Tracing the Evolution and Economic Potential of Konjac Glucomannan in *Amorphophallus* species (*Araceae*) using Molecular Phylogeny and RAPD Markers

ORACHORN MEKKERDCHOO1,6, CHALEEDA BOROMPICHAICHARTKUL2*, ALLISON L. PERRIGO3, GEORGE SRZEDNICKI4, CHEUNJIT PRAKITCHAIWATTANA2 & ALEXandre ANTONELLI5

1Program in Biotechnology, Chulalongkorn University, Bangkok, 10330 Thailand
2Department of Food Technology, Chulalongkorn University, Bangkok, 10330 Thailand
3Forest Cat Editing, Uppsala, Sweden, and Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg 405 30, Sweden
4Food Science and Technology, School of Chemical Engineering, The University of New South Wales, Sydney, NSW 2052, Australia
5Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg 405 30, Sweden, and Gothenburg Botanical Garden, Carl Skottsbergs gata 224, 41319, Gothenburg, Sweden
6Division in Industrial Fermentation Technology, Faculty of Agro-Industry, King Mongkut’s Institute Of Technology Ladkrabang, Bangkok, 10520, Thailand

*Corresponding author, e-mail: chaleedab@hotmail.com

Abstract

The genus *Amorphophallus* is an economically important taxon that is abundant in Old World tropical forests (Asia, Oceania and Africa). It includes many species that are used with increasing frequency as a source of food and pharmaceutical products worldwide. *Amorphophallus konjac* is an important economic crop and has been used widely in China and Japan for commercial konjac glucomannan (KGM) production. However, the species’ range does not extend to Thailand, where other closely related species may be more suitable for commercial KGM production. Present understanding of genetic relationships among Thai *Amorphophallus* species is still limited, and the connection between evolutionary history and KGM content is unknown. Here, the genetic relationships among various accessions of *Amorphophallus* spp. collected in Thailand are investigated using the chloroplast *trnL-trnF* spacer, nuclear ribosomal internal transcribed spacer (ITS) region and the second intron of *LEAFY* (*FLint2*) together with genome-wide DNA variation analysis, Randomly Amplified Polymorphic DNA (RAPD) technique. RAPD primers are also developed to quickly and efficiently identify species producing high levels of KGM. This study finds that two monophyletic clades include high KGM content species. RAPD analyses indicate that primer AC-10 generates specific bands identifying species belonging only to the high and medium KGM content clades. These primers can be used as a screening tool for economical species, aiming at improving the industrial production of KGM in Thailand and the world.

Introduction

*Amorphophallus* Decaisne (1834: 366) is a perennial, herbaceous plant genus that belongs to the family Araceae (Figure 1). There are over 170 species occurring from West Africa, through subtropical and tropical Asia and south into the tropical portions of the western Pacific and north-eastern Australia. The genus includes a number of economically important species, many of which are known by the broad common name “konjac”. The most frequently cultivated among these is *A. konjac* Koch (1858: 262), which forms tubers containing polysaccharides. The latter are used in a wide range of commercial products throughout Asia and increasingly throughout the rest of the world (Chua et al. 2010). One of the main commercial products derived from some *Amorphophallus* species, especially *A. konjac*, is the polysaccharide konjac glucomannan (KGM). KGM is a high molecular weight, water-soluble and neutral polysaccharide (Fang & Wu 2004, Nishinari 2000). The molecules are rich in hydroxyl groups that make it easily dissolved in water, leading to high viscosity that forms a thick hydrocolloid even when used in low concentrations (Li et al. 2005). This property makes it one of the most versatile and economically useful hydrocolloids with industrial applications including the manufacture of foods, pharmaceuticals and chemicals (Douglas et al. 2005, Chua et al. 2010, Luo et al. 2013). Thailand is both a center of diversity for *Amorphophallus* as well as an emerging producer of...
economically important *Amorphophallus* products. At present, 58 species have been recorded in Thailand, of which at least 36 (ca. 65%) are endemic. Among the Thai *Amorphophallus* are a number of species that have the potential to become highly profitable crops in South East Asia, as they produce significant amounts of KGM. *Amorphophallus konjac*, the main species used for KGM production in China, does not occur in Thailand and therefore other native species may be more suitable as sources of KGM in the region.

**FIGURE 1.** Morphological variation in *Amorphophallus* spp.
The economic potential of KGM has led to an increased focus on the study of economically significant *Amorphophallus* species (Diao *et al*. 2014, Gille *et al*. 2011, Zheng *et al*. 2013). These studies have addressed the relationship between genetic markers and KGM production in *A. konjac*, and have focused on a transcriptomics approach to identifying potentially useful regions in the genomes of several other *Amorphophallus* species for further study. However, the association between genetic diversity and KGM content in a broader population of *Amorphophallus* species has not yet been systematically assessed.

*Amorphophallus* has been studied previously in relation to its morphology (Hetterscheid & Ittenbach 1996, Hetterscheid 2006, Hetterscheid *et al*. 2012), palynology (Giordano 1999, Punekar & Kumaran 2010) and inflorescence odor biochemistry (Kite & Hetterscheid 1997, Kite *et al*. 1998). However, because the morphological and palynological characters are highly variable therefore much more difficult to determine species relationships based on these characters (Grob *et al*. 2002, Punekar & Kumaran, 2010). For this reason, a number of molecular markers have been employed to determine relationships in the genus. These include the *LEAFY (FL)* gene and the chloroplast regions *rbcL*, *matK* and *trnL* (Grob *et al*. 2002, 2004, Sedayu *et al*. 2010, Wahyudi *et al*. 2013). However, phylogenetic studies based on these regions do not produce consistent cladograms, due to a high level of conflicting signal in the informative characters. Consequently, further variable regions as well as other non-sequencing molecular methods are needed to elucidate the evolutionary history of *Amorphophallus*. This may lead to useful insights into the relationships within the genus, as well as the evolutionary history of important traits such as KGM production.

One non-sequencing method that can be valuable in identifying phylogenetically significant groupings of taxa is Randomly Amplified Polymorphic DNA (RAPD) analysis. RAPD can be used for a wide range of applications because of its sensitivity, simplicity, cost-effectiveness and because it does not require sequencing reactions (Williams *et al*. 1990, Bardakci 2001, Li *et al*. 2006). Unlike standard phylogenetic markers that are made up of nucleotide sequences from a small section of genomic DNA, RAPD segments are generally made up of larger, untargeted fragments from throughout the genome that span both coding and non-coding DNA regions, and therefore may be more representative of overall genetic patterns and can be subsequently more informative in phylogenetic analyses (Atienzar & Jha 2006, Lopes *et al*. 2012). This technique is most frequently used in population genetics studies to estimate affiliations between closely related plants, though it can also be useful at a higher taxonomic level. RAPD has been successfully applied for species-level studies in *Amorphophallus*, including work on *A. albus* (Hu *et al*. 2008), *A. titanum* Arcangeli (1879: 46) (Poerba & Yuzammi 2008) and *A. muelleri* Blume (1837: 143) (Poerba & Martanti 2008).

The main aims of this study are to determine the relationships among *Amorphophallus* species found in Thailand based on multiple molecular markers along with RAPD analysis, correlations between species relationships and KGM content and to investigate the utility of the newly generated molecular data as markers to be used as screening tools for the identification of *Amorphophallus* species in Thailand with high KGM production and potential economic value.

**Materials and Methods**

**Plant material**

Forty-seven accessions of *Amorphophallus* belonging to 35 species were used in this study. These accessions were composed of both field collections (12 species) and cultivars (23 species) (Table 1; Appendix 1). Sample material was collected from various natural habitats throughout Thailand and China (Yunnan province) (Figure 2A). Fresh tubers were taken from wild specimens (20 accessions of 12 taxa), as well as two cultivated specimens: *A. bulbifer* Blume (1837: 138) and *A. xiei* Li & Dao (2006: 240). All material from cultivated species was obtained from the leaf tissue of living plants in the Hamburg Botanical Garden (Germany), originally from the private collection of W. L. A. Hetterscheid, from Gothenburg Botanical Garden (Sweden) or from researchers’ collections in Yunnan, China. *Arisaema fimbriatum* (Masters 1884: 680) and *Gonatopus angustus* (Brown 1901: 778), collected from Gothenburg Botanical Garden, were also included in the sequencing analysis as outgroup taxa. *Pycnospatha arietina* (Gagnepain 1941: 512) was used in both the sequencing and RAPD analyses as an outgroup.
<table>
<thead>
<tr>
<th>Accession</th>
<th>Amorphophallus Species *</th>
<th>Locality</th>
<th>Voucher no.</th>
<th>Origin</th>
<th>Collection Part</th>
<th>GenBank accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>A. muelleri</em> Blume</td>
<td>Northern Thailand, Mae Hong Son</td>
<td>ME05P1T1 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534380, KR534482, KR534417</td>
</tr>
<tr>
<td>2</td>
<td><em>A. muelleri</em> Blume</td>
<td>Northern Thailand, Tak</td>
<td>TK04P1T1 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534379, KR534483, -</td>
</tr>
<tr>
<td>3</td>
<td><em>A. muelleri</em> Blume</td>
<td>Northern Thailand, Mae Hong Son</td>
<td>ME03P1T1 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534381, KR534481, KR534415</td>
</tr>
<tr>
<td>4</td>
<td><em>A. muelleri</em> Blume</td>
<td>Northern Thailand, Tak</td>
<td>TK08P2T1 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534378, KR534484, KR534414</td>
</tr>
<tr>
<td>5</td>
<td><em>A. muelleri</em> Blume</td>
<td>South-Western Thailand, Kanchanaburi</td>
<td>KC2010-05GZ (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534382, KR534480, KR534416</td>
</tr>
<tr>
<td>6</td>
<td><em>A. muelleri</em> Blume</td>
<td>South-Western Thailand, Kanchanaburi</td>
<td>KC2010-02GZ (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534383, KR534479, KR534418</td>
</tr>
<tr>
<td>7</td>
<td><em>A. bulbifer</em> (Roxb.) Blume</td>
<td>China, Yunnan</td>
<td>CN2010-01GZ</td>
<td>cultivated</td>
<td>Leaf/tuber</td>
<td>KR534405, KR534467, KR534430</td>
</tr>
<tr>
<td>8</td>
<td><em>A. bulbifer</em> (Roxb.) Blume</td>
<td>China, Yunnan</td>
<td>CN2010-02GZ</td>
<td>cultivated</td>
<td>Leaf/tuber</td>
<td>KR534404, - , KR534431</td>
</tr>
<tr>
<td>9</td>
<td><em>A. xiei</em> Li &amp; Dao</td>
<td>China, Yunnan</td>
<td>CNX2010-01GZ</td>
<td>cultivated</td>
<td>Leaf/tuber</td>
<td>KR534366, KR534496, KR534412</td>
</tr>
<tr>
<td>10</td>
<td><em>A. kachinensis</em> Eng. &amp; Gehrmann</td>
<td>Northern Thailand, Chiang Mai</td>
<td>CM10P1T6 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534395, KR534473, KR534425</td>
</tr>
<tr>
<td>13</td>
<td><em>A. macrorhizus</em> Craib</td>
<td>Northern Thailand, Lampoon</td>
<td>LO03P2T5 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534385, - , KR534445</td>
</tr>
<tr>
<td>Accession</td>
<td>Amorphophallus Species *</td>
<td>Locality</td>
<td>Voucher no.</td>
<td>Origin</td>
<td>Collection Part</td>
<td>GenBank accession numbers</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------</td>
<td>----------</td>
<td>-------------</td>
<td>--------</td>
<td>-----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>20</td>
<td>(A. \text{ napiger}) Gagn.*</td>
<td>South-Eastern Thailand, Sa Kaeo</td>
<td>H.AM. 0708</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534377</td>
</tr>
<tr>
<td>21</td>
<td>(A. \text{ corrugatus}) N. E. Br.</td>
<td>Northern Thailand, Lampang</td>
<td>LA01P1T3 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534401</td>
</tr>
<tr>
<td>23</td>
<td>(A. \text{ kachinensis}) Engl. &amp; Gehrm.</td>
<td>Northern Thailand, Mae Hong Son</td>
<td>ME07P1T6 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534394</td>
</tr>
<tr>
<td>24</td>
<td>(A. \text{ temuispadix}) Hett.</td>
<td>Eastern Thailand, Chaiyaphum</td>
<td>CP2010-C5 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534369</td>
</tr>
<tr>
<td>26</td>
<td>(Pycnospatha arietina) (outgroup)</td>
<td>South-Eastern Thailand, Prachin Buri</td>
<td>JIEL01 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534399</td>
</tr>
<tr>
<td>27</td>
<td>(A. \text{ pygmaeus}) Hett.</td>
<td>South-Eastern Thailand, Chanthaburi</td>
<td>JPM01 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534371</td>
</tr>
<tr>
<td>29</td>
<td>(A. \text{ putii}) Gagnepain *</td>
<td>Central Thailand, Saraburi</td>
<td>H.AM. 0697</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534372</td>
</tr>
<tr>
<td>34</td>
<td>(A. \text{ carneus}) Ridl. **</td>
<td>Peninsular Thailand</td>
<td>2010-2819 pZ</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534402</td>
</tr>
<tr>
<td>37</td>
<td>(A. \text{ excentricus}) Hett. **</td>
<td>Peninsular Thailand, Nakhon Si Thammarat</td>
<td>2010-2824 pG</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534398</td>
</tr>
<tr>
<td>38</td>
<td>(A. \text{ fuscus}) Hett. **</td>
<td>Northern Thailand</td>
<td>2010-1652 pZ</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534397</td>
</tr>
</tbody>
</table>
TABLE 1. Details of *Amorphophallus* samples used in Sequencing and RAPD analysis (cont.)

<table>
<thead>
<tr>
<th>Accession</th>
<th><em>Amorphophallus</em> Species<em>a</em></th>
<th>Locality</th>
<th>Voucher no.</th>
<th>Origin</th>
<th>Collection Part</th>
<th>GenBank accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Part</td>
<td>trnL-trnF FLnt2 ITS</td>
</tr>
<tr>
<td>40</td>
<td><em>A. xiei</em> Li &amp; Dao</td>
<td>China, Yunnan</td>
<td>CNX2010-02GZ</td>
<td>cultivated</td>
<td>Leaf/tuber</td>
<td>KR534365 - KR534413</td>
</tr>
<tr>
<td>43</td>
<td><em>A. bulbifer</em> (Roxb.) Bl *</td>
<td>Northern Thailand, Mae Hong Son</td>
<td>H.AM. 1451</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534403 - KR534429</td>
</tr>
<tr>
<td>46</td>
<td><em>A. amygdaloides</em> Hett. &amp; M. Sizemore</td>
<td>Northern Thailand, Tak</td>
<td>TK08P1T11 (H)</td>
<td>wild</td>
<td>Leaf/tuber</td>
<td>KR534409 KR534463 KR534458</td>
</tr>
<tr>
<td>47</td>
<td><em>A. linearis</em> Gag. *</td>
<td>South-Western Thailand, Kanchanaburi</td>
<td>CP</td>
<td>cultivated</td>
<td>Leaf</td>
<td>KR534387 - - -</td>
</tr>
</tbody>
</table>

*a* Samples with the one asterisk represent plant samples collected from Hamburg Botanical Garden (Germany) and species with double asterisks represent plant samples collected from Gothenburg Botanical Garden (Sweden)
**KGM content determination**

The fresh tubers of wild plants (250 g) were sliced and dried at 50 °C for 6-8 h to reduce the moisture content. The dried tuber slices were ground and sifted through a 120 µm mesh sieve to separate starch from glucomannan and other particles ≥ 125 µm. The resulting crude konjac flour samples were subjected to analysis of the KGM content through a 3, 5-dinitrosalicilic acid colorimetric assay (DNS) according to a sugar-reduction hydrolysis extraction method (adapted from Liu et al. 2002, Zhao et al. 2010 and Chua et al. 2012). In order to remove the reducing sugars, 85% ethanol was added to the crude konjac flour samples. This was followed by the extraction of KGM with distilled water and the subsequent hydrolysis of KGM with sulfuric acid. The KGM content was determined by DNS and calculated using equation (1), which determines the absorbency of the KGM hydrolysate at 550 nm wavelength:

\[
KGM (\%) = \frac{\varepsilon T \times 100}{m} \times 100
\]

where \( \varepsilon \) is residual molecular weight and hydrolysis molecular weight ratio of mannose and glucose in the glucomannan (molecular weights of mannose and glucose are both 180, residual molecular weight is 162, \( \varepsilon = 162/180 = 0.9 \)), \( T \) is absorbance of samples in the glucose standard curve corresponding to the number of milligrams of glucose and \( M \) is mass of sample (mg).

Each analysis was performed in triplicate and mean values were calculated. The differences between means were estimated using Duncan’s Multiple Range Test with a level of significance of \( p < 0.05 \) using the SPSS 16.0 software (IBM SPSS, Chicago, IL, USA).

**DNA amplification and sequencing analysis**

Total genomic DNA was isolated from fresh and dried leaflets using the DNeasy Plant Mini Kit (QIAGen, Valencia, CA, USA) following the manufacturer’s protocol. The DNA concentration was estimated by spectrophotometry (Nanodrop 1000, Thermo Scientific, USA). The chloroplast trnL-trnF spacer was amplified and sequenced with universal primers “c” and “f” (Taberlet et al. 1991). A section of the internal transcribed spacer (ITS) was amplified using the primer pair P17/26S-82R (Popp & Oxelman 2001). The second intron of LEAFY (FLInt2) was amplified with primers FLInt2 F1 and FLInt2 R1 (Grob et al. 2004). PCR products from each region were separated on 1% agarose gels at 100 Volt for 1 h in TAE buffer to indicate the quantity of the fragments. Multiscreen PCR (Millipore, Billerica, Massachusetts, U.S.A.) was used to purify the PCR products, according to the manufacturer’s instructions. All sequencing reactions were carried out by Macrogen (South Korea) using Applied Biosystems 3730xl DNA Sequencer (Thermo Scientific, USA).

**Random amplified polymorphic DNA (RAPD) analysis**

RAPD-PCR was used to generate banding patterns for the different Amorphophallus accessions using thirteen RAPD primers, which were selected from previous primer screening tests based on their ability to generate DNA bands in all Amorphophallus accessions (Table 4). PCR amplification conditions for long RAPD primers (ERIC1R, ERIC2, BOXA1R, RPO1) were an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 65 °C for 8 min, followed by 65 °C for 5 min. Amplification conditions for the decamer primers were an initial denaturation at 94 °C for 5 min, followed by 45 cycles of 94 °C for 1 min, 40 °C for 1 min, and 72 °C for 2 min, followed by 72 °C for 4 min. Amplifications were done using a MyGenie™ 96 Thermal Block (Bioneer, Korea) and Bio-Rad My Cycler™ Personal Thermal Cycler (Bio-Rad, USA). PCR products were separated by electrophoresis in a 1.0% (w/v) agarose gel in TAE buffer at 80 V for 1.30 hours with GeneRuler™ DNA Ladder Mix (SM0331, Thermo Scientific, USA) for size estimation. The RAPD fragments were photographed using a UV transilluminator and analyzed with a gel documentation system (Sysgene, England).

**Data analysis**

For sequence analysis, forward and reverse sequence reads that were assembled and trimmed were created using GENEIOUS Pro R6 (Version 5.4, Bio matters, New Zealand) (Kearse et al., 2012) and manually corrected. Multiple sequences alignments were created using MAFFT, with the L-INS-i algorithm. Indels were coded using SeqState V.1.4.1 (Müller 2005), under the simple indel coding option (Simmons & Ochotorena 2000). The best substitution model for each alignment was selected using jModelTest v.2.1.1 (Posada 2008), under the Bayesian information criterion (BIC) to find the evolutionary model that uses the best-fit model in each of the three regions analyzed. Bayesian inference (BI) analyses of the individual genes were performed using MrBayes v.3.2.1 (Ronquist & Huelsenbeck, 2003), with
default prior settings, for ten million MCMC generations with eight chains under the increased temperature of $t = 0.1$.

For RAPD analysis, each amplified product was sized and scored according to the presence/absence of the DNA band for each specimen and each primer, as performed with GeneTools v3.06.04 (Sysgene, England). After pre-analysis using default settings, a sizing profile of all samples was checked and where necessary manually corrected. Polymorphism information content (PIC) was calculated per primer (Nagy et al., 2012). The RAPD scores obtained were pooled to create a single data matrix and were analyzed for genetic distance using the method described by Nei & Li (1979). Dendrogram was constructed using the RAPD data.

**FIGURE 2.** Collection sites of *Amorphophallus* spp. (A) with sample numbers on the map showing each province. KGM content in *Amorphophallus* tubers (B) with different letters on the bar graph as per results of Duncan’s multiple range tests ($p \leq 0.05$) indicate significant differences between samples. Geographic distribution (N, North; NE, North-eastern; SW, South-western; C, Central; SE, South-Eastern; Pen, Peninsular).

**Results**

**KGM content in Amorphophallus tubers**

KGM is the main polysaccharide component in the tuber and occurs in varying levels in different *Amorphophallus* species. The KGM content was investigated in the tubers of plants that were growing in their natural habitat (with
exception of those from Yunnan in China). The analysis of these wild germplasm collected in their natural habitat may show how plants adapt to climatic variation under natural selection—a strategy that has been employed in plant breeding for climate resilience (Henry & Nevo, 2014; Brozynska et al. 2016). Amorphophallus tubers from 23 samples representing 14 species were selected for KGM content analysis. These samples included two economically important species from China. The dry-weight KGM content of the samples is shown in Figure 2B along with information on the collection locality. The results show that the analyzed specimens had KGM contents ranging from 1.53 to 68.9% of the tuber’s dry weight, depending on the species and collection area. The highest KGM content was found in A. muelleri (60.16–68.93%) followed by A. krausei, A. kachiensis, A. bulbifer, A. xiei and A. corrugatus respectively. Among the remaining species, KGM content was low (<10% of dry tuber weight) and the results indicate no significant variation in KGM content among these samples. The species were divided into three categories of KGM content: high (40–70%), medium (20–39%) and low (<20%), based on the dry weight KGM content of the tubers (Table 2) which agrees with the Chinese Ministry of Agriculture for the classification of common konjac flour (KGM content between 40 and 70%; Liu et al. 2002).

Geographic factors such as climate and altitude may have impact on KGM content variation (Figure 2). For example, the KGM content in A. muelleri samples was significantly different in the sample from Mae Hong Son, northern Thailand and from Kanchanaburi, western Thailand. However, in the same region within northern Thailand (Mae Hong Son and Tak province), KGM content in A. muelleri was not significantly different. Likewise, in A. kachiensis samples, the variation of their KGM content among samples found in Chiang Mai and Mae Hong Son (northern Thailand) were not significantly different.

Nucleotide sequence analysis
Three DNA regions (trnL-trnF, ITS and FLint2) were combined to create a data set used to investigate the phylogenetic relationship within Thai and Chinese Amorphophallus species. Models of nucleotide substitution were selected for Bayesian analysis with MrModeltest 2.1.1 (Nylander 2012). The characteristics of the regions (both separate and combined) are shown in Table 3.

The phylogeny based on the combined data set indicates five clades (clades A–E) within the Amorphophallus ingroup (Figure 3). Bayesian analysis was selected for this study because this method is better suited for tackling branch length variation and gave better resolution in previous studies (Holder & Lewis 2003, Turner et al. 2013). Posterior probability (PP) support can be defined as strong (>0.90), moderate (0.85–0.90), weak (0.75–0.84) or ambiguous (<0.75) (Antonelli 2008).

Amorphophallus obscurus is identified as the sister to the rest of Amorphophallus in both the individual ITS phylogeny, as well as in the combined phylogeny. This species differs from other species in this study by the smallest inflorescence of a mere 8 cm high, unique tubular shathe and small white spotted leaflets. Moreover, it occurs only in the eastern regions of Thailand (Hetterscheid & Van der Ham 2001). Amorphophallus paeonifolius, is clearly identified as the next diverging lineage in the individual ITS and FLint2 region phylogenies. This species belongs to the low KGM content group and is found all over in Thailand. The inflorescence peduncle of both species (A. obscurus and A. paeonifolius) is entirely or largely hidden in the soil (Hetterscheid & Van der Ham 2001; Boyce et al. 2012), a trait that is not seen in any of the other Amorphophallus clades discussed below.

Clade (A) consists of five taxa. Only A. tenuistylis in third subclade was analyzed for KGM content and was found to belong to the low KGM content group. This clade is identified clearly in all individual DNA region phylogenies: trnL-trnF spacer (PP 0.98), ITS (PP 0.95), FLint2 (PP 0.86) (Figures 4–6), as well as in the combined phylogeny, with strong support (PP 1.0). Species in this clade occur from the northern region through the southern region of Thailand. Clade (B) consists of nine taxa (PP 1.0), four of which belong to the medium and high KGM content groups. The first subclade comprises two sister groups of A. krausei and A. kachiensis, which belong to the high KGM content group. Meanwhile, the fourth subclade includes A. corrugatus that belongs to the medium KGM content group and is characterized by its strongly cerebriform appendix morphology. This clade is identified clearly in the individual ITS phylogeny (PP 0.8). The taxa in this clade occur in a diverse range of localities around Thailand, with the exception of A. konjac, which is restricted to China. Clade (C) is made up of six taxa (PP 1.0), three of which were analyzed for KGM content and found to contain only low levels of the polysaccharide. The blue-colored berry is a unique morphological feature in this clade. This clade is identified clearly in the individual ITS (PP 0.8) and FLint2 (PP 0.92) phylogenies. The taxa in this clade are found in northern and central Thailand. Clade (D) is made up of eight taxa occurring from northern Thailand down into the southern peninsula. In the combined phylogeny, the clade is only weakly supported (PP 0.81). Two taxa in this clade, A. pygmaeus and A. macrorhizus, were assessed for their KGM content and both were found to belong to the low-KGM production group. Clade (E) is a well-supported group (PP 1.0) and clearly supported...
by all individual DNA region phylogenies (trnL-trnF spacer (PP 0.97), ITS (PP 0.99), FLint2 (PP 1.0)). However, the trnL-trnF spacer and FLint2 do not indicate the internal relationships among those samples. This clade is made up of eleven samples belonging to three taxa, all of which were found to have high KGM levels.

FIGURE 3. Combined region phylogenetic tree using Bayesian inference. Numbers above branches indicate the posterior probability of the clade. Clearly identify clades under individual DNA region are indicated by different bar of trnL-trnF spacer ( ), ITS ( ) and FLint2 ( ). Character of tuber: globose/no-offset ( ), globose with offset ( ), elongate/unbranched ( ) and elongate with branching ( ). Berry color: red/orange color ( ), white color ( ), blue color ( ) and green color ( ). Peduncle character: long ( ) and short ( ). Spadix ratio and spathe color is mark as:

(A) Longer spadix and pale color of spathe
(B) Longer spadix and dark color of spathe
(C) Shorter spadix and pale color of spathe
(D) Shorter spadix and pale color of spathe

Support DNA region are indicated by different bar: trnL-trnF spacer ( ), ITS ( ) and FLint2 ( ).

90 • Phytotaxa 282 (2) © 2016 Magnolia Press MEKKERDCHOO ET AL.
FIGURE 4. Phylogenetic tree of trnL-trnF spacer region. Numbers above branches indicate the posterior probability of the clade. Red text represents high KGM taxa and the number in brackets represents KGM contents with significant differences ($p<0.05$) among samples. Geographic distribution (N, North; NE, North-eastern; SW, South-western; C, Central; SE, South-Eastern; Pen, Peninsular)
FIGURE 5. Phylogenetic tree of ITS region. Numbers above branches indicate the posterior probability of the clade. Red text represents high KGM taxa and the number in brackets represents KGM contents with significant differences ($p<0.05$) among samples. Geographic distribution (N, North; NE, North-eastern; SW, South-western; C, Central; SE, South-Eastern; Pen, Peninsular).
FIGURE 6. Phylogenetic tree of *FLORICAULA/LEAFY (FLint2)*. Numbers above branches indicate the posterior probability of the clade. Number in bracket represent of glucomannan content with differenced statistic. Red text represents high KGM taxa and the number in brackets represents KGM contents with significant differences (*p* < 0.05) among samples. Geographic distribution (N, North; NE, North-eastern; SW, South-western; C, Central; SE, South-Eastern; Pen, Peninsular).
### TABLE 2. Levels of KGM content in *Amorphophallus* tubers.

<table>
<thead>
<tr>
<th>Level</th>
<th>KGM content (% of total dry weight)</th>
<th>Sample Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>40–70</td>
<td><em>A. muelleri, A. kachinensis, A. krausei, A. bulbifer, A. xiei</em></td>
</tr>
<tr>
<td>Medium</td>
<td>20–39</td>
<td><em>A. corrugatus</em></td>
</tr>
<tr>
<td>Low</td>
<td>1–19</td>
<td><em>A. amygdaloides, A. asterostigmatus, A. macrorhizus, A. paeoniifolius, A. pygmaeus, A. tenuistylis, A. thaiensis, A. yunnanensis</em></td>
</tr>
</tbody>
</table>

### TABLE 3. Statistical parameters of individual and combined data matrices.

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th><em>trnL-trnF</em> spacer</th>
<th><em>FLORICAULA/LEAFY (FLint2)</em></th>
<th>Internal transcribed spacers (ITS)</th>
<th>Combined data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model of nucleotide substitution</td>
<td>TPM2uf+I+G</td>
<td>TPM1+G</td>
<td>TrN+G</td>
<td>TrN+G</td>
</tr>
<tr>
<td>Number of included positions in matrix</td>
<td>1224</td>
<td>372</td>
<td>1201</td>
<td>2699</td>
</tr>
<tr>
<td>Length range</td>
<td>835–986</td>
<td>177–322</td>
<td>805–964</td>
<td>1101–2153</td>
</tr>
<tr>
<td>%GC content</td>
<td>32.311</td>
<td>50.469</td>
<td>67.238</td>
<td>48.011</td>
</tr>
<tr>
<td>Percentage of variable sites (%)</td>
<td>16.83</td>
<td>36.828</td>
<td>54.397</td>
<td>30.641</td>
</tr>
<tr>
<td>Percentage of informative sites (%)</td>
<td>9.069</td>
<td>18.280</td>
<td>29.012</td>
<td>15.006</td>
</tr>
<tr>
<td>Number of indels</td>
<td>83</td>
<td>53</td>
<td>154</td>
<td>307</td>
</tr>
<tr>
<td>Percentage of number of clades in ingroup with &gt;0.85 posterior probability</td>
<td>75</td>
<td>75</td>
<td>82.6</td>
<td>75.6</td>
</tr>
</tbody>
</table>

Clades (B) and (E) are the only clades containing species with high KGM content. *Amorphophallus bulbifer* occurs in NE India and Myanmar and *A. xiei* occur only in Yunnan (China), while *A. muelleri* is found from northern to western Thailand, in Myanmar and in Indonesia.

**Random amplified polymorphic DNA (RAPD) analysis**

The random amplified polymorphic DNA (RAPD) technique was used as a tool for assessing genetic variation and relationships among *Amorphophallus* species. A total of thirteen primers that created reproducible amplified DNA fragments were selected. The primers and the respective size range of the bands they produced are listed in Table 4. This technique amplified a total of 269 bands ranging from 150 to 5000 bp with an average of 21 scored RAPD marker bands per primer. All amplified fragments were found to have 100% polymorphic bands. The highest number of RAPD bands (28 bands) was amplified by primer AB-20 while the lowest number of RAPD bands (12 bands) was amplified by primer ERIC1R. The polymorphism information content (PIC) value ranged from 0.33 (primer OPD-04) to 0.75 (primer AB-20). The values of pairwise comparisons of Nei and Lee’s genetic distance, from all set of primers among the 48 accession of *Amorphophallus* ranged from 0.075 to 0.949. Comparatively, a higher genetic distance (0.949) was observed between sample no. 1 (*A. muelleri* from northern Thailand) and no. 34 (*A. paeoniifolius* also from northern Thailand) than with other combinations. The lowest genetic distance (0.075) was found in both accessions (no. 1 and 3) from Mae Hong Son province in northern Thailand. When considering the different collection regions in relation to the different primer sets, it was found that the genetic distances among samples from the northern regions were lower (0.1972–0.2216) than the distances among samples from other regions in Thailand (0.3252–0.9323). A dendrogram was constructed using the unweighted pair group method with arithmetic mean (NJ) showing dissimilarity among random amplified polymorphic DNA (RAPD) haplotypes. Figure 7 shows the relationships among *Amorphophallus* samples. The dendrogram indicates a high KGM content clade where *A. muelleri, A. bulbifer* and *A. xiei* are grouped together, while *A. kachinensis, A. corrugatus* and *A. krusei* are grouped separately. This indicates that *A. muelleri, A. bulbifer* and *A. xiei* are more closely related, with a minimal genetic distance (0.351) among the cluster, whereas *A. paeoniifolius* was the most divergent among the studied *Amorphophallus* genotypes. In order to identify a primer that produces a
**FIGURE 7.** Dendrogram of RAPD profile data of *Amorphophallus* species from Thailand from 13 primers. Geographic distribution (N, North; NE, North-eastern; SW, South-western; C, Central; SE, South-Eastern; Pen, Peninsular), indicated to right of tree. Highlighted area indicates high KGM content groups.
unique RAPD fragment to be used as a proxy for identifying medium to high KGM content taxa, all fragments from the 13 primers were screened for presence/absence of each RAPD fragment scored against the KGM content groupings. One primer (primer AC-10) amplified a unique band of 600 bp that was only present in the seven high and medium
KGM content species, from the total 19 samples (Figure 8). These results confirmed the importance of using RAPD analysis for genotypic characterization, indicating a specific band that can distinguish medium and high KGM content species from those with only low levels of the polysaccharide.

### TABLE 4. RAPD primers with corresponding bands scored and their size range together with polymorphic bands observed in *Amorphophallus* spp.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequences (5’-3’)</th>
<th>Size ranged (bp)</th>
<th>Scored bands</th>
<th>Number of polymorphic bands</th>
<th>Polymorphic Information Content (PIC)</th>
<th>Average Nei and Li’s genetic distances</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-04</td>
<td>GGCACGCCTT</td>
<td>150–2000</td>
<td>23</td>
<td>23</td>
<td>0.56</td>
<td>0.5775</td>
</tr>
<tr>
<td>AB-20</td>
<td>CTTCTCGGAC</td>
<td>250–5000</td>
<td>28</td>
<td>28</td>
<td>0.75</td>
<td>0.5225</td>
</tr>
<tr>
<td>AC-09</td>
<td>AGAGCGTACC</td>
<td>200–1500</td>
<td>17</td>
<td>17</td>
<td>0.63</td>
<td>0.546</td>
</tr>
<tr>
<td>AC-10</td>
<td>AGCAGCGAGG</td>
<td>300–2000</td>
<td>20</td>
<td>20</td>
<td>0.55</td>
<td>0.3925</td>
</tr>
<tr>
<td>AH-18</td>
<td>GGGCTAGTCA</td>
<td>200–2000</td>
<td>23</td>
<td>23</td>
<td>0.45</td>
<td>0.514</td>
</tr>
<tr>
<td>OPB-17</td>
<td>AGGGAACGAG</td>
<td>100–2000</td>
<td>25</td>
<td>25</td>
<td>0.62</td>
<td>0.5735</td>
</tr>
<tr>
<td>OPC-02</td>
<td>GTGAGGCGTGC</td>
<td>250–2000</td>
<td>22</td>
<td>22</td>
<td>0.58</td>
<td>0.4715</td>
</tr>
<tr>
<td>OPC-07</td>
<td>CACACTCCAG</td>
<td>250–2000</td>
<td>22</td>
<td>22</td>
<td>0.69</td>
<td>0.4615</td>
</tr>
<tr>
<td>OPD-04</td>
<td>TCTGATGAGG</td>
<td>250–3500</td>
<td>25</td>
<td>25</td>
<td>0.32</td>
<td>0.445</td>
</tr>
<tr>
<td>BOXA1R</td>
<td>CTACGGCAAGGCCGCAGGCTGAGG</td>
<td>300–3200</td>
<td>20</td>
<td>20</td>
<td>0.44</td>
<td>0.3095</td>
</tr>
<tr>
<td>ERIC1R</td>
<td>ATGTAAGCTCCTGGGATTCAC</td>
<td>300–2500</td>
<td>15</td>
<td>15</td>
<td>0.63</td>
<td>0.301</td>
</tr>
<tr>
<td>ERIC2</td>
<td>AAGTAAAGTGACTGGGGTGAGG</td>
<td>250–1600</td>
<td>17</td>
<td>17</td>
<td>0.42</td>
<td>0.305</td>
</tr>
<tr>
<td>RPO1</td>
<td>AATTTTCAAAGCGTCTGAGG</td>
<td>300–4000</td>
<td>20</td>
<td>20</td>
<td>0.67</td>
<td>0.3885</td>
</tr>
</tbody>
</table>

**Discussion**

**KGM content of Amorphophallus species in Thailand**

In this study, the KGM content of the tubers of fourteen *Amorphophallus* species were assessed and divided into three KGM content groups: low, medium and high (Table 2). Those in the high KGM content group have a net KGM content of more than 40% of the dry tuber weight, and—like *A. bulbifer*—may be potentially useful taxa in the future production of commercial KGM, especially in Thailand. In order to help rapidly identify species that are likely to have a high KGM content, and which may be potentially useful for commercial production of KGM, a RAPD primer that produces a unique, diagnostic band in all high and medium KGM *Amorphophallus* species must be identified and tested. These species are found in two distinct clades in the molecular phylogeny presented here, indicating that high-
KGM levels in the tuber probably evolved at least twice in this genus. KGM content in the tuber is a useful parameter for resource evaluation, product development and quality control in these agricultural products. Of the 12 species for which KGM content was evaluated here the highest levels were found in *A. muelleri* (Figure 2B). This finding agrees with previous studies showing that tubers of *A. muelleri* exhibited a higher KGM content than other species of *Amorphophallus* found in Thailand (Akesowan 1991). Sanguanpong *et al.* (2002) and Kadprasert (2004) reported a list of species that were used in the KGM production industry in Thailand including *A. muelleri* and *A. corrugatus*, as well as three undetermined species. However, these studies mention that *A. corrugatus* has a long growth cycle and therefore this species is not ideal for use in the konjac cultivation industry. In commercial KGM production, dormant tubers of *A. konjac* contain 49–60% KGM (Liu 2004) which is similar to the KGM level in *A. muelleri* found in Thailand (60.16–68.93%). However, *A. konjac* has a limited propagation rate, long growth cycle and problems with soft rot disease and seedling blight (Zhao 2010, Diao *et al.* 2014). For these reasons, *A. bulbifer* has been cultivated in place of *A. konjac* in Yunnan Province, China, as this species is strongly resistant to disease and has a high propagation coefficient (Zhang *et al.* 2009). As *A. konjac* does not naturally occur in tropical regions like Thailand, native species adapted to the tropical climate that also have a high KGM content may therefore be more suitable for commercial KGM production in the country, as *A. bulbifer* is in southern China. Zhao (2010) studied the productivity and quality of KGM extracted from a number of *Amorphophallus* species and found that *A. muelleri* has the highest productivity and quality, followed by *A. konjac* and *A. bulbifer* (Input and output ratio, 10:1/14:1/12:1, KGM content 78%/60%/48%, viscosity 32,000/28,000/24,500 mPa, respectively). Similarly, Impaprasert (2013) found that when using a suitable extraction process, *A. muelleri* produced the highest KGM content at 88.46% and the product had a higher viscosity than commercial products made from KGM derived from *A. konjac*. *Amorphophallus kachinensis* and *A. krausei* were reported to have been found in China with KGM contents of 39.8% and 28.5% respectively (Liu 2004). In this finding, both species have a higher KGM content than that reported in previous studies conducted in China.

**Phylogenetic Relationships within Amorphophallus by sequencing analysis**

The combined molecular analysis based on the *trnL-trnF* spacer, ITS and LEAFY (FLint2) gave improved resolution among Thai *Amorphophallus* species compared to earlier studies, and shows a number of well-supported clades that are not apparent based on single region data alone. Among these regions, the ITS had the highest variation and was the most phylogenetically informative, followed by the more conservative FLint2 and *trnL-trnF* spacer (Table 3). Both the ITS and FLint2 regions have been reported as higher rate of variable sites because those regions evolve rapidly, leading to genetic changes that can differentiate closely related (Yingzhi *et al.* 2007, Feng *et al.* 2013, Mishra *et al.* 2016), also high sequence divergence due to biparental inheritance (Wilson 2003) and intragenomic uniformity (Alvarez & Wendel 2003). Grob *et al.* (2004) found that FLint2 is relatively short in *Amorphophallus* and is also highly variable, making it suitable for direct sequencing. This region yields more informative sites than the chloroplast genes (*matK*, *rbcL* and the *trnL-trnF* intron) investigated in this genus. Sedayu *et al.* (2010) also studied the evolution of *Amorphophallus* spp. based on *trnL*, *rbcL* and FLint2, and found that FLint2 had the highest level of variation among these regions. In addition, the present study shows that the addition of the ITS region further improves the resolution, as this region is quite long (805–920 bp) and has the highest percentage of phylogenetically informative sites. Previous studies also showed that the ITS has a higher discriminatory power than *rbcL*, *matK* or *trnH-psbA*, making it especially applicable in this genus-level study (Li *et al.* 2011).

The phylogeny presented in Figure 3 is largely congruent with previous findings. In the individual *trnL-trnF* and *Flint2* phylogenies, *A. obscurus* is the sister taxon to *A. sumawongi*, in agreement with previous studies which indicate the species’ position in a ‘Thai-Indochinese clade that, despite a strong phylogenetic signal, is not supported by morphology (Grob *et al.* 2002; Sedayu *et al.* 2010). However, in the ITS and combined phylogenies *A. sumawongi* is indicated as the sister taxon to *A. latifolius*, which has a similar inflorescence namely appendix entirely covered with staminodes (Heterscheid *et al.* 2012). *Amorphophallus latifolius* had not been previously included in any molecular phylogeny.

Clade B, which is strongly supported as a monophyletic group in the present study, was also recognized in a previous study that identified a unique 12 bp insertion within *matK* in the group (Grob *et al.* 2002). Furthermore, the present study found that two sister taxa in clade B, *A. krausei* and *A. kachinensis*, share a unique 22 bp insertion in the *trnL-trnF* spacer. Both of these species belong to the high KGM content group. In clade D, *A. pygmaeus* and *A. napiger* were also previously shown to be closely related by Grob *et al.* (2002, 2004). Clade E, which here is composed of the three high-KGM content species *A. muelleri*, *A. bulbifer*, and *A. xiei*, is well supported by a 4–5 bp insertion, despite considerable differences in inflorescence morphology among the species. In a previous study, Li & Dao (2006) claimed that *A. xiei* is closely related to *A. muelleri*, yet differing only in color from *A. bulbifer*. However,
the present study indicates that *A. xiei* is most closely related to *A. bulbifer*, as postulated by Hetterscheid in the Flora of China (Li *et al.* 2010). In this study, the findings of the phylogenetic analyses indicate that the species are not only largely grouped according to morphology, but can also be grouped by KGM content. This is the first study to find a correlation between KGM content and evolutionary history using genetic data.

**Genetic Relationships within Amorphophallus by RAPD analysis**

RAPD markers were used in this study because of their high potential to detect genomic polymorphisms relative to other techniques developed during the last two decades (Nishat *et al.* 2015). Moreover, it is a relatively simple and inexpensive method for examining variations in the total genome. In the present study, several long primers (LPRAPD) were employed since they enhance reproducible results and are more stable than shorter primers (Gillings & Holley, 1997). The dominance of RAPD markers indicates that each band represents the phenotype at a single allelic locus (Horita *et al.* 2005). The RAPD analysis presented in this study indicates that all these primers produced a highly polymorphic banding pattern (100%). The discriminatory power of each RAPD amplified band can be determined by the polymorphism information content (PIC) value (Nagy *et al.* 2012). For the primers used in this study, ten of the 13 loci were considered to be informative, since they had a PIC value greater than 0.5 (Ramadugu *et al.* 2015). The PIC value can be used to evaluate the level of gene variation. When the PIC was >0.5 the locus was considered to be of high diversity; when the PIC was between 0.25 and 0.5 it was considered to have intermediate diversity, when the PIC was < 0.25 the locus was considered to be of low diversity (Botstein *et al.* 1980). For genetic distance, high values confirmed the heterogeneity within *Amorphophallus* species. This high genetic variability may be due to the occurrence of molecular mutation and/or recombination in these species. Since even a single base change at the primer annealing site is manifested as the appearance or disappearance of RAPD bands, these bands may indicate the occurrence of genetic changes in the genome of species either through the loss or rearrangement of some of their nucleotides. Chromosomal crossing over during meiosis may result in the loss of primer attachment pair sites in the offspring, leading to novel molecular marker patterns in species (Noormohammadi, 2013).

In the present study, the RAPD results suggest a grouping of *A. muelleri*, *A. bulbifer* and *A. xiei* into a single high KGM content group. These three species were also regarded to be related to each other as they all produce bulbils on their leaves (Li *et al.* 2010, Boyce *et al.* 2012). The most interesting result from the RAPD analysis was the detection of a specific band that was only present in high-medium KGM content species (Figure 8). This result shows that the RAPD method can be used to improve the rapid and accurate screening of potentially high KGM species by using a meaningful genetic classification system instead of laborious and time-consuming biochemical tests (DNS assay for KGM content determination).

**Utility of molecular markers to determine relationships between KGM content and evolutionary history**

When KGM content was assessed in combination with genetic data, it was found that samples with similar KGM content belong to similar clades in the phylogenetic tree. This shows that KGM content is associated with the species’ evolutionary history. High KGM content species are found in clade B and E in the phylogeny inferred here (Figure 3), indicating that a high KGM content tuber has probably evolved at least twice in this genus. Because the production of KGM can be anticipated based on the species’ relationships, DNA markers are advantageous as a tool for economic species identification. Because of the shared evolutionary history of KGM production, it is possible that based on the three sequencing regions employed in this study it would be possible to develop DNA barcodes or design specific primers that could be used for molecular diagnostics of high value species. However, as the high KGM species are found in two separate clades therefore two unique barcodes or sets of primers would be required. For this reason RAPD analysis is more suitable for identifying species belonging to the high KGM clades. In RAPD analyses, primer AC-10 produced a unique band of around 600 bp exclusively in high and medium KGM content species. This unique amplicon can be further used to generate sequence characterized amplified region (SCAR) primers for screening and validation. Therefore, this study demonstrates that the knowledge from both analyses is useful as a first step to set up and develop a selection tool for potential economically important species of *Amorphophallus* in future.

**Other considerations for potentially useful economic species**

Unlike other high KGM content species, *A. bulbifer* and *A. muelleri* are triploid (Zhang *et al.* 2010). For this reason, reproduction by seed can occur without pollination. This gives these species the potential to have a high propagation coefficient. Moreover, these plants can produce multiple seedlings sequentially during growth, resulting in a high tuber yield and shortened growth cycle from the average 3–4 years down to 9–10 months (Santosa *et al.* 2003). KGM content can depend on other factors such as location, soil, weather, age of tuber and processing (Fang & Wu 2004, Zhang *et al.* 2010). In this study, the findings of the phylogenetic analyses indicate that the species are not only largely grouped according to morphology, but can also be grouped by KGM content. This is the first study to find a correlation between KGM content and evolutionary history using genetic data.
al., 2005, Zhang & Liu 2006). Because of this, the present study focused on identifying high KGM content species for optimization in the context of commercial production. Liu (2004) found that the KGM content of *A. konjac* grown in different areas varied slightly (ranging from 58.8%–52.1%). However, the results also indicate that the species is the main factor determining the productivity and quality of KGM flour, and that the growing conditions are a secondary factor that can later be manipulated for optimal KGM yield. The species identified here as belonging to the high and medium KGM content groups have the potential to become future commercial crops as new raw material resources for konjac flour production.

**Conclusion**

The phylogeny inferred from 47 *Amorphophallus* specimens from Thailand and China indicates the evolutionary history of KGM content, based on nucleotide region sequences and RAPD data. KGM content varies significantly by species, and the propensity for high KGM production was found to have evolved at least twice in the genus. Since the results of the molecular analysis indicate congruity between the evolutionary history and KGM content, molecular markers can potentially be used as a selection tool to identify *Amorphophallus* species that produce high levels of KGM. RAPD analysis using the primer AC-10 produced a unique 600-bp band in all medium to high KGM content species, and this fast and cost effective method can be used in the future for broad screening of *Amorphophallus* species to identify those with high KGM production and local economic potential. In the Thai species investigated here, *A. muelleri* was shown to have the highest KGM levels, making it especially promising as an alternative to *A. konjac* for commercial KGM production in this tropical country. The use of genetic markers to determine the KGM content in these species is an important contribution towards the further development of industrial-scale production of KGM in Thailand and throughout Asia.

**Acknowledgments**

This research was supported by the 90th Anniversary of Chulalongkorn University and the Swedish Research Council (B0569601), the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013, ERC Grant Agreement n. 331024), and a Wallenberg Academy Fellowship.

**References**


References


http://dx.doi.org/10.1016/S1055-7903(03)00183-0


http://dx.doi.org/10.1111/pbi.12215


http://dx.doi.org/10.1038/ng1044


http://dx.doi.org/10.1111/j.1439-0434.2005.00954.x


http://dx.doi.org/10.1093/bioinformatics/bts199

http://dx.doi.org/10.1016/S0031-9422(97)00221-5


http://dx.doi.org/10.1021/jf050751q

http://dx.doi.org/10.1073/pnas.1104551108


AMORPHOPHALLUS Phytotaxa 282 (2) © 2016 Magnolia Press • 103
http://dx.doi.org/10.1016/j.flora.2009.12.024

http://dx.doi.org/10.1016/j.scienta.2015.09.004


http://dx.doi.org/10.1093/bioinformatics/btg180


http://dx.doi.org/10.11248/jsta1957.47.190


http://dx.doi.org/10.1093/sysbio/49.2.369

http://dx.doi.org/10.1007/BF00037152


http://dx.doi.org/10.1016/j.ympev.2013.07.002


http://dx.doi.org/10.1093/nar/18.22.6531


http://dx.doi.org/10.11248/jsta.54.84


APPENDIX 1

Thirty-five species of *Amorphophallus* were used in this study. This study presented below in alphabetic order with full name taxonomy checklist of *Amorphophallus* occurring in Thailand (33 species) and China (2 species), as a supplement data to the Flora of Thailand (Boyce et al. 2012) and Flora of China (Li et al. 2010). In addition, wild voucher specimens can be cited as follows.

   THAILAND. Tak: Ban Tak, Huay Tak Waterfall, 21 June 2010, TK08P1T11 (H!).
   THAILAND. Saraburi: Pak Chong, Tham Phra Phothisat, 10 July 2010, JJ003 (H!).
7. *A. bulbifer* Blume (1837: 138)
8. *A. carneus* Ridley (1903: 47)
   THAILAND. Lampang: Wang Nua, Wangkaew Waterfall, 16 June 2010, LA01P1T3 (H!).
   Accession 10, THAILAND. Chiang Mai: Mae Tang, Pongduet Pa Pae Hotspring, 7 April 2010, CM19P2T11/2 (H!).
   Accession 11, THAILAND. Chiang Mai: Chiang Dao, Doi Luang chiang Dao, 9 April 2010, CM05P1T11/2 (H!).
   Accession 48, THAILAND. Lamphun: Mae Tha, Ban Mae Tha phatang, 6 March 2010, LO02P1T12 (H!).
   Accession 10, THAILAND. Chiang Mai: Chai Prakan, Doi Wiang Pha, 7 April 2010, CM10P1T6 (H!).
15. *A. konjac* Koch (1858: 262)
   Accession 11, THAILAND. Chiang Mai: Mae Tang, Pongduet Pa Pae Hotspring, 7 April 2010, CM19P2T11/2 (H!).
   Accession 12, THAILAND. Chiang Mai: Chiang Dao, Doi Luang chiang Dao, 9 April 2010, CM05P1T11/2 (H!).
   Accession 48, THAILAND. Lamphun: Mae Tha, Ban Mae Tha phatang, 6 March 2010, LO02P1T12 (H!).
   THAILAND. Lamphun: Lee, Mae Ping National Park, 21 June 2010, LO03P2T5 (H!).
   Accession 1, THAILAND. Mae Hong Son: Pai, Doi Mae Ya, 9 July 2010, ME07P1T6 (H!).
   Accession 2, THAILAND. Tak: Tha Song Yang, Mae Moi National Park, 21 June 2010, TK04P1T1 (H!).
   Accession 3, THAILAND. Mae Hong Son: Khun Yuan, Ban Nong Haeng Hot Spring, 9 April 2010, ME03P1T1 (H!).
   Accession 4, THAILAND. Tak: Ban Tak, Ban Mong Mai Phatthana, 23 June 2010, TK08P2T1 (H!).
   Accession 5, THAILAND. Kanchanaburi: Thong Pha Phum, Tha Khamun, 16 April 2010, KC2010-05GZ (H!).
   Accession 6, THAILAND. Kanchanaburi: Sangkhla Buri, Prangphie, 16 April 2010, KC2010-02GZ (H!).


27. THAILAND. Chiang Mai: Mae Sai, Pong Pha National Park, 7 June 2010, CR01P3T4 (H!). *A. prolificus* Hetterscheid & Galloway (2006: 69)


34. *A. xiei* Li & Dao (2006: 240)


THAILAND. Lamphun: Ban Hong, Huai Thaeng, 21 June 2010, LO03P2T2 (H!).