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Genetic diversity of water primrose (*Ludwigia hyssopifolia*) in Thailand based on morphological characters and RAPD analysis

Diversidad genética del prímula de agua (*Ludwigia hyssopifolia*) en Tailandia basada en caracteres morfológicos y análisis RAPD

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Abstract. Genetic diversity and relatedness of 17 water primrose (*Ludwigia hyssopifolia*) accessions in Thailand were estimated using morphological characters and random amplified polymorphic DNA (RAPD) markers. Eight morphological characters were diverse among the accessions. However, some accessions could not be distinguished from one another based on these morphological characters alone. Unweighted pair-group arithmetic average (UPGMA) analysis of these characters separated these 17 accessions into 2 major clusters. Among the 5 RAPD primers used, a total of 68 fragments (150 to 2000 bp) were amplified, showing a polymorphism percentage of 80%. The polymorphic information content (PIC) among accessions varied from 0.31 to 0.36 with an average of 0.33. These polymorphic RAPD markers successfully distinguished all 17 water primrose accessions. The 17 accessions were grouped into 3 clusters using UPGMA analysis. Pairwise coefficients of morphological characters and RAPD-based genetic similarity between all accessions ranged from 0.154 to 1.000 with an average of 0.693 and from 0.531 to 0.952 with an average of 0.802, respectively. A nonsignificant correlation between morphological characters and RAPD-based similarities was found as shown by the low correlation coefficient of 0.242 between the two Jaccard's similarity matrices in the Mantel test. Clustering of accessions within clusters also differed when morphological characters and RAPD-derived dendrograms from UPGMA analysis were compared. It would suggest that RAPD was more effective in determining the genetic variability and relationships among water primrose accessions. In addition, RAPD was also more useful for accessions identification since all 17 water primrose accessions can be effectively distinguished by only 5 RAPD primers.

Keywords: DNA markers; Genetic relationships; Random amplified polymorphic DNA; Seedbox.

Resumen. La diversidad genética y el parentesco de 17 accesiones de prímula de agua (*Ludwigia hyssopifolia*) en Tailandia fueron estimados usando caracteres morfológicos y marcadores de ADN polimórficos amplificados al azar (RAPD). Ocho caracteres morfológicos fueron diversos entre las accesiones. Sin embargo, algunas accesiones no podían distinguirse entre sí en función únicamente de estos caracteres morfológicos. El análisis del promedio aritmético del grupo de pares no ponderado (UPGMA) de estos caracteres separó estas 17 accesiones en 2 grupos principales. Entre los 5 cebadores de RAPDs utilizados, se amplificaron un total de 68 fragmentos (150 a 2000 bp), mostrando un porcentaje de polimorfismo de 80%. El contenido de información polimórfica (PIC) entre las accesiones varió de 0,31 a 0,36 con un promedio de 0,33. Estos marcadores polimórficos de RAPD distinguieron con éxito las 17 accesiones de prímula de agua. Las 17 accesiones se agruparon en 3 grupos utilizando el análisis UPGMA. Los coeficientes de pares de caracteres morfológicos y la similitud genética basada en RAPD entre todas las accesiones variaron a partir de la 0,154 a 1,000 con un promedio de 0,693 y de 0,531 a 0,952 con un promedio de 0,802, respectivamente. Entre los caracteres morfológicos y la similitud basada en RAPD se encontró una correlación no significativa según lo demostrado por el bajo coeficiente de correlación de 0,242 entre las dos matrices de semejanza de Jaccard en la prueba de Mantel. La agrupación de las accesiones dentro de los grupos también fue distinta cuando los caracteres morfológicos y los dendrogramas derivados de RAPD del análisis de UPGMA fueron comparados, sugiriendo que RAPD fue más efectivo en determinar la variabilidad genética y las relaciones entre las accesiones de prímula de agua. Además RAPD fue también más útil para la identificación de las accesiones, ya que con sólo 5 cebadores pudieron ser efectivamente distinguidas las 17 accesiones de prímula de agua.

Palabras clave: ADN polimórficos amplificados al azar; Marcadores de ADN; Relaciones genéticas; Seedbox.

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INTRODUCTION

Ludwigia hyssopifolia L. (Synonym: *Jussiaea hyssopifolia* G. Don, *Jussiaea linifolia* Vahlnon and *Ludwigia linifolia* Poir), commonly known as seedbox, water primrose or 'tian na' in Thai, belongs to Onagraceae family. The plant is an annual herb that grows extensively in China, South and Southeast Asia including Thailand and other tropical countries (Shaphiullah et al., 2003; Tharapreuksapong et al., 2012). Water primrose usually grows in wet places, and it can be a serious rice weed in lowland rice fields via its allelopathic effects (Ismail et al., 2015). However, it is also considered as a medicinal plant due to various compounds in leaves, fruits and roots that have medicinal properties such as saponins, tannins, polyphenols, alkaloids and flavonoids etc., which are used as astringents, anthelmintics, carminatives and diuretics. Moreover, the decoction from water primrose can be used to treat diarrhea, dysentery, leucorrhoea and spitting of blood. Recently, these compounds were also found to have negative effects on plant pathogens. Tharapreuksapong et al. (2012) found that crude extracts of *L. Hyssopifolia* (G. Don) Exell could inhibit the growth of *Erwinia carotovora* sub sp. *carotovora* and *Phytophthora palmivora* (Butl.) Butler.

Previous studies on *L. hyssopifolia* L. only described its important roles in medication and biological control in agriculture. However, reports on its genetic relationships or diversity are still limited. Evaluation of genetic diversity will provide a genetic base for collection and conservation, and it is the first essential step for evaluation of its medicinal and pathogen controlling activities. Generally, genetic diversity can be evaluated via morphological and genetic variation. Evaluation of morphological characters (e.g. leaf, stem, flower, root and seed characters etc.) is normally the easiest and cheapest way to identify genetic relationships or diversity. It also provides information on agronomic traits useful for practical application. Nevertheless, it may be unable to provide sufficient information especially with some closely related varieties/genotypes (Tantasawat et al., 2010a). Morphological characters have been used for assessment in several plants such as yardlong bean (*Vigna unguiculata* spp. *sesquipedalis*) (Tantasawat et al., 2010a), mungbean (*V. radiata* L.) (Tantasawat et al., 2010b), mandarin (*Citrus reticulata* Blanco) (Dorji & Yapwattanaphun, 2011), ginger (*Zingiber Officinale* Roscoe) (Ashraf et al., 2014) and wheat (*Triticum aestivum* L.) (Dong et al., 2014).

For the genetic evaluation, the DNA marker, which is a type of genetic marker, is usually applied. Several types of DNA markers have been used for genetic diversity analysis including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and sequence characterized region (SCAR) etc. (Semagn et al., 2006; Olvera-Mendoza et al., 2016). RAPD which is based on the polymerase chain reaction (PCR) has been one of the most commonly used DNA markers for diversity analysis. RAPD uses single, short and arbitrary oligonucleotide primers for DNA amplification. Its advantages are rapid and reliable assessment that can be performed at any developmental stage of a plant at a lower cost than many other DNA markers because multiple loci in the genome can be detected simultaneously (Fauza et al., 2007; Palai et al., 2007; Kumar & Gurusubramanian, 2011; Valera-Montero et al., 2016). For the *Ludwigia* genus, RAPD has been used in genetic studies of *L. polycarpa* (Huang et al., 2009), while AFLP was used in a reproduction study of *L. hexapetala* and *L. grandiflora* in California's wetlands (Okada et al., 2009). RAPD is also useful for the evaluation of genetic variation of the gamma irradiated rodent tuber (*Typhonium flagelliforme* Lodd.), a herbal plant from the Araceae family (Sianipar et al., 2015). However, it has not been used to access genetic diversity in *L. hyssopifolia*. The objective of this study was to evaluate the genetic relationships and diversity of water primrose in Thailand using morphological characters and RAPD markers.

MATERIALS AND METHODS

Plant material. A total of 17 water primrose accessions collected from several locations in Nakhon Ratchasima, Chaiyaphum and Prachin Buri provinces were evaluated in this study (Table 1). Fifteen accessions were collected from different districts in Nakhon Ratchasima, one accession was collected from Chaiyaphum, and one accession was collected from Prachin Buri.

Analysis of morphological characters and data scoring. Clearly visible morphological characters of six organs (stems, leaf, flower, pod, seed and root) of 17 water primrose accessions were used to assess their similarity. Eight diverse morphological characters were divided into 2-6 classes: stem color (green-light brown, dark green-brown, green-brown-red, green-purple-red and reddish brown), leaf color (light green, dark green, green, green-purple-red, green-red and green-light brown), number of petals (four and five), immature pod color (green, green-red and purple-red), mature pod color (brown and brown-red), seed shape (obovate and spherical), seed color (light brown, brown and brown-red) and leaf shape (lanceolate, ovate and linear). Different combinations of 0 and 1 were coded for each class of all morphological characters [(i.e. 2 classes of number of petals (0, four; 1, five), 3 classes of seed colors (00, light brown; 01, brown; 11, brown-red) etc.].

DNA isolation. Young leaves were freshly harvested from 17 water primrose accessions and rapidly frozen in liquid N_2. DNA extraction was performed by the cetyl trimethyl ammonium bromide (CTAB) method as described by Owen (2003). The concentration and purity of DNA were determined by an

ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) at A_{260} and A_{280} and adjusted to a final concentration of 15 ng/μL for use in PCR analysis.

Random-amplified polymorphic DNA (RAPD) analysis and data scoring. Five RAPD primers, MUNG2 (5'GTAGACCCGT3'), OPA02 (5' TGCCGAGCTG3'), OPA03 (5'AGTCAGCCAC3'), OPS03 (5'CAGAGGTCCC3') and OPV02 (5'AGTCACTCCC3') were used for the analysis. Each 20 μL PCR reaction contained 15 ng of genomic DNA template, 1× buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.01 % Triton X-100), 4.0 mM $MgCl_2$, 200 μM of each dNTPs, 0.75 unit of *Taq* DNA polymerase, and 0.4 μM of each RAPD primer. The PCR reactions were subjected to amplification with initial denaturation at 94 °C for 5 min; 50 cycles of denaturing at 95 °C for 30 sec, annealing at 36 °C for 1 min, extension at 72 °C for 2 min; and final extension at 72 °C for 8 min in a Amplitronyx™ 6 Thermal Cycler (Nyx Technik, Inc., San Diego, CA, USA). The amplified products were revealed on 6% denaturing polyacrylamide gel and detected by silver nitrate according to Sambrook & Russell (2001). Molecular weights of the bands were estimated by using 100 bp DNA ladder (Invitrogen, CA, USA) as standards. All amplifications were repeated at least twice and only reproducible bands were considered for analysis. Clearly different patterns of DNA bands for each primer-accession combination were scored as 0 and 1 for absence and presence of a DNA band, respectively.

Data analysis. Similarity coefficients between various accessions, in a pairwise comparison, were computed using Jaccard's coefficient and the resulting similarity matrix was further analyzed using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm; the computations were carried out using NTSYSpc version 2.2 (Rohlf, 1993). The goodness of fit of the accessions to a specific cluster in the UPGMA cluster analysis was determined by Mantel's correlation test (Mantel, 1967). The polymorphism information content (PIC), a measure of the allelic diversity at a locus, was determined by $PIC = 1 - \Sigma Pi^2$ where Pi is the frequency of the i^{th} allele in the examined test accessions. NTSYSpc version 2.2 was also used to perform principal coordinate analysis (PCoA), which is more informative regarding distances among major groups, to show multiple dimensions of the distribution of the accessions in a scatter-plot (Rohlf, 1993).

RESULTS AND DISCUSSION

Morphological characterization. Seventeen water primrose accessions (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1, KJ-SN-N1, NT-J-C1 and BK-K-P1) collected from different locations of Thailand had similar characters on their roots, stem shape, leaf arrangment and flower color and arrangement. They all had erect and square stems with a taproot system, leaves were arranged in an alternate fashion along a stem and their flowers were all yellow and solitary. By contrast, stem color, leaf color and shape, number of petals, immature and mature pod color, and seed color and shape were different among the accessions. These eight polymorphic morphological characters are shown in Table 1 and used to construct a dendrogram based on cluster analysis using UPGMA. Mantel's test with a cophenetic correlation coefficient value of 0.97 indicated that data in the similar matrix were well represented by a dendrogram. The dendrogram grouped the 17 accessions into two major groups at the genetic similarity level of 0.48 (Fig. 1). Cluster I was further divided into 2 subclusters and 3 individuals. Eight accessions, five from Muang district (SUT-M-N1, SUT-M-N3, SUT-M-N4, OS-M-N1 and PW-M-N1), one from Sung Noen district (KJ-SN-N1) and one from Pak Thong Chai district (PL-P-N1) of Nakhon Ratchasima province, and one from Kabin Buri district, Prachin Buri province (BN-K-P1) were grouped into subcluster IA, with similar characters (green-light brown stem, light green lanceolate leaf, four flower petals, green immature pod, brown mature pod, and light brown obovate seed) (Table 1). While three accessions, two from Sikhiu district, Nakhon Ratchasima province (PT-SK-N1 and KN-SK-N1) and one from Chaturat district, Chaiyaphum province (NT-J-C1) were grouped into subcluster IB. All three accessions shared similar characters and most of the characters in this group were similar to those of subcluster IA, but their leaves were darker in color than those of subcluster IA. In addition, three accessions from Chokchai district (KT-C-N1), Pak Thong Chai district (PL-P-N2) and Muang district (SUT-M-N2), Nakhon Ratchasima province were also grouped into cluster I. While KT-C-N2 from Chok Chai District, MK-SN-N1 from Sung Noen district and TS-SK-N1 from Sikhiu district, Nakhon Ratchasima province were grouped into cluster II, sharing similarity on four of eight characters; linear leaf shape, four flower petals, brown-red mature pod color and spherical seed shape.

Three-dimensional plots of PCoA based on morphological characters were generally consistent with the UPGMA cluster analysis. The three coordinates accounted for 56.87%, 16.49% and 10.96% with a total of 84.32% of total variance (Fig. 2). Jaccard's genetic similarity coefficients among the pairwise combinations of the accessions ranged from 0.154 (TS-SK-N1 and SUT-M-N2; TS-SK-N1 and PL-P-N2) to 1.000 (SUT-M-N1, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, KJ-SN-N1 and BN-K-P1; PT-SK-N1, KN-SK-N1 and NT-J-C1) with an average of 0.693 (Table 2). There appears to be no grouping of accessions according to the collection locations. In addition, these accessions could not be distinguished from one another based on these morphological characters alone. Similarly, using five morphologi-

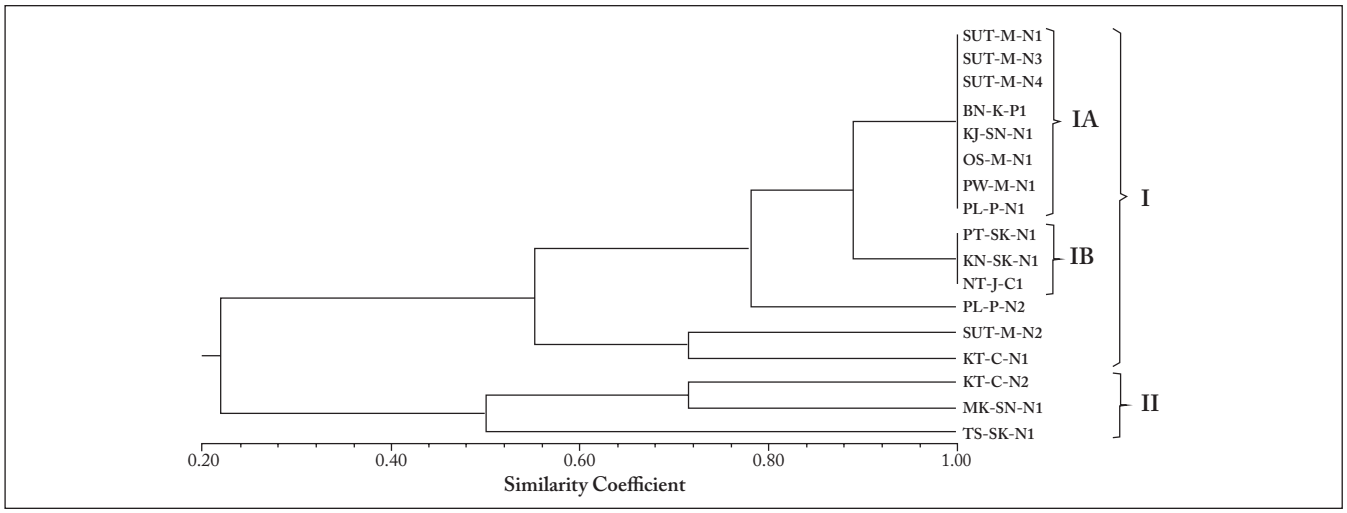

Fig. 1. Dendrogram derived from morphological characters of 17 water primrose accessions (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 and KJ-SN-N1 from Nakhon Ratchasima, NT-J-C1from Chaiyaphum, and BK-K-P1 from Prachin Buri).
Fig. 1. Dendrograma derivado de caracteres morfológicos de 17 accesiones de prímula de agua (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 y KJ-SN-N1 de Nakhon Ratchasima, NT-J-C1 de Chaiyaphum, y BK-K-P1 de Prachin Buri).

Table 1. Description of 17 water primrose accessions from several locations of Thailand.
Tabla 1. Descripción de 17 accesiones de prímula de agua de varias localidades de Tailandia.

Accessions	Sources	Stem color	Leaf color	Leaf shape	Number of petals	Immature pod color	Mature pod color	Seed color	Seed shape
SUT-M-N1	SUT, Muang Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
SUT-M-N2	SUT, Muang Dis., NR	Dark green-brown	Dark green	Lanceolate	5	Green-red	Brown	Brown	Spherical
SUT-M-N3	SUT, Muang Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
SUT-M-N4	SUT, Muang Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
OS-M-N1	Suranaree Industrial Zone, Muang Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
PW-M-N1	Prasat Hin Phanom Wan, Muang Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
PL-P-N1	Phu Luang Sub Dis., Pak Thong Chai Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
PL-P-N2	Phu Luang Sub Dis., Pak Thong Chai Dis., NR	Green-light brown	Green	Ovate	4	Green	Brown	Light brown	Obovate
KT-C-N1	Chok Chai Dis., NR	Green-brown-red	Light green	Lanceolate	4	Green-red	Brown	Brown	Spherical
KT-C-N2	Chok Chai Dis., NR	Green-brown-red	Green-purple-red	Linear	4	Green-red	Brown-red	Brown	Spherical
PT-SK-N1	Sikhiu Dis., NR	Green-light brown	Dark green	Lanceolate	4	Green	Brown	Light brown	Obovate
TS-SK-N1	Sikhiu Dis., NR	Green-purple-red	Green-red	Linear	4	Purple-red	Brown-red	Brown-red	Spherical
KN-SK-N1	Sikhiu Dis., NR	Green-light brown	Dark green	Lanceolate	4	Green	Brown	Light brown	Obovate
MK-SN-N1	Sung Noen Dis., NR	Reddish brown	Green-light brown	Linear	4	Green-red	Brown-red	Brown	Spherical
KJ-SN-N1	Sung Noen Dis., NR	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate
NT-J-C1	Chaturat Dis., CP	Green-light brown	Dark green	Lanceolate	4	Green	Brown	Light brown	Obovate
BN-K-P1	Kabin Buri Dis., PB	Green-light brown	Light green	Lanceolate	4	Green	Brown	Light brown	Obovate

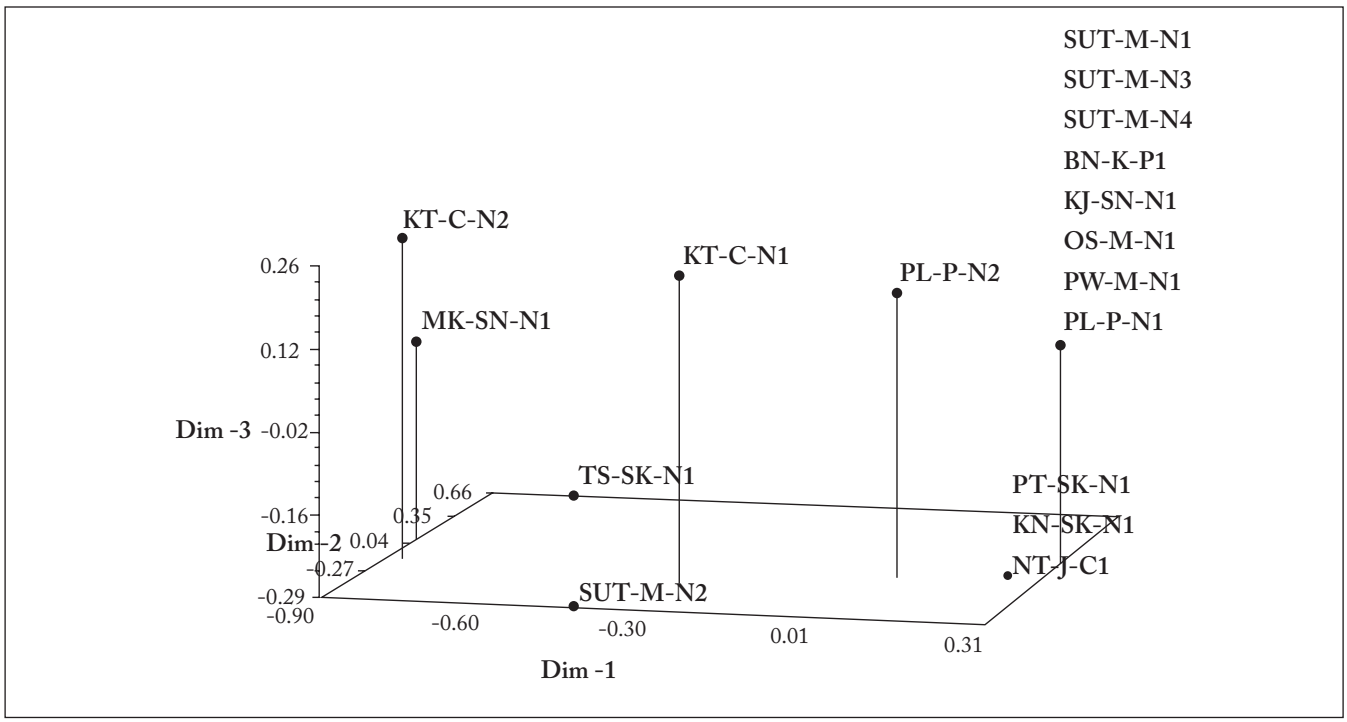

Fig. 2. Principal coordinate analysis of morphological characters (PCoA) of 17 water primrose accessions (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 and KJ-SN-N1 from Nakhon Ratchasima, NT-J-C1from Chaiyaphum, and BK-K-P1 from Prachin Buri).
Fig. 2. Análisis principal coordinado de caracteres morfológicos (PCoA) de 17 accesiones de prímula de agua (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 y KJ-SN-N1 de Nakhon Ratchasima, NT-J-C1 de Chaiyaphum, y BK-K-P1 de Prachin Buri).

cal characters including growth habits, flower and seed colors, seed luster, and shapes of leaftip were insufficient for variety identification and genetic diversity analysis of yardlong bean varieties (Tantasawat et al., 2010a).

Random amplified polymorphic DNA (RAPD) analysis. The genetic variability of 17 accessions of water primrose was analyzed using 5 RAPD primers. In total 68 RAPD fragments were amplified among the accessions of which 55 fragments were polymorphic (80%). The total number of RAPD fragments per primer varied from 12 to 15 fragments per primer with an average of 13.6 fragments per primer. The length of amplified RAPD fragments ranged from 150 to 2,000 bp. The OPA02 primer gave the highest percentage of polymorphism (100%), while the lowest one was found with OPV02 (58.3%), and the average percentage of polymorphism was 80%. The range of PIC values in this study was 0.31 to 0.36 with an average of 0.33, suggesting their usefulness in genetic differentiation. Among these RAPD markers, OPV02 (0.36) was the most informative for distinguishing among the accessions (Table 3). The RAPD polymorphic bands were used to construct a dendrogram based on cluster analysis using UPGMA. The grouping of 17 accessions in the dendrogram indicates the genetic distinctness of the accessions which were studied as they were placed in different clusters.

Mantel's test with a cophenetic correlation coefficient value of 0.64 indicated that data in a similar matrix were quite well represented by a dendrogram. The dendrogram grouped the 17 accessions into three clusters at the genetic similarity level of 0.77 (Fig. 3). Cluster I was divided into 2 subclusters, IA and IB. Two accessions from different districts of Nakhon Ratchasima (SUT-M-N1 from Muang district and KT-C-N1 from Chok Chai district) were grouped into subcluster IA, and seven accessions, six from 3 districts of Nakhon Ratchasima (SUT-M-N2, SUT-M-N3 and PW-M-N1 from Muang district, PL-P-N1 and PL-P-N2 from Pak Thong Chai district, and PT-SK-N1 from Sikhiu district) and one from Kabin Buri district, Prachin Buri province (BN-K-P1), were grouped into subcluster IB, of which two accessions (PL-P-N2 and BN-K-P1) had the highest genetic similarity. Four accessions from three districts of Nakhon Ratchasima (KT-C-N2 from Chok Chai district, KJ-SN-N1 and MK-SN-N1 from Sung Noen district, and TS-SK-N1 from Sikhiu district) were grouped into cluster II,

Table 2. Similarity matrix of 17 water primrose accessions based on morphological character analysis.
Tabla 2. Matriz de similitud de 17 accesiones de prímula de agua basadas en el análisis morfológico de caracteres.

Accessions	SUT-M-N1	SUT-M-N2	SUT-M-N3	SUT-M-N4	OS-M-N1	PW-M-N1	PL-P-N1	PL-P-N2	KT-C-N1	KT-C-N2	PT-SK-N1	TS-SK-N1	KN-SK-N1	MK-SN-N1	KJ-SN-N1	NT-J-C1	BN-K-P1
SUT-M-N1	1.000																
SUT-M-N2	0.400	1.000															
SUT-M-N3	1.000	0.400	1.000														
SUT-M-N4	1.000	0.400	1.000	1.000													
OS-M-N1	1.000	0.400	1.000	1.000	1.000												
PW-M-N1	1.000	0.400	1.000	1.000	1.000	1.000											
PL-P-N1	1.000	0.400	1.000	1.000	1.000	1.000	1.000										
PL-P-N2	0.800	0.500	0.800	0.800	0.800	0.800	0.800	1.000									
KT-C-N1	0.667	0.714	0.667	0.667	0.667	0.667	0.667	0.714	1.000								
KT-C-N2	0.182	0.462	0.182	0.182	0.182	0.182	0.182	0.308	0.667	1.000							
PT-SK-N1	0.889	0.545	0.889	0.889	0.889	0.889	0.889	0.727	0.615	0.167	1.000						
TS-SK-N1	0.182	0.154	0.182	0.182	0.182	0.182	0.182	0.154	0.267	0.429	0.167	1.000					
KN-SK-N1	0.889	0.545	0.889	0.889	0.889	0.889	0.889	0.727	0.615	0.167	1.000	0.167	1.000				
MK-SN-N1	0.182	0.462	0.182	0.182	0.182	0.182	0.182	0.308	0.533	0.714	0.167	0.571	0.167	1.000			
KJ-SN-N1	1.000	0.400	1.000	1.000	1.000	1.000	1.000	0.800	0.667	0.182	0.889	0.182	0.889	0.182	1.000		
NT-J-C1	0.889	0.545	0.889	0.889	0.889	0.889	0.889	0.727	0.615	0.167	1.000	0.167	1.000	0.167	0.889	1.000	
BN-K-P1	1.000	0.400	1.000	1.000	1.000	1.000	1.000	0.800	0.667	0.182	0.889	0.182	0.889	0.182	1.000	0.889	1.000

Table 3. Primers sequences, annealing temperature, total number of scorable DNA bands, number of polymorphic DNA bands, percentage of polymorphism and polymorphic information content (PIC) for each RAPD primer used for the analysis of 17 water primrose accessions.
Tabla 3. Secuencias de los cebadores, temperatura de hibridación, númerototal de bandas de ADN codificables, número de bandas polimórficas de ADN, porcentaje del polimorfismo y de contenido de información polimórfico (PIC) para cada cebador de RAPD usado para el análisis de 17 accesiones de prímula de agua.

Primer	Primer sequence	Annealing temperature (°C)	No. of total bands	No. of polymorphic bands	Polymorphism (%)	PIC
MUNG2	GTAGACCCGT	36	15	14	93.3	0.34
OPA02	TGCCGAGCTG	36	13	13	100	0.32
OPA03	AGTCAGCCAC	36	13	8	61.5	0.32
OPS03	CAGAGGTCCC	36	15	13	86.7	0.31
OPV02	AGTCACTCCC	36	12	7	58.3	0.36
		Total	68	55		
		Average	13.6	11	80	0.33

and four accessions, three from two districts of Nakhon Ratchasima (SUT-M-N4 and OS-M-N1 from Muang district, and KN-SK-N1 from Sikhiu district), and one from Chaturat district, Chaiyaphum province (NT-J-C1), were grouped into cluster III. Three-dimensional plots of PCoA based on RAPD were generally consistent with the UPGMA cluster analysis. The three coordinates accounted for 25.07%, 13.20% and 11.52% with a total of 49.79% of total variance (Fig. 4). Jaccard's genetic similarity coefficients among the pairwise combinations of the accessions ranged from 0.531 (NT-J-C1 from Chaturat district, Chaiyaphum province and MK-SN-N1 from Sung Noen district, Nakhon Ratchasima province) to 0.952 (PL-P-N2 from Pak Thong Chai district, Nakhon Ratchasima province and BN-K-P1 from Kabin Buri district, Prachin Buri province) with an average of 0.802, suggesting moderate genetic diversity among these Thai water primrose accessions (Table 4). Similar to the morphological characters, the genetic diversity of *L. hyssopifolia* based on RAPD were not grouped by their collection locations. By contrast, in red clover (*Trifolium pratense* L.) the genetic diversity generally corresponds to the site of cultivar origin possibly due to the influence of ecological conditions on genotypes (Grljusic et al., 2008). When the morphological and RAPD analysis were compared for the 17 accessions, the correlation coefficient (r) between similarity matrices of morphological characters and RAPD markers was 0.242 (P>0.05), indicating the unrelatedness of the two markers for genetic differentiation. Also the correlation between the dendrograms of the morphological characters and the RAPD markers was also unrelated (r = 0.045; P>0.05). Similarly, Ahlawat et al. (2016) also found a nonsignificant correlation between morphological characters and RAPD markers in Pongamia [*Pongamia pinnata* (L.) Pierre].

These results might be due to the fact that the RAPD markers mainly measured variability in non-coding sequences, which have a low impact on the morphological characters. By contrast, in red clover there is a correspondence between pairs of matrices based on the morphological and molecular markers (both markers classified the samples in a similar manner) (Grljusic et al., 2008). These markers had also been used successfully in wheat, ginger, guava and slender dwarf morning-glory (Ashraf et al., 2014; Dong et al., 2014; Naikawadi et al., 2016; Valera-Montero et al., 2016). In this study, all 17 water primrose accessions can be distinguished by using only 5 RAPD primers while the morphological characters cannot be used to separate 2 groups of 8 and 3 accessions from one another, suggesting that RAPD is more effective in accession identification.

Our results suggest no significant correlation between morphological characters and RAPD-based similarities among 17 water primrose accessions, and we found that RAPD is more effective than morphological characters for determining the genetic variability and relationships, and accession identification in this species. In addition, we reported for the first time that water primrose accessions are genetically diverse in Thailand, and that their genetic relationships are not related to collection locations.

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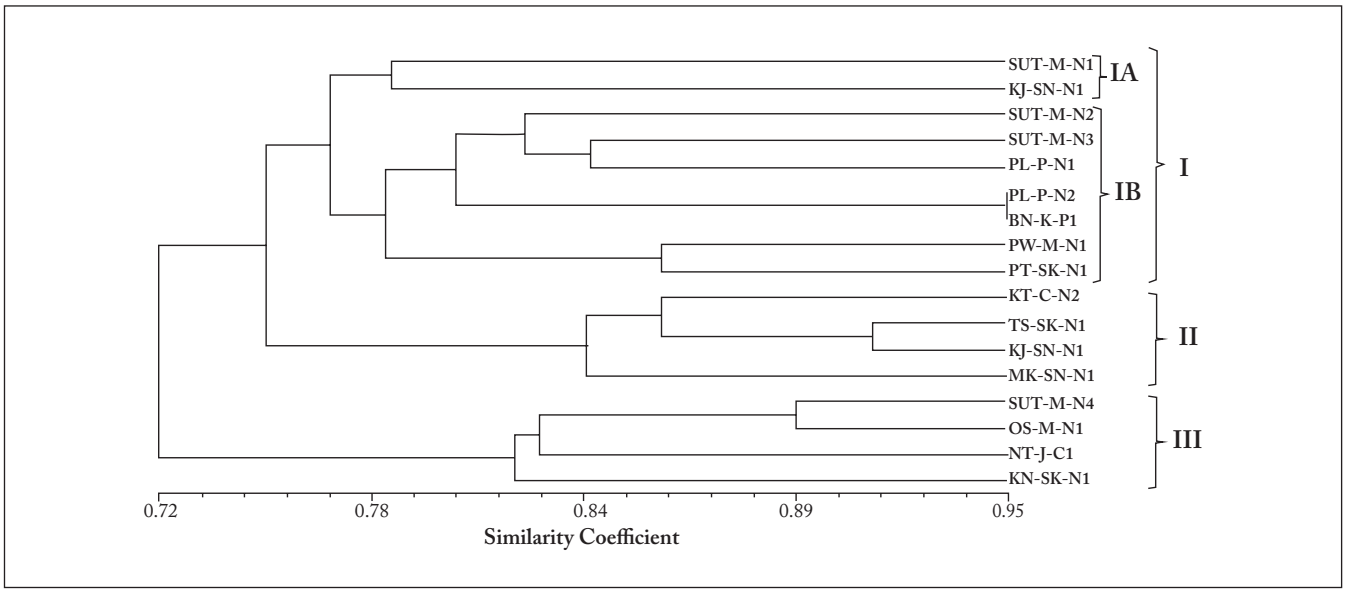

Fig. 3. Random amplified polymorphic DNA (RAPD) dendrogram derived from 17 water primrose accessions (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 and KJ-SN-N1 from Nakhon Ratchasima, NT-J-C1from Chaiyaphum, and BK-K-P1 from Prachin Buri).

Fig. 3. Dendrograma de DNA polimórfico amplificado al azar (RAPD) derivado de 17 accesiones de prímula de agua (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 y KJ-SN-N1 de Nakhon Ratchasima, NT-J-C1 de Chaiyaphum, y BK-K-P1 de Prachin Buri).

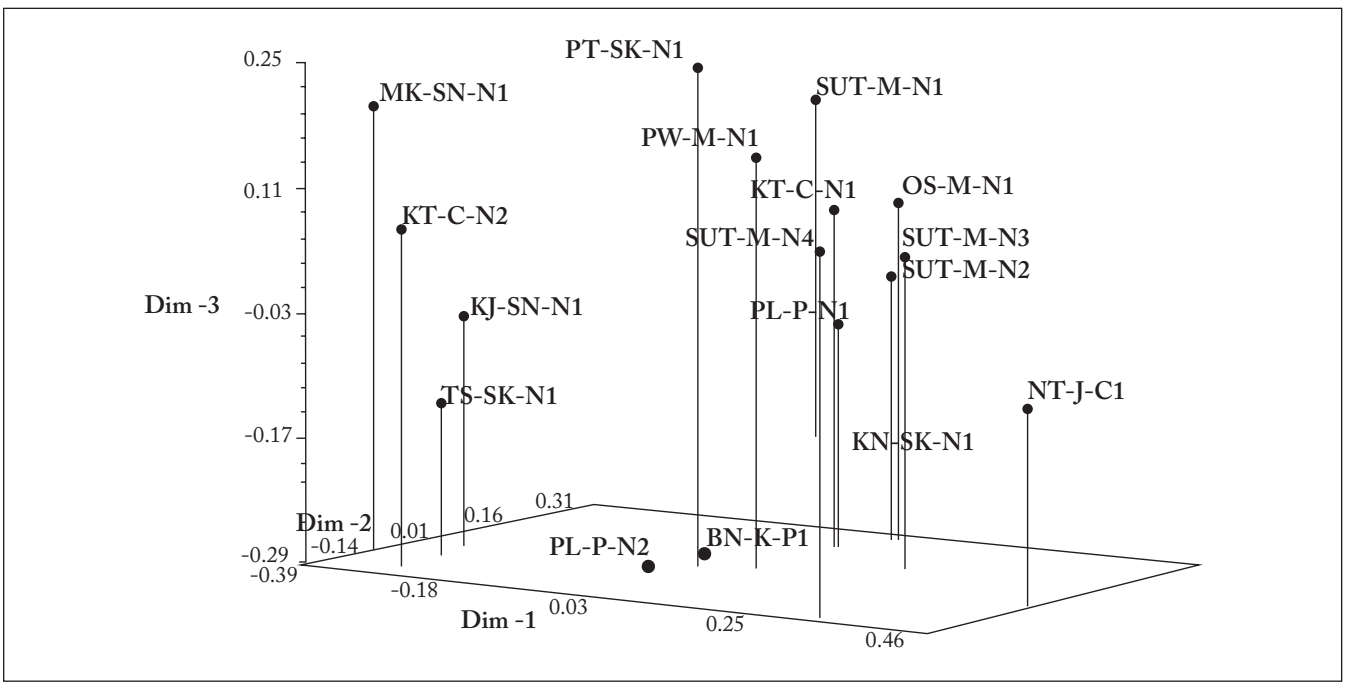

Fig. 4. Random amplified polymorphic DNA (RAPD) derived from principal coordinate analysis (PCoA) of 17 water primrose accessions (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 and KJ-SN-N1 from Nakhon Ratchasima, NT-J-C1from Chaiyaphum, and BK-K-P1 from Prachin Buri).

Fig. 4. DNA polimórfico amplificado al azar (RAPD) derivado del análisis coordinado principal (PCoA) de 17 accesiones de prímula de agua (SUT-M-N1, SUT-M-N2, SUT-M-N3, SUT-M-N4, OS-M-N1, PW-M-N1, PL-P-N1, PL-P-N2, KT-C-N1, KT-C-N2, PT-SK-N1, TS-SK-N1, KN-SK-N1, MK-SN-N1 y KJ-SN-N1 de Nakhon Ratchasima, NT-J-C1 de Chaiyaphum, y BK-K-P1 de Prachin Buri).

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