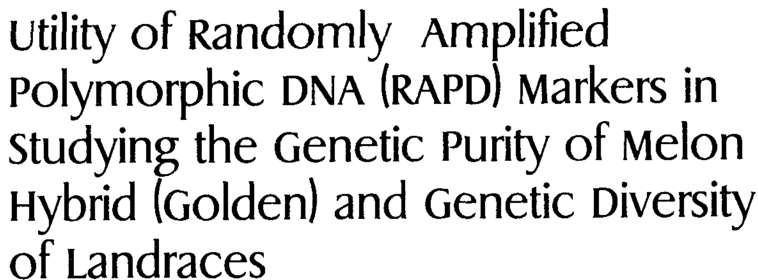

Utility of Randomly Amplified Polymorphic DNA (RAPD) Markers in Studying the Genetic Purity of Melon Hybrid (Golden) and Genetic Diversity of Landraces

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Abstract

The genetic diversity of three melon landraces of Jordan origin JO232 , JO71 and JO50 and one hybrid cultivar (Golden F1 hybrid) were evaluated at the DNA level using RAPD technique. Melon cultivar Golden F1 hybrid grown in summer 2002 showed differences in banding patterns that justified the morphological and physiological differences among plants observed by farmers.. The genetic diversity among landraces was studied using five RAPDs primers and the results showed differences in DNA amplified fragment with molecular size of 1500bp, 1000bp, 950bp and 460bp with OPA13 primer. This technique has allowed to show differences in seed lots of the hybrid provided by the farmers and the seed company. RAPD technique appears to be a useful tool in cultivar identification and in evaluating the purity of hybrids.

Key words : DNA, hyprid, landrace, melon, PCR, RAPD, genetic diversity.

ملخص

تم تقييم التنوع الوراثي لثلاثة سلا لا ت محلية من الشمام المستخدمة في الاردن 71, Jo 332 Jo و 50 Jo وصنف هجين واحد (هجين ف1 جو لدن) على مستوى المادة الوراثية باستخدام تقنية وسمات ال- DNA المضخمة عشوائيا متعددة الأشكال. صنف الشمام

جو لدنF1 الهجين زرع في صيف 2002وا ظهر اختلا فات وراثية في أشكال الحزم الوراثية مما يبرر وجود اختلافات وراثية وفسيولوجية بين النباتات التي لوحظت من طرف المزارعين. التنوع الوراثي بين السلالات المحلية درست با ستخدام خمسة بادئات لتقنية ال- RAPD و النتائج أوضحت اختلافات في المادة الوراثية للقطع المضخمة بحجم 1500 ,bp 1000 , bp950bp و450 bp باستخدام البادئة 13AOP.هذه التقنية تسمح لمشاهدة الاختلا فات في البذور المهجنة والمزروعة من قبل المزارعين والشركات المنتجة. تعتبر هذه التقنية RAPD أداة مفيدة في تحديد الاختلا فا ت بين السلالات وفي تقييم النقاوة للهجن.

الكلمات المفتاحية : المادة الو راثية ,DNA هجين, سلالة , شمام,جهاز التفاعل التسلسلي للبوليمريز RCP تقنية ال- ,RAPD التنوع الو راثي.

Résumé

La variabilité génétique de trois variétés locales (JO 232, JO 71, JO 50) et de l'hybride (Golden F1) du melon cultivés en Jordanie a été étudiée utilisant la technique du RAPD. Les résultants sur l'hybride du melon cultivé en été 2002 ont montré des différences entre les plantes pouvant supporter les différences morphologiques et physiologiques observées par les agriculteurs., De même, des différences entre et au sein des variétés locales ont été révélées par les bandes de taille 1500, 1000, 950, et 460 bp en utilisant le marqueur OPA 13. Cette technique a permis de montrer des différences entre les lots de semences des agriculteurs et de la société et des différences entre les variétés locales confirmant son utilité dans l'identification de la diversité génétique et la pureté des lots de semences des hybrides du melon.

Mots clés : ADN, hybride, variété locale, melon, PCR, RAPD, variabilité génétique

Introduction

Melon (*Cucumis melo* L.), is one of the warm season crops grown in the Mediterranean area. Most of actually used melon cultivars were introduced, but some of the growers are still using landraces. These landraces possess valuable quality attributes such as sweetness and odor that can be used in melon improvement programs; but these landraces are threatened by the rapid spread of new imported cultivars and hybrids. The landraces of melon were collected in 1995/1996 from different parts of Jordan by the National Center for Agricultural Research and Technology Transfer (NCARTT) and stored in its genebank. Growers are importing varieties based on consumers requirements of fruit size and soluble sugars concentrations. Most of the farmers are growing F1 Hybrid called "Golden" that showed significant variability in sugar concentration as determined using refractometer at NCARTT laboratories. The sugar content ranged from 2 to 20% using seed samples from the farmers and from the seed company (Hollar & Co.INC. USA). Environmental or cultural factors could affect the sugar content and produce poor quality fruits that could also be explained by, shortage of photo-assimilates (Pardossi et al., 2002). Commercial cultivars of important horticultural crops are mainly adapted to controlled environments and are generally affected by extreme environment factors (Ventura and Mendlinger, 1999). Melon cultivars and landraces are commonly distinguished by morphological traits and are generally late. Therefore, the use of molecular markers to identify and check the purity of melon cultivars and landraces is an efficient way to control the seed lot quality. Cultivars can be easily discriminated using DNA fingerprinting information based on high level of polymorphism between and within varieties. Even closely related cultivars are usually distinguished with the DNA fingerprinting methods (Nybom,1994). The most widely applied technique based on polymerase chain reaction (PCR) is RAPD which is used successfully for cultivar identification, diversity studies and genetic variation in rice (Xie et al., 2000), olive (Belaj et al., 2001), wild soybean (Li and Nelson, 2002), tomato (Villand et al.,1999), okra (Rawashdeh, 1999) and watermelon (Haschizume et al., 1996). This technique was also used for testing some melon breeding lines with aphid resistance (Klingler et al., 2001).

This paper aims to evaluate the genetic diversity of melon landraces and the seed purity of the commonly used F1 hybrid cultivar "Golden" provided by the Hollar & Co.INC.USA company.

Materials and Methods

Plant material

Eighteen seeds of the melon cultivar (Golden F1 hybrid) were taken from two samples from farmers seed lots N# 15991 and from the company seed lot n°15998 Three seeds from each of the three melon landraces (JO232, JO71 and JO50) were also used in this study. These seeds were individually planted in the greenhouse and the 14 days old seedlings were harvested individually for DNA extraction and RAPD analysis.

DNA Extraction

DNA was extracted from young leaves (100 gm) using the Wizard protocol developed by Promega, USA with minor modifications, which include addition of 25μl of 2-Mercaptoethanol to the Nuclei lysis step. Leaves were grounded in liquid Nitrogen and supernatant was recovered and mixed with 600μl isopropanol. The extracted DNA was stored at (-20 °C) until its use for PCR amplification. The DNA was diluted 1:9 with distilled water before amplification. Five random decamer oligonucleotides (Operon Technolgies, Alameda, California) OPA04, OPA13, OPF01, OPF04, and OPN16 were used for PCR amplification following the procedure of Rawashdeh (1999), with minor modifications in the amplification condition and in DNA concentration. Amplification reaction was performed in 25 μl of reaction mixture comprising 10mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton -x-100, 15 mM MgCl2 , 0.2uM each of dATP, dGTP, dCTP and dTTP, (16 ug/μl) primer, with approximately 50ng DNA template and 1 unit Taq polymerase. The reaction was incubated in a DNA Thermocycler (MJ Research, model PTC-100) programmed for 1 cycle of 5 minutes (mn)at 94°C followed by 42 cycles of 1 mn at 94°C, 1 minute at 33°C, and 3 mn at 72°C for, denaturation, annealing and extension, respectively. The last cycle was followed by incubation for 5 mn at 72°C. PCR products were stored at 4°C before analysis. The amplified products (15 ul) mixed with 3 ul loading buffer were electrophoresed on a 1.4% agarose in 0.5xTBE buffer stained with Ethidium bromide and photographed under UV light (Vilber-Lourmat, France.) Molecular sizes of amplification products were estimated using 1kb DNA ladder (Promega, USA). All reactions were repeated three times.

Results and discussion

Five RAPD primers were used for measuring the hybrid cultivar purity and the variation among and within melon landraces but three of these primers did not show any amplification (OPF04, OPA04 and OPN16). The primer OPF01 showed amplification for only one plant while OPA13 primer was able to show significant amplification (Fig.1, Fig.2 and Fig.3).

- **Genetic diversity among and within melon landraces**

RAPD patterns obtained by OPA13 (Fig.1) showed genetic variation among the three melon landraces JO232, JO71 and JO50. The later landrace showed more variation.. Amplified DNA fragments (bands) with molecular weight 1500bp, 1000bp, 950bp and 460bp were present at one plant of JO50 but absent at JO71, while bands with molecular size of 1200bp and 950bp were present at one plant of each JO50 and JO232. There is no variation within the landrace JO71 compared to JO50 and JO232 which showed within-landrace variation. More variation can be found within the landraces if more plants were used. The diversity between and within the landraces could be useful in further improvement of melon worldwide and nationally. The plants with different patterns should be characterized for consumer requirements traits and for resistance and tolerance to biotic and abiotic stresses.

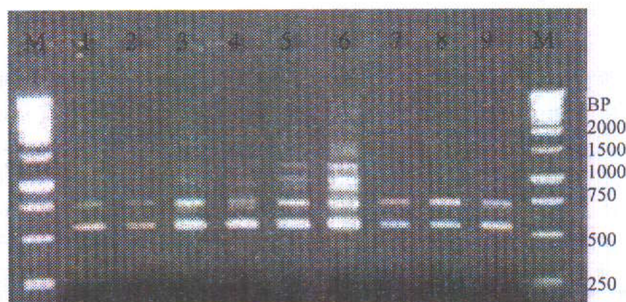

Fig.1: Agarose gel electrophoresis of DNA fragments obtained by RAPD amplification of three melon landraces with primer OPA13. Lane. 1-3 Jo232; lane 4-6: Jo50; lane 7-9: Jo71. M: 1kb ladder as molecular marker (Promega)

- **Genetic purity of hybrid seed lots**

RAPD band patterns for the eighteen seedlings of Golden F1 hybrid obtained from the farmers seed lot 15991 are shown in figure 2. Variation at the DNA level was observed among these seedlings. Band with molecular weight 1000bp was present in seven plants and five of these have a band with size 1200bp and only one plant showed band with size 700bp and another one showed band with size 450bp. Figure 3 shows differences in RAPD profiles of eighteen seedlings of Golden F1 hybrid from company seed lot (15998). Three plants out of eighteen showed the band with size 1000bp. This within and between seed lots variation could be explained by the seed segregation of the hybrid provided by the impurity of seeds that could come from mixtures. Seed source should be reliable to take advantage of the hybrids attributes. In analyzing the similarity between the Golden hybrid and the landraces. It appears that this similarity is higher in case of JO71. This similarity is confirmed by farmers on the basis of the morphology and physiology especially sweetness. Also, unique fragments in some plants could imply the presence of loci that are not present in others.

The development of traits specific RAPD markers will further facilitate cultivar identification and genetic improvement of melon varieties.

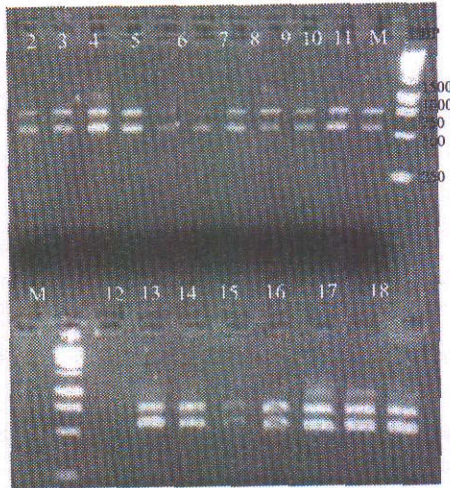

Fig.2: Agarose gel electrophoresis of DNA fragments obtained by RAPD amplification of Golden F1 hybrid melon seeds using primer OPA13. Lane 1-18: Golden F1 with lot 15991 (farmer samples). Lane M:1kb ladder as molecular marker (Promega).

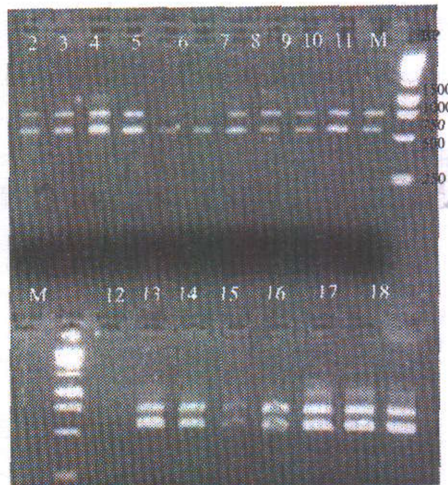

Fig.3: RAPD band profiles of Golden F1hybrid seeds obtained by using primer OPA13. Lane 1-18:Golden F1 hybrid with lot 15998 (company samples). Lane M:1kb ladder as molecular marker (Promega).

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