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Morphological and molecular characterization of watermelon genotypes using RAPD markers

Emrul Kayesh[1*]

[1]Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

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*Corresponding Author
Emrul Kayesh
e.kayesh@yahoo.com

ABSTRACT

The genotypes of watermelon showed variation in morphological as well as quantitative trait. The tallest plant (358.65 cm) was produced by CL6 genotype and the shortest plant (198.55 cm) was observed in case of CL3 genotype. The maximum number of fruit was found in CL6 (5.67) and the lowest fruit was found in CL3 (0.89). Moreover, the CL6 consistently recorded significant differences from other genotypes in all quantitative characters except in fruit weight. CL2 was also significantly different from other genotypes in all the six quantitative characters. Seven primers on six watermelon genotypes were used for their ability to produce polymorphic patterns among them only two primers (OPB06 and OPB07) gave reproducible and distinct polymorphic amplified products. This proportion of polymorphism is higher in all the selected genotypes of watermelon. The present experiment produced 1.71 scorable bands per primer and 0.85 polymorphic bands per primer. DNA markers have not been utilized well in the practice of plant identification, due to lack of analysis methods that can make the identification of plants with DNA marker easy, efficient and practical. The main advantages of this identification strategy include fewer primers used and separation of all the cultivars from each other and its helps to separate any watermelon cultivars among the 6, which can definitely be of great help to the watermelon cultivation in Bangladesh.

Keywords: Watermelon, morphology, molecular, RAPD markers

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1 Introduction

Watermelon (*Citrullus lanatus* L.) is a fruit that has a great representation in the global fruit market and the consumption is greater than that of any other cucurbit (Bisognin, 2002). It accounts for 6.8% of the world area devoted to watermelon production. In Bangladesh, during the last year watermelon was cultivated in 17,149 ha of land. Of that, 13,000 hectares of land existed in Bhola and Patuakahli district only (BBS, 2013).

Characterization of genetic resources is usually based on morphological classifications, which are easy to conduct, reliable, and have low operating costs. However, morphological descriptors are limited and influenced by environmental conditions. Another important limiting factor is the use of live plants for assessment (Zhang et al., 2011). Information on genetic diversity and relationships among and within landraces is beneficial for identification, conservation, and utilization of genetic resources for future breeding and food security. On the other hands, molecular markers can be options to characterize germplasm and protect new cultivars without environmental in-

terference. Molecular analyses using techniques of modern biotechnology, especially molecular markers, contribute significantly to these studies, which generate potentially important information for broadening the genetic base of breeding programs (Shiran et al., 2007). Another promising application of the use of molecular markers is in its use for plant variety protection and resolving trade disputes.

In recent years, various DNA-based markers have been developed and used for genetic diversity, fingerprinting and cultivar origin studies (Cheng and Huang, 2009; D¿Onofrio et al., 2009; Elidemir and Uzun, 2009; Fang et al., 2006; Melgarejo et al., 2009; Papp et al., 2010). Several molecular markers have been effectively used to assess the genetic diversity of watermelon. Isozymes (Navot and Zamir, 1987), RAPD (Levi et al., 2001a,b), AFLP (Che et al., 2003; Levi et al., 2001c; Nimmakayala et al., 2009), ISSR (Levi et al., 2001c), SSR (Jarret et al., 1997; Kwon et al., 2010; Levi et al., 2007; Sheng et al., 2012; Zhang et al., 2011; Nantoumé et al., 2013), PCR–RFLP (Dane and Liu, 2006), SRAP (Levi et al., 2007; Uluturk et al., 2011), EST–PCR (Levi et al., 2008), and HFO–TAG markers (Levi et al., 2012) have been used to estimate the genetic relationship among cultivated watermelons and different *Citrullus* species.

Among the DNA-based markers, Random Amplified Polymorphic DNA (RAPD) marker is useful for cultivar analysis with superb advantages of simplicity, efficiency, and non-requirement of any previous sequence information and also RAPD can become a prefered technique for use in plant cultivar identification. So far, RAPD marker have been widely used in the cultivar identification and genetic relationship analysis of a number of fruit species, such as apricot, pomegranate (Hasnaoui et al., 2010), cherry (Demirsoy et al., 2008), pistachio (Javanshah et al., 2007), strawberry (Wang et al., 2007), etc. Despite their popularity, the powerful DNA markers available for plant identification have not made plant variety identification an efficient, recordable, and easy exercise as anticipated. Given the above scenario, this study aimed to evaluate the variation of watermelon genotype at morphological and molecular level.

2 Materials and Methods

The study was carried out at the Horticulture Research Farm of BSMRAU, Gazipur during the period of December 2016 to May 2017 and to evaluate the six water melon genotypes (CL1, CL2, CL3, CL4, CL5, CL6) based on growth, and yield. The experiment was laid out in Randomized Complete Block Design (RCBD) in field level. The unit plot size was 4m×4m each unit plot had 5 plants each genotype. The recorded data on growth, leaf, vine, flower, fruit and seed characters were statistically analyzed with the help of MSTAT-C software and the treatment means were compared by DMRT.

2.1 DNA extraction

Total genomic DNA of each genotype was extracted from young water melon leaves using the modified cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). The extracted DNA was diluted to a final concentration of 30 ng μL-1 with1× TE buffer and stored at -20 °C for further study.

2.2 RAPD analysis

DNA samples were evaluated both quantitatively and qualitatively using spectrophotometer (SPECORD 50, Analytikjena, Germany) at 260 nm and agarose gel electrophoresis, respectively. Then, RAPD amplification reactions were performed with 2.0μL 10×buffer, 1.2μL MgCl2 (25 mM), 1.6μL dNTP (2.5 mM), 1.6μL primer (1.0μM), 0.1μL rTaq Polymerase Dynazyme (5U/μL) (10×buffer, MgCl2 , dNTP and rTaq (TaKaRa, Japan) and 1μL of genomic DNA, making a total volume of 20μL.

2.3 PCR amplification for RAPD

PCR Amplifications were carried out in a Perkin-Elmer thermal cycler 480. In each thermal cycling a negative control (water instead of template) was included to rule out amplification products due to external contamination. All amplifications were repeated twice for each sample on 1.5% agarose gel. The optimum amplification cycle was as follows:

Initial denaturation at 94 °C for 5 min
Denaturation at 94 °C for 1 min
Annealing at 36 °C for 30 sec
Extension at 72 °C for 3 min
Final extension at 72 °C for 5 min
} 45 times

After completion of cycling programme, the reactions were held at 4 °C.

2.4 RAPD data analysis

Only clear unambiguous bands in the photographic prints of gels were chosen and scored on the basis of their presence (1) or absence (0), separately for each individual varieties and each primer. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. Jaccards similarity and dissimilarity coefficients were then calculated between pairs of tracks and strains.

Table 1. Variation in qualitative characters

Genotypes	CL1	CL2	CL3	CL4	CL5	CL6
Growth habit	Runner	Runner	Runner	Runner	Runner	Runner
Leaf blade†	Medium	Medium	Medium	Medium	Medium	Shallow
Leaf shape	Pentalobate	Pentalobate	Pentalobate	Pentalobate	Pentalobate	Pentalobate
Petal colour	Deep yellow	Deep yellow	Deep yellow	Deep yellow	Deep yellow	Light yellow
Fruit shape	Round	Round	Round	Round	Elliptic	Elliptic
Main rind colour	Dark green	Light green	Light green	Gray	Green	Green
Flesh colour	Red	Yellow	Red	Pink	Red	Red
Grooves	Absent	Absent	Absent	Absent	Absent	Absent
Seed shape	Roundish	Roundish	Roundish	Roundish	Elliptical	Elliptical
Seed colour	Brown	Dark-Brown	Brown	Brown	Brown	Brown

† Depth of incisions

3 Results and Discussion

3.1 Variation in qualitative and quantitate characters

The six watermelon genotypes were evaluated for growth, leaf, vine, flower, fruit and seed characteristics. Variation in qualitative characters in the six watermelon accessions is summarized in Table 1. The morphological features of collected genotypes were very close to each other except fruit shape, main rind color, and flesh color.

Quantitative characters that were evaluated include vine length, number of branches on the main vine, fruit number, fruit weight, rind thickness and seed number. The vine length was recorded at harvesting time was significantly varied in different genotypes (Table 2). The tallest plant (358.65 cm) was produced by CL6 genotype and the shortest plant (198.55 cm) was observed in case of CL3 genotype. There was a wide variation found in number of branches on the main vine, fruit number, fruit weight, rind thickness and seed number (Table 2). The maximum no. of fruit was found in CL 6(5.67) and the lowest fruit was found in CL3 (0.89). Moreover, the CL6 consistently recorded significant differences from other genotypes in all quantitative characters except in fruit weight. CL2 was also significantly different from other genotypes in all the six quantitative characters. This genotype recorded significantly high fruit weight, thinner rind and low seed number as compared to other genotypes (Table 2).This observation had also been reported by Yaniv et al. (1999).

3.2 Primer selection and RAPD pattern

Seven primers were screened on six watermelon genotypes for their ability to produce polymorphic patterns. Of these, two primers (OPB06 and OPB07) gave reproducible and distinct polymorphic amplified products were selected (Fig. 1). DNA amplification from all the primers tested was not consistently reproducible, is a very common feature of RAPD technique. Levi et al. (2001a) provided similar results using RAPDs.

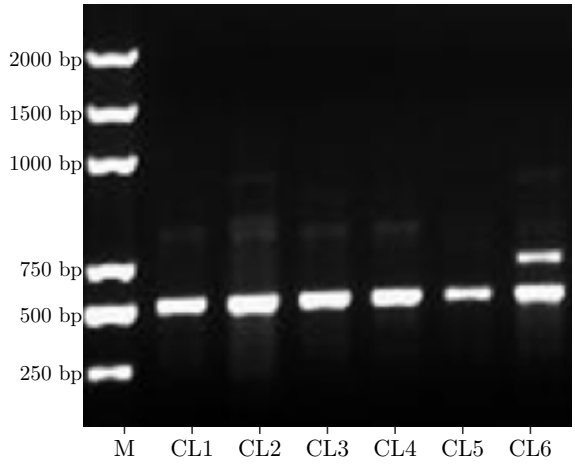

Figure 1. RAPD banding patterns with primer OPB6

A total of 12 RAPD bands were scored of which 6 (50%) polymorphic amplification products were obtained by using these arbitrary primers. The size of the amplification products ranged from 250-2000 bp (Table 3). The dissimilar numbers of bands were generated by primer OPB06 and OPB07 (Table 3).

Maximum number of band amplification was shown by the primer OPB06 (4) and the minimum number of polymorphic band by the primer OPB07 (2). A total of 6 polymorphic bands were amplified from two RAPD primers (Table 3). This proportion of polymorphism is higher in all the selected genotypes of watermelon. The present experiment produced 1.71 scorable bands per primer and 0.85 polymorphic bands per primer. These results indicate that the ability to detect polymorphism in watermelon genotypes is substantially greater with RAPD compared to isozyme (Navot and Zamir, 1987), AFLPs (Che et al., 2003) and SSR (Jarret et al., 1997; Kwon et al., 2010) analysis.

Table 2. Variation in quantitative characters

Genotypes	Main vine length (cm)	Branch no.	Fruit no.	Fruit wt. (kg)	Rind thickness (mm)	Seed no.
CL1	230.33bc	6.67cd	1.50d	1.70c	9.61b	190.91c
CL2	238.50b	9.38b	3.45b	3.01a	7.58d	126.38d
CL3	198.55d	5.13d	0.89e	1.43d	9.08bc	191.94c
CL4	221.60c	6.83c	2.38c	2.04b	8.61c	275.72b
CL5	230.18bc	6.56cd	1.50d	1.77c	9.61b	190.91c
CL6	358.65a	11.31a	5.67a	1.98bc	13.22a	372.32a
Sig. level	***	***	***	***	***	***
CV (%)	34.19	37.14	27.89	29.41	20.81	37.89

Table 3. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed

Primer code	Sequence (5′-3′)	Total no. of band scored	Size range (bp)	No. of polymorphic band	Proportion of polymorphic loci
OPB01	GTTTCGCTCCA	1	250–500	0	0%
OPB02	GTTTCGCTCCG	1	500–750	0	0%
OPB03	GTTTCGCTCCG	–	–	–	0%
OPB04	AGCGTCCTCCG	–	–	–	0%
OPB05	ACGACCGACAT	1	500–750	0	0%
OPB06	TGGTGGCGTTA	5	500–1000	4	80%
OPB07	ACCCCCGACTC	4	250–1500	2	50%
Total		12		6	50%
Average		1.71		0.85	50%

A dissimilarities matrix was used to determine the level of relatedness among the genotypes studied. The pairwise genetic dissimilarity indices indicated that the highest genetic dissimilarity was between genotypes CL02 and genotypes CL06 (45%). The lowest genetic dissimilarity among the genotype CL04 and CL05 (9%). Thus RAPD markers provide adequate power of resolution to discriminate between watermelon genotypes and it could serve as a potential tool in the identification and characterization of genetically distant genotypes from various sources. These results also confirm that the analysis of genetic dissimilarity by RAPD markers is a valid procedure (Souza sobrinho et al., 2001).

4 Conclusions

One of the main purposes of plant science is to service agriculture and the means of practical application of new biological techniques to agricultural production. However, a very little work has been done on efficient cultivar identification and genetic diversity of this economically important crop. The genotypes of watermelon showed variation in morphological as well as quantitative traits. The tallest plant (358.65 cm) was produced by CL6 genotype and the shortest plant (198.55 cm) was observed in case of CL3 genotype. The maximum no. of fruit was found in CL 6(5.67) and the lowest fruit was found in CL3 (0.89). Moreover, the CL2 and CL6 genotypes recorded significantly high branch number, high fruit number, high fruit weight, low thinner rind and seed number as compared to other genotypes.

Seven primers on six watermelon genotypes were used for their ability to produce polymorphic patterns among them only two primers (OPB06 and OPB07) gave reproducible and distinct polymorphic amplified products. The present experiment produced 1.71 scorable bands per primer and 0.85 polymorphic bands per primer. However, the results suggested that RAPD markers are useful for genetic diversity analysis and variety identification of watermelon, which will be very helpful in identification of plant cultivars, protection of cultivar-right and also beneficial for nursery industry due to early identification of seedlings.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

BBS. 2013. Statistical Pocket Book of Bangladesh. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of the People's Republic of Bangladesh.

Bisognin DA. 2002. Origin and evolution of cultivated cucurbits. Ciência Rural, Santa Maria 32:715–723. doi: 10.1590/s0103-84782002000400028.

Che K, Liang C, Wang Y, Jin D, Wang B, Xu Y, Kang G, Zhang H. 2003. Genetic assessment of watermelon germplasm using the AFLP technique. HortScience 38:81–84.

Cheng Z, Huang H. 2009. SSR fingerprinting Chinese peach cultivars and landraces (*Prunus persica*) and analysis of their genetic relationships. Scientia Horticulturae 120:188–193. doi: 10.1016/j.scienta.2008.10.008.

Dane F, Liu J. 2006. Diversity and origin of cultivated and citron type watermelon (*Citrullus lanatus*). Gen Res Crop Evol 54:1255–1265. doi: 10.1007/s10722-006-9107-3.

Demirsoy L, Demir T, Demirsoy H, Okumuş A, Kaçar Y. 2008. Identification of some sweet cherry cultivars grown in Amasya by RAPD markers. Acta Horticulturae :147–153doi: 10.17660/actahortic.2008.795.18.

D¿Onofrio C, Lorenzis GD, Giordani T, Natali L, Scalabrelli G, Cavallini A. 2009. Retrotransposon-based molecular markers in grapevine species and cultivars identification and phylogenetic analysis. Acta Horticulturae 827:45–52. doi: 10.17660/actahortic.2009.827.3.

Elidemir A, Uzun I. 2009. Assessment of genetic diversity of some important grape cultivars, rootstocks, and wild grapes in Turkey using RAPD markers. Acta Horticulturae 827:275–278. doi: 10.17660/actahortic.2009.827.47.

Fang J, Twito T, Zhang Z, Chao CT. 2006. Genetic relationships among fruiting-mei (*Prunus mume* sieb. et zucc.) cultivars evaluated with AFLP and SNP markers. Genome 49:1256–1264. doi: 10.1139/g06-097.

Hasnaoui N, Mars M, Chibani J, Trifi M. 2010. Molecular polymorphisms in Tunisian pomegranate (*Punica granatum* L.) as revealed by RAPD fingerprints. Diversity 2:107–114. doi: 10.3390/d2010107.

Jarret RL, Merrick LC, Holms T, Evans J, Aradhya MK. 1997. Simple sequence repeats in watermelon (**?** (Thunb.) Matsum. & Nakai). Genome 40:433–441. doi: 10.1139/g97-058.

Javanshah A, Tajabadipour A, Mirzaei S. 2007. Identification of a new phenotype (Siah Barg) of pistachio (*Pistacia vera* L.) with shiny-blackish green leaves using RAPD assay. Int J Agric Biol 9:307–310.

Kwon YS, Oh YH, Yi SI, Kim HY, An JM, Yang SG, Ok SH, Shin JS. 2010. Informative SSR markers for commercial variety discrimination in watermelon (*Citrullus lanatus*). Genes Genomics 32:115–122. doi: 10.1007/s13258-008-0674-x.

Levi A, Thies JA, Wechter WP, Harrison HF, Simmons AM, Reddy UK, Nimmakayala P, Fei Z. 2012. High frequency oligonucleotides: targeting active gene (HFO-TAG) markers revealed wide genetic diversity among *Citrullus* spp. accessions useful for enhancing disease or pest resistance in watermelon cultivars. Gen Res Crop Evol 60:427–440. doi: 10.1007/s10722-012-9845-3.

Levi A, Thomas C, Keinath A, Wehner T. 2001a. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Gen Res Crop Evol 48:559–566.

Levi A, Thomas C, Wehner T, Zhang X. 2001b. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. HortScience 36:1096–1101.

Levi A, Thomas CE, Wehner TC, Zhang X. 2001c. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. HortScience 36:1096–1101.

Levi A, Wechter P, Davis A. 2008. EST–PCR markers representing watermelon fruit genes are polymorphic among watermelon heirloom cultivars sharing a narrow genetic base. Plant Gen Resource 7:16–32. doi: 10.1017/s1479262108014366.

Levi A, Wechter W, Davis A, Katzir N, Tadmor Y, Ling K, Reddy U. 2007. Interspecific transferability of watermelon EST-SSR markers in cucurbit species. HortScience 42:1012.

Melgarejo P, Martínez J, Hernández F, Martínez R, Legua P, Oncina R, Martínez-Murcia A. 2009. Cultivar identification using 18s–28s rDNA intergenic spacer-RFLP in pomegranate (*Punica granatum* L.). Scientia Horticulturae 120:500–503. doi: 10.1016/j.scienta.2008.12.013.

Nantoumé AD, Andersen SB, Jensen BD. 2013. Genetic differentiation of watermelon landrace types in Mali revealed by microsatellite (SSR)

markers. Gen Res Crop Evol 60:2129–2141. doi: 10.1007/s10722-013-9980-5.

Navot N, Zamir D. 1987. Isozyme and seed protein phylogeny of the genus *Citrullus* (cucurbitaceae). Plant Syst Evol 156:61–67. doi: 10.1007/bf00937202.

Nimmakayala P, Tomason YR, Jeong J, Ponniah SK, Karunathilake A, Levi A, Perumal R, Reddy UK. 2009. Genetic reticulation and interrelationships among citrullus species as revealed by joint analysis of shared AFLPs and species-specific SSR alleles. Plant Gen Resources 8:16–25. doi: 10.1017/s1479262109990098.

Papp N, Szilvássy B, Abrankó L, Szabó T, Pfeiffer P, Szabó Z, Nyéki J, Ercisli S, Stefanovits-Bányai É, Hegedűs A. 2010. Main quality attributes and antioxidants in hungarian sour cherries: identification of genotypes with enhanced functional properties. Int J Food Sci Technol 45:395–402. doi: 10.1111/j.1365-2621.2009.02168.x.

Sheng Y, Luan F, Zhang F, Davis A. 2012. Genetic diversity within Chinese watermelon ecotypes compared with germplasm from other countries. J American Soc Hort Sci 137:144–151.

Shiran B, Amirbakhtiar N, Kiani S, Mohammadi S, Sayed-Tabatabaei B, Moradi H. 2007. Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. Scientia Horticulturae 111:280–292. doi: 10.1016/j.scienta.2006.10.024.

Souza sobrinho F, Ramalho M, Souza J. 2001. Genetic diversity and inbreeding potential of maize commercial hybrids. Maydica 46:171–175.

Uluturk Z, Frary A, Doganlar S. 2011. Determination of genetic diversity in watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] germplasm. Aust J Crop Sci 5:1832–1836.

Wang Z, Zhang Z, Li H, Gao X, Du G, Tan C. 2007. Identification of strawberry cultivars by RAPD and SCAR markers. Acta Horticuturae Sinica 34:591–596.

Yaniv Z, Shabelsky E, Schafferman D. 1999. Colocynth: Potential Arid Land Oilseed from an Ancient Cucurbit. In: J. Janick (ed.); Perspectives on new croand new uses. ASHS Press, Alexandria, VA.

Zhang H, Wang H, Guo S, Ren Y, Gong G, Weng Y, Xu Y. 2011. Identification and validation of a core set of microsatellite markers for genetic diversity analysis in watermelon, *Citrullus lanatus* Thunb. Matsum. & Nakai. Euphytica 186:329–342. doi: 10.1007/s10681-011-0574-z.

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