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Genetic diversity of gac [*Momordica cochinchinensis* (Lour.) Spreng] accessions collected from Mekong delta of Vietnam revealed by RAPD markers

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Abstract

Gac (*Momordica cochinchinensis* (Lour.) Spreng) **(**2n = 28) belongs to the Cucurbitaceae family. It is indigenous throughout in Vietnam as well as in South East Asia. To estimate the genetic diversity, 20 gac accessions collected in Mekong river delta were analyzed using 10 RAPD primers. Total of 126 fragments were obtained from ten RAPD primers, with 114 polymorphic fragments, and average of 11.4 fragments per primer. The high level of polymorphism (90.4%) was found across 20 gac accessions. The highest Nei's gene diversity value among gac accessions was H = 0.40, and the lowest was H = 0.29 with a mean of 0.36. The highest Shannon diversity index value among gac accessions was H' = 0.58 and the lowest was H' = 0.46 with an average of 0.53. Cluster analysis for genetic relationship between accessions displayed that 20 gac accessions grouped into four main clusters which genetic distance coefficient ranged from 0.29 to 0.64 and with an average of 0.54. There were defined groups according to their locations. According to these results, RAPD technique can be useful tool in genetic diversity evaluation of gac. The information achieved could also be useful for gac breeding program, cultivar differentiation and conservation.

Keywords: Cluster analysis, Gac, genetic distance, *Momordica*, Polymorphism.

Introduction

Spiny bitter gourd or Gac (*Momordica cochinchinensis* (Lour.) Spreng) **(**2n = 28) belongs to the Cucurbitaceae family. It is indigenous throughout in Vietnam as well as in South East Asia (Wimalasiri et al., 2016). The fruit is called gac in Vietnam, Fakkao in Thailand, Moc-Niet-Tu in China, Bhat Kerala in India and Makkao in Laos (Kubola and Siriamornpun, 2011). Gac fruit has been used as food and for pharmaceutical purpose (Iwamoto et al., 1985; Kubola and Siriamornpun, 2011). Gac aril is cooked along with rice to extract its red pigments and flavor oil from gac to rice for making "xoi gac" in Vietnam. Gac seed composition is of interest, because of its used in traditional Chinese medicine (Ishida et al., 2004). Recently, a pentacyclic triterpenoid ester as a medicine was isolated from the gac seeds (De Shan et al., 2001). Gac aril contained 175 µg of β-carotene and 802 µg of lycopene/g of fresh weight (Vuong et al., 2002). Lycopene is of interest, because of the correlation of reduced risk of certain cancers, such as prostate and lung (Gerster, 1997; Giovannucci, 1999; Michaud et al., 2000; Giovannucci et al., 2002). The gac aril contains high oil content and fatty acid compositions, which 69% are unsaturated and 35% of those are polyunsaturated fatty acids, and also contains the highest amount of β-carotene and lycopene (Aoki et al., 2002; Vuong and King, 2003; Vuong et al., 2006).

The crop has been cultivated in various ecological regions of Vietnam for many of years. But, there are a few studies on genetic diversity of this crop in Vietnam. The lack of information available on the genetic diversity of gac is a barrier to select genetic material for an efficient breeding program. Information on genetic diversity and relationships among populations is important for plant breeding programs as it helps to select the right genetic material to be used (Ganesh and Thangavelu, 1995). Genetic diversity in crop species can be determined by using the agro-morphological as well as biochemical and molecular markers (Koornneef, 1990; Reiter et al., 1992; Liu, 1997; Dey et al., 2006; Geleta et al., 2008; Behera et al., 2012; Bootprom et al., 2012). However, the use of agro-morphological markers is associated with a strong influenced from environmental factors. DNA markers overcome this limitation and its markers provide a relatively unbiased estimation of genetic diversity and establish genetic relationship more precisely than agro-morphological markers (Soller and Beckmann, 1983).

Random amplified polymorphic DNA (RAPD) have been widely used in genetic diversity studies in species in which there is a lack of DNA sequence information. This technique has advantage such as low development cost, low level of training and low cost for assay (Karp et al., 1997). RAPD markers have been used to assess genetic diversity among the species of sesame (Bhat et al., 1999; Ercan et al., 2004; Pham et al., 2009), bitter gourd (Dey et al., 2006), spine gourd (Rasul et al., 2007), ash gourd (Sureja et al., 2006), pumpkin (Gwanama et al., 2000), gac (Bootprom et al., 2012; Wimalasiri et al., 2016). The purpose of this study was to evaluate genetic diversity of gac accessions collected from Mekong delta of Vietnam by using RAPD markers.

Results and Discussion

Polymorphic RAPD markers for M. cochinchinensis

Gac (*Momordica cochinchinensis* (Lour.) Spreng) is native growing in Vietnam for a long time. And nowadays, it was concerned because of its nutrition and medicinal compositions. This present study is one of few report of molecular genetic diversity for gac in Vietnam. Twenty gac accessions collected in Mekong delta of Vietnam were analyzed (Table 1 and Fig. 1). Total of 10 primers amplified 126 bands, including 114 polymorphic bands and 12 monomorphic bands across the 20 gac accessions with an average polymorphism of 11.4 bands per primer. The number of band per primer varied from 9 to 16 with an average of 12.6 bands per primer. The level of polymorphism included by individual primer ranged from 72.7% to 100%. The highest polymorphism level (100%) was displayed when using four primers OPF01, OPF07, OPC20 and OPC15. The lowest polymorphism level (72.7%) was obtained with primer OPF10. The average polymorphism level was 90.4%. The size of the amplification products varied from 200 bp to 3 kb. The results are presented in Table 2 and a representative agarose gel picture is showed in Fig. 2. Genetic diversity in gac can be studied using few methods such as agro-morphological markers (Sanwal et al., 2007), RAPD markers (Bharathi et al., 2012; Bootprom et al., 2012; Wimalasiri et al., 2016). However, the use of agro-morphological markers is associated with a strong influence from environmental factors. Molecular markers overcome this limitation. Since a few DNA information in gac is a barrier to choose molecular markers for genetic diversity study. RAPD markers are one of priorities to choose because of it does not rely on previous knowledge of DNA sequencing.

In the present study, ten RAPD markers were used for assessing genetic diversity and establishing dendrogram among gac accessions collected in Mekong delta, Vietnam. This study detected a high level of polymorphism for gac accessions collected in Mekong delta. The level of polymorphism was 90.4% among 20 gac accessions. This was lower than the level of polymorphism (99%) reported earlier in gac genotypes collected in Thailand and Vietnam by Bootprom et al. (2012). It was also lower than the 98.7% of polymorphism reported by Wimalasiri et al. (2016) from a study of gac collected in Vietnam, Thailand and Australia. In contrast, this was higher than the level of polymorphism (36.5%) reported in an analysis of genetic diversity in Indian bitter gourd by Dey et al. (2006). The results were in agreement with previous gac study, which high polymorphic markers (100%) were found with primers OPC15, OPC20, OPF01, OPF07 (Bootprom et al., 2012).

Genetic diversity and cluster analysis

The genetic diversity of 20 gac accessions was estimated by using 10 RAPD primers. A high genetic diversity was observed among the 20 gac accessions, with an average of 0.54. Gac plant is a crossing pollinated, because gac is a monoecious plant bearing either male and female flowers on the same plant. Hence, the out-crossing of gac among plant and other plants could explain the high genetic variability observed. Ten RAPD markers detected enough genetic diversity among 20 gac accessions to allow for complete differentiation. Shannon diversity index (H') and Nei's gene diversity (H) for RAPD variation were estimated for the 20 gac accessions and for each primer. The highest Nei's gene diversity value among gac accessions was H = 0.40, and the lowest was H = 0.29 with a mean 0.36. The highest Shannon diversity index value among gac accessions was H' = 0.58 and the lowest was H' = 0.46 with an average of 0.53 (shown in table 2). Genetic distance coefficient ranged from 0.29 to 0.64 and with an average of 0.54 (Fig. 3). Some previous studies reported on the use of RAPD markers for estimating genetic diversity of *Momordica cochinchinensis* as well as *Momordica* genus. These results were in agreement with previous other studies based on RAPD markers which had reported high genetic diversity in gac (Bootprom et al., 2012; Wimalasiri et al., 2016). Bootprom et al. (2012) reported that high genetic diversity (0.63 – 0.90) was found in gac collected from Thailand and Vietnam by using 11 RAPD markers. Recently, genetic diversity of gac was reported by Wimalasiri et al. (2016), a high genetic diversity (0.03 – 1.65) was revealed in gac accessions collected from Thailand, Vietnam and Australia by using 5 RAPD markers. Dey et al. (2006) reported high genetic diversity between 38 genotypes of Indian *Momordica charantia* L. using 29 RAPD markers. High genetic variation of Indian *Momordica* spp. genotypes were also revealed by using 21 RAPD markers (Bharathi et al., 2012). In other crops, the use of the similar number of RAPD markers assessing genetic variation was reported. Mujaju et al. (2010) determined a genetic diversity of watermelon using 10 RAPD markers. In addition, Pham et al. (2009); Rafii et al. (2012) reported a high genetic diversity of *Sesamum indicum* and *Jatropha curcas* with 10 and 11 RAPD markers, respectively.

A dendrogram was generated using UPGMA cluster analysis, and genetic distance among the 20 gac accessions ranged from 0.29 to 0.64. The dendrogram divided the 20 gac accessions into four main groups at genetic distance mean 0.54 (Fig. 3). Cluster I was the biggest group included two sub-groups Ia and Ib, that were gathered at 0.51 distance. Sub-group Ia composed of 7 accessions, G-MKd01, G-MKd09, G-MKd03, G-MKd07, G-MKd17, G-MKd21 and G-MKd19 were joined at 0.475 distance. Sub-group Ib contained 8 accessions namely G-MKd02, G-MKd04, G-MKd08, G-MKd11, G-MKd15, G-MKd10, G-MKd18, G-MKd16 at 0.50 distance. Cluster II and cluster III consisted of only two accessions G-MKd12, G-MKd14 at 0.47 and G-MKd20, G-MKd22 at 0.50 distances, respectively. The last cluster, group IV consisted of only one accession G-MKd24. There were defined groups according to their locations. Based on dendrogram for cluster analysis, gac accessions from the same location were found to have a close genetic relationship, for example group II (G-MKd12, G-MKd14) and group III (G-MKd20, G-MKd22) from Kien Giang and An Giang provinces, respectively. Interestingly, two accessions G-MKd03 from Can Tho province and G-MKd07 from Dong Thap province were found to group together in the same sub-cluster Ia (Fig. 3). A close relation of these two accessions could be explained as a consequence of exchange by farmers between Can Tho and Dong Thap neighborhood. The results were in agreement with previous observation of Stankiewicz et al. (2001). The authors found that human factor has previously been shown to be responsible for the lack of correlation between genetic and geographical distance in some case (Stankiewicz et al., 2001).

Materials and Methods

Plant material and DNA extraction

Twenty gac accessions collected from various locations in Mekong delta of Vietnam were used for this study. The accession name codes for the accessions used in this study

Table 1. List of 20 gac (*Momordica cochinchinensis*) accessions used in this study.

ID	Accession name code	Place of collection (province)	Latitude (GPS)	Altitude (m)	Region
1	G-MKd01	Can Tho	10.02.22N 105.47.28E	3	Mekong delta
2	G-MKd02	Can Tho	10.03.12N 105.46.44E	3	Mekong delta
3	G-MKd03	Can Tho	10.08.50N 105.38.25E	2	Mekong delta
4	G-MKd04	Can Tho	10.17.31N 105.30.51E	3	Mekong delta
5	G-MKd07	Dong Thap	10.25.53N 105.38.15E	4	Mekong delta
6	G-MKd08	Dong Thap	10.28.53N 105.34.47E	10	Mekong delta
7	G-MKd09	Kien Giang	10.28.53N 105.34.47E	10	Mekong delta
8	G-MKd10	KienGiang	10.02.00N 105.05.41E	5	Mekong delta
9	G-MKd11	Kien Giang	10.05.14N 105.03.06E	5	Mekong delta
10	G-MKd12	Kien Giang	10.08.56N 104.36.28E	24	Mekong delta
11	G-MKd14	Kien Giang	10.11.12N 104.55.12E	1	Mekong delta
12	G-MKd15	Kien Giang	9.56.51N 105.07.23E	1	Mekong delta
13	G-MKd16	KienGiang	9.56.51N 105.07.23E	1	Mekong delta
14	G-MKd17	Kien Giang	9.58.34N 105.06.16E	3	Mekong delta
15	G-MKd18	Kien Giang	10.15.05N 104.49.14E	5	Mekong delta
16	G-MKd19	An Giang	10.20.23N 105.28.05E	4	Mekong delta
17	G-MKd20	An Giang	10.22.14N 105.24.57E	9	Mekong delta
18	G-MKd21	An Giang	10.24.07N 105.24.23E	2	Mekong delta
19	G-MKd22	An Giang	10.9.15N 105.20.36E	4	Mekong delta
20	G-MKd24	An Giang	10.23.53N 105.29.46E	3	Mekong delta

G-MKd: Gac Mekong delta

Fig 1. Map of Mekong delta showing collection zones of gac accessions used in this study.

Table 2. List of selected primers used in the RAPD analysis and the number of total fragments, number of polymorphic fragments, percent polymorphism, estimated molecular size range, Nei's gene diversity and Shannon information index.

Primer	Sequence (5'-3')	TF	PF	% of P	MSR (bp)	H	H'
OPF01	ACGGATCCTG	13	13	100.0	350-2,000	0.39	0.57
OPX01	CTGGGCACGA	15	11	73.3	300-2,000	0.34	0.51
OPF03	CCTGATCACC	16	15	93.8	300-1,500	0.31	0.48
OPW03	GTCCGGAGTG	12	11	91.7	200-2,000	0.29	0.45
OPF07	CCGATATCCC	15	15	100.0	250-2,500	0.37	0.56
OPC20	ACTTCGCCAC	11	11	100.0	400-2,000	0.38	0.55
OPC15	GACGGATCAG	9	9	100.0	250-3,000	0.37	0.56
OPF12	ACGGTACCAG	14	13	92.9	300-2,500	0.40	0.57
OPC19	GTTGCCAGCC	10	8	80.0	350-2,000	0.30	0.46
OPF10	GGAAGCTTGG	11	8	72.7	200-2,500	0.40	0.58
Mean		12.6	11.4	90.4	200-3,000	0.36	0.53
Total		126	114				

TF: Total fragments, *PF:* Polymorphic fragments, *% of P:* percentage of polymorphic fragments, *H:* Nei's genetic diversity, *H':* Shannon's information index, *MSR:* Molecular size range (bp).

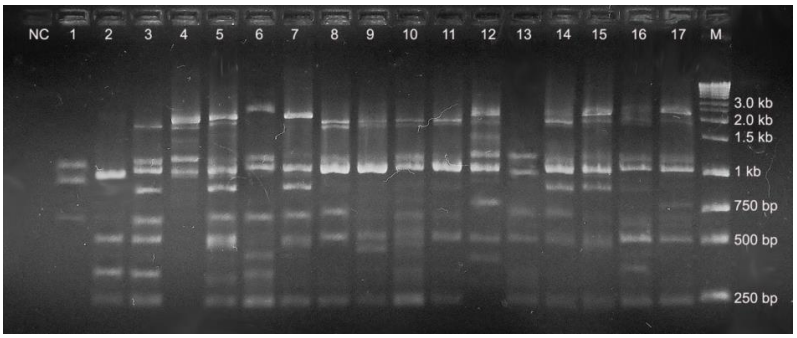

Fig 2. PCR-RAPD analysis with primer OPF07, with Lane NC: negative control, 1: G-MKd1, 2: G-MKd2, 3: G-MKd3, 4: G-MKd4, 5: G-MKd7, 6: G-MKd8, 7: G-MKd10, 8: G-MKd12, 9: G-MKd14, 10: G-MKd15, 11: G-Mkd16, 12: G-MKd18, 13: G-MKd19, 14: G-MKd20, 15: G-MKd21, 16: G-MKd22, 17: G-MKd24 and M: GeneRuler 1kb DNA ladder.

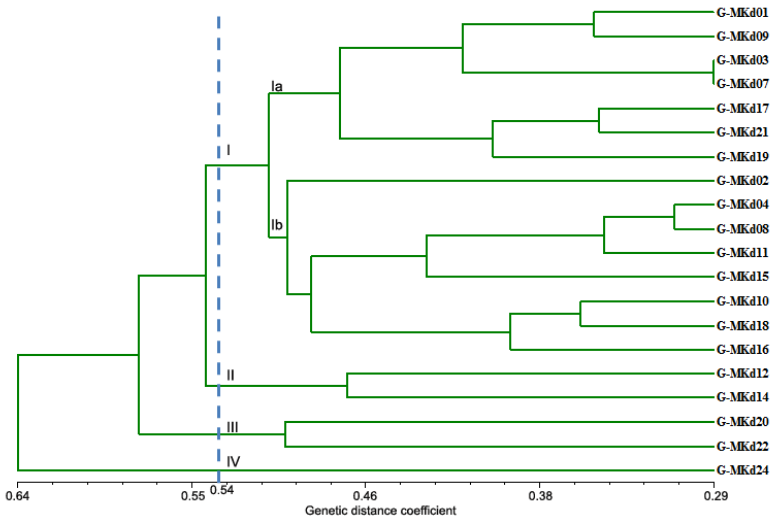

Fig 3. Dendrogram generated using UPGMA cluster analysis based on RAPD data for genetic diversity of 20 gac accessions.

correspond to the initials of the name of their collection zones (Table 1 and Fig. 1). Ten seeds from each accession were sown in plastic pots then cultivated in experimental field at Nong Lam University in Ho Chi Minh city ($10^0$52'12N $106^0$47'12E). DNA was extracted from young leaves of 6 to 8 weeks old seedling on each individual plant using a protocol described in Warwick and Gugel (2003) with minor modification described in Pham et al. (2009).

PCR-RAPD and electrophoresis

The PCR-RAPD procedure for gac was carried out as described by Williams et al. (1990). Amplification reaction was performed in a volume of 25 µ containing 1X PCR buffer (10 mM Tris–HCl, pH 8.3 and 50 mM KCl), 0.2 mM dNTP (each of dATP, dTTP, dCTP, dGTP), 3 mM MgCl2, 1U Taq polymerase (Sigma), 0.2 mM primer (Operon Biotechnologies) and about 50–60 ng DNA template. The PCR was carried out in a thermal cycler (GeneAmp PCR Apply Biosystem 9700, Singapore) using 5 min at 94 ºC for denaturation followed by 40 cycles of 60 s at 94 ºC, 90 s at 35 ºC, and 180 s at 72 ºC. The cycles were followed by a final extension period of 10 min at 72 ºC. The RAPD fragments were separated on 1% agarose gel in 1X TAE (40 mM Tris–acetate, pH 8.0, 1 mM EDTA) buffer at 80 volt and 200 mA for 1 h. GeneRuler 1kb DNA ladder ranging from 250 bp to 10 kb was used as a molecular weight marker. The reproducibility of the amplification products was checked three times for each primer. A negative control that contained all components of a typical reaction but lacked template DNA was included on each gel. After electrophoresis, the gels were stained in ethidium bromide for 30 min, and then rinsed in water for 30 min. The gels were photographed using UV light and a Sony camera. Images were later used to score amplification products and set up the binary data.

Polymorphic RAPD primers screening

Four accessions from different place of collection (G-MKd1, G-MKd8, G-MKd16 and G-MKd20) were used for evaluating amplification level of primers. Thirty RAPD primers were collected for their ability to amplify polymorphic bands, which had previously been used in investigations by Bharathi et al. (2012) in *Momordica* spp. and Bootprom et al. (2012) in *Momordica cochinchinensis*. Ten primers amplified distinctive band in four gac accessions with good polymorphic bands. Therefore, ten primers (Table 2) were selected for PCR amplification of all accessions.

Scoring and data analysis

Each amplification product was considered as an independent character (locus) and was identified by the name of the primer and the size of the DNA amplification product. The amplified fragments in each of the 20 gac accessions were scored manually for their presence (denoted as ''1'') or absence (denoted as ''0'') for each primer combination. Fragment size was compared to a molecular weight 1 kb DNA ladder.

Genetic distance and cluster analysis

The binary data was used to generate a similarity index (matrix) using the method based on Nei and Li (1979) genetic distance value. Genetic similarity $GS = \frac{2Nab}{Na+Nb}$ where *Nab* is the number of fragments shared by accession *a* and *b*, *Na* is the number of fragments in accession *a*, and *Nb* is the number of fragments in accession *b*. For the analyses of genetic distances among populations, the similarity matrix was converted to a distance matrix, using formula $GD = 1 - GS \; or \; GD = -\ln(GS)$ (Weising et al., 2005). This matrix was further employed to generate dendrogram using the unweighted pair group arithmetic average method (UPGMA).

Cluster and Neighbor joining analysis were carried out using the SAHN-UPGMA and Neighbor joining clustering method, respectively by using the Numerical Taxonomy System (NTSYSpc 2.1) software. To calculate Nei's gene diversity (H) and Shannon diversity index (H') of phenotypic diversity for the RAPD binary data. The Popgene software version 1.31 (Yeh and Boyle, 1997) was employed.

Conclusion

In conclusion, a high genetic diversity of 20 gac accessions collected in Mekong delta of Vietnam were revealed using 10 RAPD markers. The RAPD markers also revealed high level of polymorphism (90.4%) across the gac accessions. According to these results, RAPD technique can be useful tool in genetic diversity evaluation of *Momordica cochinchinensis*. The information achieved could also be used for cultivar differentiation and conservation.

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