

Full Length Research Paper

RAPD analysis of genetic diversity of Chinese F1 cultivated Melon (*Cucumis melo L*)

J.N. Wolukau, R.M. Gesimba, J.K.Nyamari, J.F. Chen and X.H. Zhou

Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536, Njoro, Kenya.

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The genetic relationships among 40 melon (*C. melo L.*) genotypes, mostly F1 hybrids of varietal group melo were assessed by analyzing 96 random amplified polymorphic DNA (RAPD) bands generated by 37 primers. A relatively high level of polymorphism (24.7%) was revealed. The mean genetic distance among the genotypes was 0.361. There was a higher level of genetic variation among the landraces (GD 0.401) than among the hybrids (GD 0.361). The lowest level of genetic variation was recorded among the PIs maintained at USDA (GD 0.262). The relatively high level of genetic variation revealed from this small sample warrants further investigation to provide information leading to more effective strategies and goals for hybridization and long term germplasm management.

Key words: Genetic relationship; genetic distance; germplasm management; RAPD, DNA.

INTRODUCTION

Genetic diversity of *Cucumis melo* has been described using molecular markers like RAPDs, RFLPs and microsatellite markers, (Aierken *et al.*, 2011; Fergany *et al.*, 2011). Melons in China are classified either as thin skin (pericarp), thick skin or hemi (Chen, personal communication). It is not known whether this characteristic is reflected at the molecular level but generally high morphological variability in the fruits has not been reflected at the molecular level, (Kerje *et al.*, 2000). Although China is considered a secondary center of diversity for melon (Chen, personal communication), few reports, Zhang *et al.*, (2005), are available regarding the genetic relationships among the vast number of commercial and landrace genotypes found in China. A world collection has been analyzed, Danesh *et al.*, (2015) with a few local Chinese genotypes while Liu *et al.*, (2001) besides analyzing a world collection included wild *Cucumis* species relatives. Akashi *et al.*, (2002), reported variation in 5 isozymes among South and East Asian melons but his focus was mainly on India. Zhang *et al.*, (2005), reported genetic assessment of *Cucumis* species using RAPD and SSR. Elsewhere, isozyme variability was reported to be very low in melon species Staub *et al.*, (1996). Other molecular markers have

shown sufficient polymorphism e.g. RFLPs, Garcia-Mas *et al.*, (2000), AFLPs, SSRs, Watcharawong paiboon & Chunwongse, (2008) in melon. The objective of the present study was to analyze the genetic relationships among a selected group of Chinese melons, mainly F1 hybrids, these were compared with plant introductions (PIs) of Chinese origin maintained at NPGRI, USDA, USA and some open pollinated landraces using RAPDs. Characterization of the level of diversity between accessions from a breeding program or commercial cultivars and a gene bank may help identify new sources of genetic diversity useful in the breeding of improved melon cultivars. The level of diversity among the hybrids and the way forward are discussed.

MATERIALS AND METHODS

Plant materials: Forty melon (*C. melo L*) genotypes (Table 1) of Chinese origin were selected. F1 hybrids purchased or donated by Prof., Ming-Zhu Wu (China) and landraces sourced from farms and fresh produce markets. PIs 532829, 157076, 420150, 157070, 167082, 194052, 323498 were provided by the North Central Regional

Center for Genetic resources Preservation, Iowa, USA. Between 10 and 12 plants of each genotype were grown under similar plastic tunnel conditions.

DNA isolation: Young leaf tissue from 6 to 10 plants was harvested, bulked and lyophilized in liquid nitrogen and stored at minus -70°C until use. Genomic DNA was extracted by a modified cetyltrimethyl ammonium bromide (CTAB) method based on Murray and Thompson (1980).

RAPD amplification: The primers (Table 2) used were purchased from Takara Biotechnology (Dalian), Co. Ltd, China. PCR products were separated by electrophoresis according to Horejsi and Staub[7]. Viewing and photographing was done using the Gel-Imaging System JS-380 (Shanghai Peiqing Scientific & Technology Co., Shanghai, China). HindIII+EcoR I digested lambda-phage DNA was used as standard marker for estimating the size of PCR products by migration distance comparison.

RAPD analysis: Five hundred and eighty primers were screened using 6 lines. Thirty seven of the primers showing polymorphism and with consistent banding were tested on the rest of the genotypes. The tests were replicated 2 times. Bright and consistent bands were scored for subsequent analysis. Polymorphic bands were scored for either presence (1) or absence (0) of fragment for each genotype giving a binary data matrix. The data were subsequently used to generate the Jaccard similarity coefficients [8] and the matrix showing genetic distances between each pair of genotypes. The binary data matrix generated was used to construct a dendrogram by un-weighted pair-group method using arithmetic average (UPGMA) using the computer program PhylipVer 3.66. A sample of PCR-agarose gel electrophoresis of RAPD products is shown in Figure 1.

RESULTS

To achieve a high level of polymorphism and bright repeatable bands, a total of 580 primers were screened using 6 representative lines. Forty nine (49) percent of the screened primers could generate scorable bands. Thirty-seven of these primers were used to analyze the 40 genotypes. The primers generated a total of 389 bands (an average of 10.5 bands per primer, range 3 to 17 bands), 96 (24.7%, 2.6 polymorphic bands per primer) of which were polymorphic among the genotypes analyzed. The fragments generated ranged in size from 200 to 3200bp. Binary data scored for the presence or absence of fragments were used to generate genetic distances shown in Table 3. Genetic distances ranged from 0.110 for the most related F1 hybrids ('Jingyu #1' and 'Jingyu #3') to 0.619 for the most distant hybrids ('Gold phoenix' and 'Jin guan'). The mean genetic distance among the 40 genotypes was 0.361. Mean genetic distance among the thin skin melons was 0.333

(range, 0.233 to 0.440), that among the thick skin was 0.375 (range 0.110 to 0.600). The F1 hybrids had a mean genetic distance of 0.361 (range, 0.110 to 0.619). The USDA accessions had a mean genetic distance of 0.262 (range, 0.150 to 0.405), while the landraces had a mean genetic distance of 0.401 (range, 0.360 to 0.428). The most genetically distant (compared to all the others) genotype was 'Gold phoenix' with a mean genetic distance of 0.580 (range, 0.533 to 0.619). Overall, the landraces were more divergent from each other followed by the thick skin while the least divergent were the USDA PIs. To represent the genetic distances among the melon genotypes graphically, UPGMA clustering analysis was performed. The resulting dendrogram (Figure 2) depicts 3 main groups. One group consisted of F1 hybrids from Xinjiang province. All genotypes from Beijing and Gansu were grouped in another group together with PI 194052. Included in this group also were thick skin hybrids from Xinjiang province. A third group which could be divided into 2 subgroups, one made up of PIs from USDA, and the other subgroup of genotypes from Anhui, Chanxi, Harbin and landraces from Nanjing.

DISCUSSION

A large number of primers were screened using a small number of genotypes. This step cuts down on cost considerably and also facilitates the selection of primers with bright and repeatable bands. In this way, it was possible to select primers that were informative. This initial step has been shown to yield good results in melon, (Mliki *et al.*, 2001) and other species, (Schnell *et al.*, 1995). The primers that were screened have been used in several other separate studies and have also been used for identifying markers linked to GSB resistance. The use of primers employed in previous studies, (Mliki *et al.*, 2001) did not yield satisfactory results in this study which is in line with work reported by Danesh *et al.*, 2015. Results with studies on level of polymorphism in *C. melo* genome have been inconsistent. On one hand, low level or no polymorphisms have been reported, (Shattuck-Eidens *et al.*, 1990) while on the other hand, sufficient polymorphisms existed as recorded by, Zhang *et al.*, (2005). A study using AFLP markers showed a percent average of polymorphic fragments for each primer pair to be 92.66% (Danesh *et al.*, 2015). In another study where SSR markers were used to evaluate the genetic diversity among Turkish melon genotypes, results showed a polymorphism rate of 97.5% among 96 genotypes and the number of alleles detected by a single primer set ranged from 2 to 12, with an average of 6.15, (Kaçar *et al.*, 2012). Despite these inconsistencies, and especially the low levels of polymorphisms reported, RAPD markers have been widely used in genetic studies of melon, (López-Sesé *et al.*, 2002). The level of polymorphism (24.7%, 2.6 bands per primer) detected by RAPDs among

Table 1. List of genotypes used in the analysis of genetic diversity among Chinese F₁ hybrids, landraces and plant introductions.

Cultivar name	Classification	Source location	Cultivar name location	Classification	Source
Danjinli	F ₁ , thick skin	Xinjiang, China	PI 420150	conomon	USDA
Jinxuelian	F ₁ , thick skin	Xinjiang, China	PI 157070	melo	USDA
Goldphoenix	F ₁ , thick skin	Xinjiang, China	PI 157082	melo	USDA
Jinguan	F ₁ , thin skin	Xinjiang, China	PI 194052	melo	USDA
Jiashi China	Landrace, thick skin	Xinjiang, China	PI 328498	melo	USDA
Lubaoshi	F ₁ , thick skin	Xinjiang, China	Mihong China	F ₁ , thick skin	Shanghai, China
Xiaojinli	F ₁ , thin skin	Xinjiang, China	Xinzhuangyuan	F ₁ , thick skin	Xinjiang, China
Xinmiza9	F ₁ , thick skin	Xinjiang, China	Yingyue	F ₁ , NA	Xinjiang, China
Snowlotus Xinjiang, China	F ₁ , thin skin		Jinguli	F ₁ , NA	Xinjiang, China
86-1	F ₁ , thick skin	Xinjiang, China	Jingyu #1	F ₁ , thick skin	Beijing, China
Huangzuixian	F ₁ , thick skin	Xinjiang, China	Jingyu #3	F ₁ , thick skin	Beijing, China
Xueli	F ₁ , thin skin	Xinjiang, China	Xiyu #1	F ₁ , thick skin	Xinjiang, China
C929-A7	Inbred line, PM ^R	USDA	Qitianyihao	Landrace, NA	Harbin, China
Newfuyu melon	F ₁ , thin skin	Nanjing, China	Taiwanxingygu	Landrace, NA	Chanxi, China
NJG1 China	Landrace, thin skin	Nanjing, China	Xiangtianyihao	Landrace, NA	Anhui, China
Huanghemi	F ₁ , NA	Gansu	Qiutianlv	F ₁ , NA	Nanjing, China
Bailangua	F ₁ , NA	Gansu	Jinbaiyu	F ₁ , NA	Nanjing, China
Yindi	F ₁ , NA	Gansu	Gaojijixue	F ₁ , NA	
PI 532829	agrestis	USDA	Hunan, China		
PI 157076	melo	SDA	Jihong	F ₁ , NA	Liaoning, China
			NJG6	Landrace, NA	Nanjing, China

^RIndicates powdery mildew resistant; NA indicates information not available.

Table 2. RAPD primers used in the analysis of genetic relationships among Chinese F₁ melon (*Cucumis melo* L.) hybrids.

Prime code	Sequence	Prime code	Sequence	Prime code	Sequence	Prime code	Sequence
A-18	AGGTGACCGT	AA-01	AGACGGCTCC	AG-02	CTGAGGTCCT	AG-03	TGCGGGAGTG
AA-03	TTAGCGCCCC	AA-08	TCCGCAGTAG	AG-04	GGAGCGTACT	AH-05	TTGCAGGCAG
AA-09	AGATGGGCAG	AA-10	TGGTCGGGTG	AH-09	AGAACCGAGG	AI-07	ACGAGCATGG
AA-11	ACCCGACCTG	AA-14	AACGGGCCAA	AI-08	AGCCCCCA	AI-09	TCGCTGGTGT
AB-09	GGGCGACTAC	AD-04	GTAGGCCTCA	AJ15	GACACAGCCC	AS15	CTGCAATGGG
AD-11	CAATCGGGTC	AD-13	GGTTCCTCTG	AQ-15	TGCGATGCGA	AX-01	GTGTGCCGTT
AF-04	TGCGGCTGA	AF-07	GGAAAGCGTC	AX-06	AGGCATCGTG	AX07	ACGCGACAGA
AF-11	CTGGGCCTC	AF-13	TGTGGACTGG	AX-09	GGAAGTCCTG	B-12	TGTTGGGCAC
AF-14	GTGCGCACT	AG-01	CTACGGCTTC	C-06	GAACGGACTC	C-13	AAGCCTCGTC
				F-04	GGTGATCAGG		

the 40 genotypes evaluated in the present study is comparable to those of López-Sesé *et al.*, 2002 (25.6%) and Barroso *et al.*, 2004 (28.3%) but slightly higher than that reported by Baudracco-Arnas and Pitrat 1996 (18.3%). The result in this study is lower than those of Zhang *et al.*, 2005 who reported 38%, 49%, 58.6% and 75.7% polymorphism respectively. Garcia *et al.*, (2006) used a much more vigorous 2-step initial primer selection process. Moreover, they examined relationships among

melons from very divergent and from distinct horticultural groups; this could explain the high level of polymorphism. It is also easy to explain the result of Liu *et al.*, (2001) who besides examining genotypes from varied geographical regions of the world, they included in their study wild relatives (*Cucumis* species) of *melon* but the uncharacteristically high level of polymorphism reported by Zhang *et al.*, (2001) who used *C. melo* is not easy to explain. On the whole, our variation is reasonably high

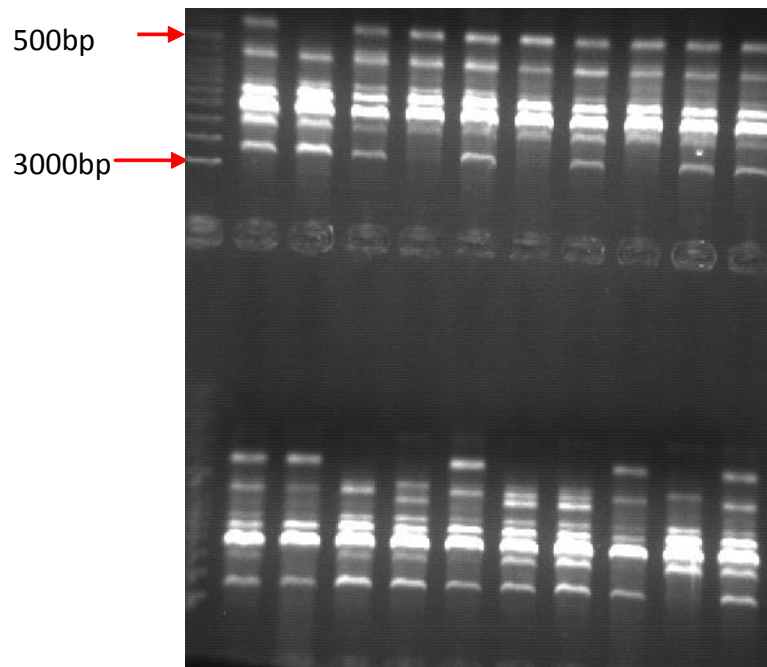


Figure 1. RAPD-PCR band profile using primer C-13 from 20 of the Chinese F1 hybrids used in the analysis of genetic relationships.

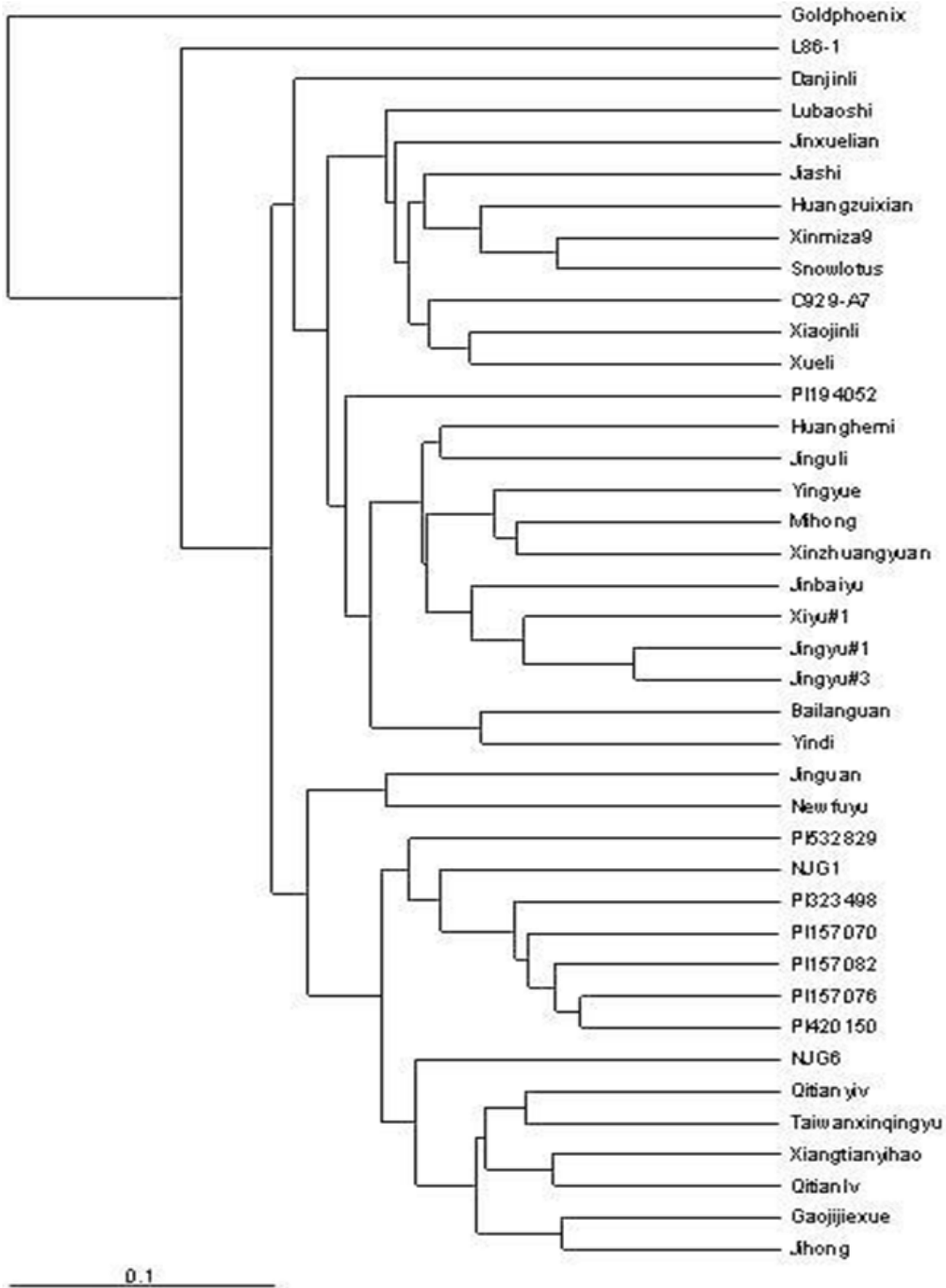
Table 3. Pair wise genetic distance values between Chinese F1 hybrids used in this study, calculations were based on RAPD markers.

Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.00																			
2	0.05	0.00																		
3	0.08	0.06	0.00																	
4	0.07	0.05	0.07	0.00																
5	0.06	0.04	0.06	0.05	0.00															
6	0.09	0.07	0.08	0.06	0.07	0.00														
7	0.08	0.06	0.07	0.05	0.06	0.08	0.00													
8	0.07	0.05	0.06	0.04	0.05	0.07	0.06	0.00												
9	0.06	0.04	0.05	0.03	0.04	0.06	0.05	0.04	0.00											
10	0.08	0.06	0.07	0.05	0.06	0.08	0.07	0.06	0.05	0.00										
11	0.07	0.05	0.06	0.04	0.05	0.07	0.06	0.05	0.04	0.06	0.00									
12	0.09	0.07	0.08	0.06	0.07	0.09	0.08	0.07	0.06	0.08	0.07	0.00								
13	0.08	0.06	0.07	0.05	0.06	0.08	0.07	0.06	0.05	0.07	0.06	0.05	0.00							
14	0.07	0.05	0.06	0.04	0.05	0.07	0.06	0.05	0.04	0.06	0.05	0.04	0.03	0.00						
15	0.09	0.07	0.08	0.06	0.07	0.09	0.08	0.07	0.06	0.08	0.07	0.06	0.05	0.04	0.00					
16	0.08	0.06	0.07	0.05	0.06	0.08	0.07	0.06	0.05	0.07	0.06	0.05	0.04	0.03	0.04	0.00				
17	0.07	0.05	0.06	0.04	0.05	0.07	0.06	0.05	0.04	0.06	0.05	0.04	0.03	0.02	0.03	0.02	0.00			
18	0.09	0.07	0.08	0.06	0.07	0.09	0.08	0.07	0.06	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.00		
19	0.08	0.06	0.07	0.05	0.06	0.08	0.07	0.06	0.05	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.01	0.01	0.00	
20	0.07	0.05	0.06	0.04	0.05	0.07	0.06	0.05	0.04	0.06	0.05	0.04	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.00

for melons that don't belong to distinct horticultural groups, (Kaçar *et al.*, 2012); most of these were *C. melo* var. *melo*. The dendrogram generated from UPGMA clustering analysis is shown in figure 2. However, results obtained by Danesh *et al.*, (2015) showed low gene

diversity values among some Iranian accessions, e.g. 0.07 and 0.09 for Sooski-e-Sabz and Khatouni respectively which may have been due to lack of intercrossing between them or high levels of inbreeding. In our study, a clear relationship between fruit skin thickness

Fig 2. Association among Chinese melon hybrids revealed by UPGMA cluster analysis on the basis of RAPD genetic distances.



of genotype and their distribution by clusters was not established, however, certain associations with region of origin were evident. Moreover, PI 532829 (an agrestis), PI 520150 (a conomon) tightly clustered together with the PIs 323498, 157070, 157082, and 157078 all of *C. melo* var. *melo* varietal group. Lack of tight clustering based on fruit characteristic has been reported. Schenell *et al.*, (1995) using RAPDs and ISSR observed that there was

no tight clustering among African accessions, chito and dudaim accessions i.e. varietal divisions were not well defined. Garcia *et al.*, (2006) reported a close relationship between varieties Makuwa and Conomon which clustered together, they shared a common genetic background or there was genetic interchange. Lopez-Sese *et al.*, (2002) observed that cluster groupings were not associated with melon market classes (based on fruit characteristics), but

were based on geographic origin. Similarly, Fernandez-Fernandez-Silva *et al.*, (2010) observed that the high morphological variability in melons is not generally reflected at the molecular level. Fernandez-Silva *et al.*, (2010) also noted that phenotypic similarities in size and shape of fruit and other agronomic features in Ghalia and Charentais varietal groups of melon did not have a phylogenetic basis. Data in the present work support these arguments. Moreover, the genotypes we examined were mainly F1 hybrids whose pedigrees were highly variable. As an example, 'Xinmiza9' and '86-1' are both hemi and thick skin melons, they share a female parent but have different divergent male parents and as such they could not cluster together. 'Gold phoenix' has an even more complex pedigree; the initial cross was between a hemi melon from Taiwan and a cantaloupe of USA origin. The resulting progeny was advanced to the F8 generation which was then backcrossed (1 backcross) to a landrace; this F1 hybrid clustered solely. A genetic interchange from the different groups e.g. thick and thin skin or movement of materials between regions could also be a source of the poor resolution observed. Generally, however, the classification of East and South Asian melons is not consistent e.g. Akashi *et al.*, (2002). Our result in the present study is consistent with these observations. Except in a few cases where genotypes were closely related (e.g. 'Jingyu #1' and 'Jingyu #3', GD 0.110), genotypes within and from different regions of China were fairly variable (Table 3, Figure 2) indicating that the genotypes used in this study have a relatively broad genetic background. The high degree of relatedness between some F1 hybrids e.g. between 'Jingyu #1' and 'Jingyu #3'; 'Xiangtianyihao' and 'Qiantianlv' is likely due to selection for superior consumer and agronomic characteristics e.g. flavor, good growth, disease tolerance, temperature adaptability etc, in which case they might share a common gene pool. A more extensive study of genetic diversity of Chinese melon would show whether the level of polymorphism observed in this study is present in larger collections making up specific or larger melon groups. In this way, hybridization programs would be managed more effectively since there is enormous evidence for a positive correlation between the genetic diversity of the parents and hybrid vigor Mliki *et al.*, (2001). Despite the broad genetic base revealed in this study, most melon F1s were highly susceptible to gummy stem blight caused by the fungus *Didymellabryoniae*. They could benefit from the introgression of *Didymellabryoniae* resistance gene(s).

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