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The Effect of Gibberellic Acid (GA3) On The *In Vitro* Seed Germination of Manggosteen (*Garcinia mangostana*)

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Abstract

Mangosteen is considered as fruit of many benefits with great popularity, rendering it valuable as an export commodity. Unfortunately, it has a relatively slow growth and long early fruiting time. Generative propagation of mangosteen by seed result in low-yielding production of qualified seeds with recalcitrant nature which means it cannot be stored for a long time. Whereas the result of vegetative propagation using grafting, branch cuttings and budding has difficulty rooting out. Tissue culture method requires growth regulators to control organogenesis and morphogenesis so it can accelerate the growth of the mangosteen explants. This study was arranged in two-factor completely randomized design (CRD). The observed variables were the strength of the medium (½ and 1 MS) and GA3 concentrations (0 ppm; 0.25 ppm; 0.5 ppm; 0.75 ppm and 1 ppm). The variatons on GA3 concentration (0 ppm; 0.25 ppm; 0.5 ppm; 0.75 ppm; 1 ppm) on different strengthMS medium (½ MS and 1 MS) did not significantly affect the growth of radicle and sooth in the in vitro seeds germination of Mangosteen (Garcinia mangostana).

1. INTRODUCTION

Mangosteen (*Garcinia mangostana*) is a tropical plant, member of Guttiferae family and genus Garcinia, which has a very distinctive and otherwise interesting scent and taste, it is therefore commonly referred as the fruit queen or "*Queen of Tropical Fruits*". Mangosteen has a considerably complex nutrient content and is often used in herbal medicine because it contains various substances which serve as anti-inflammatory, antioxidant, anti-cancer and anti-bacterial. Mangosteen also known to have neuroprotector activity for the treatment of diarrhea, abdominal pain, infection and chronic ulcer (Wulan, 2015). It is a valuable export

commodity with an ever increasing demand annually (Ministry of Agriculture, 2012). According to the Directorate General of Processing and Marketing of Agricultural Products of the Indonesian Ministry of Agriculture (2014) within the period of 2010-2013, the export on Mangosteen has reached 40.88 million US dollars, 50.52 million tons in volume.

The increasing demand over Mangosteen exports for overseas market has renewed interest on mangosteen cultivation among farmers. The first obstacle in growing Mangosteen is that it takes a relatively long time to grow and ready to bear fruits (Roostika, 2005), which could cause an unmet demand on Mangosteen exports. The slow growth of mangosteen tree is attributed to its weak root system (Nakasone, 2010). Generative plant propagation by seeds will result in low-yielding production of qualified seed with recalcitrant nature, which means it cannot be stored for a long period of time (Qosim, 2013). Whereas the products of vegetative propagation using grafting, branch cuttings and budding have difficulty to root out, even if it is already in callus state (Rugayah, 2014).

In that manner, tissue culture technique has better prospects than conventional vegetative propagation methods. It is capable to produce a large amount of highly uniform plantlets or qualified seeds in relatively fast period without being affected by time or season (Harahap, 2012). There are several factors influencing the success in doing tissue culture including explants/planting materials selection, suitable basal medium, aseptic condition, and the types and concentrations of growth regulators added into medium (Santoso, 2003).

MS (Murashige and Skoog) medium is one of the most widely used basal medium in tissue culture. According to Ritchie and Hodges (1993), medium composition is a crucial condition in *in vitro* culturing. The medium should contain macro and micro elements, vitamins, and the necessary growth regulators to ensure the growth of the explants.

Tissue culture method requires growth regulators to control organogenesis and morphogenesis in the formation of shoots, roots and callus, so it can accelerate the growth of mangosteen explants. One of the active Plant Growth Regulatory (PGR) for the initial growth of plants in tissue culture is gibberellin (GA3). This substance plays a role in replacing the necessity for light and the temperature required for seed germination (Dewi, 2015). According to Wattimena (1988) in addition to its role in germination, GA3 also stimulate stem elongation (in shoots), size enlargement on flower and leaf, leaf color alteration, inhibition of aging process and threshing on plant organs (Heddy, 1996).

Sukmadjaja (2005) stated in his study that in germination medium the somatic embryos which are the cells that undergo rapid multiplication most often to form buds, are found in ½ MS medium + GA3 0.5 mg / l. Dinarti (2010) added that germination of *Nepenthes mirabilis* seeds in the medium of ½ MS with the addition of GA3 has showed a good result. Isnaeni (2007) also reported ½ MS medium with the addition of 15 mg/l GA3 gave better result for the growth of *Nepenthes gracilis* sprouts. Similar to those reports, Mukminin (2016) also stated that the addition of 0.15-0.2 ppm GA3 into medium has significantly increased the height and the number of sprouting shoots, as well as the amount of leaves and roots.

The aim of this study is to discover the best formula for the optimum sprout growth by combining different concentration of gybberellin (GA3) growth regulators addition and MS medium strength in the *in vitro* seeds germination of mangosteen (*Garcinia mangostana* L.)

2. MATERIALS AND METHODS

The research was conducted in Plant Tissue Culture Laboratory of Biology Department, Science and Technology Faculty, Maulana

Malik Ibrahim State Islamic University of Malang.

The materials required for this research are explants of mature mangosteen seeds, Murashige and Skoog (MS) basal medium, sugar, agar, aquadest, gibberellin (GA3), 70% and 96% alcohol, disinfectants and detergents. Whereas the required tools include analytic scale, stove, pan, glass stirrer, autoclave, hot plate, stirrer, spatula, micropropet, tip, culture jar, plastic and rubber, pH paper, labels, 50 ml graduated cylinder, 1000 ml erlenmeyer, 1000 ml beaker glass, petri dish, tweezers, scissors, scalpel, plastic wrap, aluminum foil, bunsen, Laminar Air Flow Cabinet (LAFC) and hand sprayer.

The overall procedure involves preparation, tools and materials sterilization, MS medium preparation, explant initiation and observation.

All glassware and metalware that were going to be sterilized were prepared including the culture jars, petri dishes, graduated glass, erlenmeyer, beaker glass, tweezers and scalpels. The tools were dry-sterilized in the oven at 121°C for 3 hours. The petri dishes, tweezers and scalpels would then undergo subsequent sterilization using autoclave. The petri dishes should be wrapped in paper beforehand, while tweezers and scalpels in aluminum foil. All of them were put into plastic and then sterilized in autoclave at 121°C, 17.5 psi for 15 minutes.

MS medium was prepared by dissolving 2.25 gr for 1 MS and 1.175 gr for ½ MS, into 500 mL aquadest. 15 gr sugar were added into the mixture and then homogenized on the hot plate using stirrer. After all dissolved, the mixture was divided into 10 jars, 5 mL each. Exactly 0.5 gr agar was added into each bottle, followed by the addition of gibberelins of different concentrations; 0, 0.25, 0.5, 0.75 and 1 ppm successively. The pH of the medium was monitored using universal pH test. The mediums were then ready to be sterilized in autoclave.

The sterlization for the explants of the mangosteen seeds was preceded by stripping the outer tissue layer. After that, the seeds were washed in detergent for 2 mins, followed by 50% disinfectant for 2 mins. After that came seeds washing using 30% Clorox for 10 mins and then with 20% Clorox for 2 mins. Each washing was followed by rinsing in five changes of distilled water.

Explant initiation was carried out inside Laminar Air Flow Cabinet (LAFC), which has been sterilized in advance with UV-light for 2 hours. The seeds' explants were set down on a sterile petri dish and cut into two pieces using tweezers and scalpel. The seeds were planted with the flat, cut part facing the medium.

The cultures were observed daily from the first up to the 24^{th} day after initiation. This study employed two-factor completely randomized design (CRD). The first is medium types which were coded A (1 MS) and B (½ MS), as the second factor is gibberelins concentration coded G1 (0 ppm); G2 (0.25 ppm); G3 (0.5 ppm); G4 (0.75 ppm) and G5 (1 ppm). To summarize, there were 10 treatments in which each was duplicated three times. Thus, there were 30 jars total of experimental units where every jars inhabitted by one explant.

The observed variables are shoot emergence time, shoot length, radicle emergence time, radicle length (cm) and number of leaves. The obtained data were analyzed by two-way analysis of variance (ANOVA). If there were significant influence would then Duncan's test be performed at confidence level of 5%.

3. Results

Shoot and Radicle Growth of Mangosteen (Garcinia mangostana) Seeds Explants

The analysis of variance (ANOVA) has been carried out on mangosteen seeds grown at different concentration of GA3 in MS Medium of different strength. The effect of GA3 concentration variations in MS medium of

different strength on the growth of shoots and radicles can be seen on table 1.

Table 1. Analysis of Variance (ANOVA) Results Summary on the Effect of GA3 Concentration in MS Medium of Different Strength on the Shoot Growth of Mangosteen (*Garcinia mangostana*) Seeds.

No.	Observed Variable	F Count	F table 5%
1.	Mean Shoot Emergence Time	0,333*	2,223
2.	Shoot Length	0,283*	2,223
3.	Number of Leaves	0,350*	2,223

Note: * The concentration of GA3 in different strength MS medium does not have effect on all observed variables

Table 2. Analysis of Variance (ANOVA) Results Summary on the Effect of GA3 Concentration in MS Medium of different strength on the Radicle Growth of Mangosteen (Garcinia mangostana) Seeds

No.	Observed Variable	F Count	F table 5%
1.	Mean Radicle Emergence Time	0,258*	2,223
2.	Radicle Length	0,874*	2,223

Note: * The concentration of GA3 in different strength MS medium does not have effect on all observed variables

The analysis of variance (ANOVA) result on Table 1 shows that GA3 concentration in different strength MS medium levels did not affect the three observed variables; shoot emergence time, shoots length, and number of leaves. This is shown by the value of F count on all observed variables which are smaller than the F table of 5%. The highest F count belongs to number of leaves parameter, while the lowest calculated F count comes from shoot length parameter (Figure 1).

The results from analysis of variance (ANOVA) in Table 2 shows similar outcome, GA3 concentration in MS medium level did not affect two observed variables; radicle emergence time and radicle length. It is indicated by the F count of all observed variables which have smaller value than F table of 5%. F count on radicle length parameter has the highest value of 0.874 compared to that of radicle emergence time parameter (Figure 1).

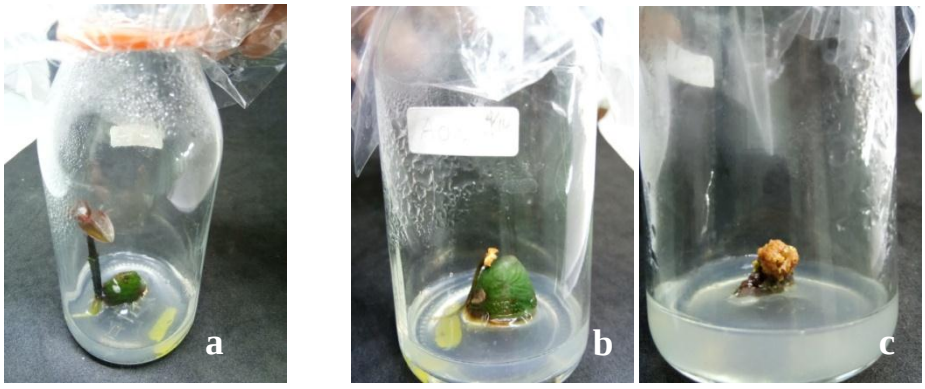

Figure 1. The growth of mangosteen (*Garcinia mangostana*) seeds explants in *in vitro.* a. The highest shoot length was observed in the treatment of 0.5 MS + 0.75 ppm GA3, whereas b. Highest radicle length was observed in the treatment of 0.5 MS + 0.5 ppm GA3, c. Callus growth was found in 1 MS + 1 ppm GA3 treatment

4. DISCUSSION

The Shooth Growth of Mangosteen Seeds Explants

The combination between the strength of the MS medium and GA3 concentration on the treatment of ½ MS with the addition of 0.75 ppm GA3 gave the best result for mean shoot emergence time parameter, that is 23.33 day after initiation. However, the results were not significantly different from the other treatments with different GA3 concentration and MS medium strength. The slowest record on shoot emergence time parameter was observed in the treatment of 1 MS + 0 ppm GA3, 1 MS + 0.5 ppm GA3, and ½ MS + 1 ppm GA3, in which shoot appeared 36 days after initiation. This results suggest that mean shoot emergence time was not influenced by GA3 concentration in different strength MS medium. This is presumably because the addition of 1 ppm GA3 is too low of concentrantion to spur shoot formation in mangosteen seed explants. As stated by Sitanggang (2015) the addition of gibberellin must be carried out on correct dose. Too much gibberellin in medium could turn it into toxin, thus inhibit the growth of the plant. Whereas in inadequate concentration, it does not have significant effect on plant growth.

The observation results on shoot length and number of leaves (Table 1) shows that there were no significant effect on the addition of varied GA3 concentration on different strength MS medium to those parameters. This result does not fit the statement of Sundahri (2014), that gibberellin serves a role in cell division process, stem elongation, leaves enlargement, and total leaves increase. This might due to the recalcitrant nature of the mangosteen seeds. According to Goh (1994), recalcitrant seeds have high endogenous auxin content which is responsible in shoot growth inhibition. In agreement to that Murni (2008) said, seeds contain both growth regulator such as auxin, cytokinin, gibberellin, and growth inhibitor which serves in germination and seedling growth. According to Arianto (2018), high concentration of gibberellin will affect the height of sprouting shoots and root length in nutmeg seeds. Sumampow (2002) reported in his study that at gibberellin concentration of 200 ppm the explant produced the highest result of shoot length and root length.

As stated by Campbell (2002), there are activities of other growth hormones besides gibberellin, known as auxin and cytokinin, which also influence the growth of shoots. The optimum content of endogenous auxin and cytokinin will stimulate cell divisions and differentiations to form new shoots. Kusumawati (2009) said the addition of GA3 to the plants would increase auxin content as well through the formation of proteolytic enzymes in which tryptophan is released as the precursor for auxin.

The Radicle Growth of Mangosteen Seed Explants

The observation results on several treatments of GA3 addition in different strength MS medium show that radicle growth was found in 0.5 MS medium. Radicle is the part seedling that will develop into the roots of mangosteen seeds. The emergence of the radicle is indicating that explants are being responsive to the medium. According to Heddy (1996), the mechanism of gibberellin to spur sprout (radicle) emergence is preceded by gibberellin initiating the synthesis of amylase, a digestive enzyme, in aleurone cells whish is found in the outermost layer of endosperm cells. Besides amylase, gibberellin is also involved in the synthesis activation of protease and other hydrolytic enzymes. The alfaamilase would then converts starch into sugar. The products from amylase and protease activity will be transported to the embryo to support embryonic development and germination.

The combination between GA3 concentration and the strength of the MS medium on the treatment of half strength MS medium and 0.5 ppm GA3 gave the best result regarding the mean radicle emergence time, in which radicle appeared at 26.33 days after

initiation. However, the result was not particulary different from other treatments. This suggests that different concentrations of GA3 in different strength MS medium did not affect mean radicle emergence time. Anwarudin, et al. (1996) said GA3 only triggers the start of growth process, as for the next growth stage it depends on other factors such as nutrient availability, water and environmental conditions. If the concentration of gibberellin in growth medium is too high, the excess gibberellin will accumulate in the seeds to saturation. Seed that is saturated on gibberellin will experiennce a decrease on its germination rate. According to Taiz and Zeiger (1998), gibberellin inhibition is possible because of feedback regulation (feedback control). The inhibition process occurs because of Gibberelin oxidase transcription factors, which is an enzyme that catalyze the transformation of gibberellin into its active form. In excessive concentration, the products will bind on the enzymes, causing feedback inhibition. The active side of the enzyme will be inactive because of the inhibitor, thus blocking substrate to bind on the enzyme. The blocking on gibberellin biosynthesis process will lead to a decrease on gibberellin activity.

Some explants were also found to grow callus. The mean callus emergence time is different for each explant. The difference in callus induction time on explants of different treatments is influenced by several factors, including the concentration of growth regulator added into the medium, the level of endogenous hormone which varies in each explant and the difference in cell competency. According to Santoso (2004), the level of endogenous hormone which varies in each explant will affect its response toward the addition of growth regulators, eventhough the explants are planted in the same culture medium. The balance interaction between the added growth regulators (exogenous) and explant endogenous hormones will trigger the growth and morphogenesis of a cultured tissue. As stated by Reinert and Bajaj (1989), cell competency is a characteristic possessed by each cell to interact with the environmental conditions to produce physiological responses which stimulate cell growth.

5. CONCLUSION

The treatment of GA3 addition in varied concentration (0 ppm; 0.25 ppm; 0.5 ppm; 0.75 ppm; 1 ppm) in different strength MS medium (½ MS and 1 MS) did not significantly affect shoot and radicle growth in the *in vitro* seeds germination of mangosteen (*Garcinia mangostana*).

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