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INTRASPECIFIC CLASSIFICATION OF CANTALOUPE (*Cucumis melo* L. var.*recticularis Naudin.*) AND THAI MELON (*Cucimis melo* L. var.*conomon*) IN MOLECULAR VARIATION

Teeraporn Tongdeenok*[1], ArakTira-umphon[2] and Mariena Ketudat-Cairns[3]

[1,2]*School of Crop Production Technology, Institute Agricultural Technology,*
[3] *School of Biotechnology,Institute Agricultural Technology,*
Suranaree University of Technology, Nakhon Ratchasima, Thailand

Abstract

Genetic variation of 14 melon varieties (2 Thai melon and 12 cantaloupes) was studied molecular maker techniques. Three primers; from 13 primer sets showed polymorphism of the melon varieties. The results from these primers form only two cluster groups (Thai melon group and Cantaloupe group)with the R^2= 0.87. These results will further help support the efficiency of parent lines selection in cantaloupe and Thai melon breeding program.

Keywords: Cantaloupe, Melon, RAPD, ISSR

INTRODUCTION

Cantaloupe (*Cucumis melo* L.) belong to the genus Cucumis in the family Cucurbitaceae. Within the genus Cucumis, it belongs to the subgenusmelo, having 2n=24 chromosomes. It's origin was thought to be in Africa, but recent data suggest that melon and cucumber may be of Asian origin (Sebastian P. et al, 2010). Melons are classified into 16 groups, 5 of which (conomon, makuwa, chinensis, momordica, and acidilus) can be assigned to the subspagrestis and 11 (cantalupensis, reticulatus, adana, chandalak, ameri, inodorus, flexuosus, chate, tibish, dudaim, and chito) to the subsp.melo. (Pitrat M. et al, 2000) Cantaloupe is an important horticultural crop across wide areas of the world, with 26 million tons produced worldwide in 2009 (FAO, 2011). Cucumis is an attractive model for studying valuable biological characters, such as fruit ripening (Pech J.C. et al, 2008) Great morphological variation exists in fruit characteristics such as size, shape, color and texture, taste and composition, *C. melo* is considered the most diverse species of the genus Cucumis. Sensitive DNA fingerprinting techniques have been used to resolve taxonomicrelationships, providing an objective and quantitative measure for genetic diversity between taxa, e.g. among genera and species (Schierwater B., 1995 ; Campos L. et al, 1994 and Millan T. et al, 1996). The sensitivity of the new methodologies also allows genotyping of varieties or cultivars within the species (Fang D.Q. et al, 1997 and Lee S.J. et al, 1996). Molecular markers have been shown to be useful to assess genetic diversity in a number of plant species (Bretting P.K. and Widerlechner M.P., 1995 and StaubJ.E. et al, 2000). Molecular characterization of cantaloupe has been performed using techniques including cleaved amplified polymorphic sequences. (Zheng J. et al, 1999) Random amplified polymorphic DNA (RAPD) profiles are obtained using decamer-primers of arbitrary sequence (William J.G.K. et al, 1993) Inter-simple-sequence-repeat (ISSR) PCR involves longer (16-18 nucleotides) primers encoding microsatellite elements that amplify DNA segments between microsatellite repeats (Gupta M. et al, 1994 and Zietkiewicz E. et al, 1994)

The aim of this study was to determine the genetic diversity of melon varieties of cantaloupe and Thai Melon genotypes collected from different provinces of Nakhonratchasima in Thailand, using reference genotypes. ISSR and RAPD analysis were done and molecular data were subjected to cluster analysis.

Corresponding Author;* teeraporn9706@gmail.com

MATERIAL AND METHODS

This study was carried out in the School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Plant materials

A total of 14 cultivars of *Cucumis melo* L. were used. Twocultivars of Thai melon and 12 cultivars cantaloupe.(Thai melon01, Thai melon03, Madhurima, Nun 2002, Green Jam 1361, ML201, ML196, ML052, Sun Lady 227, Golden Sun TA088, Sweetie 1823, Honey Sweet 1846, Pot Orange T1957 and Sophy 1899) were showed. Seedlings of each cultivar were grown in a greenhouse andgenomic DNA was extracted from freeze-dried fresh leaves of 15 day old seedlings. DNA was extracted using the method described by Gusmini G. et al, 2004.

RAPD and ISSR analyses

DNA fingerprinting were performed using eight ISSR primer and five RAPD primer (Table 1). PCR reaction mixture contained: 0.4 mM DNA template, 0.2 mMdNTP, 2 mM $MgCl_2$, 10µM Primer, 5X PCR buffer (Applichem: Germany), 1.0 mL Taq DNA (Vivantis: Malaysia) in a total volume of 50 mL. The amplification program was as follows: Step 1: 5 min at 94°C. Step 2: 30 sec at 94°C, Step 3: 30 sec at 37-47.9°C, Step 4: 45 sec 72°C,Step 2-4 for 40 cycles and a final extension of 2 min at 72°C. DNA fingerprinting by the reaction products were subjectedto electrophoresis on 3% agarose gels in 1xTAE buffer, stained with ethidium bromide and visualised under UV light.

Band scoring and cluster analysis

ISSR and RAPD DNA polymorphic fragments were scored as present (1) or absent (0) and the binary matrix obtained was used to calculate the Dice similarity coefficient among the genotypes utilized. The UPGMA cluster analysis, analyses were made using the NTSYS-pc vs. 2.20 software. The correlation coefficient was also calculated.

Table 1 Primer and Annealing Temperature used.

Primer name	Sequence (3'---->5')	Annealing Temp. (°C)	Reference
ISSR_$(AC)_8$YC	ACACACACACACACYC	47.9	Stepanskyet al, 1999
ISSR_$(GA)_8$YG	GAGAGAGAGAGAGAGAYG	47.9	
ISSR_$(ATG)_6$	ATGATGATGATGATG	46.9	
ISSR_$(TG)_8$G	TGTGTGTGTGTGTGTGG	48.0	
ISSR_$(AC)_8$T	ACACACACACACACACT	57.2	
ISSR_$(CA)_8$CG	CACACACACACACACACG	54.8	
ISSR_$(CA)_8$GT	CACACACACACACACAGT	57.2	
ISSR_$(GA)_8$TC	GAGAGAGAGAGAGAGATC	57.2	
RAPD_C43	GGCGGCACAGGA	37.0	Matsui et al, 2002
RAPD_C48	GGAGGATGGCCC	37.0	
RAPD_A20	TTGCCGGGACCA	37.0	Tanaka et al, 2007
RAPD_A41	TGGTACGGTATA	37.0	
RAPD_OPL07	AGGCGGGAAC	37.0	UBC primer set

RESULTS

Genetic distance-based phylogeny: Out of 8 ISSR and 5 RAPD primers,amplification was successful with 2 ISSR and 1 RAPD primers.The results from were use ISSR_$(GA)_8$YG, ISSR_$(ATG)_6$ and RAPD_OPL07 used in clustering. In Table 2. The polymorphism rate was 93% which was higherthan report in other literature[86%, Danin P.et al 2001 and 66.7%, Lopez S. et al, 2002]. The obtained data were analyzed by the president dendrogram. Genetic similarity values were calculated from the Dice similarity index for all 14cultivars. These values were used to compute the NTSYSpc program. Our results show that the ISSR and RAPD method is highly informative in melon, although the combination between molecular data and agronomic traits could help in detecting the differences among these genotypes that belong to the same variety type. The

analyzed genotypes are well visible on agarose gel. In the dendrogram 14cultivars were grouped into two main clusters, The analysis classified the melon at R^2= 0.87. Interprertationof the correlation coefficient was as follows: r ≥ 0.9, very good; 0.8 ≤ r < 0.9, good; 0.7 ≤ r <0.8, poor; r < 0.7, very poor. The first cluster (cluster I) contained 2 cultivars (Thai melon01 and Thai melon03). The second cluster (cluster II) contained 12 cultivars (Madhurima, Nun 2002, Green Jam 1361, ML201, ML196, ML052, Sun Lady 227, Golden Sun TA088, Sweetie 1823, Honey Sweet 1846, Pot Orange T1957 and Sophy 1899). (Fig. 1) confirming the usefulness of these markers in better understanding the genetic relationships among Thailand's melon populations.

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Table 2 Marker bands used to assess the genetic diversity of Cantaloupes and Thai melon.

Primer name	Sequence (5'---->3')	Total bands	Polymorphic	Percentage
ISSR_$(GA)_8$YG	GAGAGAGAGAGAGAGAYG	18	16	89
ISSR_$(ATG)_6$	ATGATGATGATGATGATG	10	10	100
RAPD_OPL07	AGGCGGGAAC	20	18	90
Total		**48**	**44**	**93**

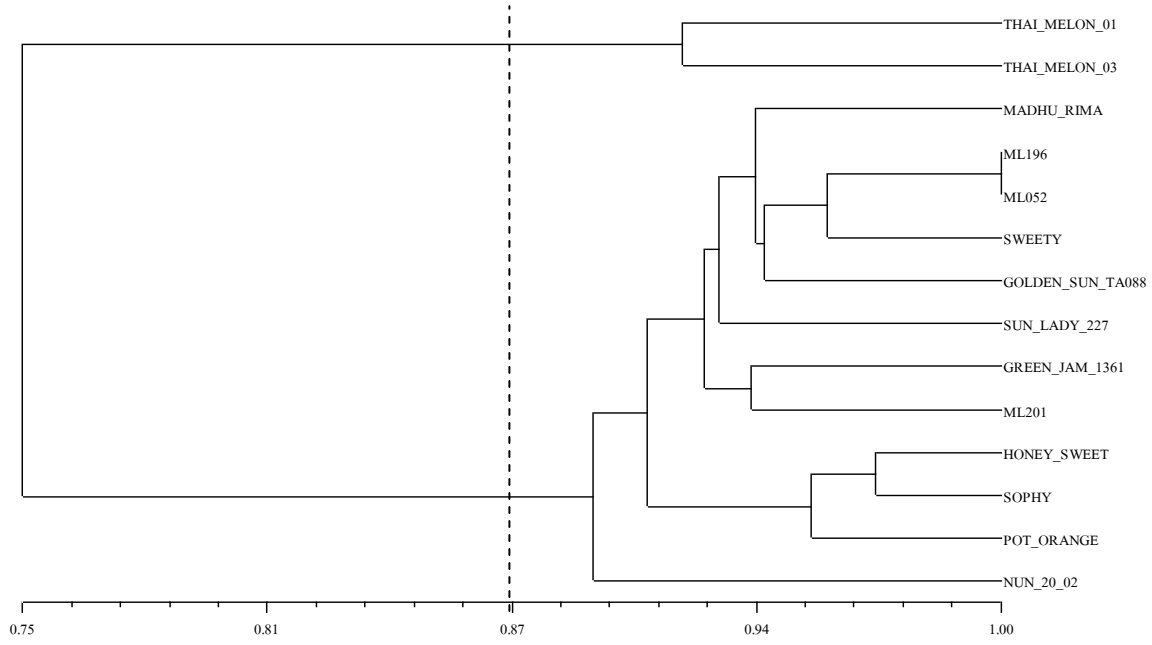

Figure 1 In the dendrogram 14cultivars were grouped into two main clusters from ISSR_$(ATG)_6$, ISSR_$(GA)_8$YG and RAPD_OPL07 by the NTSYSpc program

CONCLUSION

This study confirmed the efficacy of the approach used for distinguishing closely related melon landraces Itcan contribute to preserving genetic variability in germplasm conservation and breeding programs. An in depth study on the agronomic traits will be of great importance to clearly define the genetic similarities.

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