Evaluation in Genetic Variation of Krachai by Sequence Related Amplified Polymorphism (SRAP) and Random Amplified Polymorphic DNA (RAPD) Techniques

Sukalya Poothong[1], Waranya Mangphae[2], Wuttikon Chanhom[3], Maliwan Nakkuntod[4*]

[1]*School of Agriculture and Natural Resources, University of Phayao, Phayao 56000, Thailand*

[2,3,4]*Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand, E-mail: maliwann@nu.ac.th*

Abstract: Krachais are local name of Thai herb which are identified within 2 genera, namely *Bosenbergia* (white or red krachai) and *Kaempferia* (black krachai). The rhizome of *Bosenbergia rotunda* (L.) Mansf. contains major aromatic compounds against COVID19 but its many morphological characteristics such as leaves, stems and rhizome are like other species in same genus or family. In this study, a total of 13 accessions containing 5 samples of *B. rotunda*, 2 samples of *Boesenbergia* spp. and 3 samples of *Kaempferia parviflora* including 3 samples of other species in same family as outgroups were analyzed and clustered using sequence-related amplified polymorphism (SRAP) and random amplified polymorphism DNA (RAPD) markers. The results showed that 8 SRAP primers (M1E1, M1E2, M1E7, M2E10, M3E9, M5E1, M5E2 and M6E10) could generate 79 polymorphism bands with an average of 92.15% whereas a total of 5 RAPD primers (OPY04, OPY02, OPY04, JAT11 and JAT12) could give 64 polymorphisms with 100% as a percentage of the polymorphism band. The PIC of the SRAP marker (0.470) has a higher value than the RAPD marker (0.264). The highest similarity coefficients within genus *Boesenbergia* of 1.000 and 0.952 were obtained from SRAP and RAPD markers, respectively. The UPGMA dendrogram of SRAP and RAPD information among krachai presented 2 and 4 groups, respectively. The cluster of *Boesenbergia* was separated from *K. parviflora* and other species in same family. Furthermore, the results also pointed that *Boesenbergia* sp. from Phayao is in correct genus and is *B. pandurata* (Roxb.) Schltr. because of having red leaf while *Boesenbergia* sp. from Chiang Rai showed confusion between SRAP and RAPD data. It was concluded that SRAP and RAPD have great potential for the study of genetic diversity of *Boesenbergia* and other species in family Zingiberaceae.

Keywords: Boesenbergia / Kaempferia / SRAP / RAPD

1. INTRODUCTION

The genus *Bosenbergia* belongs to the Zingiberacea family and comprises over 80 recognized species. It is widespread in tropical regions in Southeast Asia, Sri Lanka and southern China. Thailand is one of the richest habitats with over 20 species. *Boesenbergia rotunda* or fingerroot has attracted attention as a preventive medicine and food [1]. It is a small herbaceous plant with short, fleshy, or slender rhizomes that are shaped like fingers and grow from the mother rhizome. The rhizome contains two important aromatic compounds, flavanones and chalcones. The flavanones include alpinetin, pinostrobin and pinocembrin. The group of chalcones included boesenbergine, cardamonine, panduratin A and 4-hydroxypanduratin A. In addition, the phytoconstituents from the rhizomes of *B. rotunda* can be utilized for pharmaceutical activities such as antibacterial, antiallergic, antitumor, antimutatorial, antifungal, anti-anxiety, and anti-inflammatory activities [2]. More importantly, Kanjanasiirirat and colleagues [3] reported that the effect of panduratin A in *B. rotunda* had inhibited SARS-CoV-2 or COVID-19 and prevented virus replication in the pre- and post-infection phase [4]. The demand for high-yielding and high-quality medicinal plants, especially *B. rotunda*, has increased in the food and pharmaceutical industries in the post-COVID era. Morphological traits are limited as they are influenced by environmental factors and the developmental stage of the plant. Therefore, good phenotypes were considered for commercial cultivation of high-value medicinal plants and individual natural products for the industry. Their morphological characteristics were closely related within the genus and other genera such as *Scaphochlamys*, *Caulokaempferia*, *Curcuma* and *Kaempferia*. The genus *Kaempferia* is a medium-sized, rhizomatous herb belonging to the Zingiberaceae family. *Kaempferia* comprises about 40 species distributed in monsoonal tropical Asia, 29 of which occur in Thailand. In Thailand, *Kaempferia* plants are used ethnomedicinally to treat flatulence, fever, stomach ulcers, leucorrhea, edema and to heal wounds. However, the taxonomic identification and classification of these plants based on morphological characteristics is not always satisfactory [5].

Genetic diversity between individuals or populations can be assessed using molecular markers. Molecular markers, which are based on information about the DNA sequence or polymorphisms, are independent of environmental conditions and the developmental stage of the plant. Molecular marker technology is widely used to study genetic diversity between and within species, including DNA sequencing, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), sequence related amplified polymorphism (SRAP) and random amplified polymorphic DNA (RAPD). SRAP and RAPD are molecular markers based on PCR amplification and contain no prior sequence information. The SRAP marker is a new molecular marker technology based on PCR amplification, which was discovered by Li and Quiros [6] for the genetic analysis of Brassica. The primer of the SRAP marker was developed for an open reading frame. The SRAP marker has been shown to be high throughput, reproducible, stable, and less complicated. In addition, the SRAP marker has a high efficiency in genetic differentiation between and within species. In recent days, SRAP information has been widely used for genetic analysis of numerous plants such as *Elaeis guineensis* [7], *Ricinus communis* [8], *Coffea arabica* [9], *Cucumis melo* [10], *Lavandula angustifolia* [11] and *Triticum durum* [12]. The RAPD marker is relatively simple, easy to use and requires no sequence information. It has also been shown to amplify based on the non-coding region [13]. Therefore, several studies of RAPD markers have shown that they have a high potential for polymorphism and are also successful for genetic diversity of many plants, including *Crocus sativus* [14], *Calycophyllum spruceanum* [15], *Muntingia calabura* [16], *Ricinus communis* [17]. In this study, SRAP and RAPD markers were used to assess the genetic variation of 13 accessions including krachai and other species from five provinces in Thailand.

2. MATERIEL AND METHODS

2.1. Sample Collection

Thirteen accessions belonging to 5 genera were collected from different areas of Thailand. The seven *Boesenbergia* includes 5 of *B. rotunda* and 2 of *Boesenbergia* spp. and were collected in Phayao, Nakhon Pathom, Mae Hong Son, Lampang, and Chiang Rai. Three accessions of *Kaempferia* were collected in Chiang Rai and Nakhon Phanom. Three species of *Zingiber, Globba* and *Curcuma* were obtained from Phayao University as shown in Table 1. The fresh leaves were collected between May and October 2023 and used for genomic DNA extraction.

Table 1. Thirteen accessions and locations were used in this study

No.	Species	Locality	Accessions
1	*Boesenbergia rotunda* (L.) Mansf.	Phayao (PYO)	BR_PYO
2	*B. rotunda* (L.) Mansf.	Nakhon Pathom (NPT)	BR1_NPT
3	*B. rotunda* (L.) Mansf.	Nakhon Pathom (NPT)	BR2_NPT
4	*B. rotunda* (L.) Mansf.	Mae Hong Son (MSN)	BR_MSN
5	*B. rotunda* (L.) Mansf.	Lampang (LPG)	BR_LPG
6	*Boesenbergia* sp.	Chiang Rai (CRI)	B_CRI
7	*Boesenbergia* sp.	Phayao (PYO)	B_PYO
8	*Kaempferia parviflora* Wall. ex Baker	Chiang Rai (CRI)	KP1_CRI
9	*K. parviflora* Wall. ex Baker	Chiang Rai (CRI)	KP2_CRI
10	*K. parviflora* Wall. ex Baker	Nakhon Phanom (NPM)	KP_NPM
11	*Zingiber officinale* Roscoe	Phayao (PYO)	ZO_PYO
12	*Curcuma longa* L.	Phayao (PYO)	CL_PYO
13	*Globba malaccensis* Ridl.	Phayao (PYO)	GM_PYO

2.2. DNA Extraction

Total genomic DNA extraction was extracted from fresh leaves by modified Cetyl trimethyl ammonium bromide (CTAB) method [18]. Briefly, fresh leaves 0.5-1 g were ground in liquid nitrogen by a pestle and resuspended in 5 mL of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris, 0.3% β-mercaptoethanol) and incubated in a water bath at 60 ˚C for 60 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added the sample and centrifuged at 6,000 rpm for 15 minutes. The supernatant was transferred to new 1.5 mL microcentrifuge tubes and mixed with 2/3 volume of ice-cold isopropanol. The supernatant was incubated at -20 ˚C overnight and centrifuged 6,000 rpm for 15 minutes. The solution was discarded, and the pellets were washed with 70% ice-cold ethanol and centrifuged 6,000 rpm for 3 minutes. The pellets were allowed to air dry at room temperature for 15 minutes. The pellets were re-suspended in RNase buffer (10 mM Tris, 15 mM NaCl) and transferred to new 1.5 mL microcentrifuge tubes. The solution was added 10 mg/mL RNase A and incubated at 37 ˚C for 60 minutes. The supernatant was treated with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuged at 10,000 rpm for 5 minutes at 4 ˚C. After decanting the supernatant, an equal volume of chloroform: isoamyl alcohol (24:1) was added to the supernatant and centrifuged at 10,000 rpm for 5 minutes. The supernatant obtained was mixed with 1/10 volume of 3 M sodium acetate (pH 5.2) and 3 volumes of ice-cold absolute ethanol kept at -20°C for overnight. The pellet was rinsed with 1 mL of 70% ice-cold ethanol and dried at room temperature for 15 minutes. The pellet was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA). Total genomic DNA was separated by electrophoresis on 0.8% agarose gel and stained with SafeView™ FireRed (amb, Canada). DNA purity and concentration were determined with absorbance 260 and 280 nm by spectrophotometer. Total of genomic DNA was stored in -20 ˚C for further used.

2.3. Sequence Related Amplified Polymorphism (SRAP) Amplification

One hundred combination primer of SRAP technique were screened *for B. rotunda*. The amplification of primer was performed in two stages. The primer pairs were selected for PCR amplification followed by produced clear bands and polymorphic bands among species. The PCR reactions was performed in a volume of 30 μL mixture containing 1X PCR Buffer (2 mM $MgCl_2$), 0.2mM dNTPs, 2mM Mgcl2, 0.4 μM forward primer, 0.4 μM reverse primer, 1U *Taq* DNA polymerase (BIO-HELIX, Taiwan), 100 ng DNA template and distilled water. The PCR reaction was produced two stages. The genomic DNA was denatured at 94 ˚C for 5 minutes before first and secondary stage. The first stage was subjected to amplification for 5 cycles consisting of denaturation of 94°C for 1 minute, annealing of 35 °C for 1 minute and extension 72 °C for 1 minute. The last stage followed by 35 cycles consisting of denaturation of 94°C for 1 minute, annealing of 52 °C for 1 minute, extension 72°C for 1 minute with a final extension at 72°C for 10 minutes. PCR was performed with Biometra TONE thermal cycle (Analytik Jena, Germany). Amplification products were separated by electrophoresis in 1.25% (W/V) agarose gel (stained with SafeView™ FireRed (amb, Canada)) in 1 × TAE buffer at a constant voltage (80 V) for approximately 40 min. The SRAP fragment was visualized under UV light.

2.4. Random Amplified Polymorphic DNA amplification

Fifty-five decamer primers were used for screening of RAPD technique. The decamer primers that clear amplification and polymorphic profiles were selected for amplification in all samples. The PCR reaction contained 1X PCR buffer (2 mM $MgCl_2$), 0.2 mM dNTPs, 0.6 μM decamer primer, 1U *Taq* DNA polymerase (BIO-HELIX, Taiwan), 100 ng DNA template and distilled water. The reactions were performed with Biometra TONE thermal cycle (Analytik Jena, Germany). PCR amplifications were denatured at 94 ˚C for 5 minutes, followed by 40 cycles of denaturation at 94 ˚C for 45 seconds, annealing at 37 ˚C for 1 minute and extension at 72 ˚C for 2 minutes with final extension at 72 ˚C for 7 minutes. RAPD products were separated by electrophoresis in 1.25% (W/V) agarose gel (stained with SafeView™ FireRed (amb, Canada)) in 1 × TAE buffer at a constant voltage (80 V) for approximately 40 min. The SRAP fragment was visualized under UV light.

2.5. Data Analysis

DNA fragment from SRAP and RAPD techniques were scored as 1 for presence and 0 for absence to generate a binary data matrix. Polymorphic information content (PIC) values were calculated for each polymorphic marker according to Botstein et al. [19]. To evaluate the degree of similarity index was determined using NTSYSpc 2.0 [20]. Clustering analysis was also performed using NTSYSpc 2.0 [20] based on unweighted pair-group method with arithmetic mean algorithm (UPGMA).

3. RESULTS AND DISCUSSIONS

3.1. SRAP and RAPD amplifications among *Boesenbergia* and *Kaempferia*

For SRAP, the screening of 100 primers combination including 10 forward (M) and 10 reverse (E) SRAP primers was evaluated for *Boesenbergia*. Finally, eight combination primers that produced yielded clear and detected polymorphic band among *B. rotunda* and *K. parviflora* were selected for other DNA samples. The total number of bands scored per primer combination ranged from 8 (M1/E7, M2/E10) to 15 (M1E2) bands with an average of 9.875 bands per primer combination. Total of 79 fragments were obtained with 8 primers combination (M1/E1, M1/E2, M1/E7, M2/E10, M3/E9, M5E1, M5/E2 and M6/E10) including the polymorphic bands of 73 bands with an average of 9.125. Among these primers' combination, M1/E1 generated the lowest percentage of polymorphic bands (80%) while M3/E9, M5/E1 and M6/E10 yielded the highest fragment of polymorphic bands (100%). Size of DNA fragments ranged from 100 to 1500 bp. The highest PIC value of 0.499 was obtained with M5/E2 and M6/E10 primer combinations, followed by 0.495 with M2/E10. The lowest PIC value of 0.426 was obtained with M1/E7 primer combination, as shown in Table 2. For RAPD, out of fifty-five decamer primers, five decamer primers including OPF04, OPY02, OPY04, JAT11 and JAT12 were used from RAPD-PCR amplification. The amplification generated a total of 64 clear fragments. The number fragments varied from eight (OPY04) to eighteen (OPY02) fragments with an average value of 12.8 per primer. Among 64 fragments, percentage of polymorphic band per decamer primer of 100% were obtained by all primers. The PIC value ranged 0.205 (JAT12) to 0.355 (JAT11) with an average of 0.264, as shown in Table 3.

Table 2. DNA fragment generated by SRAP with 8 primer combinations

Primers	Total number of bands	Monomorphic bands	Polymorphic bands	Percent of polymorphic	PIC value
M1E1	10	2	8	80.00	0.457
M1E2	15	1	14	93.33	0.471
M1E7	8	1	7	87.50	0.426
M2E10	8	1	7	87.50	0.495
M3E9	9	0	9	100.00	0.473
M5E1	10	0	10	100.00	0.437
M5E2	9	1	8	88.89	0.499
M6E10	10	0	10	100.00	0.499
Total	79	6	73	-	-
Mean	9.875	0.75	9.125	92.15	0.470

Table 3. DNA fragments were obtained by RAPD-PCR amplification using five decamer primers

Primers	Total number of bands	Monomorphic bands	Polymorphic bands	Percent of polymorphic	PIC value
OPF04	10	0	10	100.00	0.254

OPY02	18	0	18	100.00	0.244
OPY04	8	0	8	100.00	0.260
JAT11	13	0	13	100.00	0.355
JAT12	15	0	15	100.00	0.205
Total	64	0	64	-	-
Mean	12.8	0	12.8	100.00	0.264

In general, molecular markers are used to improve the resolution of genetic analysis in plants. In the past, the combination of morphological and molecular markers has been used to study genetic variation and phylogenetic relationships. In another study, molecular markers and phytochemicals were analyzed together [17]. In this study, two PCR-based dominant molecular markers, including SRAP (8 primers combination) and RAPD (5 decamer primers) markers, were prepared for the genetic analysis of 7 *Boesenbergia*, 3 *Kaempferia*, and other species (*Z. officinale, G. malaccensis,* and *C. longa*). Both SRAP and RAPD markers produced high polymorphism bands with an average of 92.15% and 100%, respectively. The polymorphism level of SRAP marker was ranged aproximately 80% (M1/E1) to 100% (M3/E9, M5/E1 and M6/E10), while the RAPD marker has 100% of polymorphism level in all primers. Compared with previous studies, the results conducted on the genetic variation of many plants, dominant marker such as SRAP and RAPD marker usually produced high polymorphic bands about 70-100%. SRAP marker were generated high polymorphism of 78.57% in *Cuminum cyminum* [21], 94.69% in *Polygonatum* [22], 100% in *Ricinus communis* [8], 100% in *Nigella sativa* [23] and 100% in *Coffea arabica* [9]. RAPD marker were produced 90-100% of polymorphism in *Clerodendrum* [24], *Ricinus communis* [17], and *Calycophyllum spruceanum* [15].

3.2. Cluster Analyses Among *Boesenbergia* and *Kaempferia* by SRAP and RAPD Techniques

Based on SRAP data, similarity coefficient of 13 accessions varied from 0.481 (KP2_CRI with B_PYO and KP2_CRI with B_CRI) to 0.987 (BR1_NPT with BR2-PYO and BR1_NPT with BR-MSN), as shown in Table 4. The UPGMA dendrogram obtained from cluster analysis of SRAP information at 75% similarity cut-off. The dendrogram of the relationship among 13 accessions was generated to 4 cluster (ingroup 2 and outgroup 2). The first group contained 5 of *B. rotunda* accessions from Phayao, Nakhon Pathom, Mae Hong Son, and Lampang provinces and 2 of *Boesengergia* spp. from PhaYao and Chiang Rai. The second group confined to 3 accessions of *K. parviflora* from Chiang Rai and Nakhon Pathom. The outgroup divided 2 groups of *Z. officinale* - *C. longa* clade and *G. malaccensis* clade, which was placed as the basal group, as shown in Figure 1.

Based on RAPD information, the similarity coefficient of 13 accessions ranged from 0.444 (BR2_NPT with KP2_CRI and CL with unknown) to 0.952 (BR1_PYO with BR1_NPT). The highest coefficient was 0.952 between *B. rotunda* 1 from Phayao province (BR1_PYO) with *K. parviflora* 2 from Nakhon Pathom province (KP2_NPT) as shown in Table 5. A dendrogram was assembled by UPGMA analysis. Thirteen accessions were separated into 5 clades at 75% similarity cut-off. The first clade had the largest number of 5 of *B. rotunda* accessions from Phayao, Nakhon Pathom, Mae Hong Son and Lampang province. Next group contains a accession of *Boesenbergia* sp. from Phayao province. The third cluster included 3 accessions of *K. parviflora* from Chiang Rai and Nakhon Pathom. The fourth cluster was contained *Boesenbergia* sp. from Chiang Rai province. The rest of outgroup (*Z. officinale*, *G. malaccensis* and *C. longa*) were presented closely related with *Boesenbergia* sp. from Chiang Rai province, as shown in Figure 2.

Table 4. Similarity coefficient among 13 accessions were obtained by SRAP technique

	BR_PYO	BR1_NPT	BR2_NPT	BR_MSN	BR_LPG	B_CRI	B_PYO	KP1_CRI	KP2_CRI	KP_NPM	ZO_PYO	CL_PYO	GM_PYO
BR_PYO	1.000												
BR1_NPT	0.975	1.000											
BR2_NPT	0.962	0.987	1.000										
BR_MSN	0.962	0.987	0.975	1.000									
BR_LPG	0.873	0.873	0.886	0.886	1.000								
B_CRI	0.785	0.759	0.747	0.772	0.709	1.000							
B_PYO	0.797	0.797	0.810	0.785	0.722	0.785	1.000						
KP1_CRI	0.532	0.506	0.519	0.519	0.557	0.595	0.506	1.000					
KP2_CRI	0.506	0.481	0.494	0.494	0.532	0.570	0.481	0.975	1.000				
KP_NPM	0.608	0.582	0.570	0.595	0.582	0.696	0.532	0.873	0.873	1.000			
ZO_PYO	o.671	0.646	0.658	0.658	0.772	0.633	0.570	0.633	0.633	0.633	1.000		
CL_PYO	0.620	0.620	0.633	0.608	0.671	0.582	0.620	0.658	0.633	0.633	0.671	1.000	
GM_PYO	0.544	0.544	0.532	0.532	0.570	0.456	0.468	0.481	0.456	0.481	0.544	0.620	1.000

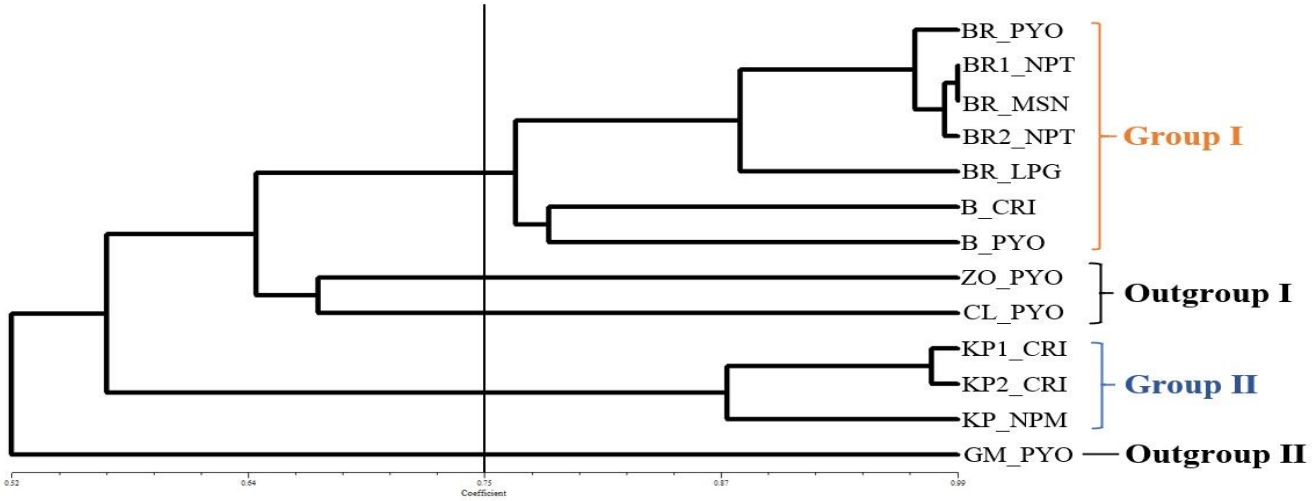

Figure 1. UPGMA dendrogram from SRAP data of 13 samples of Zingiberaceae family

The similarity coefficient of SRAP and RAPD were higher relationship within *Boesenbergia* than other species, which indicated a close relationship. Furthermore, the UPGMA dendrogram of SRAP and RAPD marker produced similar cluster among 13 accessions. The comparison of the clustering pattern revealed *B. rotunda* from various localities were grouped into together cluster by SRAP and RAPD, while 3 accessions of *K. parviflora* were clearly separated from *Boesenbergia*. White and red krachai (*Boesenbergia* spp.) from northern and central in Thailand were high similarity coefficient. This result indicated *Boesenbergia* species were collected from same germplasm and widely propagated using vegetable of rhizome parts than breeding part. Similar research, wild *B. rotunda* in northern Thailand are genetic linkage with cultivated *B. rotunda*. RAPD marker revealed that wild type of *B. rotunda* from Lamphun and Lampang were closely related with cultivated type of 5 of *B. rotunda* from Chiang Mai more than 68-70% similarity. Their morphological characteristics are small rhizome, green color on both sides of laminar and midrib. Nonetheless, the level of Phytochemical (pinostrobin and panduratin) contained in rhizome among *B. rotunda* was no correlation with the morphological characters and location [4]. A wide range of phenotype is depending on environment factors. Previous research, three types (Krachai Pha, Krachai Ban, Krachia Deang) of *B. rotunda* from Chiang Mai, Pathumthani, and Ratchaburi province, Thailand were collected for study the essential oil compositions and genetic variation based on internal transcribed spacer sequences. Based on essential oil composition and sequences profile in their rhizome were clearly categorized Krachai Pha and Krachai Ban into the same cluster, while Krachia Deang was completely separated from the others [25]. *K. parviflora* were clearly

grouped into together cluster and separated from *Bosenbergia* species. The co-dominant marker such as SSR and ISSR were used to evaluate the genetic diversity of *Kaempferia species*. Information of ISSR marker were grouped *Kaempferia* species into the same cluster [26]. Other molecular marker, SSR marker completely separated four zingiber species (*Z. montanum, C. aromatica, Alpinia galanga* and *B. rotunda*) from *the Kaempferia* species [5]. The RAPD based profiles usually widely distributed regions of genome and SRAP based profiles arise from coding region target of the genome. Accordingly, SRAP and RAPD marker methods involve those regions of genome, which were covered different genome as well as different evolution history [14].

Table 5. Similarity coefficient among 13 accessions were obtained by RAPD technique

	BR_PYO	BR1_NPT	BR2_NPT	BR_MSN	BR_LPG	B_CRI	B_PYO	KP1_CRI	KP2_CRI	KP_NPM	ZO_PYO	CL_PYO	GM_PYO
BR_PYO	1.000												
BR1_NPT	0.952	1.000											
BR2_NPT	0.825	0.873	1.000										
BR_MSN	0.857	0.905	0.841	1.000									
BR_LPG	0.857	0.905	0.937	0.873	1.000								
B_CRI	0.698	0.730	0.635	0.698	0.635	1.000							
B_PYO	0.698	0.667	0.571	0.603	0.635	0.651	1.000						
KP1_CRI	0.492	0.524	0.460	0.492	0.492	0.635	0.540	1.000					
KP2_CRI	0.508	0.540	0.444	0.508	0.476	0.714	0.524	0.889	1.000				
KP_NPM	0.571	0.603	0.508	0.571	0.540	0.778	0.587	0.857	0.937	1.000			
ZO_PYO	0.556	0.587	0.556	0.587	0.556	0.730	0.476	0.556	0.603	0.635	1.000		
CL_PYO	0.603	0.651	0.651	0.619	0.619	0.655	0.444	0.619	0.603	0.635	0.683	1.000	
GM_PYO	0.540	0.571	0.508	0.571	0.508	0.714	0.556	0.571	0.683	0.683	0.698	0.698	1.000

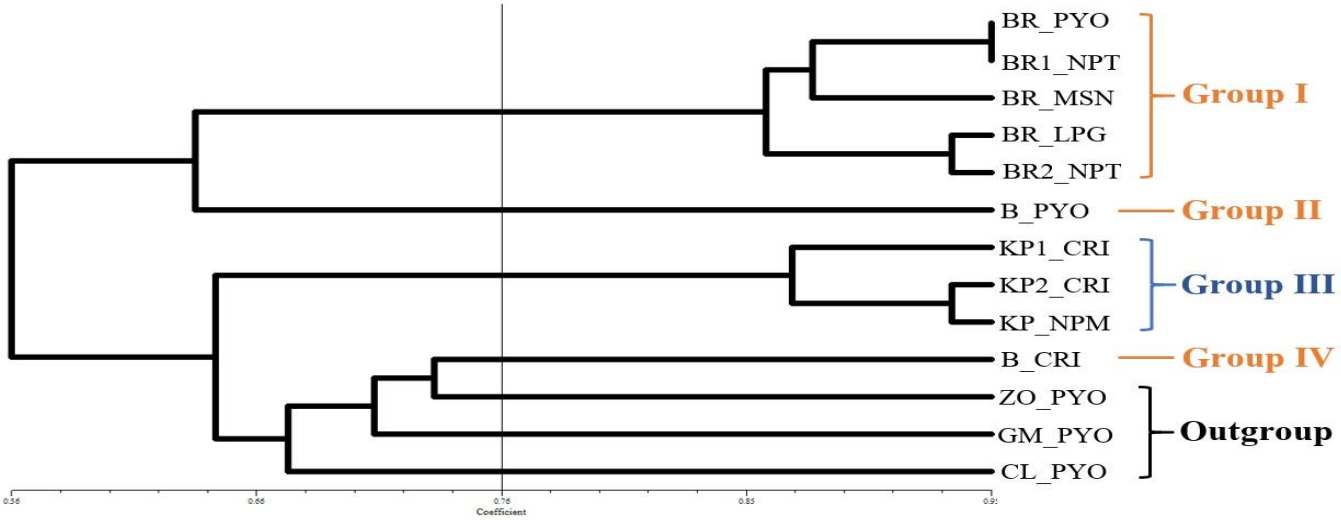

Figure 2. UPGMA dendrogram from RAPD data of 13 samples of Zingiberaceae family

CONCLUSIONS

This study showed that SRAP and RAPD is effective in taxonomy identification of *B. rotunda* from different location and can be detect the genetic variation in intraspecies of *B. rotunda* and interspecies in *Boesenbergia* and *Kaempferia*. Moreover, *Boesenbergia* sp. from Phayao may be another species called red krachai or Krachai Deang belonging to *B. pandurata* (Roxb.) which its leaves show reddish green on dorsal side while *Boesenbergia* sp. from

Chiang Rai still confuse to identify because of SRAP and RAPD ambiguity. However, krachai in Thailand should be separated into two genera, namely *Boesenbergia* and *Kaempferia*.

ACKNOWLEDGEMENTS

We are so deep to thank the University of Phayao for financial support (FF-RIM004) and Naresuan University for equipment support in molecular laboratory.

REFERENCES

[1] N. D. Yuliana, S. Budijanto, R. Verpoorte, and Y. H. J. Choi, "NMR metabolomics for identification of adenosine A1 receptor binding compounds from *Boesenbergia rotunda* rhizomes extract," Journal of Ethnopharmacology, vol. 150, pp. 95–99, 2013.

[2] M. M. Noor Diyana, "Flavonoid Pathway Gene Discoveries in *Boesenbergia rotunda* through RNA-SEQ Transcriptome Profiling of Cell Suspension Cultures in Response to Phenylalanine/Noor Diyana Md Mustafa," Doctoral Dissertation, University of Malaya, Kuala Lumpur, Malaysia. 2017.

[3] P. Kanjanasirirat, A. Suksatu, S. Manopwisedjaroen, B. Munyoo, P. Tuchinda, K. Jearawuttanakul, S. Seemakhan, S. Charoensutthivarakul, P. Wongtrakoongate, and N. Rangkasenee, "High-content screening of Thai medicinal plants reveals *Boesenbergia rotunda* extract and its component Panduratin A as anti-SARS-CoV-2 agents," Scientific Reports, vol. 10, pp. 1-12, 2020.

[4] S. Thomya, M. Wongkaew, W. Bundithiya, C. Lumsangkul, P. Jantrawut, and S. R. Sommano, "Relationship between Phenotypes and Chemical Profiling of *Boesenbergia rotunda* Collected from Different Habitats of Northern Thailand," Agronomy, vol. 13, no. 6, p. 1, 2023.

[5] O. Theanphong, T. Jenjittikul, W. Mingvanish, and K. Runhsihirunrat, "Phylogenetic relationships of Kaempferia plants based on inter-simple sequence repeat fingerprints," Songklanakarin Journal Science Technology, vol. 40, no. 3, pp. 617-622, 2018.

[6] G. Li, and C. F. Quiros, "Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*," Theoretical and Applied Genetics, vol. 103, pp. 455–461. 2001.

[7] L. Zhou, R. Yarra, H. Cao, and Z. Zhihao, "Sequence-Related Amplified Polymorphism (SRAP) Markers Based Genetic Diversity and Population Structure Analysis of Oil Palm (*Elaeis guineensis* Jacq.)," Tropical plant Biology, vol. 14, pp. 63-71. 2021.

[8] K. G. Agyenim-B0ateng, J. Lu, Y. Shi, D. Zhang, and X. Yin, "SRAP analysis of the genetic diversity of wild castor (*Ricinus communis* L.) in South China," PLoS ONE, vol. 14, no. 7, pp .1-16, 2019.

[9] K. Al-Ghamedi, I. Alaraidh, M. Afzal, M. Mahdhi, Z. Al-Faifi, M. D. Y. Oteef, T. Tounekti, S. S. Alghamdi, and H. Khemira, (2023). Assessment of Genetic Diversity of Local Coffee Populations in Southwestern Saudi Arabia Using SRAP Markers. Agronomy, 13(2), 1-16.

[10] R. R. Aziz, and N. A. R. Tahir, "Genetic diversity and structure analysis of melon (*Cucumis melo* L.) genotypes using URP, SRAP, and CDDP markers," Genetic Resources and Crop Evolution, vol. 70, pp. 799-813, 2023.

[11] T. Zagorcheva, A. Stanev, K. Rusanov, and I. Atanassov, "SRAP markers for genetic diversity assessment of lavender (*Lavandula angustifolia* Mill.) varieties and breeding lines," Biotechnology and Biotechnological Equipment, vol. 34, no. 1, pp. 303-308, 2020.

[12] N. B. Almarri, S. S. Alghamdi, M. H. Elshal, and M. Afzal, "Estimating genetic diversity among durum wheat (*Triticum durum* desf.) landraces using morphological and SRAP markers," Agricultural Sciences, vol. 22, no. 5, pp. 273-282. 2023.

[13] Y. Risjani, and G. Abidin, "Genetic diversity and similarity between green and brown morphotypes of *Kappaphycus alvarezii* using RAPD," Journal of Applied Phycology, vol. 32, pp. 2253-2260, 2020.

[14] M. A. Mir, S. Mansoor, M. Sugapriya, M.N. Alyemeni, L. Wijaya, and P. Ahmad, "Deciphering genetic diversity analysis of saffron (*Crocus sativus* L.) using RAPD and ISSR markers," Saudi Journal of Biological Science, vol. 28, no. 2, pp. 1308-1317, 2021.

[15] C. L. Saldana, J. D. Cancan, W. Cruz, M. Y. Correa, M. Ramos, E. Cuellar, and C. I. Arbizu, "Genetic Diversity and Population Structure of Capirona (*Calycophyllum spruceanum* Benth.) from the Peruvian Amazon Revealed by RAPD Markers." Forests, vol. 12, no. 8, pp. 1-12. 2021.

[16] F. Nasution, A. A. Theanhom, P. Bhuyar, and J. Chumpookam, "Genetic diversity evaluation in wild *Muntingia calabura* L. based on Random Amplified Polymorphic DNA (RAPD) markers," Gene Reports, vol. 25, pp. 1-5, 2021.

[17] H. Kim, P. Lei, A. Wang, S. Liu, Y. Zhao, F. Huang, Z. Yu, G. Zhu, Z. He, D. Tan, H. Wang, and F. Meng, Genetic Diversity of Castor Bean (*Ricinus communis* L.) Revealed by ISSR and RAPD Markers," Agronomy, vol. 11, no. 3, pp. 1-14, 2021.

[18] G. K. Agrawal, R. N. Pendey, and V. P. A. Agrawal, "Isolation of DNA from *Choeropondias asillris* leaves," Biotechnology and Biodiversity Letters, vol. 2, pp. 19-24. 1992.

[19] D. Botstein, R.L. White, M. Skolnick, and R. W. Davis, "Construction of a genetic linkage map in man using restriction fragment length polymorphisms," American Journal of Human Genetics, vol. 32, pp. 314–331. 1980.

[20] F. J. Rohlf, "NTSyS-p.c. Numerical Taxonomy and Multivariate Analysis System (Version 2.0)," Exeter Software Publishers Ltd., Setauket, 1998

[21] M. A. A. Mousa, K. A. M. Abo-Elyousr, and O. H. M. Ibrahim, "Evaluation of Genetic Variability within a Collection of Cumin Genotypes Using RAPD, ISSR, SRAP and SCoT Markers and Variability of *In Vitro* Callus Induced Therefrom," Horticulturae, vol. 9, no. 7, pp. 1-15, 2023.

[22] T. Feng, Q. Jia, X. Meng, X. Chen, F. Wang, W. Chai, and Z. Liang, "Evaluation of genetic diversity and construction of DNA fingerprinting in *Polygonatum* Mill. based on EST-SSR and SRAP molecular markers," 3 Biotech, vol. 10, pp. 1-13, 2020.

[23] P. Golkar, and V. Nourbakhsh, Analysis of genetic diversity and population structure in *Nigella sativa* L. using agronomic traits and molecular markers (SRAP and SCoT)," Industrial Crops and Products, vol. 130, pp. 170-178, 2019.

[24] B. Gogoi, S. B. Wann, and S. P. Saikia, "Comparative assessment of ISSR, RAPD, and SCoT markers for genetic diversity in *Clerodendrum* species of North-East India," Molecular Biology Reports, vol. 47, pp. 7365-7377, 2020.

[25] O. Theanphong, W. Mingvanish, T. Jenjittikul, and K. Rungsihirunrat, “Genetic variation of *Boesenbergia rotunda* (L.) Mansf. from Thailand based on essential oil compositions and internal transcribed spacer sequences,” Songklanakarin Journal Science Technology, vol. 43, no. 6, pp. 1620-1627, 2021.

[26] I. Siriluck, T. Ratchanok, H. Worakij, and K. Thitamin, “Identification of 24 Species of Zingiberaceae in Thailand Using ISSR Technique,” Thai Journal of Agricultural Science, vol. 47, pp. 1-6, 2014.

DOI: https://doi.org/10.15379/ijmst.v10i2.3287