CHAPTER 4.0

GLUCOMANNAN (GM) CONTENT IN SELECTED *Amorphophallus* spp. OF PENINSULAR MALAYSIA.

4.1 INTRODUCTION

The glucomannan (GM) content varies with age, species and different environment. It is very important for us to identify the GM content of *Amorphophallus* spp. found all over the country. It is one of the objectives of this study to determine the GM content of various *Amorphophallus* spp. at different periods of the year (a more detail description is written under chapter 2).

4.2 QUANTIFICATION OF GLUCOMANNAN (GM)

The principle for the quantification of GM requires several enzyme reactions and a treatment at high pH to remove the acetyl-groups from the polysaccharide. The first enzymic reaction involves depolymerisation of acetylated-glucomannan-oligosaccharides (Ac-GlcManol) (Step 1). For some samples (those with a high GM content), this is performed before deacetylation, while for other samples (lower concentration of GM), it is more convenient to perform deacetylation (Step 2) first.

(1) $\text{Ac-GlcMan} + H_2O \xrightarrow{(\beta\text{-mannanase})} \text{Ac-GlcManol}$

After depolymerisation into acetylated glucomanno-oligosaccharides of degree of depolymerisation (DP) 2-6, the oligosaccharides are deacetylated by increasing the pH to 12.5 (Step 2).

(2) Ac-GlcManol $\xrightarrow{\text{(pH 12.5)}}$ GlcManol + acetate

After the acetyl-group had been removed, the glucomanno-oligosaccharides are quantitatively hydrolysed to D-glucose and D-mannose by the combined action of β-glucosidase (β-Gos) and β-mannosidase (β-mos) (Step 3).

(3) GlcManol + H_2O $\xrightarrow{\text{(β-Gos + β-Mos)}}$ D-glucose + D mannose

D-glucose and D-mannose are phosphorylated by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) (Step 4), to glucose-6-phosphate (G-6-P) (Step 6) and mannose-6-phosphate (M-6-P), respectively, with the simultaneous formation of adenosine-5'-diphosphate (ADP).

(4) D-Glucose + ATP $\xrightarrow{\text{(HK)}}$ G-6-P + ADP

(5) D-Mannose + ATP $\xrightarrow{\text{(HK)}}$ M-6-P + ADP

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidised by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (Step 6)

(6) G-6-P + $NADP^+$ $\xrightarrow{\text{(G6P-DH)}}$ gluconate-6-phosphate + NADPH + H^+

The amount of NADPH formed in this reaction is stoichiometric with the amount of D- glucose. It is the NADPH which is measured by the increase in absorbance at 340 nm.

On completion of the reaction (Step 6), M-6-P is converted to fructose-6-phosphate (F-6-P) and then to G-6-P by the sequential action of phosphomannose isomerise (PMI) and phosphoglucoe isomerise (PGI) (Step 7).

(7) M-6-P $\xrightarrow{\text{(PMI)}}$ F-6-P $\xrightarrow{\text{(PGI)}}$ G-6-P

G-6-P reacts in turn with NADP+ forming gluconate-6-phosphate and NADPH, leading to a further rise in absorbance that is stoichiometric with the amount of D-mannose. Method followed Megazyme glucomannan assay procedure K-GLUM 05/11 (Megazyme International Ireland, 2011)

4.3 MATERIALS AND METHOD

4.3.1 Corm Sampling

Matured corms from four *Amorphophallus* species, namely, *A. elegans, A. oncophyllus, A. paeoniifolius* and *A. prainii* were chosen for the initial study of Glucomannan (GM) content. For *A. paeoniifolius* and *A. prainii*, additional samples were also taken from young corms for comparison. Ten samples (corm) were randomly collected at each location during the ecological survey of the earlier study in 2009 for *A. paeoniifolius* and *A prainii* while only five samples were taken for *A. elegans* and *A. oncophyllus.* This was due to limited samples available during collection. The locations and time of sampling of corms for each species are as shown in Table 4.1

Table 4.1: List of species collected for Glucomannan (GM) study.

Species	Location	Latitude & Longitude	Date Collected (2009)
A. elegans	Dungun, Terengganu	N4°41.911' E103°25.015'	April
A. oncophyllus	Dengkil, Selangor	N2°85.84.15' E101°678.375'	May
A. paeoniifolius	Jerantut, Pahang	N 3°56.003' E 102°22'	June
A. prainii	Kuala Kangsar, Perak	N4°32.435' E 100°46.073'	October

All samples were brought back to the Biology laboratory, University of Malaya for powder preparation and determination of GM content. Five samples of each species were used for this purpose.

Materials of selected raw corms of *Amorphophallus* spp. were washed with water and scrubbed to remove surface dirt. Their skins were peeled and small roots were cut off. The corms of *Amorphophallus* spp. were then sliced and their sprouts were removed. The slices were heated in an oven at 60ºC for 3 days to remove all moisture. The dried slices were placed in a grinding machine and were crushed to fine powder to less than 0.125 mm in size. Samples for GM determination were prepared and analysed using the Megazyme glucomannan assay procedure developed by Megazyme International Ireland Limited (2004).

The other five samples of *A paeoniifolius* and *A. prainii* were each transplanted to the other location in Dengkil, Selangor. The samples were put into a separate 30 x 45 cm black polythene bag containing organic soil. The polythene bags were placed under a shade whereby the light penetration given was only between 10-15 %. Daily watering was given to the corm. A 10 gm of 15:15:15 fertilizer was given to each bag every 10 days so as to allow the corm to grow into a full grown plant.

Each plant was allowed to grow for 12 months, after which each plant was harvested for the corm at intervals during February, April, June, August, October and December, to determine the variation in GM content of the *Amorphophallus* species with time.

4.3.2 Purified Glucomannan (GM) Powder

Purified GM powder from *Amorphophallus* spp. were prepared in accordance to the method developed by Wang et al., (1998). The GM powder from 4 local *Amorphophallus* spp. was prepared to determine GM content and compared with the commercial purified GM powder.

A 0.15 g of powdered sample was put in a 150 ml beaker. In order to separate free sugar, it was filtered and heated to remove the ethanol. The sample was dissolved in purified water in a 150 ml beaker at 35°C under continuous agitation with a stirrer for 12 hours. The digested solution was then centrifuged for 20 minutes at 4000 rpm. The purified GM solution was heated and digested for 1.5 h with boiling water, and then cooled. 0.5 ml 6M NaOH was added and the solution was analysed by spectrophotometric analysis using a UV-VIS spectrophotometer.

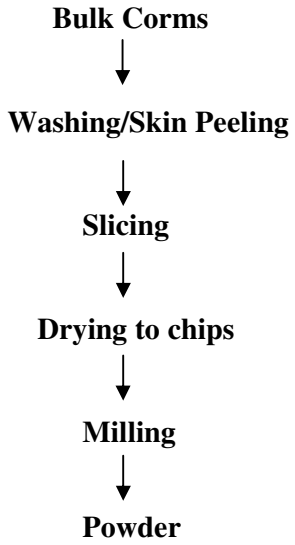

Figure 4.1: Steps involved in the preparation of powder samples from *Amorphophallus* spp.

4.3.3 Statistical Analysis

Data were statistically analysed for Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS). Similarly, all data were subjected to further Highest Significant Difference (Tukey's) Test.

4.4 RESULTS

4.4.1 Glucomannan (GM) Content in *Amorphophallus* spp Investigated.

An analysis of the GM content in purified powder of four species of *Amophophallus*, namely, *A. elegans, A. oncophyllus, A. paeoniifolius* and *A. prainii* was conducted using a method developed by Wang et al. (1998)

Result from the purified GM study as in Table 4.2., indicated that *A. oncophyllus* had the highest GM content (58.65 %). This was followed by mature *A. paeoniifolius* (50.22%), mature *A. prainii* (29.71 %) and *A. elegans* (17.01 %) respectively. The GM content was lowest in young *A. prainii* (9.23 %) followed by young *A. paeoniifolius* (14.90 %).

This study also indicated that the GM content was higher in mature corms than in young corms of *A. paeoniifolius* and *A. prainii,* respectively. For *A. paeoniifolius,* the GM content of the mature corm was 50.22 % while that of the young corm was only 14.90 %. Similarly, for *A. prainii,* the GM content of the mature corm was 29.71 %, and that of a young corm was only 9.23 %.

T**able 4.2**: Glucomannan (GM) content in purified powder of several *Amorphophallus* spp.

Species	Mean GM Content (%)
A. elegans	17.0103 (± 0.35)
A. oncophyllus	58.6486 (± 1.13)
A. paeoniifolius **(mature)**	50.2194 (± 0.41)
A. paeoniifolius **(young)**	14.9014 (± 0.10)
A. prainii **(mature)**	29.7146 (± 0.17)
A. prainii **(young)**	9.2266 (± 0.10)

Note: Standard Deviations are in parenthesis

4.4.2 Glucomannan (GM) Content from Original and Transplanted Corms of *A. paeoniifolius* and *A. prainii*

A comparative study of GM content was made between corms from original samplings and transplanted samples. Results from the ANOVA as in Table 4.3 indicated that there were highly significant differences in the GM content of the original and transplanted corm species (F = 31.85 **). Results from further ANOVA as in Table 4.4 also indicated that there were highly significant differences of the GM content detected between the two species (F = 49.53 **) and locations of sampling (F = 44.73 **). However, there was no significant difference detected among the interaction between species and locations (F = 1.3 n. s).

Table 4.3: Analysis of Variance (ANOVA) of the Glucomannan (GM) content of original and transplanted corms of *A. paeoniifolius* and *A. prainii*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	**3**	**80.9579**	**26.986**	**31.85****	**< .0001**
Error	**16**	**13.5557**	**0.8472**		
Corrected Total	**19**	**94.5137**			

R- Square Estimate	Coefficient of Variation (%)	Root MSE	GM Mean
0.8566	**7.8193**	**0.9205**	**11.7715**

Note: ****** indicated highly significant at p <0.001

Table 4.4: The Analysis of Variance (ANOVA) of the Glucomannan (GM) content of *A. paeoniifolius* and *A. prainii* from two locations of sampling.

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Species	**1**	**41.963**	**41.963**	**49.53 ****	**< 0.0001**
Location	**1**	**37.895**	**37.895**	**44.73 ****	**< 0.0001**
Spe x Loc	**1**	**1.0998**	**1.0998**	**1.3 n. s**	**0.2713**

Note: n. s indicated not significant at p < 0.005
** indicated highly significant at p < 0.001

The mean and standard deviation of the GM content of the two species taken from two sources, namely original and transplanted samples are as shown in Table 4.5. Results indicated that the mean GM content was high in *A. paeoniifolius* from the original source sample (14.36 %), followed by *A. paeoniifolius* from the transplanted

source (12.08 %), *A. prainii* from the original corm sample (11.93 %), and lastly the lowest GM content in *A. prainii* from the transplanted corm sample (8.71 %).

Table 4.5: The mean Glucomannan (GM) content of *A. paeoniifolius* and *A. prainii* from two sources of sampling

Samples	Source of the Plant	N	Mean Glucomannan (GM) %
A. paeoniifolius	Original	5	14.362 (± 1.11)
	Transplanted	5	12.0780 (± 1.15)
A. prainii	Original	5	11.934 (± 0.56)
	Transplanted	5	8.712 (± 0.72)

Note: Standard Deviations are in parenthesis

A further comparison of the mean GM content using the Tukey's Studentized Range Test (HSD) was conducted. Results indicated as shown in Table 4.6, that there was a highly significant difference in the GM content of the two species of *Amorphophallus.* Similarly, the Studentized Range (Tukey's) Test as shown in Table 4.7 indicated that there was a highly significant difference in the GM content of both species in two different locations.

Table 4.6: Tukey's Studentized Range (HSD) test for Glucomannan (GM)

Mean Glucomannan Content	N	Species
13.22**a**	10	*A. paeoniifolius*
10.323**b**	10	*A. prainii*

Note: Means with the same letter are not significantly different at $p < 0.001$

Table 4.7: Tukey's Studentized Range (HSD) test for Glucomannan (GM) content in different locations.

Mean Glucomannan Content	N	Location
13.14**a**	10	Original
10.39**b**	10	Transplanted

Means with the same letter are not significantly different at $p < 0.001$

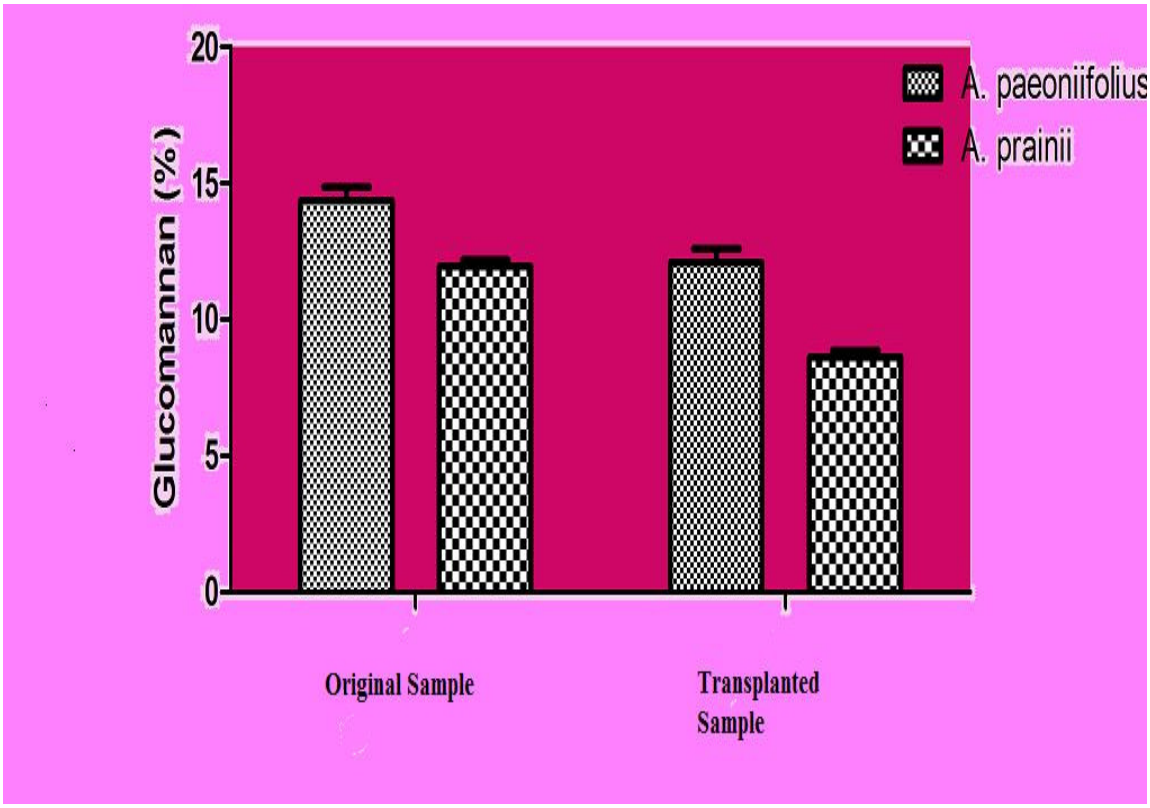

Figure 4.2: Comparison of Glucomannan (GM) content in raw corm from original and transplanted materials of *A. paeoniifolius* and *A. prainii*.

4.4.3 Variations of Glucomannan (GM) Content in Raw Corms of the Two *Amorphophallus* spp. With Time (Months)

An analysis of variance (ANOVA) conducted on the data as shown in Table 4.8 indicated that there was a highly significant difference in the GM content at different time of sampling (F = 87.56 **). Similarly, result as in Table 4.9 indicated that there was a highly significant interaction between time and species of *Amorphophallus* (F = 5.6 **). A closer look at the trend of GM content with time as shown in Table 4.10 and Figure 4.3, was that the GM content was highest when the corms were harvested in December and lowest when harvested in February. There was an upward trend from low GM content in February and increasing to maximum in December. The dry season falls during February while December marks the end of the wet monsoon period. Result from Tukey's Test as shown in Table 4.10 indicated that there was no significant difference detected in the GM content when the corms were harvested in June and August.

Table 4.8: The Analysis of Variance (ANOVA) of the Glucomannan (GM) content of the two *Amorphophallus* spp. with time

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	**19**	**682.4539**	**35.9186**	**87.56****	**< .0001**
Error	**40**	**16.4083**	**0.4102**		
Corrected Total	**59**	**698.8623**			

R- Square	Coeff Var	Root MSE	GM Mean
0.9765	**6.4679**	**0.6405**	**9.9023**

Note: ** indicated highly significant at $p < 0.001$

Table 4.9: The Analysis of Variance (ANOVA) of *A. paeoniifolius* and *A. prainii* with time

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Replicate	**4**	**2.4209**	**0.6052**	**1.48 n. s**	**0.2278**
Time	**5**	**322.0731**	**64.4146**	**157.03 ****	**0.0001**
Species*Time	**5**	**12.6272**	**2.5254**	**6.16 ****	**0.0003**

Note: n. s indicated not significant at $p < 0.05$
** indicated highly significant at $p < 0.001$

Table 4.10: Tukey's Studentized Range (HSD) test for variation of Glucomannan (GM) content with time (month)

Mean	N	Time (2009)
13.2370**a**	10	December
12.0430**b**	10	October
10.8220**c**	10	August
8.6870**d**	10	June
7.9320**d**	10	April
6.6930**e**	10	February

Note: Means with the same letter are not significantly different at $p < 0.001$

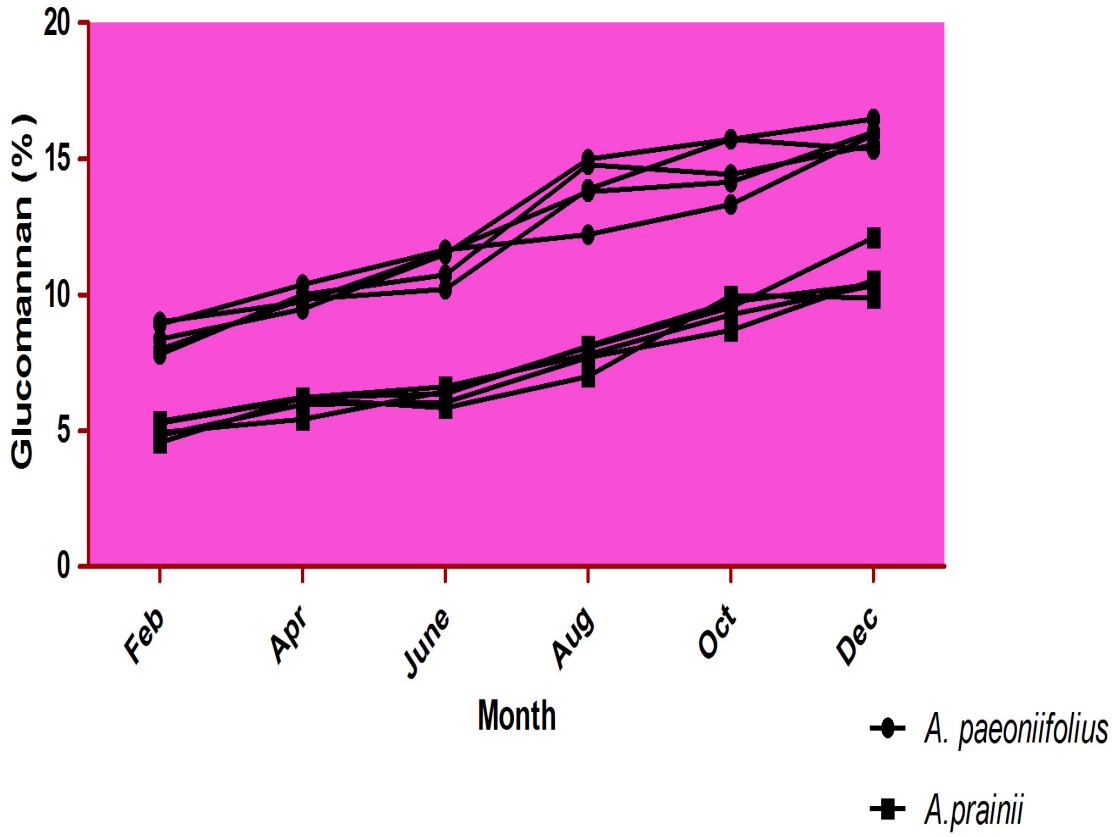

Figure 4.3: Glucomannan (GM) content in *A. paeoniifolius* and *A. prainii* against time.

Table 4.11: The variation of mean Glucomannan (GM) content of raw corms of *A. paeoniifolius* and *A. prainii* with time

Time of Sampling	N	Mean Glucomannan %	
		A. paeoniifolius	***A. prainii***
February	5	8.402 (± 0.54)	4.984 (± 0.33)
April	5	9.87 (± 0.33)	5.994 (± 0.34)
June	5	11.122 (± 0.64)	6.252 (± 0.31)
August	5	13.92 (± 1.1)	7.724 (± 0.45)
October	5	14.656 (± 1.04)	9.43 (± 0.49)
December	5	15.832 (± 0.43)	10.642 (± 0.85)

Note: standard deviations are in parenthesis

4.5 DISCUSSION

Results of the GM content of the purified powder from four species, namely, *A. elegans*, *A. oncophyllus*, *A. paeoniifolius* and *A. prainii* have been statistically analysed. A detailed study on the GM content of two species, namely, *A. paeoniifolius* and *A. prainii* were conducted based on age, time of harvesting, and location of sampling. Results indicated that there were highly significant variations in GM content between species; *A. oncophyllus* had the highest GM content (58.65 %). This was followed by mature *A. paeoniifolius* (50.22%), mature *A. prainii* (29.71 %) and *A. elegans* (17.01 %). The GM content was lowest in young *A. prainii* (9.23 %) followed by young *A. paeoniifolius* (14.90 %). According to the research made by Nguyen et al. (2009), GM content of *A. paeoniifolius* collected from Vietnam was about 8%, which was approximately 46% lower than what was found in this study. This might be due to different methods of isolation and purification. In their research, they directly separated the GM from the crude flour by dispersion of the crude flour in water. While in this study, the isolation process were carried out using *A. paeoniifolius* corm in the dried form, that means the corm of *A. paeoniifolius* were dried at 60 °C before the GM was isolated.

This study showed that the GM content was higher in mature corms than in young corms. The result showed that the highest glucomannan content recorded was from the mature tuber collected from the second vegetative phase of the growth stage. This finding is supported by Brown (2000) who stated that the content of glucomannan within the developing corm changes throughout the growing season and was highest just before the foliage died off, prior to dormancy. *A. paeoniifolius* had an appreciable GM content of an average of 50.22 % in December as compared to February, which was only 8.402 %. This might be due to environmental conditions

that support the growth and production of GM content during that season. Zhang et al. (1998) reported that the concentration of GM in raw corm increase with growing time.

As listed in Table 4.7 and shown in Figure 4.2, the GM content in the original corm collected from Kuala Kangsar, Perak for *A. prainii* and from Jerantut, Pahang for *A. paeoniifolius* had a mean value of 11.934 %, and 14.362 %, respectively. While the GM content in the transplanted corms of *A. prainii* and *A. paeoniifolius* in Dengkil, Selangor in this study had a mean value of 8.712 % and 12.0708 %, respectively. The GM content in the transplanted corms was slightly lower by approximately 15 – 18 % from the original corm. Disturbing the plant as in the case of transplanting the plant to a new environment may disrupt the physiological process of the plant and may result in lower GM content in the corm. The result was similar to the study carried out by Fang and Wu (2004) that showed that the GM content might be reduced after the plant is transplanted to a new environment. This may be due to the alteration of the ecological environment for *Amorphophallus* spp., which could lead to lower productivity in GM. *Amorphophallus* spp. require certain climatic and ecological conditions, and one of the factors that influence the amount of GM content is time of harvesting and planting site.

According to my results, *A. oncophyllus* exhibited the highest GM content in its purified powder with a mean value of 58.65 %. This result implies that *A. oncophyllus* could become a potential plant to be domesticated in Peninsular Malaysia. Although the species is native to Java, Indonesia and Thailand, it is scarcely found in Peninsular Malaysia except in the northern part of Peninsular Malaysia (Hetterscheid & Ittenbach, 1996). From the study, it can be said that mature *A. paeoniifolius* could also be the next highly potential plant to be domesticated in Peninsular Malaysia since the GM content is quite high with a mean value of 50.22 %. *Amorphophallus* spp. may be

developed as an economic crop for production of GM as in China and Japan, China being the largest country in producing GM in the world (Chua et al., 2010).