



## ***IN VITRO* GERMINATION OF ANTI-DIABETIC PLANT LOQUAT (*Eriobotrya japonica* Lindl.) TO PRODUCE GOOD SEEDLING**

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### **ABSTRACT**

The aim of the study is to obtain good seedling of anti-diabetic plant Loquat (*Eriobotrya japonica* Lindl.) through *in vitro* germination by using growth stimulator of gibberellic acid (GA<sub>3</sub>) and kinetin. The study is carried out using a completely randomized design with two factors, the concentrations of GA<sub>3</sub> (0, 250 and 500 mg/L) and the kinetin (0, 4, 8 and 12 mg/L). The results showed that the technique has successfully shortened the germination time of loquat seed and stimulate the growth development of shoots, leaves and roots. The GA<sub>3</sub> and kinetin at optimum condition are found very effective to stimulate seed germination and growth of loquat seedlings. Combination of 500 mg/L GA<sub>3</sub> and 8 mg/L kinetin, followed by 250 mg/L GA<sub>3</sub> and 4 mg/L kinetin was observed to be the best experimental condition to produce good quality seedling. It was also observed that high concentration of 500 mg/L GA<sub>3</sub> alone is very effective to improve the growth of loquat root. The study is promising to obtain good seedling of loquat plants.

**KEYWORDS:** *In Vitro*, Loquat (*Eriobotrya japonica* Lindl.), Germination, Anti-diabetic plant, Seedling



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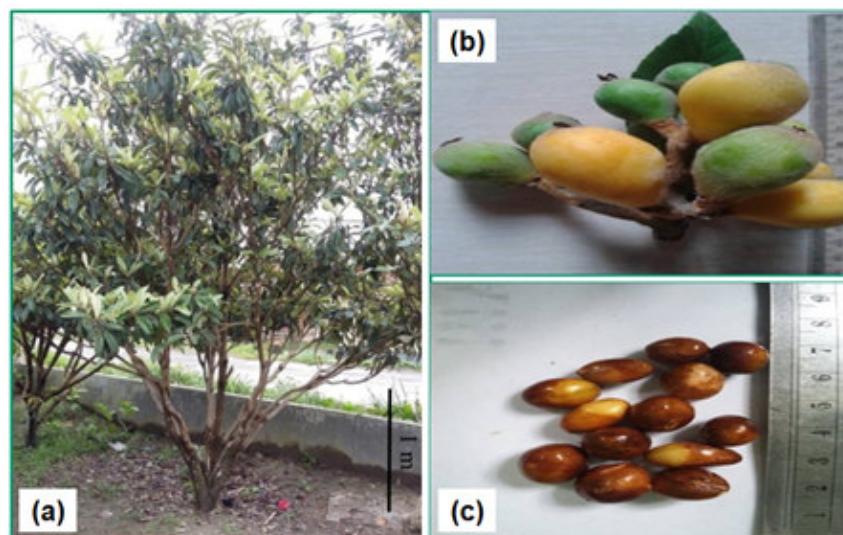


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## INTRODUCTION

The trend in the exploration of medicinal plants is very important to obtain plants contains bioactive that is potential to cure diseases. The use of plant drugs as a therapy has widely used in developing countries for some reasons, such as less toxic, free from side effects, and relatively cheap. Diabetes mellitus is one of the targets in the therapeutic by using of herbal medicines. Many people, including Indonesians, are using traditional medicines particularly in the rural area, and mostly are obtained from important parts of the plants. It brings a consequent to the propelled search for pharmaceutical remedies against diabetes from plants. Various types of plants have been identified to be potential as anti-diabetic such as *Zizyphus lotus* L (Desf.),<sup>1</sup> *Nauclea latifolia*, *Oxytenanthera abyssinica* and *Picralima nitida*,<sup>2</sup> *Ambrosia maritima*, *Ammi visnaga*, *Acacia senegal*, *Sesamum indicum*, *Nigella sativa*, *Foeniculum vulgare* of Sudanese,<sup>3</sup> the Indian ayurvedic plants from Australia,<sup>4-6</sup> and *Sorbus decora* C.K. Schneid. (Rosacea).<sup>7</sup> Loquat (*Eriobotrya japonica* Lindl.) is a typical plant in Indonesia, it becomes the interest in this study for it was commonly used as herbal traditional medicine to cure diabetic diseases. Various strategies have been conducted to produce good quality seedlings of traditional medicinal plants, including through seed, budding, cutting, or layering,<sup>8</sup> cutting bud propagation,<sup>9</sup> *in vitro* propagation,<sup>10-11</sup> and the combination of cutting bud and *in vitro* propagation,<sup>12-14</sup> and sub culture technique.<sup>15-16</sup> *In vitro* propagation is the most commonly used. It has been used to propagate rare medicinal plants.<sup>17-19</sup> *In vitro* germination has also become a very good strategy in the production of plant seedling. It has successfully applied to obtain seedling of cherry laurel *Prunus*

*laurocerasus*<sup>20</sup> and indigenous grain legume Bambara Groundnut (*Vigna subterranea* (L.) Verdc. (Fabaceae)).<sup>21</sup> The strategy is believed to be an effective to obtain good seedling for Loquat plant. Loquat plant (*Eriobotrya japonica* Lindl.) is one of the plants from family Rosaceae that is potential as anti-diabetic plant. Loquat is grown well in Kabanjahe, Kabupaten Karo, North Sumatera Indonesia. The plant which is called *Biwa* has potential genetic compared with other loquat, where the fruit is large in size, has very sweet taste, and becomes multifunction plants as sources of fruit, and the leaves, peels and seeds are containing bioactive compounds that are commonly used for Karo traditional medicine to cure diabetic diseases and for expectorant to relieve cough.<sup>22</sup> Loquat is a small tree with a rounded crown, short trunk and woolly new twigs, short branches with sweet fruits as shown in Figure 1. Loquat plant is an evergreen fruit crop of subtropical regions originally from China and has been grown worldwide, including Indonesia.<sup>23-24</sup> The plant is 2.5 – 5 m tall, with diameter 3.9 – 9.1 cm, have green leaves 8-15 cm long, leathery in texture and densely velvety-hairy below with thick yellow-brown pubescence (Figure 1a). Loquat fruit are oval, rounded-pear shape 3-5 cm long, with a smooth, orange when ripe, sweet in taste (Figure 1b). The fruit contains one to three large brown seeds (Figure 1c). Loquat leaves has been used as traditional herbal medicine to cure diabetes because it contains phytochemical compounds. Traditional treatment by using loquat plants becomes an alternative as it has been reported to be able to control diabetes mellitus.<sup>22</sup> However, the cure is still suffered from the lack of scientific evidence, but the information is valuable to add the list of plants as sources of potential anti-diabetic drugs.



**Figure 1**  
**Loquat tree grown at Kabanjahe, Kabupaten Karo, North Sumatera Indonesia:**  
**(a) Mature loquat tree variety Kabanjahe, (b) Mature fruits, and (c) seeds.**

Propagation of loquat seedling brings high attention in this study as a strategy to obtain mass production of good quality seedling for plantation, in order the plants are sufficient to produce high quantity and quality leaves to be used as raw material for traditional medicine. Loquat fruit has been known to have high economic potential, but it has not been grown in mass plantation due to the difficulties to obtain good quality seedlings. In

the present time, the peasants in Kabanjahe only use wild type seedling that are grown close to trees due to the difficulty of germination of the seedlings. This study is conducted to reduce germination time in the production of good seedlings. It is also intended to introduce loquat to the farmers in order they are interested to domesticate loquat trees for mass plantation. As a final result, the program will provide

sufficient loquat plants to produce high quantity of good quality fruits, and the leaves are available in larger quantity to be used as raw material for anti-diabetic medicine. *In vitro* germination becomes a good strategy to overcome the problem in the germination to produce high quality of loquat seedlings. Propagation of loquat cultivar Mardan via *in vitro* cultures is the only report by Abbasi been available<sup>25</sup>. The aim of the study is to apply *in vitro* germination Loquat (*Eriobotrya japonica* Lindl.) by using growth stimulator, Gibberellic acid (GA<sub>3</sub>) and kinetin to obtain the best condition for germination and growth development of loquat plants. The optimum experimental condition is used as a model for the production of good quality of loquat seedlings.

## MATERIALS AND METHODS

Research procedures are consisted of sterilization of equipments, preparation of medium solution, *in vitro* germination, growth development of seedlings, and data collection are carried out as per reference.<sup>8, 22</sup> The research was conducted in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Indonesia. Loquat seeds were collected from farmer plantation that was grown in Kanbanjahe, Kabupaten Karo, North Sumatera, Indonesia.

### Sterilization of Equipments

Sterilization of the equipments was conducted by soaking them in Sun Light detergent, followed by rinsing with sterile water flow and keep to dry. They are then wrapped in aluminum foil and sterilized in hot air oven at 180° C for 2 hours. Glasswares are sterilized by using autoclave at 121 °C, 15 psi for 15 minutes. The laminar air flow cabinet (LAFB) was sterilized using UV light and alcohol (70%).

### Preparation of Medium Solution

The culture media used in this study was Murashige and Skoog (MS) medium,<sup>26</sup> containing sugar, of nutrients (macro, micro, and trace) supplemented with various combination concentrations of gibberellic acid (GA<sub>3</sub>) and kinetin.<sup>22</sup> The solution was prepared in sterile water and transferred into Erlenmeyer, and the stock solution was then stored in the fridge when not in used. The solution was adjusted to pH 5.8, and it was then sterilized at 121 °C, 15 lb for 20 minutes. A two complete factorial experiment with (0-500 mg/L) GA<sub>3</sub> and (0-12 mg/L) kinetin was conducted to study their effects on *in vitro* germination of anti-diabetic plant loquat (*Eriobotrya japonica* Lindl.). Variation composition combinations of GA<sub>3</sub> and Kinetin are presented in Table 1 with six replicates.

**Table 1**  
**Composition combination of gibberellic acid (GA<sub>3</sub>) and kinetin (K) supplemented into solution for *in vitro* germination of anti-diabetic plant Loquat (*Eriobotrya japonica* Lindl.).**

	Kinetin (K/mg/L)			
	0	1	2	3
Gibberellic acid (G/mg/L)	0 G0K0	G0K1	G0K2	G0K3
1	G1K0	G1K1	G1K2	G1K3
2	G2K0	G2K1	G2K2	G2K3

G0 = GA<sub>3</sub> 0.0 mg/L; G1 = GA<sub>3</sub> 250 mg/L; G2 = GA<sub>3</sub> 500 mg/L; K0 = Kin 0.0 mg/L; K1 = Kin 4.0 mg/L; K2 = Kin 8.0 mg/L; K3 = Kin 12.0 mg/L.

### *In vitro* Germination of Loquat

*In vitro* germination of loquat was carried out followed the procedure explained by Sinaga<sup>22</sup> and Koyuncu<sup>27</sup> with modification. Loquat seeds were selected, rinsed in flow water, and then was immersed in sodium hypochlorite NaOCl 2% for 5 minutes and rinsed again in aquadest. Repeated immersions into NaOCl 1% solution for 5 minutes were then conducted followed by rinsing with distilled water. The sterile seeds were then immersed in a medium solution containing of GA<sub>3</sub> for 24 hours, followed by plantation in the culture medium containing different concentrations of GA<sub>3</sub> and kinetin. The culture was incubated (25 ± 2°C) and regularly sprayed with alcohol 70% every day.

### Experimental Design and Data Analysis

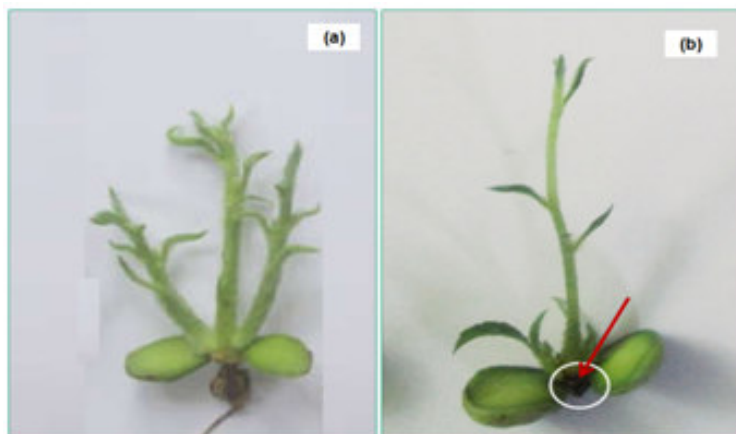
The experiments were carried out in a two factor randomized complete design with six replicates. Germination data for the length of germination time, the number of shoots, the height shoots, the weight of wet shoots, the weight of dry shoots, the number of leaves, the weight of wet sprout, the weight of dry sprout, the weight of wet roots and the weight of dry roots are recorded. The data analysis were conducted with

ANOVA at 5 significance level, and the means were compared by Duncan's multiple range analysis.

## RESULTS AND DISCUSSION

### Typical Germination of Loquat Seedling

Germination of loquat seeds to become sprouts has been observed, and growth development of the plant was monitored in medium culture to produce seedling. The germination was proceeded differently depends on the variation of experimental conditions, where growth stimulator of GA<sub>3</sub> and kinetin act to speed up the germination time and influenced the seedling viability. Typical germination was vary, some of them were grown in normal growth in which the plumula accompanied with green leaves with hypocotyls with normal roots are obtained (Figure 2a), and few was abnormal where the plant with unsymmetrical leaves, twisted plumula, hypocotyls, epicotyls was developed, or a plant with big cotyledon without root was observed as shown with the arrow in Figure 2b. Similar in the germination type with normal and abnormal growths were also reported in other plants by the influence of growth stimulator in culture medium.<sup>28</sup>



**Figure 2**  
**Germination growth of loquat seedling after 40 days: (a) normal growth with shoot, leaves and roots, and (b) abnormal growth containing shoot, leaves, and without root. The circle in the figure showed the root was not developed.**

It has been observed that variation of growth stimulator concentration in medium culture resulted in the variation of type of germination (Table 2). The results have shown that the GA<sub>3</sub>, kinetin, and the combination of GA<sub>3</sub> and kinetin did not influence the germination viability of loquat seed. The seed in culture without GA<sub>3</sub> and kinetin are able to germinate (G0K0 66, 66%). Some cultures containing of GA<sub>3</sub> and kinetin are successfully germinated the seeds (100%), those are in G1K0, G1K1, G2K1, and G2K2. However, at a very high concentration of 12.0 mg/L kinetin in the medium as in G0K3 was found not improve the germination viability of loquat seed, but the kinetin speed up the germination time of the seed become 14.66 days compare to 32 days for

control (G0K0). This result is similar to that reported for barley and lettuce seeds where at high concentration of kinetin will slow down the germination time, and it would be overcome by the addition of GA<sub>3</sub>.<sup>29</sup> The effect of GA<sub>3</sub> and kinetin interactions on to the germination time has also studied. The time for germination was calculated start from the day of plantation until the germ comes out from the seed as required by International Seed Testing Association (ISTA).<sup>30</sup> The results showed that variation in the germination time are significantly influenced by the variation in the concentration of GA<sub>3</sub> and kinetin in the medium culture. The seed viability and germination time of loquat seeds under the variation of growth stimulator are summarized in Table 2.

**Table 2**  
**The effect of GA<sub>3</sub>, kinetin and the interactions on the germination of Loquat (*Eriobotrya japonica* Lindl.)**

Experimental Treatments	Seed Viability (%)	Germination Time (Day)
G0K0	66.66	32.00 <sup>d</sup>
G0K1	33.33	20.00 <sup>a</sup>
G0K2	33.33	13.33 <sup>a</sup>
G0K3	66.66	14.66 <sup>a</sup>
G1K0	100.00	18.00 <sup>a</sup>
G1K1	100.00	15.66 <sup>a</sup>
G1K2	66.66	14.66 <sup>a</sup>
G1K3	66.66	19.33 <sup>a</sup>
G2K0	66.66	12.66 <sup>a</sup>
G2K1	100.00	13.33 <sup>a</sup>
G2K2	100.00	12.00 <sup>a</sup>
G2K3	66.66	15.00 <sup>a</sup>
Average	<b>83.33</b>	<b>15.80<sup>a</sup></b>

Data shown are mean of six experiments, followed by notation letter are significant according to Duncan's multiple range analysis ( $P = 0.05$ ).

The cultures containing GA<sub>3</sub>, kinetin and the interactions are observed to have shorter germination time (12-20 days) compare to that in the control without growth stimulator (32 days). Increasing the concentration of GA<sub>3</sub> resulted in the shorter germination time for loquat seeds. The cultures containing of high concentration of 500 mg/L GA<sub>3</sub> are having very short seed germination time. It appears that the concentration of 500 mg/L GA<sub>3</sub> and 8 mg/L kinetin (G2K2) is the best condition for fast germination time. Furthermore, increasing the concentration of kinetin resulted in the shorter of

germination time until optimum at 8 mg/L kinetin. However, at a very high concentration of 12 mg/L kinetin did not shortened the germination time, where the germination time was observed longer than the culture containing 8 mg/L kinetin. Similar result has been observed that by using high concentration of 500 mg/L GA<sub>3</sub> results in very short germination time for apple seed.<sup>31</sup> The results is in agreed with those applied for many types of plants where the concentration of growth stimulator such as GA<sub>3</sub> is a key factor to induce germination.<sup>32</sup> The interaction of GA<sub>3</sub> and kinetin at

optimum condition shorten the germination time as observed for barley seed.<sup>33</sup>

### Growth of Shoot and Leaves

The growth of shoot has been studied under the influence of variation combination in the concentration of growth stimulator. The influence of supplementation of GA<sub>3</sub> and kinetin in the culture medium on to the growth and development of shoots and leaves of loquat plants have been observed. The parameters in the number of shoots, the height shoots, the weight of wet shoots, the weight of dry shoots, and the number of leaves in respect to the variation concentration of GA<sub>3</sub> and kinetin and the interaction are summarized in Table 3. The results showed that shoots and leaves are obtained in all experiments in the absence and in the presence of GA<sub>3</sub> and kinetin. The number of shoots and leaves are vary in culture medium. The highest number of shoots was observed n the medium culture containing of 250 mg/L GA<sub>3</sub> and 12 mg/L kinetin (G1K3) with 4 shoots. Very

high number of leaves are grown in G0K3 with 12 mg/L kinetin without GA<sub>3</sub>. The best condition for the growth of leaves are observed in G0K3 (12.66 leaves) with very high concentration of 12 mg/L kinetin, followed by G1K1 (12.33) that was supplemented 1 mg/L GA<sub>3</sub> and 4 mg/L kinetin, and G0K2 (11.66) with 8 mg/L kinetin in the absence of GA<sub>3</sub>. There is no consistent trends are observed in the growth development of shoots and leaves under the variation in the concentration of GA<sub>3</sub> and kinetin in culture medium. It was different with loquat plant that formation of shoots and leaves are observed in all culture in the absence and in the presence plant hormone. The kinetin stimulate the formation of leaves of loquat plant. This results was similar to that observed for black pepper.<sup>33</sup> It has been reported that GA<sub>3</sub>, cytokinin and auxin are able to stimulate formation of shoots and leaves due to its ability to activate the induction of proteolysis enzyme to produce tryptophan to be known as a precursor of auxin that potential to the formation of shoots and leaves.<sup>34</sup>

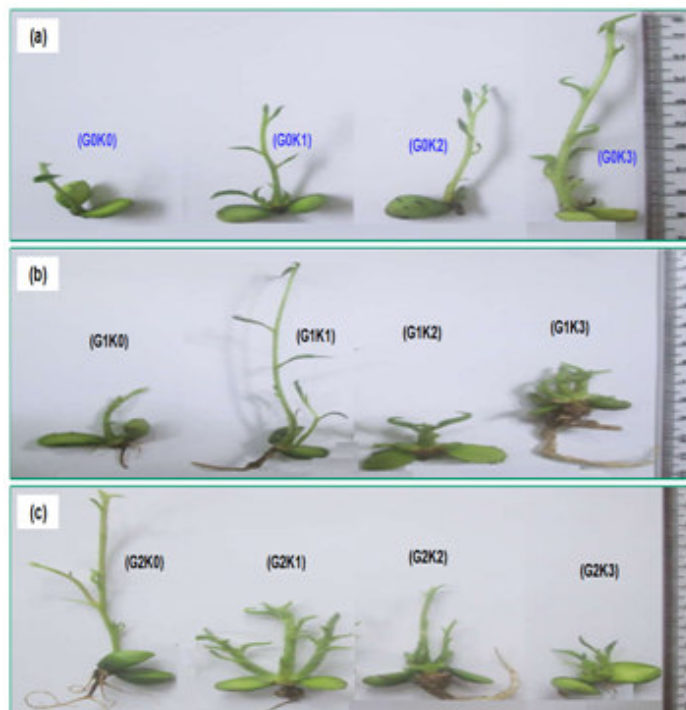
**Table 3**  
**The effect of GA<sub>3</sub> and kinetin interactions on growth and development of shoots and leaves of loquat (*Eriobotrya japonica* Lindl.).**

Experimental Treatments	Number of Shoot	Height of Shoot (Cm)	Number of Leaves
G0K0	3.00	2.53	9.66
G0K1	2.33	4.60	10.66
G0K2	1.00	4.10	11.66
G0K3	3.66	6.20	12.66
G1K0	2.00	4.93	7.00
G1K1	3.00	2.26	12.33
G1K2	3.00	2.46	9.33
G1K3	4.00	2.30	8.00
G2K0	2.66	3.96	7.33
G2K1	3.66	2.80	11.33
G2K2	2.33	2.66	7.66
G2K3	2.66	1.60	5.66
<b>Average</b>	<b>2.91</b>	<b>2.87</b>	<b>8.58</b>

*Data shown are mean of six experiments.*

Growth development of shoots under the influence of growth stimulator in the medium culture has also been studied as summarized in Table 3. Addition of GA<sub>3</sub> and kinetin did not influence the height of the shoots. The development of the shoots has been observed after 60 days as shown in Figure 3. The results in Figure 3a demonstrated the effect of variation concentration of kinetin alone on to the height of the shoots. It appears that increasing the concentration of kinetin in the medium culture resulted in the taller of shoots obtained. The longest shoots is obtained in experimental condition

of G0K3 (6.20 cm) in the medium contains 500 mg/L kinetin without GA<sub>3</sub>. The effect of combination of low concentration of 250 mg/L GA<sub>3</sub> with the variation concentration of the kinetin is proven in Figure 3b. There are no increasing trends in the height of shoots obtained by the variation in the concentration of kinetin in the medium. Medium culture containing of 250 mg/L GA<sub>3</sub> and 4.0 mg/L (G1K1) produce the longest shoots. However, when increasing the concentration of the kinetin did not improve the growth development of shoots.



**Figure 3**

**The growth and development of shoots of loquat seedling under the influence of growth stimulator: (a) The shoots with variation of kinetin and in the absence of GA<sub>3</sub>, (b) The shoots at medium concentration of 250 mg/L GA<sub>3</sub> with variation of kinetin, (c) The shoots with high concentration of 500 mg/L GA<sub>3</sub> combine with variation in the concentration kinetin.**

Furthermore, growth development of shoots at a very high concentration of 500 mg/L GA<sub>3</sub> combine with variation the concentration of kinetin has also been investigated, and the results is showed in Figure 3c. The interaction of GA<sub>3</sub> and kinetin was found influence the length of shoots. It is revealed that increasing the concentration of kinetin (at very high concentration of GA<sub>3</sub>) was resulted in decreasing the height of shoots. Therefore, the concentration of GA<sub>3</sub> has to be controlled to obtain the best result in the growth development of shoots. Kinetin alone play its role in the growth development of shoots and it also act to stimulate the growth of roots. However, when the GA<sub>3</sub> are mixed with kinetin, there is antagonist role been the two are observed, where the higher the concentration of the kinetin, the shorter the shoots are obtained. The results is in agree with the study done for crop plants where the activity of kinetin has been transformed to the development of roots instead of the growth development of shoots.<sup>33</sup>

#### **Effect of Growth Stimulator on the Weigh of Sprout**

The weight of sprout of loquat resulted from *in vitro* development of the plant was calculated for both wet sprout and dry sprout as summarized in Table 4. The results have showed that supplementation of GA<sub>3</sub> and kinetin or their interactions has no significant effect on to the development of sprout. The heaviest wet sprout was

obtained in G0K3 (1056.66 mg) that was the experiment treatment using high concentration of 12 mg/L kinetin in the absence of GA<sub>3</sub>. The higher the concentration of kinetin without GA<sub>3</sub> increases the weight of wet sprout. When the concentration GA<sub>3</sub> added into the mixture, the weight of fresh sprout has also improve as improving the concentration of kinetin. However addition of very high concentration of GA<sub>3</sub> did not improve the weight of sprout with increasing the concentration of kinetin. Kinetin plays an important role on the multiplication of loquat cell.<sup>36</sup> The weight of dry sprout is used as a parameter for the growth development of plants,<sup>37</sup> and therefore it was also observed in this study. It was obtained that supplementation of GA<sub>3</sub> and kinetin or their interactions has no significant effect on to the weight of dry sprout. The heaviest dry sprout was obtained in G0K2 (153.91 mg) by using 8 mg/L kinetin in the absence of GA<sub>3</sub>. The growth development of culture has also obtained in the absence of growth stimulator as in G0K0 (137.76 mg), however the weight of dry sprout was lower compare to those culture with addition of the GA<sub>3</sub> and kinetin. This results demonstrated that growth stimulator influence the growth intensity of shoots. Similar to that wet- dry sprout, the growth development of the loquat plant is effected by the supplementation of GA<sub>3</sub> and the kinetin that can stimulate the cell multiplication that resulted in the weight of the plant.<sup>38</sup>

**Table 4**  
**The Effect of GA<sub>3</sub> and kinetin interactions on growth and development of shoots and leaves of Loquat (*Eriobotrya japonica* Lindl.).**

Experimental Treatments	Weight of Fresh Sprout (mg)	Weight of Dry Sprout (mg)	Weight Proportion of Dry to Fresh Sprout (%)
G0K0	646.66	137.76	21
G0K1	736.66	118.33	16
G0K2	906.66	153.91	17
G0K3	1056.66	121.80	12
G1K0	643.33	105.35	16
G1K1	790.00	108.45	14
G1K2	933.33	133.78	14
G1K3	933.33	126.06	14
G2K0	913.33	144.56	16
G2K1	920.00	113.13	12
G2K2	813.33	105.81	13
G2K3	650.00	89.30	14
<b>Average</b>	<b>824.58</b>	<b>115.81</b>	<b>14</b>

Data shown are mean of six experiments followed by notation letter are significant according to Duncan's multiple range analysis (P = 0.05).

#### Growth Development of Roots

The quality of root is used as indicator for the successful growth and development of the plant.<sup>39</sup> The effect of GA<sub>3</sub> and the kinetin supplemented in the culture media onto the development of roots of loquat plant has been investigated based on the weight of wet roots and the weight of dry roots as summarized in Table 5. The results have shown that the presence of GA<sub>3</sub> has significant effect on the growth development of the root. The weight of roots in the culture supplemented with high concentration of (250 and 500 mg/L) GA<sub>3</sub> was found different to those obtained in the standard (G0K0) without GA<sub>3</sub>. The heaviest roots was obtained in G1K1 (130.00 mg) by using 250 mg/L GA<sub>3</sub> and 4 mg/L kinetin. Multiplication of the root cell was found very intense in this condition, it was almost ten time compare to control G0K0 (13.80 mg). When the concentration of kinetin was improved, the weight of root was also improved until it was optimum at 8 mg/L kinetin. The weight of root decreases when the concentration of kinetin was added at 12 mg/L kinetin. This results was similar to that observed in *Phaseolus vulgaris* (Dwarf bean) plant<sup>39</sup> that obtained optimum condition on the multiplication of root

when using 0.5-10 mg/L kinetin, and slow down root development at very high concentration of the kinetin.<sup>40</sup> The weight of dry roots has also been measured for all experiment treatments condition as summarized in Table 5. The results showed that the heaviest dry root is obtained in G1K1 (12.13 mg), it is the same condition for the heavy wet roots. Improvement of dry ash was observed when using very high concentration of 500 mg/L GA<sub>3</sub> when it was combined with kinetin. In this condition, the higher the concentration of kinetin supplemented in the medium containing 500 mg/L GA<sub>3</sub> results in the higher weight of dry roots until optimum, followed by declining the weight of dry roots when using very high concentration of kinetin. All experiments with kinetin are having heavier dry roots compare to control G0K0 (0.12 mg). The combination of GA<sub>3</sub> and kinetin in culture medium contributed to the development of root in loquat plant. This results supported by previous study where GA<sub>3</sub> has important role in the development of roots due to its contribution to activate the enzyme in the metabolism of amylase, lipase and protease in the seed those are needed in the biosynthesis of plant cell.<sup>37</sup>

**Table 5**  
**The Effect of GA<sub>3</sub> and kinetin interactions on growth and development of roots Loquat (*Eriobotrya japonica* Lindl.).**

Experimental Treatments	Weight of Fresh Roots (mg)	Weight of Dry Roots (mg)	Weight Proportion of Dry to Fresh Roots (%)
G0K0	13.80 <sup>ab</sup>	0.12	0.87
G0K1	13.33 <sup>ab</sup>	1.16	8.70
G0K2	10.00 <sup>ab</sup>	2	20.00
G0K3	0	0	0
G1K0	53.33 <sup>bc</sup>	5.46	10.24
G1K1	130.00 <sup>c</sup>	12.13	9.33
G1K2	10.66 <sup>ab</sup>	1.86	17.45
G1K3	23.66 <sup>ab</sup>	7.53	31.83
G2K0	30.00 <sup>ab</sup>	4.76	15.87
G2K1	63.33 <sup>bc</sup>	9.83	15.52
G2K2	116.66 <sup>c</sup>	9.96	8.54
G2K3	9.33 <sup>ab</sup>	2.56	27.44
<b>Average</b>	<b>54.62<sup>ab</sup></b>	<b>6.76</b>	<b>12.38</b>

Data shown are mean of six experiments followed by notation letter are significant according to Duncan's multiple range analysis (P = 0.05).

A better understanding of germination and seedling establishment for loquat plant has been developed. The effect of GA3 and kinetin in the media on seed germination and subsequent seedling growth has been examined. The use of GA3 has been demonstrated to produce high germination rate and good seedling morphology for *Gentiana lutea* L. var. *aurantiaca*.<sup>42</sup> It has been showed in the study that GA3 and kinetin regulated the growth and multiplication of plant organs.<sup>43</sup> The role of phytohormone such as gibberellic acid in the stimulation of seed germination, stem elongation, meristematic tissue development and differentiation of floral organs has been documented as a trigger transitions from meristem to shoot growth, juvenile to adult leaf stage.<sup>43</sup> The results for loquat plant has revealed that the combination GA3 and kinetin plays an important role in the development of shoot and root. The GA3 is required to break seed dormancy leading to its germination. This study observed for *Arabidopsis thaliana* has proven that gibberellic acid and abscisic acid controlled and promote seed germination.<sup>44</sup> The combination of two hormones change the environmental conditions encountered by the seed in the germination process. The results showed that dry weight showed synergism with fresh weight. The GA3 and kinetin improve the weight of culture that contributed to dry weight accumulation.<sup>45</sup> Increases in GA3 and kinetin levels lead to increases in the numbers of leaves and shoots, and in the rooting percentage by triggering cell division and elongation.<sup>46</sup> It revealed that phytohormone of GA3 and kinetin plays an important role in the growth development of loquat plant because they are able to stimulate cell division to produce normal plant.

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## CONCLUSION

In vitro germination of anti-diabetic plant of Loquat (*Eriobotrya japonica* Lindl.) has successfully conducted to produce good seedling. The study has contributed to the role of phytohormone of gibberellic acid and kinetin to the germination and seedling establishment for loquat plant. The concentration of GA3 and kinetin has been formulated to obtain optimum condition in the production of loquat seedling. It has revealed that combination concentration of GA3 and kinetin at optimum condition stimulate seed germination, stem elongation, and meristematic tissue development to become shoot, leaves and roots. The concentrations of GA3, kinetin and its combination showed significant effect to the length of germination time, weight of cultures, number of roots and shoots. The best condition was obtained when using combination of 500 mg/L GA3 and 8 mg/L kinetin, followed by 250 mg/L GA3 and 4 mg/L kinetin where all produce high quality loquat seedling containing shoots, green leaves and roots. Supplementation of high concentration of 500 mg/L GA3 is effective to improve the growth of root of loquat (*Eriobotrya japonica* Lindl.).

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## CONFLICT OF INTEREST

Conflict of interest declared none.

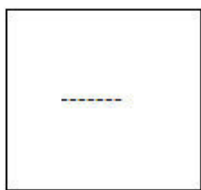
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