Proceeding of International
Conference on Science and Technology
Lembaga Penelitian Pengabdian kepada Masyarakat dan Dakwah Islamiyah, Universitas Islam Kuantan Singingi, Indonesia, November 10th 2022
DOI : https://doi.org/10.36378/internationalconferenceuniks.v0i0.2816
ISSN 2985-8739
Page: 1-6

Kinetin Concentration Test On Growth Castury Orange (Citrus microcarpa B.) On Medium Woody Plant Medium (WPM)

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Abstract

The development of kasturi oranges is still little done because it is easily attacked by citrus plant pests, especially in humid conditions. Therefore, another solution can be to multiply the seeds with the tissue culture technique. The success of implementing tissue culture techniques is determined, among others, by the use of an appropriate media composition. Kinetin is one type of cytokinin growth regulator which is widely used for shoot propagation because it can stimulate the formation of shoots, with high concentrations not easily damaged when the media is sterilized. The purpose of this study was to examine the effect of kinetin administration on the growth of explants of Kasturi orange (Citrus microcarpa B). The design used in this study was a non-factorial Completely Randomized Design (CRD) consisting of 7 levels of treatment with 3 replications, namely: K0 (Without Kinetin administration), K1 (Kinetin administration 1.5 mg/I), K2 (Kinetin administration) 2 mg/I), K3 (Giving Kinetin 2.5 mg/I), K4 (Giving Kinetin 3 mg/I), K5 (Giving Kinetin 3.5 mg/I), and K6 (Giving Kinetin 4 mg/I). Thus this study consisted of 21 experimental units. Each unit consisted of 1 culture bottle, each containing 4 explants, 3 of which were sampled. The results showed that the administration of kinetin to explants of musk kasturi orange had a significant effect on root length parameters with the best treatment at K0 (without kinetin administration) with an average of 8.22 cm. However, there was no significant effect on the parameters of the age of shoot emergence, number of shoots, and number of leaves.

Keywords: Explants, Kasturi Orange, Kinetin, Concentration

1. Introduction

Kasturi orange (Citrus microcarpa B) is a type of fruit plant that has a fragrant aroma, and has a sour taste when ripe, and bitter when unripe. In some areas it is often referred to as fish lemon or lemon cui, this orange is an agricultural product that is used more as a spice or flavor enhancer in various foods such as kitchen spices, food preservatives, and can also be used as an ingredient in syrup. In addition, kasturi oranges have benefits for the health of the body, such as helping to increase blood circulation, maintaining healthy teeth, helping to lose weight, maintaining bone health, and helping maintain kidney health. Kasturi orange contains 12 calories, with little fat, 1.2 grams fiber, 37 mg potassium, 7.3 mg vitamin C, 54.4 mg vitamin A, 8.4 mg calcium and 15.5 ml water. This plant has the advantage of adapting well in the low to medium lands (Ramli et al, 2012).

Kasturi orange propagation can be done through generative and vegetative ways. The generative propagation of citrus fruit is propagation carried out through the process of marriage or pollination, namely by using seeds, while vegetative propagation is the process of plant reproduction which is carried out asexually or without the need for fusion between male and female sex cells. Kasturi orange belongs to the Rutaceae family and has a relatively long growth characteristic with its generative development having a productive period after 5-6 years, while vegetatively around 3-4 years. The development of Kasturi orange (Citrus microcarpa B) is still little done because it is easily attacked by citrus plant pests, especially in humid conditions. Although it has been developed with a graft technique. However, the results of this technique are not very satisfactory because the trees fall easily and their production life is shorter. One way that can be used to overcome this problem is to use tissue culture techniques (Gusti, 2017).

Tissue culture is a technique for cultivating a plant tissue or plant part which includes stems, roots, leaves, flowers, callus, cells, protoplasts and embryos into small plants that have the same properties as their parents. The plant parts used are called explants, isolated from in vitro conditions, then cultured on sterile media so that they can regenerate and differentiate into complete plants (Aini, 2012). The advantages of tissue culture techniques are that it can produce healthy and uniform seeds in a short period of time, its propagation does not require a very large place, it can be done throughout the year regardless of the season, so that the availability of seeds can be guaranteed (Zulkarnain, 2009). The success of implementing tissue culture techniques (In-Vitro) is determined, among others, by the use of an appropriate media composition. Media is a determining factor in plant propagation by tissue culture (Sundari et al, 2015).

The culture media used specifically for woody plants is WPM (Woody Plant Medium) which is a medium with low ion concentration. This medium is consistent as a medium for woody plants developed by other experts, but the sulfate used is higher than sulfate in other woody plant media, so this WPM medium is very good for hardwood plants such as musk oranges in in vitro propagation (Sundari et al. , 2015).

Planting by tissue culture generally also experiences obstacles such as slow growth of explants, so it is necessary to add PGR to stimulate explant growth, one of the influential PGRs is cytokinins. The addition of ZPT which is classified as cytokinin in tissue culture, one of which is kinetin. Kinetin in culture media is a growth regulator that can stimulate explant growth, leaf formation, shoot height and root formation (Dwi and Ellok, 2016).

Kinetin is one type of cytokinin PGR that is widely used for shoot propagation because it has the ability to stimulate the formation of shoots with high concentrations that are not easily damaged when the media is sterilized (Wahyuni, 2020).

The results of Wahyuni's research (2020), Kinetin has a significant effect on the parameter of the number of new shoots at a Kinetin concentration of 3 mg/l with an average of 1.67 shoots as the highest number of shoots in Kasturi Orange plants. Meanwhile, according to researchers Dewi and Dyah (2010) have used Kinetin to see its effect on shoot growth on plant propagation of Jatropha. The results showed that kinetin administration of more than 1.00 ppm could increase shoot growth, especially at a concentration of 2.00 ppm.

The purpose of this study was to determine the Kinetin Concentration Test on the Growth of Kasturi Orange (Citrus microcarpa B) Explants on WPM (Woody Plant Medium) Media.

2. Research Methods

The tools that have been used in this research are laminar air flow cabinet, measuring cup, beaker, petridish, pipette, autoclave, analytical balance, erlenmayer, magnetic stirrer, glass stirrer, tweezers, scalpel, spritus lamp, hand sprayer, pH meter, knife , culture bottles, gas stoves, measuring flasks, test tubes, plastic rubber, pans, scissors, aluminum foil, stationery and washing equipment that support activities in tissue culture research.

The materials used in this study were explants of musk oranges in the form of seeds obtained from musk oranges, WPM media chemicals, Kinetin Growth Regulators, alcohol, agar powder, sterile distilled water, detergents, prokline, rubber bands, label paper and other materials. others who support his research.

The design used in this study was a non-factorial Completely Randomized Design (CRD) consisting of 7 treatments and 3 replications so that there were 21 experimental units. Each unit (bottle) consisted of 4 explants, thus this study consisted of 84 explants. Each experimental unit consisted of 3 sample plants. The level of treatment in this study are as follows:

K0: Without Kinetin administration, K1: Kinetin administration 1.5 mg/l, K2: Kinetin administration 2 mg/l, K3: Kinetin administration 2.5 mg/l, K4: Kinetin administration 3 mg/l, K5: Kinetin administration 3.5 mg/l, K6: Administration of Kinetin 4 mg/l. Parameters observed were age of shoot emergence, number of shoots, number of leaves, and root length.

3. Results and Discussions

Age Of Shooting (Days)

Based on the results of observations on the age parameter of the emergence of musk orange explant shoots, after statistical analysis showed that the Kinetin treatment had no significant effect on the emergence of musk orange explant shoots. The results can be seen in table 1.

Table 1. Average age of emergence of musk orange explant shoots with Kinetin administration on WPM . Media

Treatment	Average
K0 (0 mg/l)	8,89
K1 (1,5 mg/l)	9,00
K2 (2 mg/l)	9,11
K3 (2,5 mg/l)	8,44
K4 (3 mg/l)	9,22
K5 (3,5 mg/l)	9,89
K6 (4 mg/l)	10,11
KK= 7,83 %	

Based on the data in table 1, it can be seen that the administration of kinetin alone had no significant effect on the age of emergence of musk orange explant shoots. This is presumably due to the inability of the explants to absorb the nutrients and growth regulators provided, meaning that the kaffir lime seeds have not been fully able to utilize the Kinetin added in WPM media, because the explant seeds still get hormones from their endosperm (Wahyuni, 2020). According to Gunawan (2009), the interaction and balance of growth regulators added to the media and produced by plant cells endogenously determines the speed and direction of development of a culture, including the age of shoot emergence. When the shoots appear, it is influenced by three factors, namely explants, media, and environmental factors (Nisa and Rodinah 2005).

When viewed from the average, the treatment with

the fastest shoot emergence was obtained in the K3 treatment (administration of 2.5 mg/l kinetin) which was 8.44 days, followed by K0 (without treatment) 8.89 days, K1 (1.5 mg/l). l) 9.00 days, K2 (2 mg/l) 9.11 days, K4 (3 mg/l) 9.22 days, K5 (3.5 mg/l) 9.89 days, and K6 (4 mg /l) 10.11 days.

K6 treatment (administration of Kinetin 4 mg) was not able to give a good response to the number of explants of musk citrus plant. This is because cytokinins cannot work alone, they must be combined with auxin to be able to produce good budding time. This is in accordance with the opinion of Karjadi (2008), who said that auxin is a hormone found in the apical that can stimulate shoot growth. The ratio between auxin and cytokinin will determine the speed of cell division that can trigger shoots to appear in the explants of musk orange seeds. The interaction between auxin and cytokinin at optimal concentrations will stimulate the growth of roots and shoots quickly.

The results of this study when compared with research conducted by Mahadi et al, (2015) obtained different results. Mahadi et al, (2015) concluded that administration of 5 mg/l of kinetin into MS media had a significant effect on the growth of bud explants of Kasturi orange (Citrus micocarpa B) with an average age of 5 days. While in this study, giving Kinetin 2.5 mg/l on WPM media, was able to produce the fastest shoots with an average age of 8.44 days. The results of Mahadi et al, (2015) are 3.44 days faster than this study. The difference in the response of the explants was due to the use of different types of concentration so that the resulting response was also different. This is supported by the opinion of Wahyuni and Fitrianingsih (2009) that the administration of hormones with several concentrations gives a good percentage of shoot growth, because each concentration contains different levels of vitamins and calcium.

Number of Shoots (Fruit)

Based on the results of observations on the parameters of the number of shoots explants of Kasturi orange, after statistical analysis showed that the administration of Kinetin had no significant effect on the parameters of the number of shoots of explants of Kasturi citrus. The results can be seen in table 2.

Table 2. The average number of shoots (fruit) of Kasturi orange explants with Kinetin administration on WPM Media

Treatment	Average
K0 (0 mg/l)	2,33
K1 (1,5 mg/l)	2,00
K2 (2 mg/l)	2,56
K3 (2,5 mg/l)	2,22
K4 (3 mg/l)	2,44
K5 (3,5 mg/l)	2,11
K6 (4 mg/l)	1,89
KK= 8,25	

Based on the table above, it can be seen that the administration of kinetin had no significant effect on the number of buds explants of Kasturi orange. It is suspected that the calcium contained in WPM media is quite high. Calcium contained in WPM media plays a very important role in plant cell growth even without the addition of growth regulators. In addition to the calcium content contained in WPM media, endogenous cytokinins or hormones present in explants have been able to encourage shoot growth. So that the addition of exogenous cytokinins such as Kinetin did not have a significant effect on shoot growth.

Giving Kinetin as much as 2 mg/I into WPM media was able to produce more shoots when compared to K0, meaning that adding Kinetin to WPM media could increase the number of shoots in musk citrus plant explants. According to Dewi and Dyah (2010) Kinetin is one type of cytokinin ZPT which is widely used for shoot propagation because it has the ability to stimulate the formation of shoots with high concentrations that are not easily damaged when the media is sterilized. The provision of cytokinins to a certain extent has an effect on spurring shoot formation time, this is in accordance with the function of cytokinins to stimulate shoot formation.

The treatment that produced the highest number of shoots was found in treatment K2 (given Kinetin 2 mg/I) with an average of 2.56 pieces, while the least amount was found in treatment K6 (given Kinetin 4 mg/I) with an average of 1.89 pieces.

The results of this study when compared with research conducted by Mahadi et al, (2015) there are different results. Where based on the results of research by Mahadi et al, (2015) the results were obtained, namely the administration of Kinetin 3 mg/l on MS media was able to produce the highest number of shoots on kasturi citrus plants with an average number of shoots of 2.4 pieces. The results of this study were 0.16 more shoots than the study of Mahadi et al, (2015).

This is presumably because the nutrient content contained in WPM media was able to be optimized by explants of Kasturi Orange (Citrus microcarpa) for shoot formation. Macro elements contained in WPM media such as high magnesium elements are very supportive in the growth of plant tissue, especially in shoot growth. WPM media has sufficient nutrient content to support shoot formation.

Number of Leaves (Strand)

Based on the results of observations on the

parameters of the number of leaves of explants of kaffir lime, after statistical analysis showed that the treatment with kinetin had no significant effect on the parameters of the number of leaves of explants of citrus plants. The results can be seen in the table below.

Based on table 3, it can be seen that the administration of kinetin had no significant effect on the parameter of the number of leaves of Kasturi citrus explants. The magnesium element contained in WPM media is thought to be sufficient for leaf formation. The role of magnesium in plants is quite important because it is related to the process of photosynthesis. Even without Kinetin, it is possible that the nutrient content in WPM media is sufficient for leaf formation (Nursetiadi, 2008). In addition, it was also caused by Kinetin in WPM media not being able to respond to the number of leaves in the explants of musk oranges. Because cytokinins (Kinetin) cannot act alone, they must be combined with auxin. This is supported by the opinion of Widyastuti (2017) which states that leaf formation in in vitro culture is strongly influenced by exogenous and endogenous cytokinins and auxins.

Table 3. Average number of leaves (strands) of Kasturi orange explants with Kinetin administration on WPM Media

Treatment	Average
K0 (0 mg/l)	2,44
K1 (1,5 mg/l)	2,56
K2 (2 mg/l)	3,67
K3 (2,5 mg/l)	2,33
K4 (3 mg/l)	3,22
K5 (3,5 mg/l)	3,00
K6 (4 mg/l)	2,89
KK= 11,70	

When viewed from the mean value of the number of leaves explants of the citrus plant, the most widely found in K2 treatment (Given Kinetin 2 mg/I) with an average of 3.67 leaves. While the least was found in the K3 treatment (administration of Kinetin 2.5 mg/I) with an average of 2.33 strands.

Administration of Kinetin as much as 2 mg/l into WPM media was able to produce more leaves than control (K0), meaning that by adding Kinetin to WPM media, the number of leaves in musk citrus explants was increased. This is supported by the opinion of Widyastuti (2017) that leaf formation in in vitro culture is strongly influenced by cytokinins both exogenous and endogenous.

The results of this study are different from the results of research conducted by Maisarah and Isda Novaliza (2021), namely the administration of Kinetin significantly affected the parameter of the number of leaves of kaffir lime explants, with the average number of leaves of explants of Kasturi citrus plants which was mostly found in the administration of Kinetin 1.5 mg / I, with an average number of 3.6 leaves. While the lowest mean number of leaves was given with Kinetin 1 mg/l with an average number of 1.0 leaves. The results of this study produced 0.07 more leaves than the research of Maisarah and Isda Novaliza (2021). This is presumably due to differences in the amount of concentration, types of explants and media used, because each explant has a different ability to absorb and optimize the given growth regulators.

Root Length(cm)

Based on the results of observations on the root length parameters of musk citrus explants, after statistical analysis showed that the treatment with kinetin had a significant effect on the root length of musk citrus explants. The results can be seen in the table below.

Table 4. Average root length (cm) of musk orange explants with Kinetin administration on WPM . Media

Treatment	Average
K0 (0 mg/l)	8,22 a
K1 (1,5 mg/l)	7,00 ab
K2 (2 mg/l)	6,89 ab
K3 (2,5 mg/l)	6,33 bc
K4 (3 mg/l)	5,67 bc
K5 (3,5 mg/l)	5,89 bc
K6 (4 mg/l)	5,00 c
KK= 8,81	BNJ = 1,62

Note: The numbers in the row followed by the same lowercase letter are not significantly different according to the further test of honest significant difference (BNJ) at the 5% level.

Based on table 4, it can be seen that the single administration of kinetin significantly affected the root length parameters of musk orange explants. This is because the nutrient content contained in WPM media can be optimized by explants for root growth. Because WPM media is consistent as a medium for woody plants developed by other experts, because the sulfate used is higher than sulfate in other woody plant media, so this WPM medium is very good for hardwood plants such as musk oranges in in vitro propagation (Sundari et al. al, 2015).

If it is seen from the average root length of kasturi citrus plant explants, the K0 treatment without kinetin was able to produce the best root length with an average of 8.22 cm. Meanwhile, the most unfavorable results were found in K6 treatment with Kinetin 4 mg/I with an average of 5.00 cm. This treatment was seen from the results of the further test with a significantly honest difference (BNJ) of 5% indicating that treatment K0 was not significantly different from treatment K1, K2, and significantly different from treatment K3, K4, K5, K6.

K0 treatment without Kinetin was able to produce

better root length than other treatments. Because this kinetin belongs to the cytokinin group. These cytokinins play a role in regulating cell division and morphogenesis. Cytokinins function to stimulate the formation of shoots, have an effect on cell metabolism, and stimulate cells and their main activity is to encourage cell division (Karjadi, 2008). The higher the level of kinetin administration, the shorter the root length. This is because if kinetin is used in high concentrations it will inhibit root formation. The inhibition of root formation will cause disruption of the process of cytokinin synthesis in the roots, so that it will affect the root extension process (Karjadi, 2008). This is supported by Mahadi et al, (2015) medium without cytokinins is better than medium containing cytokinins for root formation. A good growth regulator for root growth is the type of auxin. Auxin is widely used in tissue culture for cell elongation, adventitious root formation, and inhibiting the formation of adventitious shoots and axillary buds.
The results of this study are different from the results of research conducted by Hardiyati et al (2021). Based on the research of Hardiyati et al, (2021), stated that K2 treatment by giving Kinetin 2 mg/I to two bunches of Ambon banana plants on MS media was able to produce the best root length with an average root length of 2.58 cm. As for the lowest root length, treatment K1 with Kinetin 1 mg/I was only able to produce an average root length of 1.56 cm. Hardiyati et al, (2021) concluded that the increase in root length was caused by the higher concentration of kinetin given.

The results of this study resulted in the longest root length being 8.22 cm without kinetin (K0) on WPM media, while the study by Hardiyati et al (2021) resulted in the longest root length being 2.58 cm with Kinetin 1 mg/I administration in MS media. So that there is a difference of 5.64 cm in root length in this study which is longer than the study of Hardiyati et al (2021). It is suspected that WPM media is a special medium for woody plants, and the nutrients contained in WPM media have sufficient nutrients for the growth of the number of roots and Kinetin is only effective for the formation of shoots, number of shoots and number of leaves.

4. Conclusion

Based on the results and discussion above, it can be concluded that the administration of kinetin had a significant effect on the root length of Kasturi orange explants with the best average being in treatment K0, which was 8.22 cm, but had no significant effect on the parameters of shoot emergence age, number of shoots and number of leaves.

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