Agriculture and Natural Resources 52 (2018) 497–502

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Genetic variation in cucumber (*Cucumis sativus* L.) germplasm assessed using random amplified polymorphic DNA markers

Hadsaya Panyanitikoon,[a] Chanuluk Khanobdee,[b] Chatchawan Jantasuriyarat,[a, c] Sompid Samipak[a, c, *]

[a] Department of Genetics, Faculty of Science, Kasetsart University, Bangkhen Campus, Bangkok 10900, Thailand
[b] Rajamangala University of Technology Lanna, Lampang Agricultural Research and Training Centre, Lampang 52000, Thailand
[c] Center for Advances Studies in Tropical Natural Resources, National Research University-Kasetsart University (CASTNAR, NRU-KU), Kasetsart University, Bangkok 10900, Thailand

ARTICLE INFO

Article history:
Received 12 July 2017
Accepted 2 August 2017
Available online 2 November 2018

Keywords:
Cucumber
Downy mildew
Random amplified polymorphic DNA (RAPD) marker
Genetic diversity
Agro-economic trait

ABSTRACT

In total, 26 random amplified polymorphic DNA (RAPD) markers were used to assess the genetic diversity of 38 cucumber accessions curated at Rajamangala University of Technology Lanna, Lampang, Thailand. Polymorphic (140) and monomorphic (10) fragments were detected with this set of markers. The polymorphic information content value ranged from 0.04 to 0.45, with an average value of 0.27. The dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic mean algorithm classified cucumber germplasm into clusters A and B which corresponded well with plant disease reaction to downy mildew. Cluster A was divided into subgroups A1 and A2. The A1 subgroup had a higher yield, longer fruit length and longer flesh pith length than the A2 subgroup and B cluster, while fruit width was uniform across accessions. This grouping was in good agreement with country of origin, with A1 members being from Far East Asia, A2 members from Southeast Asia and B members from South Asia. The close genetic relationship between A1 and A2 suggested more mobile seed transfer between Far East Asia and Southeast Asia while separation of the B cluster suggested limited genetic transfer from South Asia to other parts of the Asia continent.

Introduction

Cucumber (*Cucumis sativus* L.; 2n = 2x = 14) is a member of the gourd family Cucurbitaceae that is widely cultivated in many areas around the world and was first domesticated 300 yr ago (Whitaker and Davis, 1962; Jeffrey, 1980). It is the fourth most valuable vegetable crop being sold in the world (FAO, 2016). The genus *Cucumis* contains more than 55 species globally including 25 Asian and Australian and approximately 30 African species as revealed by DNA sequences from plastid and nuclear markers (Renner et al., 2010). The two species, *Cucumis sativus* (cucumber) and *Cucumis melon* (melon, muskmelon and Persian melon) are economically important vegetable crops in many countries especially where the cucumber is widely consumed fresh (Dhaliwal, 2017). The top producers of cucumber are China, India, Russia and USA, respectively (Food and Drug Administration, 2016).

Common problems with cucumber plantation are damage by insects such as cucumber beetles, aphids and pickleworms, and diseases such as bacterial wilt, mosaic leaf spot, anthracnose, scab and downy mildew (DM) (Sharma et al., 2016). Thailand, being a tropical country, has high humidity that often leads to a major problem of DM infestation that can spread widely. DM is caused by a fungus *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov, which attacks the plant foliage during cool, damp periods (Shankar et al., 2014). A short latent period, rapid spread and high secondary infection cause a drastic decrease in plant health, fruit quality and total yield. The symptoms of DM on cucumber begin with the appearance of light-yellow spots on the top surface and a water-soaked appearance on the underside. As the spots expand, they appear angular as the disease does not spread past major veins. The proliferation of fungal spores causes the leaf to appear dusty gray and to eventually shrink and turn brown (Holmes et al., 2006; Savory et al., 2011).

* Corresponding author. Department of Genetics, Faculty of Science, Kasetsart University, Bangkhen Campus, Bangkok 10900, Thailand.
E-mail address: ssamipak@gmail.com (S. Samipak).

https://doi.org/10.1016/j.anres.2018.10.015

Currently, various lines of cucumbers from around the world with different agro-economic traits have been collected as the main cucumber genetic resource in Thailand at Rajamangala University of Technology Lanna in Lampang. So far, studies have been done on phenotypic evaluation, but there has been little genetic study of this germplasm. The accessions have been grown in Lampang for field evaluation, where agro-economic traits have been recorded along with disease reaction to DM. DNA marker study on this germplasm is needed to gain a better understanding of the genetic differences between the DM-resistant and the DM-susceptible groups in order to identify resistant plants in the future.

Molecular markers are common tools used to identify genetic variation in various organisms (Irshad and Idrees, 2014). A DNA marker has advantages over the other marker types due to its ability to identify individuals with great reliability and precision and also it is not affected by changes in the environment. There are many types of DNA markers such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) (Nadeem et al., 2018). RAPD and ISSR are multi-locus, dominant markers that rely on the relatively simple PCR technique; both markers are quite inexpensive, can produce highly polymorphic genotypes, and more importantly, prior knowledge of DNA sequences is not required (Williams et al., 1990; Pradeep Reddy et al., 2002). RAPD uses an arbitrary, short primer that anneals to the genome at complementary sequences, and sizes of amplified products reveal existing polymorphisms (Nadeem et al., 2018). It is less expensive, produces high polymorphism and require simple detection technology (Williams et al., 1990). ISSR detects DNA segments that are flanked at both ends by SSRs; suitable size regions are amplified and used as markers to reveal genetic variation (Ng and Tan, 2015). Both RAPD and ISSR have been used to estimate genetic diversity in many plant species, including in *Ocimum* species (Chen et al., 2013), in barley (Guasmi et al., 2012), in walnut (Ahmed et al., 2012), in *Cucurbita pepo* (Nontuthuko et al., 2015) and in potato (Rocha et al., 2010). The Lampang germplasm has been previously investigated using ISSR and SSR (Innark et al., 2014). In the current study, RAPD markers were used to analyze 38 cucumber accessions collected in Lampang germplasm to reveal their genetic diversity. This information should provide invaluable information for more effective germplasm management and for setting up a breeding program to provide new cucumber varieties to the expanding market.

Material and methods

Plant screening

Thirty-eight cucumber accessions were obtained from the core collection at Rajamangala University of Technology Lanna, Lampang, Thailand. These accessions originated from China (12), Thailand (9), India (10) and Taiwan (2), with one accession from each of Malaysia, Korea, Pakistan, Zambia and the former Soviet Union. Plant growing arrangements and care were described previously (Innark et al., 2013). Phenotypic evaluation included yield, fruit weight, fruit width, fruit length, and flesh pith length. DM disease score rating was done at 45 d after planting (Innark et al., 2014). The disease score was classified into five classes on the average of leaf symptom area described by (Kaweekijthummakul et al., 2007) in which less than 20% was considered as highly resistant (HR), 20–39% as moderately resistant (MR), 40–59% as intermediate (I), 60–79% as moderately susceptible (MS) and 80% or more as highly susceptible (HS).

DNA isolation

Cucumber seeds were planted on culture trays filled with planting soil. Fully expanded cotyledons were collected for DNA extraction using the modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). DNA quality was analyzed using agarose gel electrophoresis. The DNA samples were stored at −20 °C until used.

Random amplified polymorphic DNA analysis

The genomic DNA of each accessions was analyzed using 26 random amplified polymorphic DNA (RAPD) markers (Eurofin Genomics RAPD 10mer Kits (Genomics India Pvt Ltd; Karnataka, India)). PCR amplification was carried out in 20 μL containing 50 ng of extracted genomic DNA, 10 μM specific primer, 5U *Taq* DNA polymerase enzyme, dNTPs, 10 X $MgCl_2$ free PCR buffer, $MgCl_2$ solution and dH_2O. DNA was amplified under the following protocol: denaturation at 94 °C for 3 min; then 34 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min and extension at 72 °C for 1 min, with the final extension at 72 °C for 10 min. The annealing temperature was adjusted to optimally amplify each RAPD primer. PCR products were separated using electrophoresis on 1% (weight per volume) agarose gel in 0.5 X TBE buffer, visualized using ethidium bromide staining and photographed under ultraviolet light.

Data analysis

RAPD fragments were scored as discrete variables, using 1 to indicate the presence and 0 to indicate the absence of a fragment. The scores obtained from all the primers and all plants were then combined to create a single data matrix that was used to calculate similarity. The genetic relationship was analyzed using the NTedit version 1.03 program and NTSYSpc version 2.10 m (Rohlf, 2008). The unweighted pair group method with arithmetic mean (UPGMA) method was used to construct a dendrogram representing genetic relationships between accessions. The Excel software package (2010; Microsoft Corp.; Redmond, WA, USA) was used to calculate the polymorphic information content (PIC) using Eq. (1) for the dominant marker:

$$\mathrm{PIC} = 1 - \left[f^2 + (1-f)^2\right] \quad (1)$$

where f is the marker frequency in the dataset (De Riek et al., 2001).

Results

Random amplified polymorphic DNA analysis

Twenty-six RAPD markers (100%) could amplify all 38 cucumber accessions. In total, 150 amplified fragments were produced, of which 140 fragments were polymorphic while 10 fragments were monomorphic across all accessions. The number of amplified fragments of each RAPD marker varied from 2 in UPB-483 to 11 in primer OPAC-05 with an average of 5.8 fragments per primer (Table 1).

To determine the level of polymorphism in the analyzed germplasm, the PIC was calculated. The PIC value of each primer was calculated based on the mean of the PIC values for all fragments generated by that primer. From this experiment, the PIC value ranged from 0.04 to 0.45, with an average value of 0.27. The highest PIC was observed in primer UPB-500, while the lowest was observed in primer OPB-01. The percentage of polymorphic bands of each primer ranged from 57.14% for OPK-04 to 100% for OPA-09,

Table 1
Characteristics of the 26 random amplified polymorphic DNA markers used and the diversity detected in cucumber accessions.

No.	Marker	Sequence (5′-3′)	Tm (°C)	Allele number	Polymorphic allele number	PIC score	Polymorphism (%)
1	OPA-01	CAGGCCCTTC	35.4	5	4	0.40	80
2	OPA-09	GGGTAACGCC	35.4	5	5	0.26	100
3	OPAC-03	CACTGGCCCA	39.5	8	5	0.15	62.5
4	OPAC-04	ACGGGACCTG	43.6	6	6	0.21	100
5	OPAC-05	GTTAGTGCGG	39.5	11	8	0.20	72.73
6	OPAH-01	TCCGCAACCA	35.4	7	6	0.21	85.71
7	OPAH-02	CACTTCCGCT	35.5	5	4	0.20	80
8	OPAH-03	CTCCCCAGAC	39.5	4	3	0.30	75
9	OPAH-04	CTCCCCAGAC	43.6	5	5	0.26	100
10	OPAH-05	TTGCAGGCAG	39.5	4	4	0.40	100
11	OPB-01	GTTTCGCTCC	39.5	3	2	0.04	66.67
12	OPB-04	GGACTGGAGT	35.4	9	9	0.25	100
13	OPB-05	TGCGCCCTTC	35.5	5	5	0.42	100
14	OPE-03	CCAGATGCAC	39.5	9	8	0.23	88.89
15	OPE-04	GTGACATGCC	43.6	6	6	0.30	100
16	OPE-05	TCAGGGAGGT	39.5	5	4	0.21	80
17	OPJ-13	GGTTGTACCC	35.5	5	5	0.38	100
18	OPJ-15	TGTACCCGGG	39.5	6	6	0.32	100
19	OPK-04	CCGCCCAAAC	43.6	7	4	0.34	57.14
20	OPK-07	AGCGAGCAAG	39.5	8	5	0.27	62.5
21	UPB-483	GCACTAAGAC	35.4	2	2	0.18	100
22	UPB-485	AGAATAGGGC	35.4	4	4	0.24	100
23	UPB-486	CCAGCATCAG	39.5	6	6	0.27	100
24	UPB-489	CGCACGCACA	43.6	6	4	0.17	66.67
25	UPB-499	GGCCGATGAT	39.5	7	7	0.36	100
26	UPB-500	TTGCGTCATG	35.4	3	3	0.45	100

PIC = polymorphic information content.

OPAC-04, OPAH-04, OPAH-05, OPB-04, OPB-05, OPE-04, OPJ-13, OPJ-15, UPB-483, UPB-485, UPB-486, UPB-499 and UPB-500 with a mean of 87.61% (Table 1).

Dendrogram construction and principal component analysis

For the detection of genetic relationship, a dendrogram was constructed from RAPD information based on the UPGMA clustering method (Fig. 1). The 38 cucumber accessions were divided into two main clusters, A and B with a coefficient of 0.63. Incorporating the phenotypic data, this clustering had good correspondence with variation in DM resistance and also cucumber yield. All DM resistant accessions (intermediate, moderately and highly resistant) were grouped into cluster A while most DM-susceptible accessions were grouped into cluster B except for the accessions PI217644 (MS), PI500360 (HS) and PI504566 (HS) that were susceptible but were included in the resistant cluster. Besides the differences in cucumber resistance to DM, accessions in cluster A had higher yield than in cluster B (22.93 ± 8.50 and 6.50 ± 3.75 t/ha, respectively) when grown in the field at Lampang.

Two main subgroups were identified within the resistant cluster A (A1 and A2). Subgroup A1 contained 11 accessions while A2 had

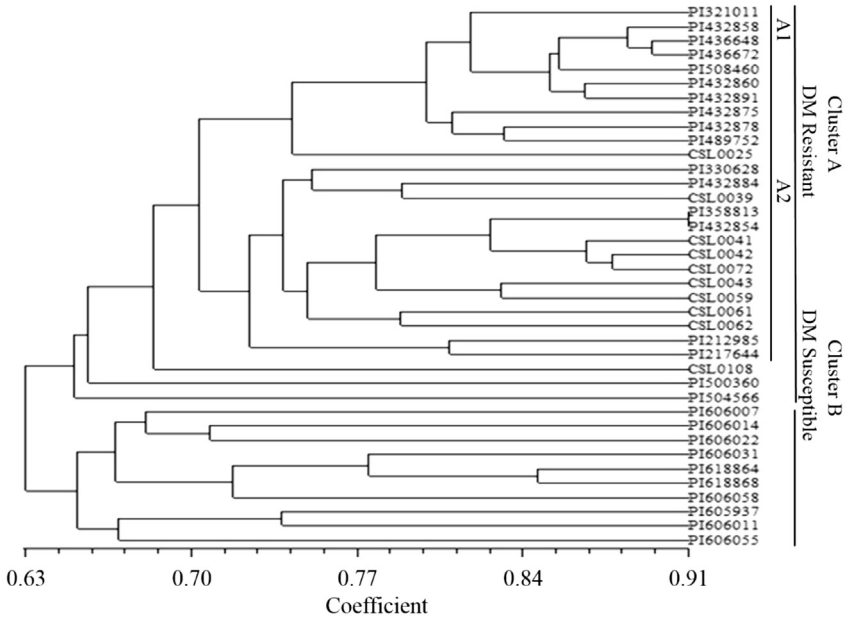

Fig. 1. Dendrogram of 38 cucumber accessions using 26 random amplified polymorphic DNA primers with the unweighted pair group method with arithmetic mean algorithm method. A1 subgroup = Far East Asia, A2 subgroup = Southeast Asia, Cluster B = South Asia.

14 accessions (Fig. 1). This subdivision, based on the RAPD markers, coincided with diversity in the fruit weight, fruit length and flesh pith length. The mean fruit weight for the A1 subgroup was 151.78 ± 26.98 g and 84.30 ± 24.27 g for the A2 subgroup (Table 2). The mean fruit length and mean flesh pith length for the A1 subgroup were 26.20 ± 3.80 cm and 20.08 ± 2.42 cm, respectively, while for the A2 subgroup, the means were 12.03 ± 5.97 cm and 9.27 ± 4.59 cm, respectively. In general, for the three recorded traits (fruit weight, fruit length and flesh pith length), the A1 subgroup had heavier and longer fruit characteristics than the A2 subgroup.

The clustering of these cucumber accessions based on RAPD markers was also in good agreement with the country of origin of each accession (Fig. 1). Cluster A included accessions mainly from Far East Asia and from South Asia. On the other hand, cluster B included accessions mainly from South Asia. Three accessions that were not included in any subgroup were CSL0108 from Taiwan, PI500360 from Zambia and PI 504566 from the former Soviet Union. Two of these (PI500360 and PI504566) were highly susceptible to DM while CSL0108 was moderately resistant to DM. The fruit width was quite uniform across all accessions having a mean of 3.47 ± 0.33 cm.

The PCA results further confirmed the grouping identified from the constructed dendrogram in which cucumber accessions were placed into group 1 and group 2 with three accessions placed in between groups (Figs. 1 and 2). Group 1 contained DM-resistant accessions originating from the Far East and Southeast Asia corresponding to cluster A from the dendrogram. Group 2 contained DM-susceptible accessions originated from South Asia corresponding to cluster B from the dendrogram. Three accessions situated between groups 1 and 2 were PI504566 from the former Soviet Union and PI217644 and PI212985 from India. One of these accessions (PI504566) was placed in cluster A but not in any subgroups, showing less genetic similarity to the other members.

Discussion

In this study, 38 cucumber accessions from the collection at Rajamangala University of Technology, Lanna, Lampang, Thailand were evaluated for the genetic diversity using 26 RAPD primers along with agro-economic traits. The sampled accessions constituted mainly cucumbers from China, India and Thailand. From the results, 26 RAPD markers were effective in analyzing 38 cucumber accessions. In total, 140 polymorphic alleles were obtained with the average percentage of polymorphism of each primer of 87.61%. The usefulness of these markers was demonstrated by measurement of the PIC value. PIC provides an estimate of the discriminatory power of each marker by taking into account not only the number of alleles, but also their relative frequencies in the population. The PIC values of these RAPD markers ranged from 0.04 to 0.45 with an average of 0.27. With RAPD being a dominant marker, the highest possible PIC value is 0.5; thus, the identified PIC values were considered to have medium discriminating power. This same germplasm was previously analyzed using 17 ISSR primers and the results showed a similar average PIC value of 0.25 (Innark et al., 2014). Though the majority of research showed that ISSR markers usually gave higher PIC values than RAPD markers (Manimekalai et al., 2006; Tonk et al., 2014), comparable or higher PIC values identified by RAPD over ISSR markers when used to analyze the same plant germplasm has been reported. Evaluation of 100 cashew germplasm accessions using RAPD and ISSR markers revealed similar PIC values of 0.31 and 0.295, respectively (Thimmappaiah et al., 2009), while evaluation of *Cymbopogon winterianus* collected across West Bengal, India using RAPD and ISSR markers had a higher PIC value using RAPD (0.25) than from ISSR (0.22) markers (Bhattacharya et al., 2010).

The RAPD markers could separate the germplasm into two main clusters of A and B. This clustering corresponded well with the disease reaction to DM, with cluster A containing all accessions with intermediate, moderate and high resistance to DM, while the majority of susceptible lines were placed in cluster B. Cluster A accessions had a higher yield per area than cluster B accessions. This appeared to be quite logical since being resistant to DM allowed cucumber accessions to have higher yields due to lower plant damage by the DM disease as it has been shown that DM can cause a drastic reduction in cucumber yield (Holmes et al., 2006).

The dendrogram based on RAPD markers further subdivided cluster A into A1 and A2 subgroups. This subdivision further demonstrated the power of the marker set in this germplasm, as the A1 and A2 subgroups showed clear differences in yield and yield components between the subgroups namely fruit weight, fruit length and flesh pith length. This confirmed previous work done on this germplasm which showed that the yield trait correlated strongly with fruit weight, flesh pith length and fruit length (Innark et al., 2013). Furthermore, the country of origin also coincided well with the RAPD-based grouping. The majority of subgroup A1 accessions was obtained from countries in Far East Asia and subgroup A2 accessions were from Southeast Asia, while cluster B accessions were from South Asia.

The results obtained from analysis using RAPD markers was similar with ISSR markers with minor differences. From the RAPD work, all DM-resistant accessions were grouped together in cluster A, while from the ISSR work two resistant accessions (CSL0108 and PI212985) were misplaced into the susceptible group C (Innark et al., 2014). On the other hand, the constructed dendrogram based on RAPD markers showed that two susceptible accessions (PI500360 and PI504566) were also misplaced in the DM-resistant cluster A. Even though both PI500360 from Zambia and PI504566 from the former Soviet Union were placed in cluster A, they were not included in any subgroups, suggesting that they were genetically distant from other members in that cluster. The minor displacements observed in analysis by either ISSR or RAPD markers stressed the fact that both ISSR and RAPD markers are suitable for surveying the polymorphisms existing in the genome, and often that the places where these primers annealed to might be far from any gene controlling desirable traits and thus causing unclear clustering. Possible ways to address this situation are to increase the number of markers or to try different types of DNA marker to effectively resolve the genetic relationship.

From the dendrogram based on ISSR analysis (Innark et al., 2014) and the RAPD analysis, accessions from Far East Asia (mainly from China) were genetically closer to accessions from Southeast Asia (mainly from Thailand) than accessions from South

Table 2
Average ± SD of yield component traits.

Group	Yield/ha(t)	Fruit weight (g)	Fruit widht (cm)	Fruit length (cm)	Fresh pith length (cm)
A1	27.88 ± 7.38	151.78 ± 26.98	3.20 ± 0.20	26.20 ± 3.80	20.08 ± 2.42
A2	21.75 ± 5.75	84.30 ± 24.27	3.63 ± 0.34	12.03 ± 5.97	9.27 ± 4.59
B	6.50 ± 3.75	85.04 ± 30.03	3.52 ± 0.33	13.21 ± 5.61	10.58 ± 3.66

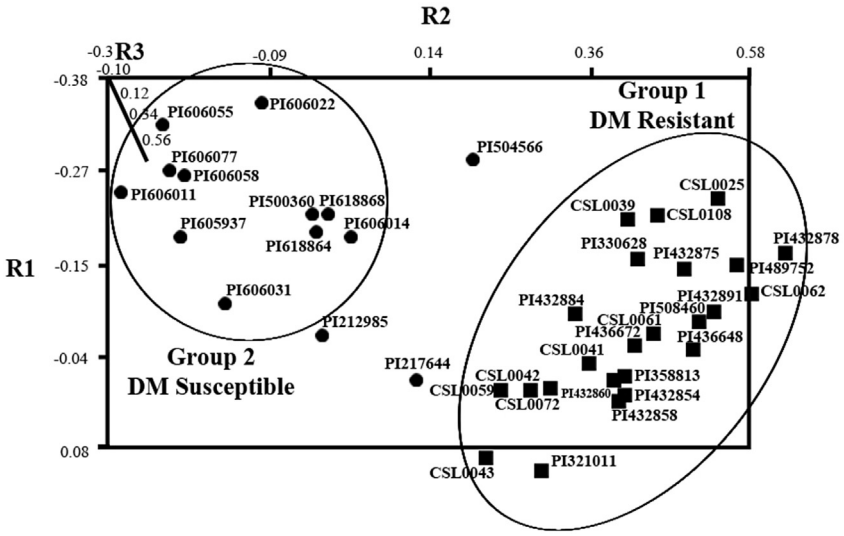

Fig. 2. Principal component analysis of cucumber accessions using random amplified polymorphic DNA fingerprinting data (2 dimensions).

Asia (mainly India). This could reflect the way that various goods including vegetable seeds were transferred between geographically feasible areas. The presence of a geographical barrier such as the high Himalayan mountain range separating South Asia from Northeast Asia could prevent cucumber seed transfer between India and China while the lower altitude terrain alongside the Mehkong river may have allowed greater seed transfer between China and Thailand, resulting in cucumber accessions in Far East Asia and Southeast Asia being closer genetically.

Unlike the dendrograms that were quite similar between RAPD and ISSR marker results, the PCA from the ISSR analysis showed three groups based on the country of origin (Innark et al., 2014) while the PCA from the RAPD analysis showed two main groups corresponding to disease reactions to DM. This difference between grouping by dendrogram and PCA could be due to the fact that all amplified DNA fragments were used for dendrogram construction while only a few fragments showing the best separation were used in PCA making the latter more sensitive to the types of DNA markers used.

In conclusion, the RAPD marker proved to be a convenient tool to assess genetic diversity in plants. The information obtained should be beneficial to germplasm curators or plant breeders worldwide. As demonstrated in this study, the RAPD marker showed good association regarding a plant's reaction to DM disease and other important agro-economic traits such as yield, fruit weight, fruit length and flesh pith length. Further study using DNA markers would allow for the precise selection of plant genotypes with high potential for future plant improvement programs.

Conflict of interest

None.

Acknowledgments

The authors are grateful to the Faculty of Science at Kasetsart University, Bangkok, Thailand (PRF1/2552). This research was supported by funding from the National Center for Genetic Engineering and Biotechnology (BIOTEC) and also by the Graduate School, Kasetsart University through a Graduate Research Fellowship for Works Published in International Academic Journals (2011).

References

Ahmed, N., Mir, J.I., Mir, R.R., et al., 2012. SSR and RAPD analysis of genetic diversity in walnut (*Juglans regia* L.) genotypes from Jammu and Kashmir, India. Physiol. Mol. Biol. Plants 18, 149–160.

Bhattacharya, S., Bandopadhyay, T.K., Ghosh, P.D., 2010. Efficiency of RAPD and ISSR markers in assessment of molecular diversity in elite germplasms of *Cymbopogon winterianus* across West Bengal, India. Emir. J. Food Agric. 22, 13–24.

Chen, S.Y., Dai, T.X., Chang, Y.T., et al., 2013. Genetic diversity among *Ocimum* species based on ISSR, RAPD and SRAP markers. Aust. J. Crop. Sci. 7, 1463–1471.

De Riek, J., Calsyn, E., Everaert, I., et al., 2001. AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. Theor. Appl. Genet. 103, 1254–1265.

Dhaliwal, M.S., 2017. Cucurbits. In: Handbook of Vegetable Crops, third ed. Kalyanis publishers. Darya Ganj, New Delhi, India, pp. 77–147.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

FAO, 2016. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy.

Food and Drug Administration, 2016. Cucumbers. The Western Institute for Food Safety and Security. University of California-Davis, Davis, CA, USA.

Guasmi, F., Elfalleh, W., Hannachi, H., Feres, K., Touil, L., Marzougui, N., Triki, T., Ferchichi, A., 2012. The use of ISSR and RAPD markers for genetic diversity among South Tunisian barley. ISRN Agronomy 2012, 1–10.

Holmes, G., Wehner, T., Thornton, A., 2006. An old enemy re-emerges: downy mildew rears its ugly head on cucumber, impacting growers up and down the Eastern U.S. Am. Veg. Grow. 14–15. February.

Innark, P., Khanobdee, C., Samipak, S., Jantasuriyarat, C., 2013. Evaluation of genetic diversity in cucumber (*Cucumis sativus* L.) germplasm using agro-economic traits and microsatellite markers. Sci. Hortic. 162, 278–284.

Innark, P., Ratanachan, T., Khanobdee, C., Samipak, S., Jantasuriyarat, C., 2014. Downy mildew resistant/susceptible cucumber germplasm (*Cucumis sativus* L.) genetic diversity assessment using ISSR markers. Crop Prot. 60, 56–61.

Irshad, M., Idrees, M., 2014. Molecular markers in plants for analysis of genetic diversity: a review. Eur. Acad. Res. 2, 1513–1540.

Jeffrey, C., 1980. A review of the Cucurbitaceae. Bot. J. Linn. Soc. 81, 233–247.

Kaweekijthummakul, W., Smitamana, P., Kanobdee, C., 2007. Rapid screening technique for downy mildew resistant cucumber. In: Proceedings of the the 8th National Plant Protection Conference. Amarin Lagoon Hotel, Pitsanulok, Thailand, p. 19e.

Manimekalai, R., Nagarajan, P., Kumaran, P.M., 2006. Comparison of Effectiveness of RAPD, ISSR and SSR Markers for Analysis of Coconut (*Cocos nucifera* L.) Germplasm Accessions. Eighteenth Annual Congress of the PGIA, pp. 16–17.

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., et al., 2018. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol. Biotechnol. Equip. 32, 261–285.

Ng, W.L., Tan, S.G., 2015. Inter-simple sequence repeat (ISSR) markers: are we doing it right? ASM Sci. J. 9, 30–39.

Nontuthuko, R.N., Pangirayi, B.T., Alpheus, M.Z., 2015. Genetic diversity in landraces revealed by RAPD and SSR markers. Sci. Hortic. 189, 192–200.

Pradeep Reddy, M., Sarla, N., Siddiq, E., 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128, 9–17.

Rocha, E.A., Paiva, L.V., Carvalho, H.H.D., Guimaraes, C.T., 2010. Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. Crop Breed. Appl. Biotechnol. 10, 204–210.
Rohlf, F.J., 2008. NTSYSpc: Numerical Taxonomy System, Ver. 2.10m. Exeter Publishing, Ltd. Setauket, NY, USA.
Savory, E.A., Granke, L.L., Quesada-Ocampo, L.M., Varbanova, M., Hausbeck, M.K., Day, B., 2011. The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Mol. Plant Pathol. 12, 217–226.
Shankar, R., Harsha, S., Bhandary, R., 2014. A Practical Guide to Identification and Control of Cucumber Diseases. Tropica seed pvt. Ltd. Basavangudi, Bangalore, India.
Sharma, A., Rana, C., Shiwani, K., 2016. Handbook of Cucurbits: growth, cultural practices and physiology. In: Pessarakli, M. (Ed.), Important Insect Pests of Cucurbits and Their Management, first ed. CRC Press, London, England.
Thimmappaiah, Santhosh, W.G., Shobha, D., Melwyn, G.S., 2009. Assessment of genetic diversity in cashew germplasm using RAPD and ISSR markers. Sci. Hortic. 120, 411–417.
Tonk, F.A., Tosun, M., Ilker, E., Istipliler, D., Tatar, O., 2014. Evaluation and comparison of ISSR and RAPD markers for assessment of genetic diversity in triticale genotypes. Bulg. J. Agric. Sci. 20, 1413–1420.
Whitaker, T.W., Davis, G.N., 1962. Cucurbits: Botany, Cultivation, and Utilization. Interscience Publishers, New York, NY, USA.
Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18, 6531–6535.

Further reading

Sebastian, P., Schaefer, H., Telford, R.H., Renner, S.S., 2010. Cucumber (Cucumis sativus) and melon (C. melo) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. Proc. Natl. Acad. Sci. U. S. A 107 (32), 14269–14273.