The Usage of Gibberellin-Rich Seed-Waste for Vegetable Growth Enhancement: A Case Study of Rambutan Seed

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Abstract

This research aims to study the feasibility of plant growth enhancement by Extracted Gibberellic Compounds (EGCs) of rambutan seed-waste, with 3 extracted solvents: 80% methanol, 80% ethanol and rice whisky, and at the considered ratios (wt./vol.) of rambutan seeds (grams) and extracted solvent (milliliters) of 1:1, 1:2 and 1:3. The growth enhancement was identified through the Lettuce Hypocotyl Bioassays (LHB) method. Among the 5 species of lettuce (Grand rapids, Red cos, Red oak, Red coral, and green oak) for bioassays, the Green Oak lettuce was finally determined to be the representative lettuce for LHB. The 2.1 % of diluted EGC (methanol solvent, ratio 1:1) performed better as a growth enhancer than the other EGAs. Moreover, the same EGC type revealed a non-significant difference of growth enhancement with standard GA_3 at 0.1 ppm. ($p > 0.05$). Therefore, the 2.1% EGC (methanol, 1:1) had gibberellic acid equivalent (GAE) to 0.1 ppm standard GA_3. In application to Water Morning Glory growth enhancement, the introduction of EGC (Methanol,1:1) with the dilution ratio 1:1 with water performed significantly different ($p<0.05$) in height and no-significant difference ($p>0.05$) in fresh weight with the control (water).

Keywords*:* Extracted Gibberellic Compounds(EGCs)/Rambutan seed/Gibberellic acid equivalent/ Lettuce Hypocotyl Bioassays /Water Morning Glory

1. Introduction

Currently, land is becoming a more and scarcer resource, particularly for agricultural production, therefore, competition for available land for different uses is creating more conflicts and complexities (FAO, 1997). As of now, the farming systems development (FSD) is considered to be a potential strategy for improving food security. In particular, the usage of plant growth regulators (PGRs), defined as "a substance used for controlling or modifying plant growth processes without appreciable phytotoxic effects at the dosage applied" (Lessenger, 2006; Moore, 1998). PGRs are mainly composed of variations of gibberellic acid (GAs), ethylene, auxin, abscisic acid, gibberellins, cytokinins, jasmonates, brassinosteroids and salicylates. But the most widely used is gibberellic acid (GA_3). However, it has to be extracted from cultured fungi, which consequently makes it quite expensive. So, the local farmer and gardener cannot access this improvement for their food production system. However, several studies have reported the gibberellic-like substances (GLS) are available in seeds, tips and roots at various amounts and growing stages (Ortega- Baesa, 2007; Çetinbaş, 2006; Niran, 1993; Bachelard, 1968).

Rambutan (*Nephelium lappaceum Linn*.) is a seasonal fruit native to Southeast Asian countries. Thailand has become the leading producer and exporter of Rambutan. This fruit is generally consumed fresh, and sometimes is industrially processed to obtain juice, jams, jellies and marmalades, as well as with a chunk of pineapple and canned in syrup. In the preparation process, the rambutan fruits are deseeded during processing and these seeds (~ 4-9 g/100 g) are considered as a waste by-product (Wannee, 2011). Furthermore, there are reports that plants in the *Sapindaceae* family and related genus, such as rambutan and longan seeds, possess a relatively high amount of GLS (Sunee & Aussanee, 2009). However, whether or not this GLS will be available for local usage depends on the extraction process, which needs locally available solvents to become a viable option. Thus, this study will consider edible ethanol (40 degree rice whisky) in association with such novel solvents as methanol, ethanol (Jones,1968; Asen,1960) and investigate whether these EGCs or GLS from rambutan seeds with the aforementioned solvents can enhance plants and vegetables growth or not. Hence, in the study it was applied to water morning glory. Can it enhance the growth and quality of the product such as: uniform length, fleshy weight, as well as reducing the harvesting time?

2. Methodology

To approach the research questions, whether the gibberellin-rich seed waste is feasible for plant and vegetable growth enhancement or not, three main objectives were setup as illustrated by the conceptual framework in Fig. 1. They include i) the effectiveness of ECGs from Rambutan seeds when compared with standard gibberellic acid, ii) the growth enhancement of plants and such as as water morning glory (WMG).

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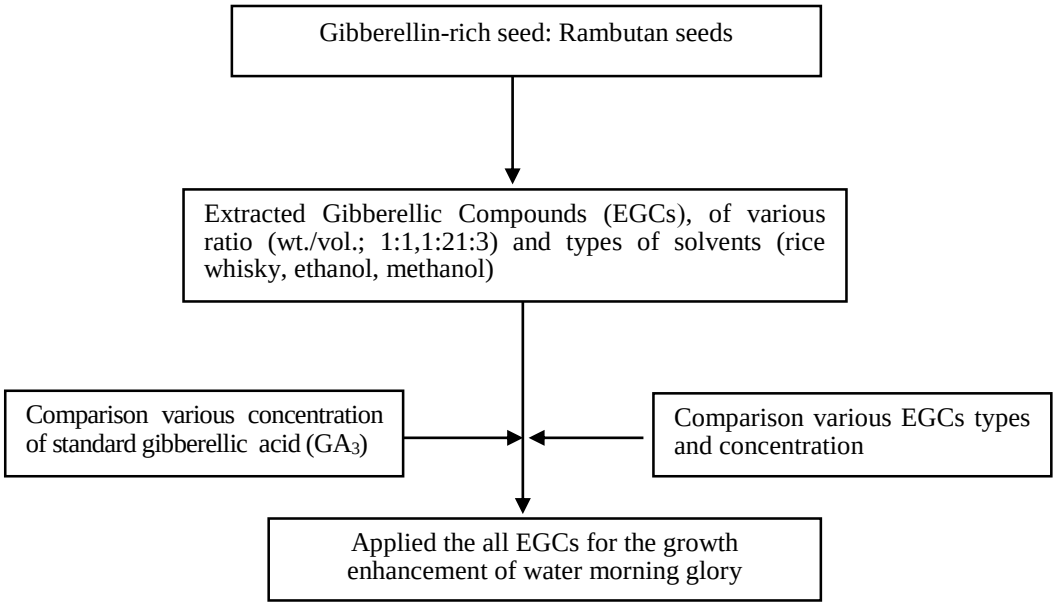

Figure 1: Diagram of study

2.1The preparation of extracted gibberellic compounds (EGCs) from rambutan seed

To investigate the sensitivity of EGCs from various types, the technique of Lettuce Hypocotyl Bioassays (LHB) (Kaewladdakorn, 2002, 2003; Donald, 1981; Rai,1976; Fletcher, 1966; Frankland, 1960) was applied as follows.

2.1.1 Rambutan seed extracts and standard GA_3 preparation

The procedure was conducted by putting 200 grams of well-washed rambutan seeds into a blender. Then the various solvents were poured (in milliliters) into the blender at the studied ratios 1:1(a_1), 1:2 (a_2) and 1:3 (a_3). For example, 1:1 was composed of 200 gram of rambutan seeds and solvents of 200 ml. The studied solvents were 80% rice whisky (b_1), 80% ethanol (b_2) and 80% methanol (b_3). Hence, there were 9 types of EGC as shown in Table 1. Then the mixture was blended until it had a homogeneous performance (mix approximately 5 minutes), and poured into a light brown bottle that was then closed with a lid. The bottle was kept at 4 °C for 18 hours. After that, the bottle was moved to be kept at room temperature for 6 hours.

Table 1: Types of EGC

Ratios of rambutan seed and solvents	**Solvent types**		
	80% Rice whisky (b_1)	**80% Ethanol (b_2)**	**80% Methanol(b_3)**
1:1 (a_1)	a_1, b_1	a_1, b_2	a_1, b_3
1:2 (a_2)	a_2, b_1	a_2, b_2	a_2, b_3
1:3 (a_3)	a_3, b_1	a_3, b_2	a_3, b_3

Remarks: a_1b_1 refers to the ratios of Rambutan seeds (grams) and solvent (milliliters) at 1:1 in rice

Finally, a centrifuge was used to separate the solution. The obtained solution (as much as possible) was separated and moved to a rotary evaporator at 39 °C (to eliminate the excess alcohol) until it produced a dense fluid (approximately less than 1 ml. remained, but lower and higher volumes remained, which resulted in EGC concentration ratios which effected the comparison growth study. All samples were then adjusted to be 1 ml. by water. The 1 ml obtained here were the stock of various types of EGA for the study. Therefore, many extraction samples are needed to get enough EGCs for the experiment.

2.1.2 Various EGC concentrations

Each stock EGCs type (see 2.1.1) was diluted into 17 concentrations as seen in Table 2. Example 0.5% EGC (C_1 type) is composed of such stocked EGCs 10 µl and water 1990 µl, or 0.6% EGC (C_2 type) is composed of such stocked EGCs 12 µl and water 1988 µl). Totally 153 types were arranged for the study.

2.1.3 Standard GA_3 preparation

The commercial Gibberellic acid (GA_3) at concentration of 90.61% (wt. /wt., BDH Chemical LtD.) was calculated to be 1.11 gm and was weighed and dissolved with 1000 ml of distilled water, for 1000 ppm concentration. Then, it was further diluted to various concentrations of 10 ppm (1 ml of stocked GA_3 and distilled water 100 ml), 1 ppm (0.1 ml of stocked GA_3 and distilled water 100 ml), 0.1 ppm (0.01 ml of stocked GA_3 and distilled water 100 ml), 0.01ppm (0.001 ml of stocked GA_3 and distilled water 100 ml), and 0.001 ppm (0.0001 ml of stocked GA_3 and distilled water 100 ml).

Table 2: Attributes of 153 types of PGRs

Adjusted EGCs %	Extracted Gibberellic Compounds (EGCs) solutions								
	a_1 b_1	**a_2 b_1**	**a_3 b_1**	**a_1 b_2**	**a_2 b_2**	**a_3 b_2**	**a_1 b_3**	**a_2 b_3**	**a_3 b_3**
0.5% (c_1)	a_1 b_1c_1	a_2 b_1 c_1	a_3 b_1 c_1	a_1 b_2 c_1	a_2 b_2 c_1	a_3 b_2 c_1	a_1 b_3 c_1	a_2 b_3 c_1	a_3 b_3 c_1
0.6% (c_2)	a_1 b_1 c_2	a_2 b_1 c_2	a_3 b_1 c_2	a_1 b_2 c_2	a_2 b_2 c_2	a_3 b_2 c_2	a_1 b_3 c_2	a_2 b_3 c_2	a_3 b_3 c_2
0.7% (c_3)	a_1 b_1 c_3	a_2 b_1 c_3	a_3 b_1 c_3	a_1 b_2 c_3	a_2 b_2 c_3	a_3 b_2 c_3	a_1 b_3 c_3	a_2 b_3 c_3	a_3 b_3 c_3
0.8% (c_4)	a_1 b_1 c_4	a_2 b_1 c_4	a_3 b_1 c_4	a_1 b_2 c_4	a_2 b_2 c_4	a_3 b_2 c_4	a_1 b_3 c_4	a_2 b_3 c_4	a_3 b_3 c_4
0.9% (c_5)	a_1 b_1 c_5	a_2 b_1 c_5	a_3 b_1 c_5	a_1 b_2 c_5	a_2 b_2 c_5	a_3 b_2 c_5	a_1 b_3 c_5	a_2 b_3 c_5	a_3 b_3 c_5
1.0% (c_6)	a_1 b_1 c_6	a_2 b_1 c_6	a_3 b_1 c_6	a_1 b_2 c_6	a_2 b_2 c_6	a_3 b_2 c_6	a_1 b_3 c_6	a_2 b_3 c_6	a_3 b_3 c_6
1.1% (c_7)	a_1 b_1 c_7	a_2 b_1 c_7	a_3 b_1 c_7	a_1 b_2 c_7	a_2 b_2 c_7	a_3 b_2 c_7	a_1 b_3 c_7	a_2 b_3 c_7	a_3 b_3 c_7
1.2% (c_8)	a_1 b_1 c_8	a_2 b_1 c_8	a_3 b_1 c_8	a_1 b_2 c_8	a_2 b_2 c_8	a_3 b_2 c_8	a_1 b_3 c_8	a_2 b_3 c_8	a_3 b_3 c_8
1.3% (c_9)	a_1 b_1 c_9	a_2 b_1 c_9	a_3 b_1 c_9	a_1 b_2 c_9	a_2 b_2 c_9	a_3 b_2 c_9	a_1 b_3 c_9	a_2 b_3 c_9	a_3 b_3 c_9
1.4% (c_{10})	a_1 b_1 c_{10}	a_2 b_1 c_{10}	a_3 b_1 c_{10}	a_1 b_2 c_{10}	a_2 b_2 c_{10}	a_3 b_2 c_{10}	a_1 b_3 c_{10}	a_2 b_3 c_{10}	a_3 b_3 c_{10}
1.5% (c_{11})	a_1 b_1 c_{11}	a_2 b_1 c_{11}	a_3 b_1 c_{11}	a_1 b_2 c_{11}	a_2 b_2 c_{11}	a_3 b_2 c_{11}	a_1 b_3 c_{11}	a_2 b_3 c_{11}	a_3 b_3 c_{11}
1.6% (c_{12})	a_1 b_1 c_{12}	a_2 b_1 c_{12}	a_3 b_1 c_{12}	a_1 b_2 c_{12}	a_2 b_2 c_{12}	a_3 b_2 c_{12}	a_1 b_3 c_{12}	a_2 b_3 c_{12}	a_3 b_3 c_{12}
1.7% (c_{13})	a_1 b_1 c_{13}	a_2 b_1 c_{13}	a_3 b_1 c_{13}	a_1 b_2 c_{13}	a_2 b_2 c_{13}	a_3 b_2 c_{13}	a_1 b_3 c_{13}	a_2 b_3 c_{13}	a_3 b_3 c_{13}
1.8% (c_{14})	a_1 b_1 c_{14}	a_2 b_1 c_{14}	a_3 b_1 c_{14}	a_1 b_2 c_{14}	a_2 b_2 c_{14}	a_3 b_2 c_{14}	a_1 b_3 c_{14}	a_2 b_3 c_{14}	a_3 b_3 c_{14}
1.9% (c_{15})	a_1 b_1 c_{15}	a_2 b_1 c_{15}	a_3 b_1 c_{15}	a_1 b_2 c_{15}	a_2 b_2 c_{15}	a_3 b_2 c_{15}	a_1 b_3 c_{15}	a_2 b_3 c_{15}	a_3 b_3 c_{15}
2.0% (c_{16})	a_1 b_1 c_{16}	a_2 b_1 c_{16}	a_3 b_1 c_{16}	a_1 b_2 c_{16}	a_2 b_2 c_{16}	a_3 b_2 c_{16}	a_1 b_3 c_{16}	a_2 b_3 c_{16}	a_3 b_3 c_{16}
2.1% (c_{17})	a_1 b_1 c_{17}	a_2 b_1 c_{17}	a_3 b_1 c_{17}	a_1 b_2 c_{17}	a_2 b_2 c_{17}	a_3 b_2 c_{17}	a_1 b_3 c_{17}	a_2 b_3 c_{17}	a_3 b_3 c_{17}

Remark: $a_1b_1c_1$ means ratios of Rambutan seeds (grams): solvent (milliliters) = 1:1, with rice whisky and adjusted concentration to be 0.5% with water

2.2 Analysis of available GLS in such EGCs

The stock EGC was analyzed for available gibberellin by the HPLC system (the reversed–phase C_{18} column µ Bondapak 10 µm (3.9×300 mm, mobile phase of 35% methanol, flow rate 1.2 ml/min, detector wavelength 206 nm, injection volume 20 µl, running time 10 mins.) (Lee, 1998) in comparison with standard GA_3.

2.3 Sensitivity test by Lettuce Hypocotyl Bioassays (LHB) method

The study outlined the many biological tests that are used as quantitative assays for gibberellins (Fletcher, 1966). The lettuce hypocotyl test was the simplest to carry out, but the lowest concentration that can be reliably assayed was only 0.1 mg/1. The applied procedure in this study was conducted by soaking the lettuce seeds (included 5 species of lettuce: Grand rapids, Red cos, Red oak, Red coral and Green oak) in the 10% sodium hypochlorite for 15 minutes and then washing them with the distilled water 3 times (Waycott, 1991; Donald,1981). After that, they were laid out separately and completely covered by wet filter paper (Whatman No. 1) in a petri dish (Wet germination tray, water-saturated filter paper). They were then placed in a dark growth chamber at 28±2 °C for 48 hours. Next, a piece of filter paper with existing lettuce (of 3 mm. hypocotyl in length) was selected and cut out, transferred into a cultivation tray. Then 2 ml of standard GA_3 (1 ppm) was dropped on 500 seeds of each species. Next, the cultivation box was closed and placed in a growth chamber at a constant 28±2 °C. with a fluorescent light at 3,000 lux. The length of hypocotyls was measured after 72 hours.

2.4 Selection the most sensitive lettuces

The same procedure as above (Waycott, 1991; Donald, 1981) was applied to the 2 most sensitive of the lettuce species. (These were found to be Red cos and Green oak). The experiments were separated into 2 steps. The first step was to check the growth of lettuce at the considered concentrations of standard GA_3. Where it's growth performance could be used to determine the gibberellic acid equivalent (GAE) of such EGCs types. The study was conducted by dropping 2 ml of the various concentrations of standard GA_3 (0.000 – 1.000 ppm) on each 100 Red cos and Green oak. Next, the hypocotyl length was measured at 72 hours. The species with best variation in growth performance (this being Green oak) was selected for a further step. In the second step, 2 ml of each arranged EGCs ($a_nb_nc_n$) with various concentration (0.5–2.1 %) was dropped and measured the hypocotyl length at 72 hours. It was noticed that the introduction of EGCs exceeding 2.1 percent on the studied lettuces, showed deterioration of the structure, and finally death. 30 Lettuce sprouts, with 3 replications, had tests conducted on them for each type of EGA. These studied lettuce sprouts were cultivated at 28±2 °C in a growth chamber with the fluorescent light at 3,000 lux. The outcome of the growth was recorded by measuring the length of the hypocotyls of the lettuce sprouts at 72 hours or 3 days.

2.5 Growth enhancement of water morning glory

1)The various treatments of EGCs

To determine the most suitable EGC types and dilutions for growth enhancement of the water morning glory (WMG) was selected as the best representative of vegetables. The dilution ratios of EGCs (a_nb_n) with water were considered at 1:1 (d_1), 1:2 (d_2), and 1:4 (d_3). Therefore, 27 treatments were carried out as shown in Table 3.

Table 3: The EGC type and dilution treatments

Various Types of EGC	Ratios of EGC to water		
	1:1 (d_1)	**1:2 (d_2)**	**1:4 (d_3)**
a_1b_1	$a_1b_1d_1$	$a_1b_1d_2$	$a_1b_1d_3$
a_2b_1	$a_2b_1d_1$	$a_2b_1d_2$	$a_2b_1d_3$
a_3b_1	$a_3b_1d_1$	$a_3b_1d_2$	$a_3b_1d_3$
a_1b_2	$a_1b_2d_1$	$a_1b_2d_2$	$a_1b_2d_3$
a_2b_2	$a_2b_2d_1$	$a_2b_2d_2$	$a_2b_2d_3$
a_3b_2	$a_3b_2d_1$	$a_3b_2d_2$	$a_3b_2d_3$
a_1b_3	$a_1b_3d_1$	$a_1b_3d_2$	$a_1b_3d_3$
a_2b_3	$a_2b_3d_1$	$a_2b_3d_2$	$a_2b_3d_3$
a_3b_3	$a_3b_3d_1$	$a_3b_3d_2$	$a_3b_3d_3$

Note: $a_1b_1d_1$ refers to the treatment ratios of Rambutan seeds (grams): solvent (milliliters) = 1:1 with rice whisky (milliliters), and diluted with water (milliliters) to ratio 1:1.

2) Growth enhancement determination by using water morning glory

The procedure to determine the growth enhancement was conducted with the Kamchai (1993) methodology**.** A set of pots (11 x 14 niches) with saturated wet media composed of soil: husk: manure: ash and coconut dust 0.5: 2 : 0.5 : 2 : 2) were arranged. This set was composed of 81 pots (27 EGC solutions in Table 3 and 3 replications). Then an amount (50-60) of WMG seeds (Chia-Tai Trade mark) were scattered to cover the whole surface media in pot. Each pot was sprayed with 1 liter of water and 0.05% urea solution at daily intervals. On the 6th day, after the unhealthy sprouts were eliminated and only 40 sprouts were left for one pot, 100 ml of diluted EGC was sprayed at various considered ratios as 1:1 (50 ml EGC with 50 ml water), 1:2 (33.3 ml EGC with 66.6 ml water) and 1:4 (25 EGC with 75 ml water). And on the 9th and 12th day, 100 ml of the same dilution of EGC was repeatedly sprayed into the same pot again. The height of the WMG seedling was measured every 3 days (in the morning) until the harvest day or 30 days. The fresh weight after harvesting was recorded.

3.Results

The results to fulfill the research questions were found step-by-step as follows.

3.1 The available of gibberelin-like substances (GLS) in rambutan seeds

The results showed that there was no detected peak of GA_3 in all EGCs methanol, ethanol and rice whisky). However, there were unknown peaks in the analysis process, as shown in Figure 2, and of which more details are given in the discussion section.

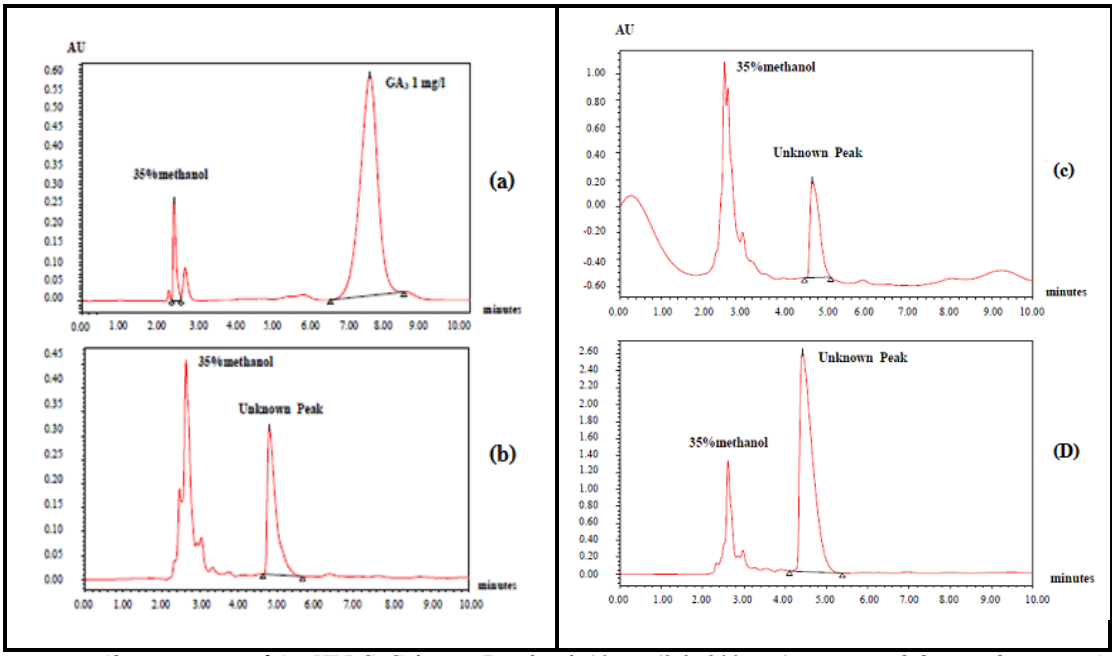

Figure 2: Chromatogram of C_{18} HPLC. Column µBondapak 10 µm (3.9×300 mm). Mobile phase: 35% methanol, Flow rate 1.2 ml/min, Detection at 206 nm, Injection volume 20 µl and Run time 10 mins. Performed (a) Standard GA3 of 1 mg/litre, (b) EGCs with 80% methanol (c) EGC with 80% rice whisky (d) EGCs with 80% ethanol

3.2 Sensitivity test by Lettuce Hypocotyl Bioassays (LHB) method

The study found that 500 seeds of 5 species of lettuce, Grand rapids, Red cos, Red oak,Red coral and Green oak, had the sensitivity with standard gibberellic acid (GA_3) as shown in Table 4

Table 4: The length performance of hypocotyls in 5 species of lettuce

Species of lettuce	Amount	The length of hypocotyls	
		mm.($\bar{X}$±S.D)	Ranking
Red coral	500	6.46±2.23	5
Red oak	500	6.69±2.36	4
Grand rapids	500	7.54±2.16	3
Green oak	500	8.87±2.38	2
Red cos	500	11.39±3.58	1

Remark: Sensitivity test by 2 ml of 1 ppm standard GA_3

The data in Table 4 shows that the length of hypocotyls (sensitivity indicator) was the highest in Red cos (11.39±3.58 mm), followed in descending order by Green oak (8.87±2.38 mm), Grand rapids (7.54±2.16 mm), Red oak (6.69±2.36 mm) and Red coral (6.46±2.23 mm) respectively.Therefore, the Green oak and Red cos were considered for further study on the correlation of plant growth with standard GA_3 as shown in Table 5.

Table 5: The hypocotyl length of Red cos and Green oak in various GA_3 concentrations

Standard GA_3 Conc. (ppm)	Amount	Length of hypocotyls different (mm)	
		Red cos($\bar{X}$±S.D.)	Green oak ($\bar{X}$±S.D.)
0.000	90	10.39±3.22	6.69±1.66
0.001	90	9.33±3.10	7.91±1.68
0.010	90	11.93±4.07	8.32±1.27
0.100	90	12.00±3.20	9.88±1.81
1.000	90	13.30±2.82	11.05±2.11

The GA_3 at 0.000 ppm. (control or water only) produced the Red oak hypocotyls length at 10.39±3.22 mm, while the other concentrations showed the increasing order from 9.33±3.10 mm. in 0.001 ppm. to 11.93±4.07 mm. in 0.010 ppm., 12.00±3.20 mm. in 0.100 ppm. and 13.30±2.82 mm. in 1.000 ppm. Red cos showed obvious better growth or sensitivity than Green oak. But the change of increment at the lower concentration of Red cos was not distributed well. Therefore, the study selected only Green oak for Lettuce Hypocotyls Bioassay of the various EGC concentration and types.

3.3 Sensitivity of ECGs by Lettuce Hypocotyls Bioassay

EGC types at difference solvents (80% of methanol, 80% of ethanol, 80% of rice whisky) and different ratios (Rambutan seeds: solvents: 1: 1 , 1 : 2 , 1 : 3) produced growth enhancement as shown in Table 6.

Table 6: The hypocotyls length at various EGCs

Conc. (%)	Hypocotyls length (mm) $\bar{X}$±S.D								
	Ethanol			Rice Whisky			Methanol		
	(1:1)	(1:2)	(1:3)	(1:1)	(1:2)	(1:3)	(1:1)	(1:2)	(1:3)
0.0					6.80±1.18				
0.5	7.09±1.54	6.54±1.19	6.25±1.11	6.52±0.78	6.52±1.52	6.20±0.95	7.27±1.06	7.22±1.81	6.49±1.38
0.6	7.37±1.11	6.81±1.15	6.43±1.04	6.85±0.88	6.77±1.75	6.35±0.94	7.89±1.76	7.56±1.44	6.52±1.29
0.7	7.47±1.35	7.04±1.10	6.94±0.96	7.13±0.90	6.96±1.45	6.46±0.88	7.95±1.24	7.75±1.61	6.94±1.00
0.8	7.54±1.64	7.19±1.08	7.01±0.83	7.33±0.86	7.15±1.34	6.48±1.00	8.16±1.54	7.83±2.89	7.09±1.27
0.9	7.56±1.77	7.29±1.30	7.14±0.98	7.47±0.93	7.25±1.08	6.55±1.30	8.25±1.26	7.96±1.45	7.20±1.36
1.0	7.71±1.52	7.46±1.26	7.24±0.84	7.48±0.93	7.35±1.38	6.67±1.23	8.29±1.46	8.01±1.48	7.23±1.23
1.1	7.80±1.34	7.67±1.57	7.28±1.31	7.78±0.92	7.56±1.74	6.69±0.89	8.37±1.62	8.04±1.55	7.27±1.17
1.2	7.81±1.35	7.72±0.82	7.39±1.45	7.88±0.84	7.66±1.42	6.72±1.28	8.46±1.05	8.22±1.54	7.40±0.73
1.3	7.84±1.65	7.87±0.90	7.39±1.01	7.89±0.75	7.74±0.97	6.90±1.00	8.51±1.34	8.30±1.78	7.44±0.83
1.4	8.02±1.74	7.89±0.87	7.40±1.03	7.93±0.82	7.84±1.45	6.93±1.12	8.75±1.37	8.35±1.02	7.50±0.89
1.5	8.12±1.63	7.96±1.11	7.55±1.29	8.00±0.71	7.89±1.41	6.96±1.12	8.81±1.35	8.54±1.79	7.70±0.96
1.6	8.23±1.42	8.07±0.73	7.55±1.43	8.12±1.09	7.91±1.33	6.98±1.43	8.95±1.03	8.72±1.81	7.73±0.98
1.7	8.41±1.29	8.12±1.33	7.61±1.45	8.21±1.05	8.05±0.88	7.01±1.24	9.08±1.17	8.86±1.75	7.87±0.72
1.8	8.52±1.40	8.12±1.27	7.61±1.34	8.41±1.24	8.08±1.38	7.16±1.70	9.23±0.96	8.86±1.38	7.92±0.84
1.9	8.69±1.43	8.24±1.04	7.70±1.35	8.48±1.49	8.13±1.33	7.17±1.10	9.27±1.28	8.90±1.07	8.05±1.07
2.0	8.78±1.30	8.27±1.21	7.71±1.47	8.71±1.07	8.24±1.19	7.17±1.00	9.32±1.18	8.97±1.27	8.12±1.23
2.1	8.88±1.48	8.49±0.83	8.03±0.83	8.77±1.03	8.34±1.84	7.42±1.38	9.54±1.30	9.26±1.40	8.23±1.37
> 2.1	**Onset of deterioration , some die**								

The data showed that the growth enhancement increased as the concentration of EGCs increased. But when that concentration increased to over 2.1%, all lettuces deteriorated in shape and finally died. Hence, 2.1 % EGC was considered the optimum amount to produce the greatest enhancement of growth than any other concentration. However, regarding among the best concentration (2.1%) for growth enhancement, EGCs from methanol (1:1) gave the highest enhancement, while rice whisky (1:3) gave the lowest, as shown in Table 7.

All had a significant difference with control ($p<0.05$), except rice whisky (1:3) ($p>0.05$).

However, when making a comparison with standard GA_3, only the EGC (methanol, 1:1) type gave the growth enhancement nearly the same as 0.1 ppm of standard GA_3, and the rice whisky (1:2) also gave nearly the same as 0.01 ppm GA_3. But, in statistic analysis, only EGC (methanol, 1:1) type had the significant difference ($p<0.05$) of growth enhancement with 0.01 ppm GA_3, but not as significant as with 0.1 ppm GA_3.While EGCs (methanol, 1:2, ethanol, 1:1) had lower enhancements capability, or they did not produce any significant growth enhancement ($p>0.05$) with standard 0.01 ppm GA_3. Presumably, EGCs (methanol, 1:1) had the enhancement capability of equivalent to 0.1 ppm.

Table 7: Comparison of the growth enhancements of standard GA_3 and 2.1% EGC

Standard GA_3		**EGCs: Hpocotyls length different**		
Concentrations (ppm)	**Growth mm($\bar{X}$±S.D)**	**Solvents**	**Ratios (Seed : Solvent)**	**mm ($\bar{X}$±S.D)**
		Control	0	6.80±1.18
0.001	7.94±1.15	Rice Whisky	1:3	7.42±1.38
		Ethanol	1:3	8.03±0.83
		Methanol	1:3	8.23±1.37
0.01	8.32±1.27	Rice Whisky	1:2	8.34±1.84
		Ethanol	1:2	8.49±0.83
		Rice Whisky	1:1	8.77±1.03
		Ethanol	1:1	8.88±1.48
		Methanol	1:2	9.26±1.40
0.1	9.88±1.81	Methanol	1:1	9.54±1.30

3.4 Growth enhancement of water morning glory

The study considered the growth enhancement by measuring the plant height and weight of WMG. The results seen in Table 8 and 9 are described as follows.

Table 8: Water Morning Glory Growth enhancement by EGCs (plant height)

Types of EGCs	**Ratios of EGC to water**		
	1:1 (d_1)	**1:2 (d_2)**	**1:4 (d_3)**
Control(Water)		31.01±3.36	
Rice Whisky (1:1)	33.03±3.57	32.71±3.54	32.17±3.91
Rice Whisky (1:2)	32.34±3.78	31.91±3.75	31.03±3.57
Rice Whisky (1:3)	31.92±3.68	30.95±3.80	30.41±3.57
Ethanol (1:1)	33.28±3.66*	32.44±4.10	32.38±3.10
Ethanol (1:2)	33.13±4.02	32.21±3.65	31.36±4.04
Ethanol (1:3)	32.32±3.73	30.29±3.44	29.77±4.52
Methanol (1:1)	33.36±3.70*	32.36±3.91	31.97±3.78
Methanol (1:2)	32.29±3.82	32.26±3.93	31.78±3.82
Methanol (1:3)	32.13±3.94	32.08±3.69	31.76±4.60
Std.GA_3 0.001 ppm	31.85±3.20	32.51±2.81	31.09±3.66
Std.GA_3 0.01 ppm	33.03±3.21	32.69±3.11	32.77±2.90
Std.GA_3 0.1 ppm	33.84±3.39*	33.36±3.00*	33.05±3.47
Std.GA_3 1 ppm	34.38±3.12*	34.24±2.75*	34.11±2.87*

Remark: * performed the significant difference with control or water only

Table 9: Average fresh weight (gm; per/ tree) of water morning glory by various EGC concentrations and dilutions

Types of PGRs	Ratios of EGCs to water		
	1:1 (d_1)	**1:2 (d_2)**	**1:4 (d_3)**
Control(Water only)		13.58±2.75	
Rice Whisky (1:1)	14.59±3.15	14.30±2.91	13.97±2.84
Rice Whisky (1:2)	14.29±2.51	14.19±3.02	13.87±2.50
Rice Whisky (1:3)	14.27±3.21	14.01±2.71	14.86±3.05
Ethanol (1:1)	15.08±3.02	13.87±2.90	13.59±3.10
Ethanol (1:2)	13.47±2.40	14.11±3.75	15.00±2.50
Ethanol (1:3)	13.54±2.00	14.19±3.12	14.02±2.81
Methanol (1:1)	14.19±2.80	15.07±2.61	14.95±2.81
Methanol (1:2)	15.09±2.62	14.82±3.12	14.73±2.45
Methanol (1:3)	14.98±3.00	14.60±3.02	14.47±3.12
Std.GA_3 0.001 ppm	14.01±3.10	14.05±3.00	13.09±2.75
Std.GA_3 0.01 ppm	14.27±2.73	14.41±2.94	13.90±2.65
Std.GA_3 0.1 ppm	14.94±2.31	13.19±2.00	13.42±2.91
Std.GA_3 1 ppm	14.38±3.20	14.97±2.54	14.87±2.01

3.4.1 Plant height of water morning glory

The results showed that GA_3 performed significant ($p<0.05$) growth enhancement with control. Also, only methanol (1:1) and ethanol (1:1) EGCs, at the dilution with water of 1:1, had a significant growth enhancement with control ($p<0.05$).

3.4.2 Fresh weight of water morning glory

There was no significant difference ($p>0.05$) of average fresh weight of WMG, which enhanced by such EGCs and control (water). In particular, the standard GA_3 1 ppm, was performed non-significant difference ($p>0.05$) with control. Therefore, the study would like to assume that the growth enhancement by such EGCs was only cell division, elongation or seed germination. It was not cell weight enhancement.

4. Discussion

The study had to give some discussion as followed

4.1Gibberellin-rich seed:Rambutan seed

Gibberellins (GAs) are tetracyclic diterpenoid growth factors that are essential for normal growth and that affect a wide variety of plant developmental processes. The number of identified gibberellins is now over a hundred, but only a few of these have known to have biological activity (Hooley, 1994; Richards, 2001; Hartweck, 2008). However, there has been a history of gibberellins discovery, which includes Jones (1980), who extracted the endogenous gibberellin in immature seeds of *Pharbitis nil* L., and found GA_3, GA_5, GA_{17}, GA_{19}, GA_{20}, GA_{29} and GA_{44}. Furthermore, Bottini (1985) extracted gibberellins from immature seeds of Apricot (*Prunus armeniaca* L.) found GA_1, GA_5, GA_{29} and GA_{32}. as also, Nakayama (1990) extracted gibberellins in mature seeds of Radish (*Raphanus sativus L*. cv. Taibyo-sobutori), and found GA_1, 3-epi-GA_1, GA_8 GA_9, GA_{17}, GA_{19}, GA_{20}, GA_{24} 12β-hydroxy-GA_{24}, GA_{25} and GA_{77}. More recently, Patrick (2000) extracted gibberellins from sweet cherry (*Prunus avium* L. cv. Stella) 1-year sprouts, 2-year sprouts, 3-year sprout mature seeds and flowers, and found GA_1, GA_3, GA_5, GA_8, GA_{19}, GA_{20}, GA_{29}, GA_{32}, GA_{85}, GA_{86} and GA_{87}. A year later, Nakayama (2001) found GA_3, GA_9, GA_{17}, GA_{19}, GA_{30}, GA_{44}, GA_{61}, GA_{63}, GA_{87}, GA_{95}, GA_{97}, GA_{118}, GA_{119}, GA_{120}, GA_{121}, GA_{122}, and GA_{126} in immature seeds of *Prunus persica* . And Patrick (2002) found abscisic acid (ABA), indole-3-acetic acid (IAA), jasmonic acid (JA), indole-3-acetonitrile (IAN), methyl jasmonate (MeJA) and gibberellins (GAs): GA_1, GA_3, GA_8, GA_9, GA_{12}, GA_{15},GA_{17}, GA_{19}, GA_{20}, GA_{24}, GA_{29}, GA_{44}, GA_{51} and GA_{53} in seeds of ash (*Fraxinus excelsior*). Moreover, the functions of gibberellin to higher plant growth and development, including germination, hypocotyl elongation, stem growth, reproductive, organ and seed development etc. were defined (Olszewski *et al.* 2002). Therefore, gibberellins are very useful for people who are attempting to control their crop cultivation in farming systems development (Altman, 1999; Taiz, 2010).

In particular, there were studies, concerned with the amount of gibberellins or gibberellin-like substances (GLS) in seeds, such as Chen (1990), which reported high gibberellin in the xylem sap at the stage of leaf expansion of lychee, a sub-tropical evergreen tree of the *Sapindaceae* family. Even though there are no studies concerned with the GLS in rambutan, there are studies, such as Suryawati (1997), on the effects of storage condition and gibberellin concentration on the deterioration of rambutan seed. Sunee (2009) suggested that the plants in *Sapindaceae* family, such as rambutan and longan seed, contained gibberellin in high enough

quantities for plant development and enhancement. Therefore, the study had modified the mentioned results into this study, whether the EGCs (rambutan seed) had the potential of plant growth enhancement in comparison with standard GA_3. Also, it has further application for vegetable growth enhancement.

4.2 EGCs extraction and purification

Currently, recognized endogenous levels of each plant's hormones (including ethylene, auxin, abscisic acid, gibberellins, cytokinins, jasmonates, brassinosteroids and salicylates) occurred at very low concentrations or even lower than the concentration of other types of such plant secondary metabolites as flavonoids. In addition, many plant hormones coexist with other endogenous organic compounds in plant extracts, which can interfere with the final assay of the plant hormones. It is therefore the challenge for researchers to enrich these low-level compounds from plant extracts, in particular, the enrichment method through proper solvent types and purification process, etc. The following 3 factors were considered for such target hormone extraction as gibberellin in this study. The first was the rambutan seed preparation. It is recommended to keep this seed or fine powder (grinding) in cold temperatures at 4 °C during extraction process to avoid enzymatic induction of metabolic change or chemical degradation of the GLS. Second, the extraction process that depended on solvent selections, which had to be compatible with the physicochemical properties of target-extracted compounds, as well as, the part of plant materials had to be considered from the literature reviews. However, the ideal solvents that are currently being widely used in methods are methanol, ethanol, acetone, acetone/water, propanol, and propanol/water. Of these organic solvents, methanol has become the preferred solvent and is widely used for extraction of plant hormones. This is because of its small size and low molecular weight which allow it to efficiently penetrate into plant cells during extraction. Moreover, the study had considered the other solvents which had the same lower molecular weight, the intoxicating, and feasibly self–produced practice by local people as rice whisky alcohol. Third, the ratio of plant material and solvents through the solid to liquid phase extraction had to be adjusted on a case-by-case basis. However, through Lettuce Hypocotyls Bioassay, the study had specifically found that the methanol solvent at ratio 1:1 (wt. by vol.) performed the most appropriately for EGCs of rambutan seed. Otherwise, the study would like to note that the efficiency of extraction and purification might depend on the particulate size of plant material, according to the different physicochemical properties of such types of GLS (Ji Hong, 2011; Hoyerová, 2006; Harborne, 1998; Bieleski, 1964). Thus, further investigation is required.

4.3Available of gibberellin in rambutan seed

In this study, the available GLS was not in the form of GA_3 (unfortunately, only GA_3 was used as standard in HPLC). As well, the unknown peak in the graph of figure 1 might be the existence of GLS in the other types as GA_1, GA_4 and GA_7, which could react and cause the same growth enhancement in plants (Chen, 2008; Talon, 1990). However, a study (Brain, 1964) had compared the potency in bioassays of nine gibberellins (A_1 to A_9) based on extension of stems of dwarf garden pea (*Pisum sativum*), dwarf bean (*Phaseolus vulgaris*), *Lunaria annua*, hypocotyls of cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), and of leaf sheaths of three dwarf mutants of maize (*Zea mays*). Gibberellins (A_3: gibberellic acid) and A_7 were the high potency in most bioassays. While, the A_8 gibberellin was negligible potency and was probably not a functional hormone. The other gibberellins (A_1, A_2, A_4, A_5, A_6, A_9) showed a more or less marked tendency to specificity. Therefore, the explanations in relation to the obtained results of HPLC study, if the GLS in this study was not A_3. In referring to this, Brain (1964) mentioned that it should definitely be A_7. Which, it should be the further study to determine it again. Moreover, the performance of low-quantity GLS was confirmed by the assumptions in many studies (Kumar, 2014; Kojima, 1996; Tongumpai, 1991; Niran, 1993; Peeradej, 1994; Goldschmide, 1976; Bhalla, 1971) that the quantity of GLS would be high during seed germination (young seeds). The mature or old seeds (used in this study) would unsuspectedly contain low-quantity GLS.

4.4 Sensitivity of EGCs to plant growth

Sensitivity of EGCs to plant growth was identified by Lettuce Hypocotyl Bioassay. The hypocotyls performed the respondence to EGCs at the concentrations of 0.5% to 2.1%. The concentrations of more than 2.1% introduced a slow down and eventually the death of lettuce. This might be due to the higher concentrations of EGCs which remained as residual alcohol, and consequently deteriorate the lettuce. However, it could be presumed that the amount of GLS in 0.5-2.1% ECGs had at least the amount of GLS higher than the sensitivity level of the Lettuce Hypocotyl Bioassay method, and that was defined to be as low as 0.1 mg/1 (Fletcher, 1966). Hence, its capability could still enhance the growth of lettuce.

4.5 The gibberellic acid (GA_3) equivalents gibberellin-like substances in EGCs

Even the study method could not define the exact concentration and types of GLS in such EGC types due to unknown typical gibberellic acid in the HPLC investigation. The interpretation of GLS could be presented in other terms as "gibberellic acid equivalents (GAE)" (Hill, 1969), in which the quoted value was derived from a comparison of the observed responses in the bioassay based on the response from the known

quantities of gibberellic acid (GA_3). Hence, through the Lettuce Hypocotyls Bioassay data (Table 7), the best 2.1 % EGCs (methanol, 1:1) gave the equivalent to 0.1 ppm standard GA_3 at the same volume (2 ml. in this study).

4.6 EGCs applications

Actually, the gibberellins (GAs) in agriculture have been applied for growth enhancement (Rappaport, 1980; Nickell, 1976; Pharis, 1976). But, there was the argument that gibberellin enhanced plant growth by affecting either the cell expansion or cell division, or both. Cell division alone cannot result the growth in plant, but it might contribute to growth by producing more cells which later expand cell-by-cell and enhance the growth of the plant. Afterwards, studies (Rappaport, 1980; Wittwer, 1958) observed that GA_3 promoted elongation and increased the fresh weight of the petioles of certain celery *(Apium graveolens)*. The finding was not totally consistent with this study when EGCs was applied to water morning glory (WMG). When the data showed significant growth in hypocotyls length enhancements with control ($p<0.05$), but did not show significant growth in fresh weight enhancement with control ($p>0.05$). The study would like to assume that growth enhancement was only cell division, increasing cell numbers and then elongation or expansion. Hence, the growth enhancement was only cell elongation, not weight enhancement of plants. That might be due to the limitation of biogeochemicals supplement of the area and/or the other attributes of the area. The usage of GLS for agriculture in such areas need the supplements of other biogeochemials, such as calcium, phosphorus etc., as well as the area attributes.

5. Conclusions

This research aimed to investigate whether EGCs from gibberllin–rich seeds, such as rambutan seeds, was feasible to be used as a growth enhancement compound for plants or not. Through the studied methodology, it would like to conclude that EGC 2.1% (the best performance), or only 40-42 µl of total EGC 2 ml (extracted from rambutan seed 200 grams) by methanol solvent (at 1:1 ratio wt/vol) had a growth enhancement equal to standard GA_3 at 0.1 ppm. In application for agriculture through water morning glory, the study concludes that the usage of GLS for growth enhancement also needs the supplement of the other biogeochemicals, in particular the area attributes for such types of agriculture.

As well as, the study would like to note that at least 2 findings can be applied into the food security management and waste minimization. 1) For the food security management, as the world population is still increasing , expectedly to be as high as 9 billion people in the year 2050.(PRB, 2009), this amount of population comes with enormous challenges for agricultural products. However, the "Green Revolution" with new agronomic practices had consequently doubled grain production from the year 1960 to the year 2000 (Khush 2001). But in actual, the new agronomic practices were introduced through i) the large application of nitrogenous fertilizers needed, which eventually cause environmental problems, ii) the non-transgenic and transgenic techniques which find it very important to cautiously observe potential damage to new plants. The performance in the potential feasibility of extracted Gibberellic Compounds (EGCs) for plant growth enhancement, can be introduced to avoid such unwanted practices of the green revolution, but the economical, practical, and impact issues need more research . 2) For waste minimization, this practice of using rambutan seed utilizes a standard organic by product, and therefore reduces the financial burden of farmers and minimizes ecological damage. The EGCs in the study is a strategic example, of how to reduce waste through a closed-loop pattern of waste management and moves away from the current, linear and wasteful pattern. If a closed-loop system can be adapted into such production systems, our limited resources will have multiple uses and waste will be significantly reduced.

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7. References

Asen, S.and Cathey, H.M. 1960. Enhancement of Gibberellin Growth-Promoting Activity by Hydrangenol Isolated from Leaves of Hydrangea Macrophylla. **Plant Physiol**. 35(6): 816–819.

Altman, A. 1999. Plant biotechnology in the 21st century: the challenges ahead. Plant Biotechnology 2(2) - **EJB Electronic Journal of Biotechnology** [Online]. Available:http://www.ejbiotechnology.info/content/vol2/issue2/full/1/ [Accessed 14 March 2014].

Bachelard, E.P. 1968. Effects of Seed Treatments with Gibberellic Acid on Subsequent Growth of Some Eucalypt Seedlings. **New Phytol.** 67: 595-604.

Bhalla, P. 1971. Gibberellin-like Substances in Developing Watermelon Seeds **Physiologia Plantarum** 24(1):106–111.

Bieleski, R. L. 1964. The problem of halting enzyme action when extracting plant tissues. **Anal. Biochem.** 9:431–442.

Bottini, R., De Bottini, G., Koshioka, M., Pharis, R. P., Coombe, B.G. 1985. Identification of Gibberellins A_1, A_5, A_{29} and A_{32} from immature seeds of Apricot (*Prunus armeniaca* L.). **Plant Physiol** 78:417-419.

Brian, P.W., Hemming, H.G., Lowe, D. 1964. Comparative potency of nine gibberellins. **Annals of Botany** 28(3):369-389.

Çetinbaş, M., Koyuncu, F. 2006. Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. **Hort.Sci. (Prague)** 33(3):119–123

Chen, S-Y., Kuo, S-R., Chien, C-T. 2008. Roles of gibberellins and abscisic acid in dormancy and germination of red bayberry (*Myrica rubra*) seeds. **Tree Physiology** 28: 1431–1439

Donald, W. W. 1981. EPTC Effects in the Lettuce (Lactuca sativa) Hypocotyl Bioassay for Gibberellins.**Weed Science** 29(4):490-499.

Fletcher, R. A., Osborne, D.J. 1966. A Simple Bioassay for Gibberellic Acid. **Nature** 211:743 – 744.

Frankland, B., Wareing, P. F. 1960. Effect of GA on hypocotyl growth of lettuce seedlings. **Nature (Lond.)** 185:255-256

Goldschmide, E. E. 1976. Endogenous Growth Substances of Citrus Tissues. **Hortscience II** (2)**:** 95-99.

Gupta, A. B., Agarwal, P. R. 1973. Extraction, Isolation, and Bioassay of a Gibberellin-like Substance from *Phormidium foveolarum*. **Ann. Bot.** 37(4): 737-741.

Harborne, J.B., 1998. **Phytochemical Method. A Guide to Modern Techniques of Plant Analysis**. 3rd Edn., Springer (INDIA) Pvt. Ltd., New Delhi, ISBN-10: 0412572702, pp: 5-12.

Hartweck, L. M. 2008. Gibberellin signaling . **Planta** 229:1–13.

Hill, T.A., Wimble,R.H. 1969. A Note on the Precision of Estimates of Gibberellin Concentration from Regression Lines Calculated from Bioassay Data. **Planta (Berl.)** 87:20-25.

Hooley, R. 1994. Gibberellins: perception, transduction and reponses. **Plant Mol. Biol**. 26:1529–55

Hoyerová, K., Gaudinová, A., Malbeck., J., & et al. 2006. Efficiency of different methods of extraction and purification of cytokinins. **Phytochemistry** 67: 1151–1159.

FAO/UNEP. 1997. ***Negotiating a Sustainable Future for Land.*** **Structural and Institutional Guidelines for Land Resources Management in the 21st Century**. FAO/UNEP, Rome.

Ikuma, H., Thimann,K.V. 1960. Action of Gibberellic Acid on Lettuce Seed Germination. **Plant Physiol.** 35(5): 557–566.

JiHong, F., XiaoHong, S., JiDe,W., JinFang, C., CunYu, Y.2011. Progress in quantitative analysis of plant hormones. **Chinese Sci Bull.** 56(4-5):355-366.

Jones, R.L. 1968. Aqueous Extraction of Gibberellins from Pea. **Planta** 881: 97-105.

Jones, M. G., Metzger, J. D., Zeevaart, A. D. 1980. Fractionation of Gibberellins in Plant extracts by Reverse phase High Performance Liquid Chromatography. **Plant Physiol** 65:218-221.

Kamchai Pilikaew 1993 . **Influenceing of GA_3 on harvesting age and quality of water morning glory**. Bangkok: Kasetsart University. (In Thai).

Kaewladdakorn, V., A. Pichakum**,** K. Krisanapook and N. Juntawong. 2003. Effect of GA_3 on fruit growth and level of GA, ABA-like activity in mango fruit cv. Nam Dok Mai'. **Agricultural Sci. J.** 34: 1-3 (Suppl.): 219-222.

Kaewladdakorn, V. 2002. **Relationship between endogenous gibberellins-like substance level and growth of seed and fruit in loquat (*Eriobotrya japonica* Lindl.).** Special topic. M.Sc. (Agriculture). Bangkok: Kasetsart University. Thailand.

Kaewladdakorn, V. 2002. **Effect of GA3 on fruit set, fruit growth and levels of GA, ABA-like substances in mango fruit cv. Nam Dok Mai**. M.sc Thesis. Bangkok:Kasetsart University.

Khush, G. S. 2001**.** Green revolution: the way forward. **Nature Reviews Genetics** 2: 815-822

Kojima, K.1996. Changes of abscisic acid, indole-3-acetic acid and gibberellin-like substances in the flowers and developing fruitlets of citrus cultivar ' Hyuganatsu'. **Scientia Horticuhurac** 65: 263-272.

Kumar, M., Ponnuswami1,V., Kumar, P. J. and Saraswathy, S. 2014. Influence of season affecting flowering and physiological parameters in mango. **Sci. Res. Essays** 9(1):1-6.

Lee, I.J., Foster, K., Morgan, P.W.1998. Photoperiod control of gibberellin levels and flowering in sorghum. **Plant Physiol** 116: 1003–1011.

Lessenger, J. E.(editor) 2006. **Agricultural Medicine A Practical Guide**. New York:Springer Science Business Media, Inc. 541 pp.

Moore, G. M. 1998. Tree Growth Regulators: Issues of Control, Matters of Management. Journal of Arboriculture 24(1):10-18.

Nakayama, M., Koshioka, M., Matsui, H., Ohara, H., Mander, L. N., Leitch, S. K., Twitchin, B., Kraft-Klaunzer, P., Pharis, R. P., Yokota, T. (2001). Endogenous gibberellins in immature seeds of *Prunus persica* L.: identification of GA(118), GA(119), GA(120), GA(121), GA(122) and GA(126). **Phytochemistry** 57(5): 749-58.

Nakayama, M., Yamane, H., Yokota, T., Yamaguchi, I., Murofushi, N., Takahashi,N., Nishijima, T., Katsura, N., Nonaka, M., Gaskin, P., MacMillan, J., Mander, L. N., Chu, A. 1990. Endogenous gibberellins in mature seed of *Raphanus sativus* L. cv. Taibyo-sobutori. **Agricultural and Biological Chemistry** 54(3):837-840

Nickell, L. G. 1976. Chemical growth regulation in sugar cane. **Outlook on Agriculture** 9:57-61.

Nirun Chunvongse. 1993. Chapter 5 ; Plant Growth Regulators. Bangkok: Kasetsart University Research and Development Institute. 137 pp.(inThai)

Noppadon, J. 1994**. Hormones and Plant Growth Regulators**. Bangkok : Rouykwey Publishing, 128 pp (In Thai).

Noppadon, J.1999. **Plant Growth Regulators Substances**. Chiangmai: Faculty of Agricultural Production, Maejo University Bangkok.110 pp(In Thai).

Ortega-Baesa,P. , Rojas-Arechiga, M. 2007. Seed germination of Trichocereus erscheckii(Cactaceae): Light, temperature and gibberellic acid effects. **Journal of Arid Environments** 69:169–176.

Olszewski, N., Sun,T. P., Gubler, F. 2002. Gibberellin signaling:biosynthesis,catabolism, and response pathways. **Plant Cell** 14:S61–S80.

PRB(Population Reference Bureau staff. 2009. World Population Highlights: Key Findings From PRB's 2009 World Population Data Sheet, **Population Bulletin** 64, no. 3.

Patrick S. B., June, M. T., William, E. Finch-Savage.(2002). Identification of abscisic acid, indole-3-acetic acid, jasmonic acid, indole-3-acetonitrile, methyl jasmonate and gibberellins in developing, dormant and stratified seeds of ash (*Fraxinus excelsior*). **Plant Growth Regulation** 37(2):119-125.

Patrick, S. B., Gordon, B., Lynda, J. B. and Lewis, N. M. 2000. Gibberellins in seedlings and flower trees of *Prunus avium* L. **Phytochemistry** 53:519-528.

Peeradej Thongampai 1994. **Plant Hormones : Guideline for Usage in Thailand**. Bangkok: Vichai Printing Co. LtD. 196 pp (In Thai)

Pharis. R. P, Ross, S.D. 1976. Gibberellins: their potential in forestry. **Outlook Agric.** 9: 82-87.

Rappaport, L. 1980. **Applications of Gibberellins in Agriculture**: Plant Growth Substances 1979. Proceedings in Life Sciences. pp 377-391

Rai, V. K. and Laloraya, M. M. 1967. Effect of Different Gibberellins on the Growth of the Hypocotyl. **Physiologia Plantarum** 20: 879–885.

Richards, D. E., King, K. E. Ait-ali,T., Harberd, N. P. 2001. How Gibberellin Regulates Plant Growth and Development: A Molecular Genetic Analysis of Gibberellin Signaling. **Annu. Rev. Plant Physiol. Plant Mol. Biol.** 52:67–88.

Ruben Bottini, Guillermina De Bottini, Masaji Koshioka, Richard P. Pharis and Bryan G. Coombe. 1985. Identification of Gibberellins A_1, A_5, A_{29} and A_{32} from immature seeds of Apricot (*Prunus armeniaca* L.). **Plant Physiol** 78:417-419.

Suryawati, A. 1997. **The effects of storage condition and gibberellin concentration on deterioration of rambutan seed.** Indonesian Center for Agricultural Library and Technology Dissemination, Pusat Perpustakaan dan Penyebaran Teknologi Pertanian. [Online]. Available:http://agris.fao.org/agris-search/search.do?recordID=ID2002001386 [Accessed on 1 March 2014]

Sunee, B., Aussanee, P. 2009. **The Plants in Family *Spindaceae* and related genus for Thai's Economic Supporting.** [Onlne]. Available*:*www.vcharkarn.com/project/view/134 [Accessed on 15 July 2010].

Sunanta R., Sumalee S., Supatra, M. 1978. **Lipid production from Rambutan Seed and Usage Study**. Bangkok : Thailand Institute of Scientific and Technological Research.

Wanrada, S.,Talon, M., Koornneef,M., Zeevaart, J. A. D. 1990. Endogenous gibberellins in Arabidopsis thaliana and possible steps blocked in the biosynthetic pathways of the semidwarf ga4 and ga5 mutants. **Proc. Natl. Acad. Sci**. 87:7983-7987

Taiz, L., Zeiger, E. 2010. **Plant Physiology** (5 th ed.) ; Chapter 20 : Gibberellins-Regulators of Plant Height. Sinauer Associates, Inc 782pp.

Tongumpai P, Jutamanea K, Sethapakdi R, Subhadrabandhu, S 1991. Variation in level of gibberellins–like substances during vegetative growth and flowering of mango cv.Khiew Sawoey. **Acta Hort.** 291:105 107.

Vimon Kaewladdakorn. 2002. **Effect of GA3 on fruit set, fruit growth, and levels of GA-. ABA-like substances in mango fruit cv. 'Nam Dok Mai'**. M.Sc. (Agriculture). Bangkok:Kasetsart University. Thailand

Waycott, W., Taiz, L. 1991. Phenotypic Characterization of Lettuce Dwarf Mutants and Their Response to Applied Gibberellins. **Plant Physiol.** 95:1162-1168.

Wittwer, S.H., Bukovac, M.J.1958. The effect of gibberellin on economic crops. **Econ. Bot.** 12: 213-255.

Wannee, J., Utai, K. 2011. Response surface optimization and characteristics of rambutan (*Nephelium lappaceum* L.) kernel fat by hexane extraction. **Food Science and Technology** 44:1946 – 1951

Wall, M. M. 2006. Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. **Journal of Food Composition and Analysis** 19: 655–663.