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Molecular, morphological and biochemical characterization of some Turkish bitter melon (*Momordica charantia* L.) genotypes

Kevser Karaman[a], Akife Dalda-Şekerci[b], Halit Yetişir[b], Osman Gülşen[b], Ömer Faruk Coşkun[b]

[a] Erciyes University, Faculty of Agriculture, Department of Agricultural Biotechnology, Kayseri, Turkey
[b] Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Turkey

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ABSTRACT

Bitter melon or bitter gourd (*Momordica charantia* L.) of the *Cucurbitaceae* family is valuable plant for their nutritive and medicinal properties. In this study, molecular, morphological and some biochemical properties of 12 bitter gourd genotypes from different parts of Turkey were determined. Considerable morphological variation among the genotypes was observed for fruit properties such as fruit length (9.33 to 14.33 cm) and time for maturity (late June to late September). For molecular caharacterization fifteen ISSR primers were produced, on average, 113 bands in the accessions were examined and 59 (52%) of them were detected as to be polymorphic, which resulted in a Jaccard's similarity range from 0.80 to 0.98. According to biocehmical analyses, palmitic, stearic, oleic, linoleic and eleostearic acids were detected as the major fatty acids in the studied bitter gourd seeds. Eleostearic acid had the highest proportion among the fatty acids (42.66-61.99%). The bitter gourd genotypes showed different fractions of vitamin E. G1 contained alpha, gamma and delta tocopherol, while G2, G18, G19 and G24 contained only gamma and delta tocopherols. Beta carotene was also present in all genotypes and the ratio was between 56.57 and 116.75 mg/kg. In addition, the genotypes were evaluated for their mineral composition and phosphorus was the major mineral for the genotypes and only sodium was not observed in all genotypes among the studied minerals. This study provided useful information for nutritional perspective of bitter melon and also breeding studies.

1. Introduction

Momordica charantia L. known as bitter melon, bitter gourd or bitter cucumber belongs to the *Cucurbitaceae* family. It is a vegetable with many culinary uses particularly in Asia and Africa and is widely cultivated in India, China, Malaysia, Africa, and South America (Singh et al., 1990; Behera et al. 2008a). Asian and Indian people eat the immature fruit, young stems, leaves and flowers of this plant as a vegetable (Reyes et al., 1993). In Turkey, it is known as "odd apple" locally and consumed as oil, paste by seeds and vegetable. Among the cucurbits, *M. charantia* L. is different from the other members because of its higher nutritive value such as ascorbic acid and iron (Behera et al. 2008a, 2008b, 2008c; Bharathi et al., 2012), the other vitamin and minerals (Reyes et al., 1993; Kendrick et al., 2004) and medicinal properties. Mature fruits, aril membrane and seed pericarp of *Momordica* species have reported to be contained high levels of carotenoids (Kubola and Siriamornpun, 2011; Nagarani et al., 2014). Several studies related to therapeutic effects of *M. charantia* L. such as antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, antimycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties (Ng et al., 1992; Raman and Lau, 1996; Basch et al., 2003; Grover and Yadav, 2004) have been carried out intensively. Saponins are important bioactive phytochemicals for pharmacological activities and the well-known saponins of *M. charantia* L. are momordin and momorcharside (Keller et al., 2011; Khatib et al., 2017). This type of effects was reported in some minor tropical fruits such as *Borojoa patinoi* Cuatrec (Chaves-López et al., 2018) and *Carica papaya* L. (Vij and Prashar 2015).

In addition to medicinal studies, genetical studies about *M. charantia* L. have been performed (Horejsi and Staub, 1999; Decker-Walters et al., 2001; Levi et al., 2001; Paris et al., 2003; Levi et al., 2004). Genetic diversity among populations can be characterized using molecular and morphological markers. Morphological characters have restrictions since they are influenced by environmental conditions and the developmental phase of the plant. In contrast, molecular markers, based on DNA sequence polymorphisms, indicate higher levels of polymorphism without depending on ecological factors (Dey et al., 2006). DNA based molecular markers are beneficial to provide a relatively independent estimation of genetic diversity. Among these, PCR based random molecular markers like Inter Simple Sequence Repeats (ISSRs) are used extensively in species in which there is a lack of DNA sequence information (Bharathi et al., 2012). ISSR markers have been used in genetic diversity analyses (Levi et al., 2004; Ritschel et al., 2004; Bharathi et al., 2012). ISSR markers are useful in detecting genetic polymorphisms among accessions. It is simpler than SSR (Reddy

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et al., 2002) and found relatively low costs compared to Random Amplification of Polymorphic DNA (RAPD) (Yang et al., 1996).

In this study, seed-fruit morphology, fatty acid, mineral and vitamin composition of *M. charantia* L. genotypes collected from different regions of Turkey were investigated. Additionally, genetic diversity and relationships among different genotypes were assessed by ISSR markers. The genotypes were classified by using principal component analysis (PCA) technique according to their biochemical composition.

2. Materials and Methods

2.1. Materials

In this study, *M. charantia* L. genotypes were collected from different geographical regions of Turkey. Seeds of the 12 bitter gourds were sowed under unheated greenhouse. The seedlings in 2-3 true leaf stage were transplanted to the field. For multiplying seeds, each of 12 genotypes was selfed, extracted from mature fruit and dried at room temperature in laboratory for analyses. All analyses were replicated three times for each genotype.

2.2. Methods

2.2.1. Determination of morphological properties

Genotypes were evaluated for thirteen different phenotypic characteristics as follows: leaf (intensity of green color, number of lobes, stalk length, diameter and length), fruit (length, diameter, shape, color of skin, color of skin at ripe stage, flesh thickness and time of physiological maturity) and plant (attitude of petiole). Means of morphological characters were provided with three replicates.

2.2.2. Determination of molecular properties

Genomic DNA was isolated from young leaves of field grown plants of each genotype using modified CTAB method and purified. The quality and quantity of DNA isolated from these leaf samples were determined by agarose gel electrophoresis. The genomic DNA was subjected to PCR amplification using 15 ISSR primers (Table 3). PCR reaction was performed in a 15 µl volume containing 1.5 µl Taq buffer A (10 mM Tris–HCl, pH 8.3 with 15 mM $MgCl_2$), 1.2 µl of 2.5 mM dNTPs, 0.2 µl of 3 unit of Taq DNA polymerase, 2 µl (20 ng) of template genomic DNA and 1 µl (5 pM) each of ISSR primers. PCR reactions were run on a thermocycler (Bio-Rad, C1000, USA). Cycling conditions were as follow: initial denaturation at 94 °C for 5 min followed by 30 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and a final extension step at 72 °C for 5 min. The amplified products were resolved on 2% of agarose gel at 110 V for 3.5–4 h, using TBE (Tris-Boric acid-EDTA) buffer, visualized under UV light after staining with ethidium bromide and photographed using gel documentation system (Kodak EL Logic 200, USA). Then, clear and reproducible DNA fragments were scored as 1–0 binary data matrix for the presence and absence of a band, respectively. Cluster analysis among the 12 genotypes of *Momordica* species was based on Jaccard' similarity coefficient (Jaccard, 1908) using the unweighted pair-group with arithmetic average method (UPGMA) and SAHN clustering algorithm. Analyses were carried out using NTSYS-pc (Numerical Taxonomy Multivariate Analysis System, NTSYS, 2.11, USA) program. The total number of fragments (TNF), number of polymorphic fragments (NPF) and mean polymorphism (MP) for each primer combination were determined.

2.2.3. Determination of fatty acid composition

Fatty acid composition of samples was determined according to methodology described by Kaplan et al. (2017) with some modifications. Seeds of all genotypes were subjected to oil extraction process with hexane using a Soxhlet extraction system to detect the oil content of the genotypes (VELP, SER 148, Italy). To characterize the fatty acid composition of the extracted oil, a methylation process was carried out to propagate fatty acid methyl esters. For this purpose, the extracted oil was dissolved in 3 mL of n-hexane and saponification of oil samples with 2 M KOH was conducted in the mixture. The mixture was vigorously shaken using a vortex and then centrifuged at 2,516 × g for 5 min. A 1 mL of the supernatant was transferred to gas chromatography (GC) vials and injected to the GC immediately. GC (Agilent 6890), equipped with a flame ionization detector and a 100 m × 0.25 mm i.d. HP-88 column, was used. Injection block temperature was set at 250 °C. The oven temperature was kept at 103 °C for 1 min and then programmed from 103 to 170 °C at 6.5 °C/min, from 170 to 215 °C for 12 min at 2.75 °C/min and finally, 230 °C for 5 min. Helium was the carrier gas with a flow rate of 2 mL/min and split rate was 1/50. The analyses were performed in triplicate for each sample.

2.2.4. Determination of vitamin composition

The vitamin content was analyzed by the method of Beltran et al. (2005). According to the procedure, 1.5 g of oil was dissolved in the mobile phase (10 ml) and the chromatographic separation was carried out using HPLC (Agilent, 1260) equipped with a UV–vis detector. Injection volume was 20 µl and a flow rate was 1.0 ml/min. The mobile phase was composed of 0.5% isopropanol in n-hexane and the absorbance was measured at 295 nm. Tocopherols were quantified by an external standards method; the α-, δ- and γ-tocopherol obtained from Sigma Aldrich Co (St. Louis, MO, USA).

2.2.5. Determination of mineral composition

Approximately 0.5 g of *M. charantia* L. seeds was taken and 10 ml of nitric + perchloric acid mixture was added to the sample to determine some minerals in the seed. Then the samples were subjected to wet ashing until 1 ml of sample remained. After the ashing procedure was completed, the samples were diluted with distilled water and analyzed by ICP-OES spectrometer (Perkin-Elmer, Optima 4300 DV, ICP/OES, Shelton, CT 06484-4794, USA). The contents of Ca, Mg, Na, K, P, S, Fe, Mn, Zn, Cu, B, Cd, Cr, Pb and Ni were determined (Mertens, 2005a, 2005b).

2.2.6. Statistical analysis

Statistical analyses of the samples were carried out using SAS statistical software (SAS 2000). The comparative analyses were conducted using Duncan's multiple range tests. A significance level of 0.05 was used for all comparisons. Classification of genotypes was achieved by PCA using XLSTAT Software (XLSTAT, USA).

3. Results and Discussion

In this study, *M. charantia* L. seeds were characterized in terms of morphological, biochemical and molecular properties. Table 1 and 2 show the qualitative and quantitative morphological data of genotypes. Significant morphological variation was observed among the bitter gourd genotypes studied. According to the statistical comparison, the leaf lengths of G22 (11.17 cm) and G23 (11.33 cm) were found significantly larger than those of the genotypes G1 (8.17 cm), G2 (8.0 cm), and G39 (8.33 cm) ($p < 0.05$). For fruit diameters, the genotype G42 (53.37 mm) was found significantly different than the other genotypes except G2, G1 and G22 ($p < 0.05$). Also, fruit lengths were compared, the genotype G1 (14.33 cm) was found significantly taller than the other genotypes except G22, G24, G28 and G42 (Table 1). The percentage of intensity of green color in leaves was determined to be 66.6% of genotypes as medium, 16.7% of genotypes as light and 16.7% of genotypes as dark (Table 2).

The results of morphological characterization were supported by the molecular data. Fifteen ISSR primers produced, in average, 113 bands in the accessions examined and 59 (52%) of them were polymorphic (Table 3). This rate (52%) was observed as to be lower than the level of polymorphism (74.5%) reported in an earlier study with the RAPD and ISSR markers in bitter gourds (Bharathi et al., 2012). In a different