Agrotechniques in Industrial Crops

Journal Homepage: https://atic.razi.ac.ir

Study on Quantitative and Qualitative Traits Diversity in Some *Momordica charantia* L. Genotypes

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ARTICLE INFO

Original paper

Article history:
Received: 12 Sep 2023
Revised: 4 Nov 2023
Accepted: 18 Dec 2023

Keywords:
Biochemical traits
Genetic parameters
Germplasm diversity
Momordica charantia L.
Morphology

ABSTRACT

Bitter cucumber (*Momordica charantia* L., fam. Cucurbitaceae) is a good source of carbohydrates, proteins, vitamins, and minerals with the highest nutritional-medicinal value among gourds. The present study aimed at investigating the morphological and biochemical traits of some bitter cucumber genotypes. The results showed that all morphological traits except lobe width, length to lobe width ratio, and number of nodes to the first male flower, photosynthetic pigments of leaf (carotenoids only) and fruit, qualitative traits of fruit (except total acidity), and biochemical traits of leaf or fruit (except phenols) were significantly affected by genotype. The genotype "*Bankok orange*" had the lowest number of days until the appearance of the first male or female flowers or fruit. The first four components in the principal component analysis showed the total data diversity. The PC1 emphasized on the morphological traits, PC2 the leaf photosynthetic pigments, fruit flavonoids, and fruit dry weight percentage, PC3 on the fruit quality and phenology, and PC4 on the fruit photosynthetic pigments and leaf biochemical traits. Based on the results of traits correlation, internode length on main stem had a high correlation with stem thickness, leaf length, and lobe depth. Fruit weight also had a significant negative correlation with fruit dry weight percentage, leaf chlorophyll-*b*, total leaf chlorophyll, and fruit flavonoids. The results of genetic estimation of traits showed that fruit length, fruit peduncle length, fruit carotenoids, and leaf phenols had the highest coefficient of phenotypic and genetic changes, respectively, and had the highest diversity compared to the other traits. Also, fruit chlorophyll-*b* had the highest heritability (99%). Examining the hydroalcoholic extracts of the plant showed that this plant contains significant amounts of phenolics, especially total flavonoids, which confirms its medicinal value. It could be suggested to extract the species phytochemicals and examine their biological and pharmacological properties.

DOI: 10.22126/ATIC.2023.9735.1118

1. Introduction

Momordica charantia L. (2x= 2n= 22) is an annual tropical and subtropical vine of the fam. Cucurbitaceae originated from the tropics of Asia. It produces green wart-like or wrinkled fruits that look like pumpkins or cucumbers. This species is known as African cucumber, bitter gourd, bitter melon, bitter cucumber, and balsam or karela pear in different parts of the world (Basch *et al*., 2003). It is used as food and medicine in tropical regions of the world including parts of South America and the Amazon Basin like Brazil, Guyana and the Caribbean, East Africa, and parts of Asia including India, China, the Philippines, Pakistan, Nepal, and Sri Lanka (Gayathry and John, 2022; Mini Raj *et al*., 1993). Bitter cucumber fruits have a very bitter taste. In addition to the fruits, this bitterness is also found in the leaves, stems, and other parts of the plant (Platel and Srinivasan, 1995). The plant bitterness is due to the presence of saponins and mainly cucurbitacin glycosides (Tan *et al*., 2016). Production of bitter cucumber has become widespread in tropical regions of Iran such as Sistan & Baluchestan and Hormozgan provinces. For the first time, this plant was cultivated in Konarak City, Sistan & Baluchestan

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province in 2009 which yielded 18 tons ha^{-1} (Noorzai, 2009). These plant fruits are a good source of carbohydrates, proteins, vitamins, and minerals with the highest nutritional value among Cucurbitaceae plants (Gayathry and John, 2022; Desai and Musmade, 1998; Mini Raj *et al*., 1993). For centuries, bitter cucumber has been used in traditional medicine in India, China, Africa, and Latin America. Bitter cucumber extract has antioxidant, antimicrobial, antiviral, anti-hepatitis, and anti-ulcer properties and also has the ability to lower blood sugar (Raman and Lau, 1996). These medicinal properties have been attributed to the presence of biochemicals including triterpenoids, proteins, and steroids (Grover and Yadav, 2004).

Due to the high medicinal and nutritional value, comprehensive researches for proper utilization of this species of germplasm is essential. The first important step before cultivating and processing this species and using it in the country pharmaceutical industries is the introduction of valuable species or ecotypes in terms of having highly active compounds and suitable yield. The present study was conducted to investigate the diversity of morphological traits and effective compounds of different bitter cucumber genotypes and to determine the relationships between different traits in this plant.

Dey et al. (2006) studied the morphological and molecular diversity between 38 bitter cucumber genotypes including some commercial cultivars from different agricultural and ecological regions of India. In their study, 14 morphological quantitative traits and 29 molecular random primers could not show any similarity between the genotypes. Also, the genotypes grouping based on molecular markers did not match with the grouping based on morphological quantitative markers. Singh et al. (2014) observed a wide range of quantitative trait diversity between 25 different Indian bitter cucumber genotypes. Karaman et al. (2018) also found significant morphological and DNA (ISSR markers) diversity among Turkish bitter cucumber genotypes. Valyaie et al. (2021) presented the first report of this species cultivation in Iran. They studied the vegetative characteristics, phenology, fruit yield, and biochemical compositions of six bitter cucumber cultivars (named "*Iranshahr*", "*Mastisa*", "*No. 486*", "*Japan*", "*Isfahan*", and "*Ilukano*") under the climatic conditions of Karaj city, Alborz province, Iran. They observed significant differences between cultivars in vegetative characteristics, phenology, and biochemistry.

2. Materials and methods

2.1. Plant material

Some bitter cucumber genotypes introduced in Table 1 were used in the present study.

Table 1. *Momordica charantia* genotypes used in the study on quantitative and qualitative traits diversity.

Name	Code	Geographical origin
Pakan seed company	*PS*	United States
Kashan	*KN*	Iran (Kashan)
Bitter gourd F_1 hybrid by Kraft seed	*KS*	India
Bankok orange	*BO*	Thailand
Hongkong karela	*HK*	Hong Kong
Bankok white	*BW*	Thailand
Hybrid bitter gourd by ol seed	*OS*	India
Hybrid bitter gourd by Super agree green	*AG*	India
Order number 404-4841900-9975523	*ON3*	India
Order number 404-6647498-3826725	*ON5*	India

2.2. Experimental design and cultivation

The experiment was conducted as a microplot design in which the number of each genotype plant was divided into four equal parts each of which was planted in a plot in different parts of the field located in the research farm of the Faculty of Agriculture, Tarbiat Madras University in August 2019.

First, the seeds were soaked in distilled water for 24 hours. They were then placed on filter paper and stored in a germinator at 25°C and 70% humidity. After a week, with the radicle emergence, the seeds were planted into the mixture of sand, cocopeat, and perlite medium in culture trays under greenhouse conditions. After three weeks, the seedlings with three to four true leaves were planted as scaffolding in the main field with a planting distance of 90 cm between the rows and 70 cm between the plants on the rows.

2.3. Morphology, phenology, and biochemical studies

Mature leaves located between nodes 15 and 25 on branches and half-ripe green fruits were sampled. Forty-three morphological traits (23 quantitative and 20 qualitative ones) were studied with the guidelines of the International Union for the Protection of New Plant Varieties. Six, three, five, two, three, and four quantitative traits were respectively related to the leaves, stems, fruits, flowers, phenology, and fruit

quality. Quantitative traits included length and width of leaf blade, petiole length, lobe depth, lobe width, depth to lobe width ratio, main stem thickness, number of secondary stems on the main stem, internode length, fruit length, fruit diameter, fruit fresh weight, fruit dry weight percentage, peduncle length, number of nodes until the first male flower, number of nodes until the first female flower, phenology traits (number of days until the first male flower appearance, number of days until the first female flower appearance, and number of days until the first fruit formation), and fruit quality determining traits (fruit tissue firmness, fruit acidity, fruit pH, and total soluble solids (TSS)).

Dry weight percentage, acidity, and soluble solids of fruits were measured according to AOAC (The Association of Official Analytical Chemists) method.

For biochemical studies, ethanol extract to measure the amount of carbohydrates, flavonoids, and proline and methanol extract to measure the amount of phenol, chlorophyll, and carotenoid were prepared from the desired plant parts (1- fresh leaves and 2- shade-dried fruits) and were stored at 4°C in darkness until use. One observation was used to measure the biochemical traits. Measurements of the content of photosynthetic pigments by Warren (2008) method and reading at wavelengths of 665, 652, and 470 nm, anthocyanin by Lako et al. (2007) method and reading at wavelengths of 510 and 470 nm, total phenol by colorimetric method with Folin-Ciocalteu (Ainsworth and Gillespie, 2007) and reading at a wavelength of 765 nm, total flavonoids by aluminum chloride colorimetric method (Chatatikun and Chiabchalard, 2013) and reading at a wavelength of 415 nm, total carbohydrates by phenol sulfuric acid method (Masuko *et al*., 2005) and reading at a wavelength of 490 nm, proline by ninhydrin-based method (Carillo and Gibon, 2011) and reading at a wavelength of 520 nm, and protein by an absorbance ratio of 470/610 nm (in protein and blank samples) (Grintzalis *et al*., 2015) were done. Epoch nanodrop device was used for reading at different wavelengths.

2.4. Analysis of variance

Analysis of variance (ANOVA), means comparison (LSD test at 5% probability level), and correlation test between traits were performed using SAS Ver.9.2 software. Cluster analysis (CA) for grouping genotypes (based on the distance between groups by WARD method), factor analysis (FA), and principal component analysis (PCA) for the examined traits were done using SPSS Ver.16.0 software.

3. Results

The results of morphological traits evaluation in bitter cucumber genotypes showed a wide range of changes for most of the traits indicating high diversity between the studied genotypes. Internode length of the main stem, thickness of the main stem, number of lateral branches on the main stem, length and width of the leaf blade, petiole length, lobe depth (P<0.01), and number of nodes to the first female flower (P<0.05) were significantly affected by the different genotypes (Table 2).

Table 2. ANOVA of leaf, stem, and flower quantitative traits in different *Momordica charantia* genotypes.

Source of variations	d.f.	M.S.										
		Internode length on the main stem	Main stem thickness	Number of secondary stems on the main stem	Leaf blade length	Leaf blade width	Petiole length	Lobe depth	Lobe width	Depth to lobe width ratio	Number of nodes until the first male flower	Number of nodes until the first female flower
Genotype	9	1149.5**	3.0**	6.9**	778.2**	1593.2**	1512.6**	344.6**	156.3$^{n.s}$	0.0$^{n.s}$	2.6$^{n.s}$	52.7*
Experimental error	24	181.2	0.3	0.9	114.3	140.9	116.3	116.1	98.84	0.0	1.1	29.5
Sampling error	26	31.9	0.1	0.4	29.0	69.0	46.0	56.6	87.26	0.0	1.4	22.3
C.V. (%)		11.0	11.9	12.0	8.8	9.3	12.5	15.9	25.4	10.6	19.3	21.5

$^{n.s.}$, *, and **: non-significant, significant at 5, and 1% probability levels, respectively.

Based on the means comparison results (Table 3), the genotype *ON3* had the longest internode length (88.6 cm) and stem thickness (4.2 cm) compared to the other genotypes. The highest number of lateral branches was observed in the genotypes *PS* and *KN* and the lowest one in *HK* and *ON5*. Also, the genotype *AG* had the highest length and leaf width (79.9 and 117.9 mm, respectively). The genotype *BW* had the highest number of nodes on the stem (28) before the first female flower, which did not show significant

differences with the genotypes *ON5*, *KN*, *KS*, *BO*, and *AG*. The lowest number of nodes before the first female flower was also related to the genotypes *ON3*, *OS*, and *HK*.

Phenological traits such as number of days until the appearance of the first male flower, number of days until the appearance of the first female flower, and number of days until the formation of the first fruit were significantly (P<0.01) affected by different genotypes of bitter cucumber (Table 4). Based on the means comparison results (Fig. 1), the genotypes *BO* and *BW* had the lowest number of days until the first male flowers, which did not differ significantly from the genotypes *PS* and *HK*. Also, the genotypes *PS*, *KN*, *KS*, *ON5*, and *ON3* had the highest number of days until the first female flower or fruit, which were significantly different from the genotypes *BO* and *BW*. The lowest number of days until the first female flower or fruit was also observed in the genotype *BO*.

Table 3. Means comparison of leaf, stem, and flower quantitative morphological traits in different *Momordica charantia* genotypes.

Genotype	Internode length on the main stem (mm)	Main stem thickness (mm)	Number of secondary stems on the main stem	Leaf blade length (mm)	Leaf blade width (mm)	Petiole length (mm)	Lobe depth (mm)	Number of nodes until the first female flower
PS	48.9^{c-e}	3.2^{cd}	6.5^{a}	70.4^{a-c}	96.3^{b-d}	52.9^{a-b}	53.0^{a-d}	19.4^{bc}
KN	40.1^{e}	2.1^{e-f}	6.6^{a}	49.0^{e}	67.7^{e}	15.2^{c}	39.2^{de}	23.2^{a-c}
KS	65.3^{b-c}	3.8^{a-c}	4.4^{c-d}	74.3^{a-b}	110.2^{a-b}	74.1^{a}	55.9^{a-c}	20.4^{a-c}
BO	39.9^{e}	2.6^{d-f}	4.6^{c-d}	53.3^{d-e}	80.8^{d-e}	44.5^{b}	44.3^{b-e}	23.3^{a-c}
HK	52.5^{c-e}	3.4^{a-d}	4.0^{d}	55.5^{c-e}	89.7^{c-d}	46.1^{b}	43.1^{b-e}	18.8^{c}
ON5	61.1^{b-d}	3.3^{b-d}	3.8^{d}	64.6^{b-d}	100.5^{b-c}	74.9^{a}	50.5^{a-e}	27.0^{ab}
BW	38.2^{e}	2.0^{f}	5.8^{a-c}	44.5^{e}	70.3^{e}	53.3^{a-b}	36.8^{e}	28.0^{a}
OS	42.9^{d-e}	2.6^{d-e}	6.0^{a-b}	56.7^{c-e}	87.6^{c-d}	46.9^{b}	41.6^{c-e}	19.0^{c}
AG	78.7^{a-b}	4.1^{a-b}	5.0^{b-d}	79.9^{a}	117.8^{a}	60.5^{a-b}	57.4^{a-b}	23.0^{a-c}
ON3	88.6^{a}	4.2^{a}	5.5^{a-c}	78.9^{a-b}	108.5^{a-b}	65.3^{a-b}	61.2^{a}	18.0^{c}

In each column, the means with common letters are in the same statistical group at 5% probability level (LSD test).

Table 4. ANOVA of phenological traits in different *Momordica charantia* genotypes.

Source of variations	d.f.	M.S.		
		Number of days until the first male flower appearance	Number of days until the first female flower appearance	Number of days until the first fruit formation
Genotype	9	146.1**	103.3**	97.8**
Experimental error	24	29.0	14.9	9.5
Sampling error	26	5.2	2.7	2.4
C.V. (%)		4.8	2.9	2.9

**: significant at 1% probability level.

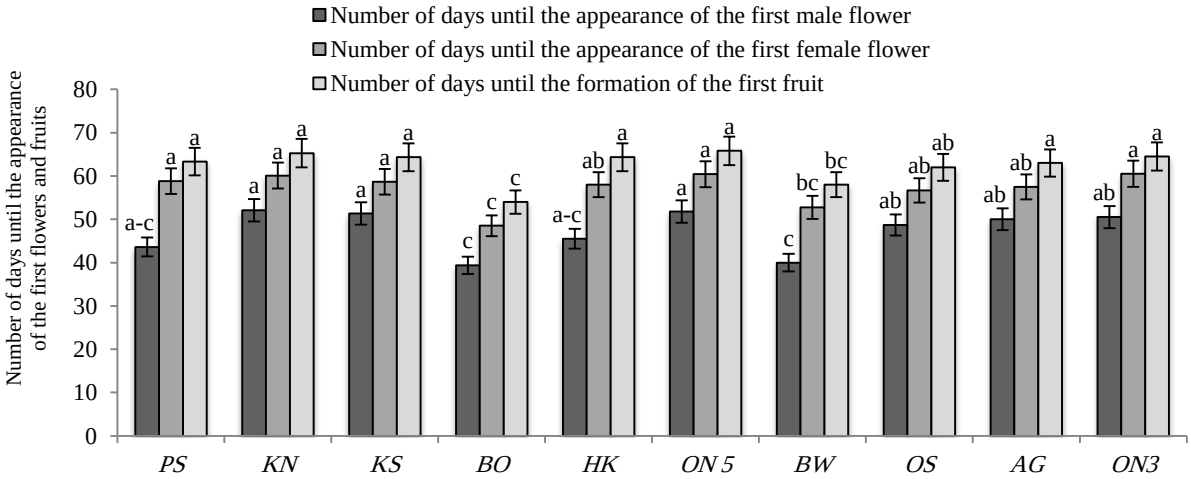

Figure 1. Means comparison of some phenological traits in different *Momordica charantia* genotypes. The means with common letters are in the same statistical group at 5% probability level (LSD test).

Among the measured leaf photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, only the amount of carotenoids differed significantly (P<0.01) between different bitter cucumber genotypes (Table 5). Also, the leaf biochemical traits such as anthocyanins, phenol, flavonoids, total soluble carbohydrates, proline, and protein were significantly (P<0.01) affected by different genotypes (Table 5).

The means comparison results (Table 6) showed that the genotypes *KS*, *ON5*, *BO*, and *PS* had the highest content of carotenoids, anthocyanins, total phenol, and total protein in the leaves, respectively. The genotype *KN* took the first place among the genotypes in terms of flavonoids and total soluble carbohydrate content. In terms of proline content, the genotypes *KS*, *ON5*, and *AG* ranked first.

Table 5. ANOVA of leaf biochemical traits in different *Momordica charantia* genotypes.

Source of variations	d.f.	M.S.				
		Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	Anthocyanins
Genotype	9	0.4$^{n.s.}$	0.2$^{n.s.}$	1.0$^{n.s.}$	0.0**	3127.9**
Experimental error	24	0.2	0.1	0.7	0.0	70.9
C.V. (%)		16.0	17.4	16.2	13.6	14.0
Source of variations	d.f.	M.S.				
		Total phenol	Flavonoids	Total soluble carbohydrates	Proline	Total protein
Genotype	9	2.1**	164.5**	69.4**	16.7*	0.0**
Experimental error	24	0.1	32.2	17.1	6.2	0.0
C.V. (%)		17.5	12.7	16.4	15.9	9.7

$^{n.s.}$, *, and **: non-significant, significant at 5, and 1% probability levels, respectively.

Table 6. Means comparison of leaf biochemical traits in different *Momordica charantia* genotypes.

Genotype	Carotenoids (mg.g^{-1} FW)	Anthocyanins (μg.mg^{-1} FW)	Total phenol (mg.g^{-1} FW)	Flavonoids (mg.g^{-1} FW)	Total soluble carbohydrates (mg.g^{-1} FW)	Proline (mg.g^{-1} FW)	Total protein (mg.g^{-1} FW)
PS	0.3^{cd}	75.6^{b}	1.4^{c}	47.0^{b}	24.1^{bcd}	13.0^{bc}	0.7^{a}
KN	0.4^{b}	34.4^{cd}	2.1^{b}	59.2^{a}	34.6^{a}	11.8^{c}	0.5^{cd}
BO	0.3^{bcd}	30.9^{d}	3.7^{a}	44.9^{bcd}	27.3^{b}	16.3^{ab}	0.6^{ab}
KS	0.5^{a}	51.9^{c}	1.8^{bc}	42.8^{bcd}	27.0^{bc}	18.1^{a}	0.6^{cd}
BW	0.4^{bc}	73.3^{b}	2.0^{b}	47.7^{b}	26.1^{bcd}	14.9^{abc}	0.6^{cd}
ON5	0.2^{e}	118.1^{a}	1.3^{c}	37.0^{cd}	25.0^{bcd}	17.8^{a}	0.5^{cd}
HK	0.2^{e}	37.4^{cd}	1.3^{c}	40.5^{bcd}	19.4^{d}	16.5^{ab}	0.5^{d}
OS	0.3^{de}	84.2^{b}	1.4^{c}	35.8^{d}	21.0^{bcd}	15.5^{abc}	0.5^{d}
AG	0.2^{e}	19.4^{d}	1.3^{c}	40.7^{bcd}	20.4^{cd}	18.3^{a}	0.5^{d}
ON3	0.3^{cd}	52.1^{c}	1.4^{c}	45.1^{bc}	21.0^{bcd}	16.6^{ab}	0.6^{bc}

In each column, the means with common letters are in the same statistical group at 5% probability level (LSD test).

Quantitative morphological traits of fruits including fruit length, fruit diameter, peduncle length, fruit fresh weight (P<0.01), and fruit dry weight percentage (P<0.05) were significantly affected by different genotypes (Table 7). The means comparison results (Table 8) showed that the genotype *ON3* had the longest fruit (233.2 mm) and *KS*, *HK*, *ON5*, and *OS* had the largest fruit diameter (50.6 mm). The longest fruit peduncle (88.7 mm) was observed in *KS* and *ON5*. The genotypes *OS* and *BW* had the highest fresh weight (112.6 g) and fruit dry weight percentage (8.5%), respectively. The lowest fruit fresh weight was also assigned to the genotypes *AG* and *BW*.

Table 7. ANOVA of fruit morphological traits in different *Momordica charantia* genotypes.

Source of variations	d.f.	M.S.				
		Fruit length	Fruit diameter	Peduncle length	Fruit fresh weight	Fruit dry weight percentage
Genotype	9	13210.2**	330.6**	2397.2**	2346.3**	2.1*
Experimental error	24	518.5	50.2	254.4	140.3	0.6
Sampling error	26	353.9	40.4	81.3	375.6	0.7
C.V. (%)		17.6	15.5	18.2	29.6	11.4

* and **: significant at 5 and 1% probability levels, respectively.

Table 8. Means comparison of fruit morphological traits in different *Momordica charantia* genotypes.

Genotype	Fruit length (mm)	Fruit diameter (mm)	Peduncle length (mm)	Fresh fruit weight (g)	Fruit dry weight percentage
PS	110.3^{e-d}	45.2^{a-b}	52.9^{a-b}	64.4^{d-e}	7.6^{a-b}
KN	50.7^{h}	32.2^{c}	15.2^{c}	50.8^{e-f}	6.9^{b-c}
KS	159.6^{b}	48.3^{a}	74.1^{a}	64.4^{d-e}	6.9^{b-c}
BO	68.0^{g-h}	33.1^{c}	44.5^{b}	58.1^{d-e}	7.7^{a-b}
HK	97.0^{e-g}	46.5^{a}	46.1^{b}	70.4^{c-d}	6.8^{b-c}
ON5	154.7^{b-c}	49.0^{a}	74.9^{a}	101.5^{a-b}	7.3^{b}
BW	79.2^{f-h}	35.5^{b-c}	53.3^{a-b}	40.0^{f}	8.5^{a}
OS	125.2^{c-e}	50.6^{a}	46.9^{b}	112.6^{a}	6.0^{c}
AG	136.4^{b-d}	32.6^{c}	60.5^{a-b}	39.2^{f}	7.8^{a-b}
ON3	233.2^{a}	36.1^{bc}	65.3^{ab}	86.6^{b-c}	7.4^{a-b}

In each column, the means with common letters are in the same statistical group at 5% probability level (LSD test).

Based on ANOVA, fruit quality-determining traits such as fruit tissue firmness, fruit pH, and TSS were significantly affected by different genotypes, but the amount of total fruit acidity did not show a significant difference in different genotypes (Table 9). The means comparison results showed that the genotypes *KN* and *ON3* fruits had the highest tissue firmness and *OS* had the lowest one (Fig. 2). The highest fruit pH was observed in *BO* and *OS* (Fig. 3) and the highest TSS in *KN* and *HK* (Fig. 4).

Table 9. ANOVA of fruit quality determining traits in different *Momordica charantia* genotypes.

Source of variations	d.f.	M.S.			
		Fruit tissue firmness	Fruit pH	Total fruit acidity	Total soluble solids
Genotype	9	8.3**	0.2**	0.1$^{n.s.}$	1.8**
Experimental error	24	0.7	0.0	0.1	0.2
Sampling error	26	0.4	0.1	0.1	0.1
C.V. (%)		13.8	3.9	19.4	11.2

$^{n.s.}$ and **: non-significant and significant at 1% probability level, respectively.

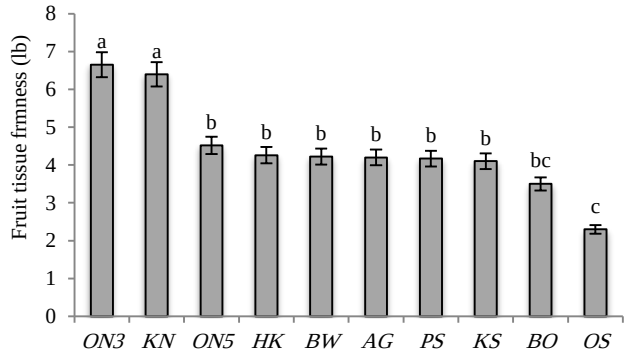

Figure 2. Means comparison of fruit tissue firmness in different *Momordica charantia* genotypes. The means with common letters are in the same statistical group at 5% probability level (LSD test).

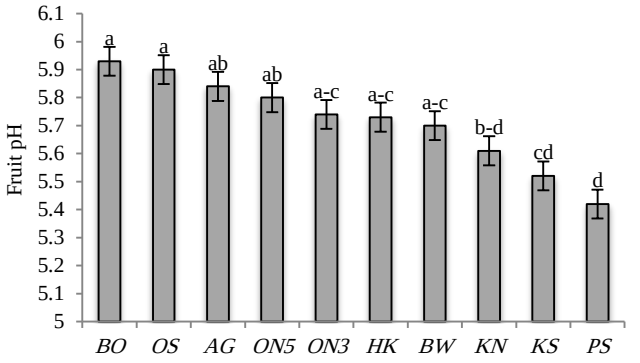

Figure 3. Means comparison of fruit pH in different *Momordica charantia* genotypes. The means with common letters are in the same statistical group at 5% probability level (LSD test).

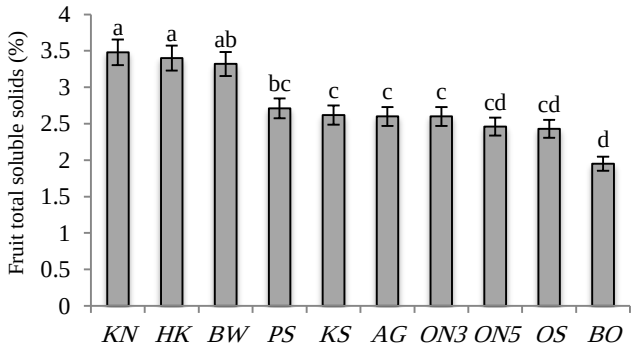

Figure 4. Means comparison of fruit total soluble solids in different *Momordica charantia* genotypes. The means with common letters are in the same statistical group at 5% probability level (LSD test).

Based on ANOVA (Table 10), the amount of photosynthetic pigments of half-ripe fruits, including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, were significantly affected by different bitter cucumber genotypes (P<0.01). Biochemical traits of different genotypes such as anthocyanins, flavonoids, soluble carbohydrates, and proline differed significantly (P<0.01), but their phenol and protein content did not differ.

The means comparison results showed that the highest contents of half-ripe fruit biochemical traits were observed in the genotypes *PS* and *AG* (Fig. 5; chlorophyll *a*), *BW* (Fig. 5; chlorophyll *b* and total chlorophyll), *PS*, *KS*, and *BW* (Fig. 5; carotenoids), *HK* (Table 11; anthocyanins), *AG* (Table 11; flavonoids), *HK* and *KN* (Table 11; proline), and *OS* (Table 11; total soluble carbohydrates).

Based on the CA results (Fig. 6), the genotypes were classified into three groups. The genotypes *PS*, *KN*, *BO*, *HK*, and *OS*, which had the highest number of secondary stems on the main stem, fruit fresh weight, fruit total soluble solids, leaf and fruit phenol, leaf

flavonoids, leaf soluble carbohydrates, and fruit proline, and the lowest percentage of fruit dry weight and fruit tissue firmness, were placed in the first group. The genotypes with the highest internode length on the main stem, stem thickness, length and leaf blade width, petiole length, depth and lobe width, number of days until flowering and fruiting (late fruit genotypes), fruit length, carotenoids, anthocyanins, and leaf proline including *KS*, *ON5*, *AG*, and *ON3* were placed in the second group. The genotype *BW* with the highest number of nodes until the formation of the first male or female flower, fruit dry weight percentage, and total fruit chlorophyll were put in the third one.

Table 10. ANOVA of half-ripe fruits biochemical traits in different *Momordica charantia* genotypes.

Source of variations	d.f.	M.S.				
		Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	Anthocyanins
Genotype	9	117.6**	53511.1**	50740.0**	2347.4**	3867.6**
Experimental error	24	22.2	1720.4	4006.7	93.3	79.2
C.V. (%)		13.1	12.0	16.6	15.96	10.7
Source of variations	d.f.	M.S.				
		Phenol	Flavonoids	Soluble carbohydrates	Proline	Protein
Genotype	9	0.6$^{n.s.}$	214.9**	19025.1**	438.0**	0.5$^{n.s.}$
Experimental error	24	0.3	9.8	5602.4	34.7	0.4
C.V. (%)		12.1	13.1	14.0	13.7	25.2

$^{n.s.}$ and **: non-significant and significant at 1% probability level, respectively.

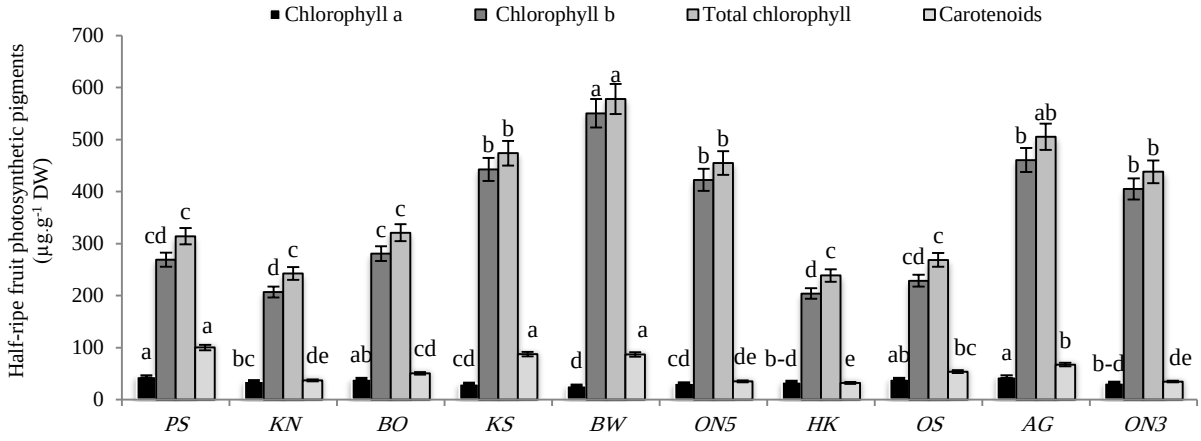

Figure 5. Means comparison of half-ripe fruit photosynthetic pigments in different *Momordica charantia* genotypes. The means with common letters are in the same statistical group at 5% probability level (LSD test).

Table 11. Means comparison of half-ripe fruit anthocyanins, flavonoids, proline, and total soluble carbohydrates content in different *Momordica charantia* genotypes.

Genotype	Anthocyanins (μg.mg^{-1} DW)	Flavonoids (μg.g^{-1} DW)	Proline (mg.g^{-1} DW)	Total soluble carbohydrates (μg.g^{-1} DW)
PS	83.8^{d}	34.0^{ab}	45.9^{b}	522.7^{b-e}
KN	110.9^{ab}	32.1^{ab}	58.1^{a}	636.6^{ab}
BO	77.2^{d}	18.3^{c}	43.0^{bc}	407.1^{e}
KS	12.5^{f}	20.4^{c}	42.4^{b-d}	540.9^{a-d}
BW	79.0^{d}	30.3^{b}	38.8^{b-d}	506.6^{c-e}
ON5	104.2^{bc}	17.9^{cd}	26.7^{e}	515.7^{b-e}
HK	122.0^{a}	12.9^{d}	64.7^{a}	581.1^{a-c}
OS	91.7^{cd}	18.8^{c}	32.9^{de}	647.2^{ab}
AG	46.9^{e}	35.7^{a}	39.2^{b-d}	449.0^{de}
ON3	117.9^{ab}	18.4^{c}	35.3^{c-e}	530.4^{a-d}

In each column, the means with common letters are in the same statistical group at 5% probability level (LSD test).

PCA results (Fig. 7) showed that length and leaf width, depth and lobe width, stem thickness, internode length on the main stem, fruit length, peduncle length, and petiole length correlated with each other, positively. These traits had the highest negative correlation with number of nodes until the first male flower, leaf soluble carbohydrates, and number of nodes until the first female flower. Also, leaf chlorophylls *a* and *b*, total leaf chlorophyll, and fruit dry weight percentage correlated with each other, positively and had a strong negative correlation with number of days until the first male flower appearance and fruit weight. Total soluble solids, total acidity, number of days until the first fruit appearance, and number of days until the first female flower appearance

correlated with each other, positively, but had a strong negative correlation with leaf and fruit phenols. Fruit chlorophyll *b*, total fruit chlorophyll, and number of nodes until the first female flower had a positive correlation with each other, but correlated with fruit proline and fruit chlorophyll *a*, negatively. Leaf or fruit carotenoids and fruit protein also showed the highest negative correlation with fruit anthocyanins.

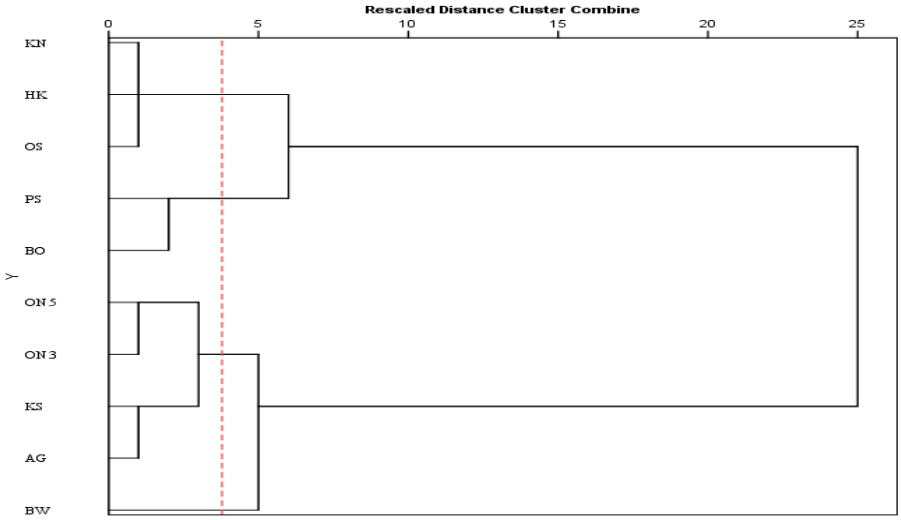

Figure 6. CA dendrogram of morphological, phenological, and biochemical traits in different genotypes of *Momordica charantia.*

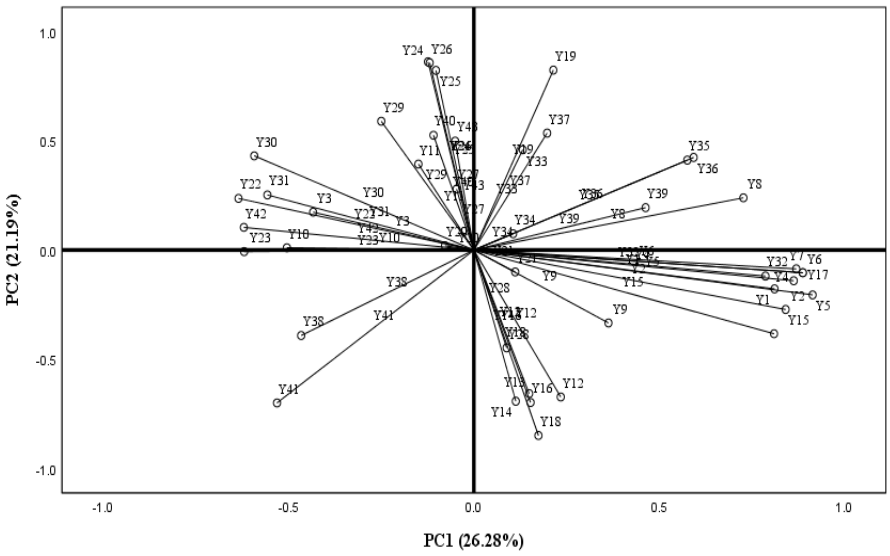

Figure 7. Bioplot of some studied traits in *Momordica charantia* genotypes. Y1: main stem internode length, Y2: main stem thickness, Y3: number of lateral branches on main stem, Y4: leaf blade length, Y5: leaf blade width, Y6: petiole length, Y7: lobe depth, Y8: lobe width, Y9 : depth to lobe width ratio, Y10: number of nodes until the first male flower, Y11: number of nodes until the first female flower, Y12: number of days until the first male flower appearance, Y13: number of days until the first female flower appearance, Y14: number of days until the first fruit formation, Y15: fruit length, Y16: fruit diameter, Y17: peduncle length, Y18: fresh fruit weight, Y19: fruit dry weight percentage, Y20: fruit tissue firmness, Y21: fruit pH, Y22: total acidity, Y23 : total soluble solids, Y24: leaf chlorophyll *a*, Y25: leaf chlorophyll *b*, Y26: total leaf chlorophyll, Y27: leaf carotenoids, Y28: leaf anthocyanins, Y29: leaf phenols, Y30: leaf flavonoids, Y31: leaf soluble carbohydrates, Y32: leaf proline, Y33: leaf soluble protein, Y34: fruit chlorophyll *a*, Y35: fruit chlorophyll *b*, Y36: total fruit chlorophyll, Y37: fruit carotenoids, Y38: fruit anthocyanins, Y39: fruit phenols, Y40: fruit flavonoids, Y41: fruit soluble carbohydrates, Y42: fruit proline, Y43: fruit soluble protein.

Based on the traits correlation results, internode length significantly positively correlated with stem thickness (r= 0.92**), leaf blade length (r= 0.90**), lobe depth (r= 0.90**), leaf blade width, lobe width, and fruit length and significantly negatively correlated with number of nodes until the first male flower. Stem thickness significantly positively correlated with leaf blade length (r= 0.93**), leaf blade width (r= 0.96**), petiole length, lobe depth (r= 0.92**), lobe width, fruit length, and peduncle length. Number of secondary branches significantly positively correlated with leaf length and significantly negatively correlated with

number of nodes until the first male flower. Leaf blade width was significantly positively correlated with petiole length, lobe depth (r= 0.92**), lobe width, fruit length, and peduncle length. Petiole length was significantly positively correlated with lobe depth, fruit length, and peduncle length (r= 1.00**) and significantly negatively correlated with leaf or fruit flavonoids. Lobe depth significantly positively correlated with lobe width, fruit length, and peduncle length and significantly negatively correlated with number of nodes until the first male flower. Lobe width significantly negatively correlated with number of nodes until the first male flower. Length to lobe width ratio also correlated with fruit length significantly positively.

Number of days until the first male flower appearance had a significant positive correlation with number of days until the first female flower appearance and number of days until the first fruit formation and a significant negative correlation with leaf chlorophylls *a* or *b* and total leaf chlorophyll. Number of days until the first female flower appearance also had a significant positive correlation (>95%) with number of days until the first fruit formation.

Also, fruit tissue firmness showed a significant positive correlation with leaf flavonoids. Total fruit acidity was significantly positively correlated with total fruit soluble solids (r= 0.91**) and leaf flavonoids and significantly negatively correlated with fruit phenols. Total fruit soluble solids showed a significant positive correlation with fruit proline and a significant negative correlation with fruit phenols (r= -0.90**). The amount of leaf chlorophyll *a* significantly positively correlated with leaf chlorophyll *b* (r= 0.96**) and total leaf chlorophyll (r= 0.99**). The amount of leaf chlorophyll *b* significantly positively correlated with total leaf chlorophyll (r= 0.98**), too. The results also showed that leaf flavonoids content had a significant positive correlation with leaf soluble carbohydrates and a significant negative correlation with leaf proline. The amount of fruit chlorophyll *b* also showed a significant positive correlation with total fruit chlorophyll (r= 0.99**) and the amount of fruit carotenoids showed a significant negative correlation with fruit anthocyanins.

The results showed that leaf anthocyanins (48%), fruit length (47%), and peduncle length (45%) had the highest coefficient of phenotypic variation. Most of the studied traits including leaf anthocyanins (47%), fruit length (46%), and peduncle length (43%) had also the highest coefficient of genetic variation. In general, the mentioned traits plus fruit carotenoids and leaf phenols had the highest coefficients of phenotypic and genetic changes compared to other traits, respectively indicating high selection efficiency for these traits and better identification of desirable and undesirable genotypes from each other in this plant breeding.

The present research results also showed that heritability in most of the studied traits such as fruit chlorophyll *b*, fruit or leaf anthocyanins, fruit length, fruit carotenoids or flavonoids or phenols, leaf phenols or carotenoids, fruit weight, petiole length, total fruit chlorophyll, fruit proline, leaf width, and fruit tissue firmness was more than 90%, probably due to the uniformity of the test medium. The highest genetic gain was obtained for leaf anthocyanins and fruit length (97% and 93%, respectively) and the lowest one was obtained for length-to-leaf width ratio and leaf chlorophyll *b*.

4. Discussion

The present research proved a high bitter cucumber genotype diversity based on most studied morphological traits. The genotype *ON3* had the highest internode length and stem thickness among the studied genotypes. *BO* is the earliest genotype with the fewest days until the appearance of the first male or female flower and fruit. In addition to the fruits, the leaves of this species are also used, so high morphological diversity in these two organs, which play a key role in determining the vegetative or reproductive yield of the plant, can be used to select and breed to achieve the desired genotypes.

Balkaya et al. (2010) reported a great diversity between the *Cucurbita maxima* populations especially based on weight, length, diameter, shape, color brightness, flesh thickness, and grain cavity length of the fruits. Aliu et al. (2011) showed that *Cucurbita pepo* fruit traits including shape, weight, peduncle length, and fruit flesh thickness had the highest diversity between the studied populations.

In general, the study of the growth characteristics of different populations of plants is one of the basic and important factors in determining the best genus and species compatible with the cultivation region (Babalar *et al.*, 2013). Vegetative and morphological characteristics diversity could be attributed to various

factors like the plant growth environment (Gul *et al*., 2021). Since bitter cucumber is propagated by seeds, factors such as mutations and natural plant selections could play a role in creating genetic diversity in this species in addition to pollination and fertilization factors.

Plant morphology is influenced by genetics and environment (Gul *et al*., 2021). The environmental effects on the plant mostly involve phenotypic changes, although in some cases they also include genetic changes. Natural selection and mutations are of the genetics and environmental interactions that affect the plants to make new genotypes and phenotypes (Crossa *et al*., 2010; Nicotra *et al*., 2010). Plants respond differently depending on the conditions and environment in which they grow. For example, to tolerate drought, salinity, high pH, and element toxicity stress conditions, plants show changes in their morphological traits like leaf area reduction and ultimately growth decrease. Texture and soil elements of each region are reported to have effects on morphological characteristics and diversity of medicinal plants (Tabrizi and Kouchaki, 2014). According to the present study results, different bitter cucumber genotypes differed based on the amount of phenolic compounds and total flavonoids. In the grouping, genotypes in the first group had the highest fruit or leaf phenols and leaf flavonoids indicating more medicinal value and therapeutic properties of this group. Also, genotypes in this group had high quality in terms of important quantitative traits such as fruit weight (highest amount) and fruit quality determining traits (highest TSS and lowest fruit tissue firmness). The latest genotypes with the highest number of days until the first fruit formation were placed in the second group which can be suitable for cultivation in areas with a long growing season.

In the present study, main stem internode length correlated positively and significantly with main stem thickness, length and leaf blade width, depth and lobe width, and fruit length. Since the shape and leaf area are the main factors in increasing the efficiency of photosynthesis, therefore, the morphology and anatomy characteristics of the leaf can affect plant growth (Wilson *et al*., 1999). A decrease in leaf area causes plant growth reduction (Li *et al*., 1998). Also, the vegetative growth and reproductive organ growth quality of plants are closely related to each other. An increase in dry matter production in leaves causes fruit growth characteristics to increase (Smith and Stitt, 2007).

As we found in our study, the values of the phenotypic and genetic diversity coefficients were almost close to each other for most of the traits, which indicates that variation between the studied genotypes is mostly due to the genetic effects. The more differences between phenotypic and genetic diversity coefficients for a trait, the more environmental effect on that trait. The genetic variation coefficient, taking heritability into account, provides a good estimate through phenotypic selection. Heritability along with genetic progress are selection parameters that, if used together, are highly effective in cultivars development. Heritability is the most important parameter in genetic studies of quantitative traits (Ajmera *et al*., 2017) and plays a vital role in choosing a specific trait (Lotfi Aghmioni *et al*., 2015). Heritability is considered an indicator of the transferability of traits from parents to children. High heritability of traits proves low environmental effects on the traits (Ajmera *et al*., 2017). High heritability of traits also indicates that these traits are controlled by fewer genes and traits with low heritability are controlled by more genes with low effects. Researchers have shown that high heritability is not always associated with high genetic progress. Therefore, since high heritability does not indicate high genetic gain, it is recommended that heritability be considered along with genetic improvement to predict the effects of superior variety selection (Ogunniyan and Olakojo, 2014).

The importance of using medicinal plants and their products, including herbal medicines and functional foods, has increased especially in the last decade in developing and developed countries. Therefore, paying attention to the quality, safety, and effectiveness of plant products requires sufficient and extensive researches in this field. A study on the hydroalcoholic extracts of bitter cucumber in the present research showed that this plant contains significant amounts of phenolic compounds, especially total flavonoids, which confirms the medicinal use of this plant in the region and can be a key clue to extracting secondary medicinal compounds by examining its medicinal effects. In Iran, which is suitable for different plant species cultivation due to the variety of climatic conditions, paying attention to medicinal plant

production and their use in factories producing medicinal products and functional foods has led to the production of high-quality products. It not only encourages domestic consumers to use them but also makes it possible to compete with global products and export them.

It could be suggested that the genotypes of this experiment, in addition to agronomic traits, be studied molecularly, and the obtained polymorphic information be used to obtain genetic parameters in breeding programs of this species.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All data are embedded in the manuscript.

Authors' contributions

All authors have an equal role in designing the idea, doing, analyzing, and writing the article.

Informed consent

The authors declare not to use any patients in this research.

Funding/Support

This study was supported by Tarbiat Modares University (TMU), Iran.

Acknowledgement

This article was achieved based on the financial support of Tarbiat Modares University (TMU) for which the authors are grateful.

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HOW TO CITE THIS ARTICLE

Looregipoor F., Hadi N., Shojaeiyan A. 2023. Study on Quantitative and Qualitative Traits Diversity in Some *Momordica charantia* L. Genotypes. *Agrotechniques in Industrial Crops* 3(4): 211-222. 10.22126/ATIC.2023.9735.1118