly different from the media treatment without the addition of BA on the time of shoot emergence as shown in Table 1.

Table 1. Time of emergence of shoots, number of shoots and number of leaves on different media

Treatment	Variable		
	Time of shoots	Number of shoots	Number of leave
M1	10,43 a	1,00 b	3,86 d
M2	12,29 a	12,29 a	27,14 a
M3	9,00 a	0,71 b	2,86 d
M4	10,64 a	4,43 b	10,57 c
M5	10,83 a	2,54 b	5,40 c
M6	12,50 a	10,14 a	19,57 b
BNT 5%	ns	3,81	6,46

Note: Numbers followed by the same letter indicate an insignificant difference based on the BNT test at the 5% level

3.2 Development of Shoots of Sachinoka Strawberry Variety

The development of sachinoka variety strawberry shoots on various types of basic in vitro culture media can be seen in Figure 1.

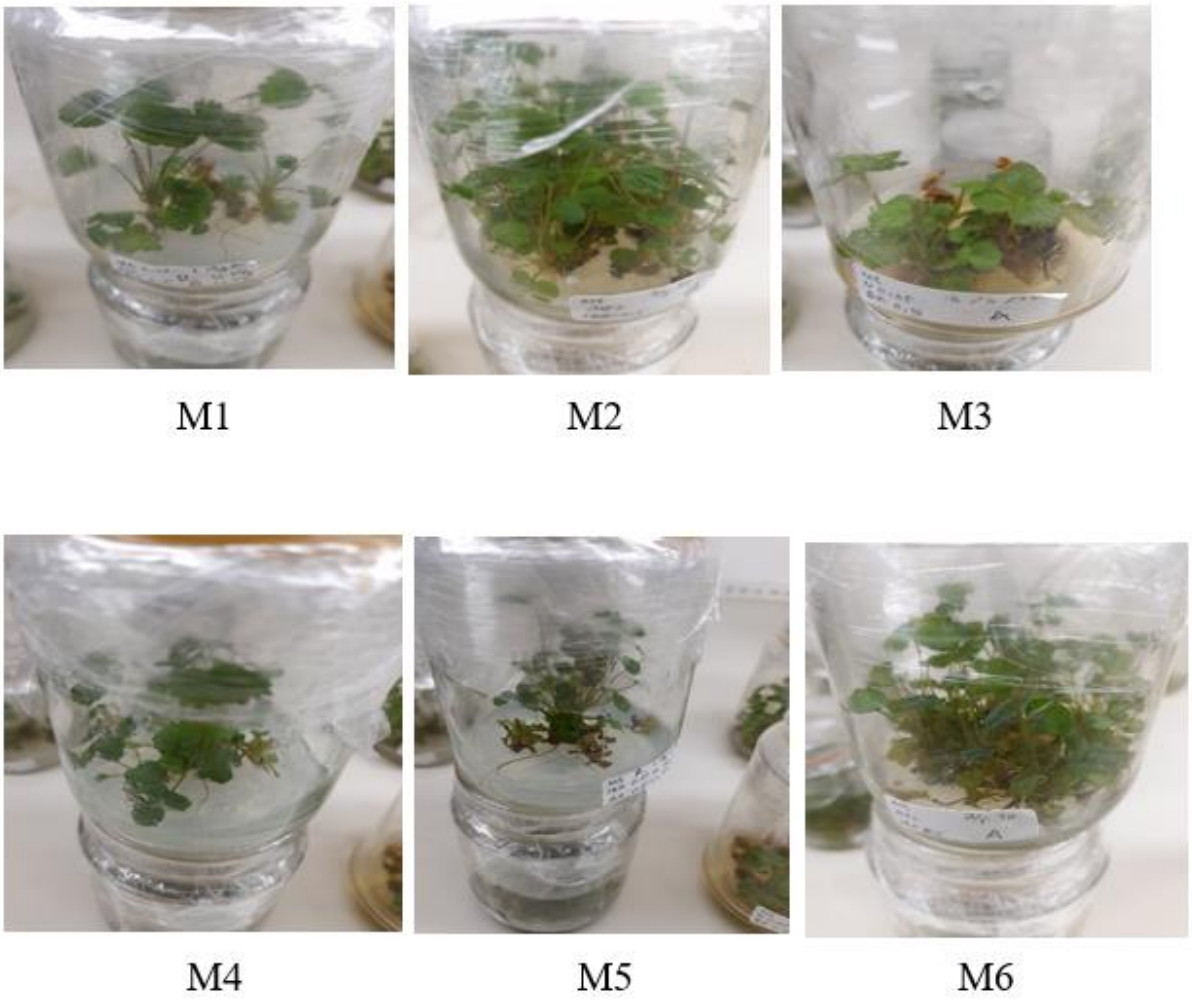

Figure 1. Development of shoots of sachinoka strawberry variety

4. Discussion

The time the shoots appear is an important factor in plant propagation using the tissue culture method. The sooner the shoots appear, the faster the material for plant propagation will be produced. The shoots that form are the result of differentiation from the explant. Explants that are relatively easier to induce shoots are explants that have meristem tissue or buds at the node. Kosmiatin *et al*. (2005) stated that the fastest shoot induction time was obtained from node explants without leaves.

The fastest shoot emergence times in this study were on WPM (Woody Plant Medium) media with the addition of 4 ppm BA (M1 = 12.29), BMM (Banana Multiplication Medium) media with the addition of 4 ppm BA (M5 = 12.50), and followed by NP media (New Phalaenopsis) with the addition of 4 ppm BA (M3 = 10.64) (Table 1). This is because the nutritional content contained in the WPM media can be optimized by the explants for shoot formation. Apart from that, WPM media is a media commonly used in tissue culture on various types of woody plants.

According to Pardal *et al*. (2004) WPM media is widely used in various woody plant species, because it has a low total ion content, but a high sulfate content. The macro elements contained in WPM media, such as high levels of magnesium, really support the growth of plant tissue. Apart from that, according to Wetherell (1982), the media must contain minerals, sugar, vitamins and hormones in the correct ratios required. It is suspected that WPM media has sufficient nutritional content to support shoot formation. Media is the main factor in propagation by in vitro culture and has a huge influence on the growth and development of the explants that will be produced (Tuhuteru *et al*., 2012).

The analysis results also showed that the slowest shoot emergence time occurred on NP media without the addition of BA (M2 = 9.00). Even though it contains macronutrients needed by plants in large and unlimited quantities, there is a certain threshold that plants can tolerate. Each type of plant requires different amounts of elements. For example, the formation of PLB (Protocorm Like Body) in explants treated with NP media without giving BAP indicates that a plant tissue contains endogenous hormones that can influence the growth and development of a tissue even if no external growth regulator is added. This is in accordance with research by Paramartha *et al*., (2012) that Dendrobium taurulinum J.J Smith seeds experienced up to 100% germination on media without the addition of NAA and BAP ZPT.

Apart from that, the media treatment in this study was given a growth regulator from the auxin group, namely 0.5 ppm NAA. The provision of NAA was intended to stimulate root formation, but in every treatment tested no roots appeared. It is suspected that the auxin given was not too high, resulting in an imbalance between cytokinin and auxin in the plant explants. According to Pardal et al., (2004) if the ratio between auxin and cytokinin is low, cytokinin will stimulate towards the shoot and conversely if the ratio between auxin and cytokinin is high, auxin will stimulate towards the root.

Based on the results of the analysis in Table 1, the variable number of shoots can be indicated as success in multiplication, where the highest number of shoots was obtained in treatment M1 = 12.29 fruit, the more shoots formed, the higher the multiplication level. Meanwhile, a small number of shoots were obtained in treatment M2 = 0.71 pieces. In the three types of media treatment with the addition of 4 ppm BA could produce better shoot growth results. This proves that cytokinins have the ability to divide cells, especially the formation of shoots (Figure 1). Mok *et al*. (2000) reported that 6-benzyl aminopurine and 6-benzyladenine (BAP, BA) are adenine-type cytokinins that increase cell division and cell enlargement in plant cultures.

The use of growth regulators in tissue culture depends on the goal or direction of desired plant growth. The growth regulator BA (benzyl adenine) is most widely used to stimulate shoot multiplication because it has strong activity compared to kinetin (Zaer and Mapes, l982). BA has the same basic structure as kinetin but is more

effective because BA has a benzyl group (George and Sherington, l984). Flick *et al*. (1993) stated that in general plants have a better response to BA than to kinetin and 2-iP so that BA is more effective for in vitro shoot production.

Observation of the number of leaves was carried out at the end of the study. The highest number of leaves from the results of this study was obtained in the WPM media treatment (M1 = 27.14 strands) and the lowest was obtained in the NP media treatment (M2 = 2.86 strands) (Table 1). Leaves are vegetative organs; their growth is influenced by the nitrogen content in the medium. The greater the number of leaves, the better the explant growth (Acima, 2006). Apart from that, leaves are an important organ in plant growth because they are the place where photosynthesis occurs, namely the process of forming carbohydrates from CO2 and H2O with the help of sunlight.

5. Conclusions

The best medium for growing meristem culture in Sachinoka strawberry varieties is: M2 = WPM + 4 ppm BA + 0.5 ppm NAA. WPM is used for culturing woody plants, so strawberry plants with woody stems are very suitable for growing on WPM media. The addition of cytokinin (BA) is needed to stimulate shoot growth.

Author Contributions

Contributions: Conceptualization, Y.F.; R.D and I.A.P.D.; methodology, Y.F. and R.D.; software, R.D. and I.A.P.D.; validation, Y.F. and R.D.; formal analysis, H.Y. and I.A.P.D.; investigation, R.D.; resources, Y.F; data curation, R.D.; writing—original draft preparation, Y.F.; H.Y.; R.D.; and I.A.P.D.; writing—review and editing, Y.F.; and R.D.; visualization, I.A.P.D..; supervision, Y.F.; project administration, Y.F. and R.D; funding acquisition, Y.F. and R.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare there no conflict of interest

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