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Genetic diversity among Iranian *Citrullus colocynthis* accessions using morphological and phytochemical characteristics

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Abstract

Objective: Colocynth (*Citrullus colocynthis* L. Schrad.) is an important medicinal and oilseed species adapted to arid and semi-arid environments. This study aimed to characterize the morphological and phytochemical diversity among Iranian *C. colocynthis* accessions and identify superior genotypes with desirable yield and nutritional traits.

Methods: Seventeen accessions, collected from different geographical regions of Iran, were evaluated under field conditions using a randomized complete block design with three replications. Morphological traits related to fruit and seed characteristics, along with phytochemical attributes- including fatty acid composition, total phenolic content, and carotenoid concentrations- were analyzed using several statistical methods, including analysis of variance, principal component analysis, and hierarchical clustering. Correlation coefficients among the morphological and phytochemical characteristics were also computed.

Results: Significant variation was observed among the colocynth accessions for all of the evaluated traits, indicating substantial genetic diversity within the Iranian colocynth germplasm. The Jiroft, Khorasgan 5, Arak, and Orzoeeh accessions exhibited superior performance for fruit yield, and fruit number. Seed oil analysis revealed the predominance of unsaturated fatty acids, particularly linoleic acid, with the highest concentration detected in the Orzoeeh accession (73%), followed by Arak (72%), Hormozgan (72%), Jiroft (71%), and Khorasgan 5 (71%). Higher total phenolic content was observed in the Kerman and Yazd accessions, while the Ahvaz, Jiroft, and Arak accessions exhibited higher total carotenoid levels, suggesting a higher antioxidant potential and adaptation to environmental stress conditions. Principal component and cluster analyses confirmed the divergence among the colocynth accessions and differentiated them based on combined agronomic and phytochemical characteristics.

Conclusion: This study represents one of the first evaluations integrating the morphological and phytochemical characteristics of Iranian colocynth germplasm. The results demonstrate substantial genetic diversity within Iranian colocynth germplasm for morphological and phytochemical characteristics. The high proportion of unsaturated fatty acids, particularly linoleic acid, further emphasizes the nutritional and pharmaceutical value of this germplasm. The identified superior accessions may serve as valuable genetic resources for breeding programs targeting drought tolerance, medicinal quality, antioxidant capacity, and seed oil improvement under arid climatic conditions.

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Introduction

Colocynth (*Citrullus colocynthis* L. Schrad.), also known as bitter melon, is one of the most important medicinal and therapeutic species of the *Cucurbitaceae* family, the largest family in the order Cucurbitales, comprising approximately 750 species (Ferriol and Pico 2008). Members of this family exhibit remarkable ecological adaptability, and despite sharing the same chromosome number (2n = 40), are distributed across diverse climatic regions (Alfawaz 2004). Colocynth seeds contain both saturated and unsaturated fatty acids, with an oil content of approximately 52%, as well as, high protein content and significant health benefits (Akobundu *et al*. 1982; Montes-Hernández *et al*. 2005). Previous studies have confirmed the abundance of polyunsaturated fatty acids, particularly linoleic acid, in colocynth seed oil (Ziyada and Elhussien 2008).

Various plant organs, especially seeds and pulp, possess pharmacological properties and are traditionally used for medicinal purposes (Alfawaz 2004). The seeds are particularly valued for their potential antidiabetic effects (Benariba *et al*. 2013). Phytochemical investigations have demonstrated that colocynth seeds contain vitamins, minerals, dietary fiber, antioxidant compounds, and bioactive secondary metabolites with free radical scavenging activity (Nayab *et al*. 2006; Tannin-Spitz *et al*. 2007; Marzouk *et al*. 2010). Phenolic and flavonoid compounds are recognized as major contributors to this antioxidant activity (Benariba *et al*. 2013). Furthermore, phenolic accumulation in cucurbit species has been reported to vary among plant organs and genotypes (López *et al*. 2016).

Morphological and phenotypic descriptors remain fundamental tools for the characterization and classification of cucurbit germplasm (Lazos 1986; Morovati *et al*. 2024). Fruit and seed characteristics, including size, shape, color, and structural attributes, are particularly valuable for distinguishing among genotypes and assessing genetic diversity (Balkaya *et al*. 2010). Considerable phenotypic variation has been reported in colocynth accessions, including differences in fruit morphology, seed dimensions, and developmental traits.

Iran represents an important center of diversity for cucurbit species due to its broad range of ecological and climatic conditions. Therefore, the evaluation of Iranian colocynth germplasm may provide valuable genetic resources for breeding and domestication programs.

The objective of the present study was to investigate morphological and phytochemical diversity among Iranian *C. colocynthis* accessions and to identify superior genotypes with desirable agronomic and medicinal characteristics.

Materials and Methods

Plant materials and experimental design

Seventeen colocynth accessions were collected from different regions of Iran (Table 1). Seeds were planted at the Research Station of the Department of Horticultural Sciences, University of Tehran, Tehran, Iran, in a field with clay-loam soil. The experiment was conducted as a randomized complete block design with three replications. Single-row plots (5.6 m^2) with four plants were used for each accession in each block. The row-to-row and intra-row spacing were 2 m and 70 cm, respectively. All four plants in a plot were used for measuring each trait. Standard agronomic practices, including drip irrigation and weed control (using 1.5 g/L paraquat dichloride at the 6-7 leaf stage), were performed. Disease management was conducted using the captan fungicide at 1.5 g/L. Fruits were harvested at physiological maturity when approximately 75% of the fruit surface had turned yellow.

Morphological evaluation

Morphological characterization was performed according to cucurbit descriptor guidelines (Esquinas-Alcazar and Gulick 1983). Fruit and seed traits in each accession were recorded weekly. The evaluated traits included the number of fruits per plant, fruit weight, fruit yield per plant, and seed weight.

Table 1. Codes and geographical collection site characteristics for the seventeen *Citrullus colocynthis* accessions evaluated in this study.

Code	Site name	Longitude	Latitude	Code	Site name	Longitude	Latitude
1	Kerman	56°54′33.05″ E	29°25′11.50″ N	10	Khojir	51°50′41.47″ E	35°28′42.58″ N
2	Khorasgan 2	51°19′41.25″ E	33°43′25.57″ N	11	Mashhad	59°24′38.27″ E	36°16′44.95″ N
3	Khorasgan 4	50°37′30.00″ E	33°01′35.44″ N	12	Hormozgan	56°01′48.98″ E	27°19′55.65″ N
4	Khorasgan 5	53°07′47.58″ E	32°59′22.78″ N	13	Ahvaz	48°44′07.27″ E	31°39′10.07″ N
5	Khorasgan 7	51°38′08.67″ E	31°34′40.62″ N	14	Roodehen	52°29′33.63″ E	35°45′50.63″ N
6	Yazd	54°08′26.25″ E	31°50′23.79″ N	15	Isfahan	51°35′30.47″ E	32°41′39.44″ N
7	Shahrkord	50°51′37.08″ E	32°19′41.55″ N	16	Orzoeeh	56°18′57.30″ E	28°17′40.40″ N
8	Shiraz	52°28′14.53″ E	29°11′23.76″ N	17	Jiroft	57°49′55.31″ E	28°22′52.07″ N
9	Arak	49°35′32.23″ E	34°00′38.56″ N				

Phytochemical analysis

After fruit harvest, phytochemical traits such as fatty acids (oleic, stearic, palmitic, and linoleic), total carotenoids, and total phenols were measured. Multiple samples were collected from each plot to ensure experimental accuracy.

Fatty acids**:** Seed oil extraction was performed using the chloroform:methanol (2:1 v/v) method described by Folch *et al.* (1957). Fatty acid methyl esters (FAMEs) were prepared according to Joseph and Ackman (1992) and analyzed using gas chromatography equipped with a flame ionization detector. The major fatty acids quantified included: linoleic acid, oleic acid, palmitic acid, and stearic acid.

Total phenols**:** Total phenolic compounds were determined using the Folin–Ciocalteu method with gallic acid as the standard (Marinova *et al*. 2005; Maran *et al*. 2013). Absorbance was measured at 725 nm using a UV/Vis spectrophotometer.

Total carotenoids**:** Total carotenoids were quantified spectrophotometrically at 470 nm after extraction with 80% acetone following the method of Thaipong *et al.* (2006).

Statistical analyses

Analysis of variance (ANOVA), principal component analysis (PCA), and cluster analysis were conducted, and correlation coefficients were calculated, using SPSS software (version 19). Ward's hierarchical clustering method, using the standardized data and Euclidean distance, was performed to classify the colocynth accessions based on combined morphological and phytochemical traits.

Results and Discussion

ANOVA showed significant differences among the evaluated accessions of colocynth for all of the morphological and phytochemical traits, including fruit yield per plant, fruit number, fruit weight, fruit length, fruit width, central mesocarp diameter, seed mesocarp diameter, pericarp diameter, seed weight, seed length, seed width, seed thickness, oleic acid, stearic acid, palmitic acid, linoleic acid, total phenols, and total carotenoids (table not shown). This indicates the presence of substantial genetic variability within the Iranian *C. colocynthis* germplasm for the evaluated characteristics and demonstrates the potential usefulness of these traits for germplasm characterization and future breeding programs.

Morphological traits

Fruit morphology varied considerably among accessions. They exhibited relatively similar shapes; however, considerable variation was observed in fruit color and size among accessions (Figure 1). A study conducted in the United Arab Emirates by Shahid and Rao (2014), reported significant variation in fruit and seed shape among 25 colocynth accessions.

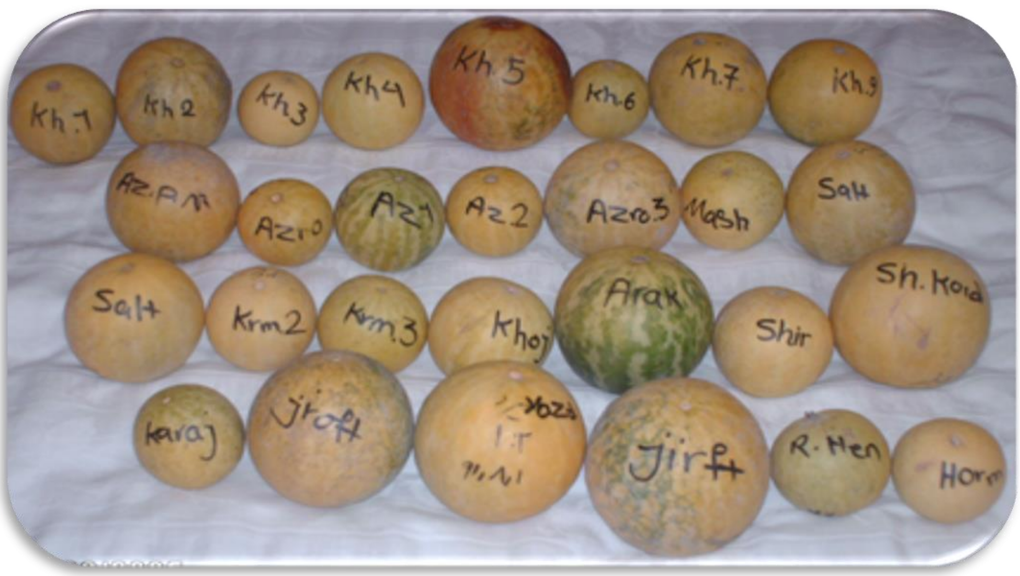

Figure 1. Variation in fruit size and color among *Citrullus colocynthis* (colocynth) accessions.

Here, we only present the mean comparisons among *C. colocynthis* accessions for the more important morphological traits, including the number of fruits per plant, fruit weight, fruit yield per plant, and seed weight (Figures 2-5). Orzoeeh, Jiroft, and Khorasgan 5 accessions produced the highest number of fruits per plant (42, 35, and 35, respectively), followed by Arak and Shiraz accessions (both 30 fruits) (Figure 2). Chawgien and Kiattisin (2021) reported that increased fruit number in desert cucurbits is frequently associated with improved assimilate translocation efficiency and enhanced drought tolerance. Khorasgan 5 exhibited the highest fruit weight of 182 g, followed by the Shahrekord (150 g), Khorasgan 7 (141 g), and Arak (140 g) accessions (Figure 3). Jiroft and Khorasgan 5 also showed the greatest fruit yield per plant (4300 and 3710 g, respectively), followed by Arak (3500 g) and Orzoeeh (3050 g) accessions (Figure 4). Although the Orzoeeh accession produced the highest number of fruits per plant, its fruit yield was lower than that of Jiroft and Khorasgan 5 accessions. In contrast, the Khorasgan 5 accession exhibited the highest fruit weight, indicating genotype-specific allocation of assimilates toward fruit enlargement rather than fruit number. Similar trade-offs between fruit size and fruit number were previously described by Achigan-Dako *et al.* (2021) in *Cucurbitaceae* breeding studies. Overall, the observed variation among accessions for fruit characteristics reflects a relatively high level of genetic heterogeneity within the studied accessions.

Significant variation was also observed in seed weight (Figure 5). The Khorasgan 4 and Hormozgan accessions showed the highest seed weight (49 and 43 g, respectively), followed by the Roodehen and Jiroft accessions (42 and 40 g, respectively).

Phytochemical characteristics

Substantial variation was observed among accessions for phenolic compounds, carotenoids, and fatty acid profiles. The Yazd accession had the highest palmitic acid content (11.4%). The Khojir accession exhibited greatest oleic acid concentration (19.6%). Khojir and Khorasan 2 had the highest stearic acid content (8.3%), followed by Roodehen (7.6%), Khorashan 5 (7.3%) and Jiroft (7.3%) accessions (Figure 6). Regarding linoleic acid content, Orzoeeh was the best-performing accession in terms of this fatty acid (72%), followed by Hormozgan (72%), Arak (72%), Isfahan (71.4%), Ahvaz (71.4%), Shahrekord (71%), Jiroft (71%), Khorasgan 5 (71%), and Khorasgan 7 (71%) (Figure 7). Similar results were previously documented by Bourhia *et al.* (2021), who reported that *C. colocynthis* seed oil is rich in unsaturated fatty acids, particularly linoleic acid. The predominance of linoleic acid in most accessions highlights the nutritional and medicinal importance of Iranian colocynth germplasm.

High accumulation of unsaturated fatty acids is considered an important adaptive mechanism in plants because these compounds maintain membrane fluidity and cellular stability under heat and drought stress. Thamer and Thamer (2023) emphasized that high concentrations of linoleic and oleic acids improve the nutritional, pharmaceutical, and industrial value of colocynth seed oil.

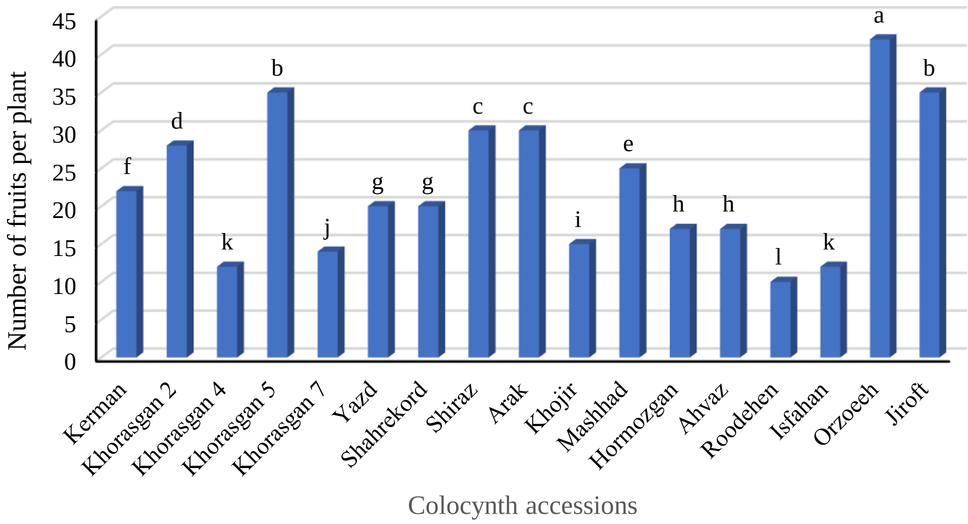

Figure 2. Fruit number per plant in *Citrullus colocynthis* accessions. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

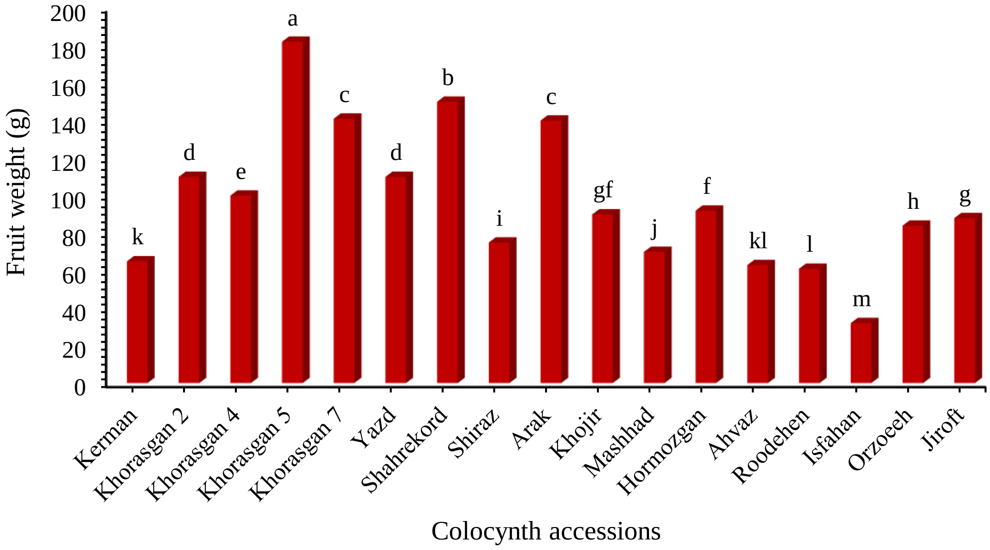

Figure 3. Fruit weight in *Citrullus colocynthis accessions.* Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

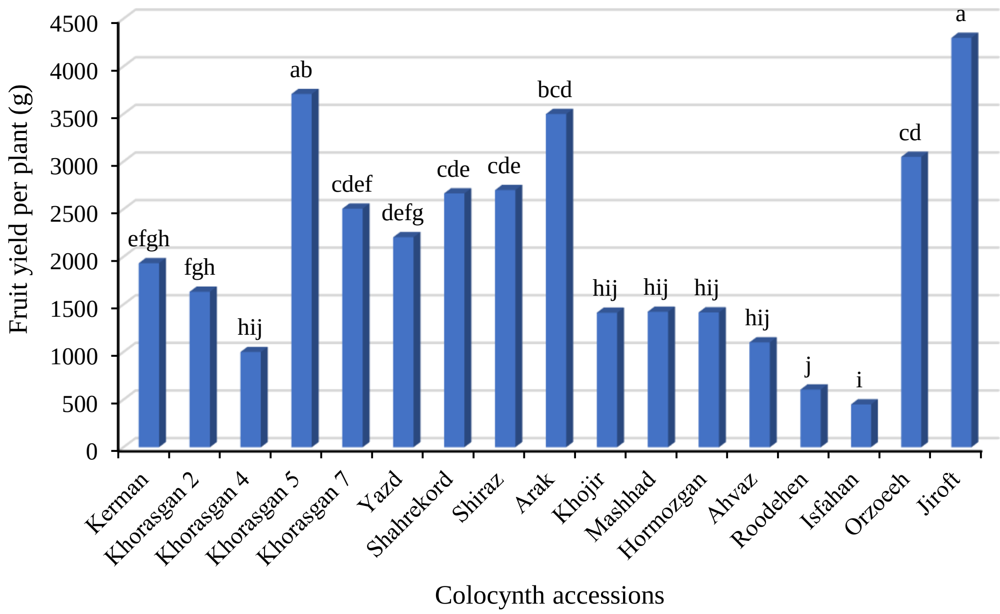

Figure 4. Fruits yield per plant in *Citrullus colocynthis* accessions. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

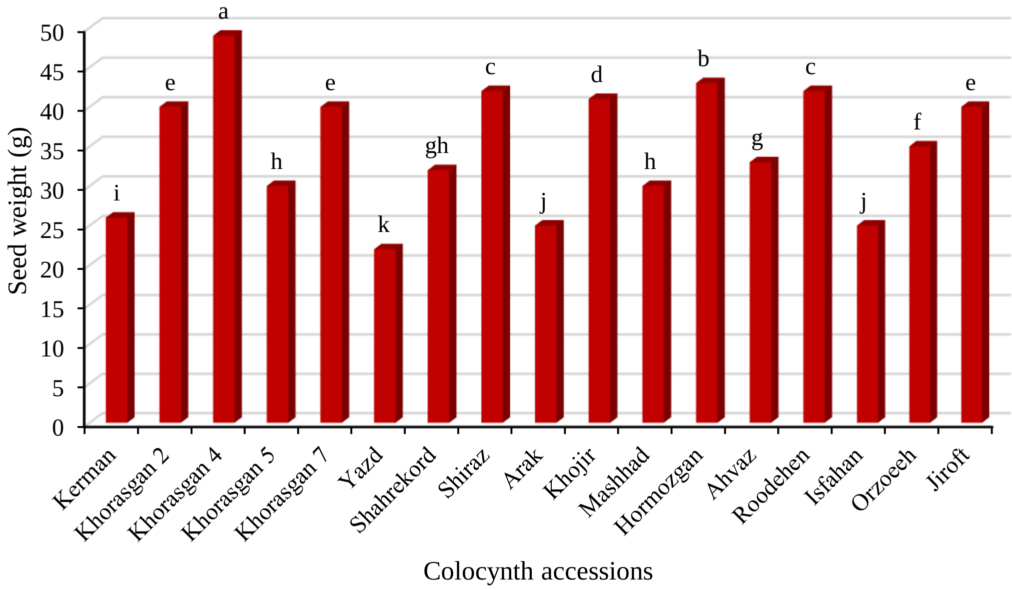

Figure 5. Seed weight in *Citrullus colocynthis* accessions. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

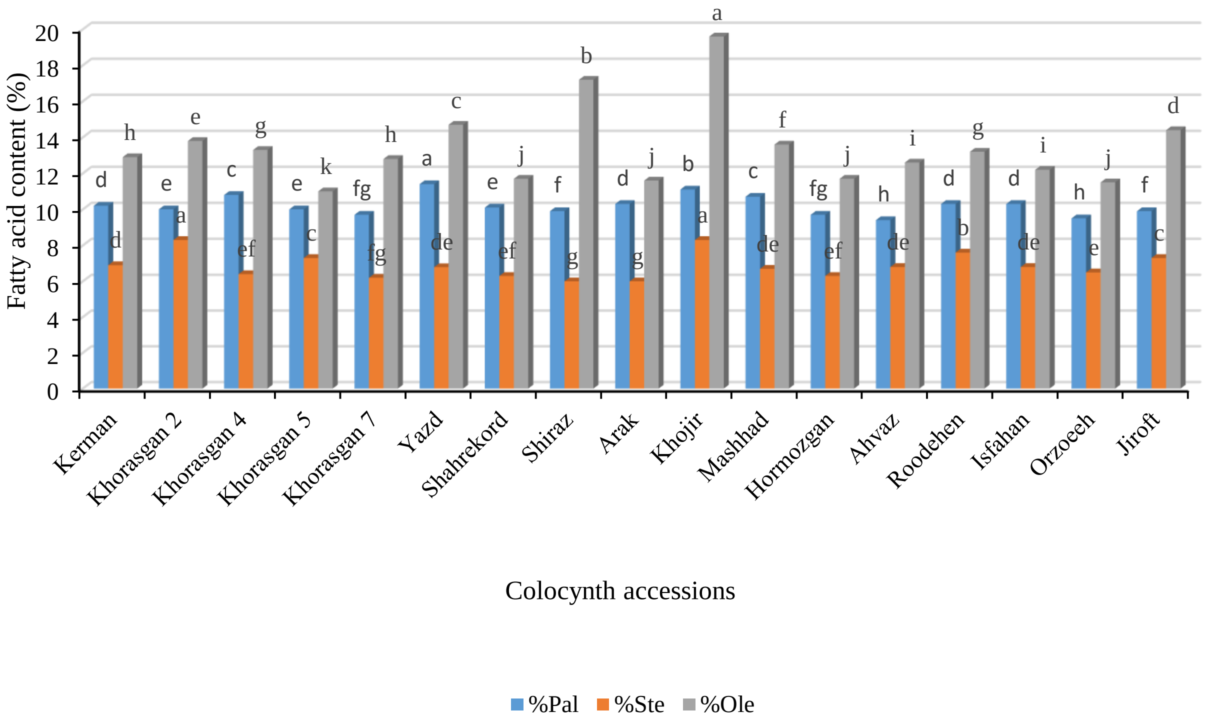

Figure 6. Fatty acid content (%) in seeds of *Citrullus colocynthis* accessions. Pal: Palmetic acid, Ste: Stearic acid, Ole: Oleic acid. Different letters within each fatty acid type indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

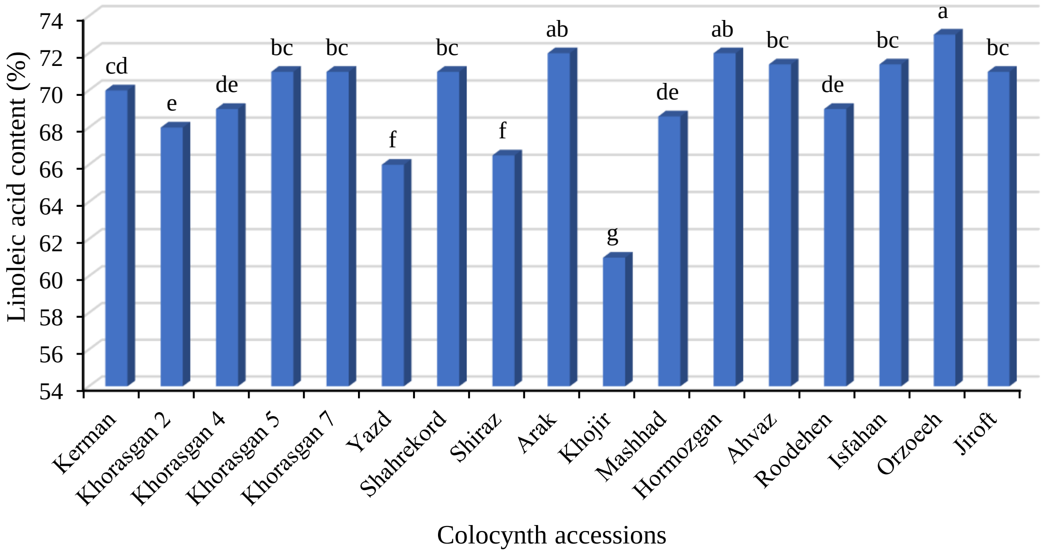

Figure 7. Linoleic acid content (%) in seeds of *Citrullus colocynthis* accessions. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

Significant variation was observed in the total phenolic content of colocynth. Phenolic compounds function as important antioxidants by scavenging reactive oxygen species generated under environmental stress conditions (Fathiazad *et al.* 2010). The Kerman and Yazd accessions exhibited the highest total phenolic content (88.10 and 70.24 mg/g GAE, respectively), reflecting their strong antioxidant capacity (Figure 8). Fathiazad *et al.* (2010) demonstrated that medicinal plants growing under severe drought and high solar radiation accumulate phenolic compounds as protective antioxidants. Environmental stress conditions activate oxidative defense pathways and stimulate phenolic biosynthesis in xerophytic species. Therefore, the higher phenolic concentration observed in the Kerman and Yazd accessions likely reflects their adaptation to harsh environments.

Ahvaz and Jiroft accessions exhibited the highest carotenoid concentrations (89.4 and 87 mg/g DW, respectively), followed by Arak (83 mg/g DW) (Figure 9). Carotenoids are essential antioxidant pigments in photosynthetic tissues, involved in photoprotection and reactive oxygen species scavenging (Ashraf *et al.* 2011). Thus, the higher total carotenoid accumulation in the Arak and Ahvaz accessions may enhance photoprotection and oxidative stress tolerance, since carotenoids play key roles in protecting plants against photooxidative damage. Elnaggar *et al.* (2024) reported that carotenoid accumulation in *C. colocynthis* increases significantly under heat and drought stress conditions, thereby enhancing oxidative stress tolerance. They concluded that high-carotenoid medicinal plant genotypes possess greater nutraceutical and pharmaceutical value due to their higher antioxidant properties.

Correlations among morphological and phytochemical traits

There were several significant relationships among the evaluated morphological and phytochemical traits (Table 2). Fruit yield per plant exhibited a high and moderately high positive correlation with fruit number (r = 0.85) and fruit weight (r = 0.58), respectively, indicating that both traits contributed substantially to fruit yield in the evaluated accessions. These traits may be used in breeding programs to indirectly select for fruit yield in colocynth. Fruit weight was positively associated with central mesocarp diameter (r = 0.69), suggesting that increased mesocarp development contributes directly to fruit mass accumulation.

According to Table 3, among phytochemical traits, linoleic acid showed a strong negative correlation with oleic acid (r = −0.93), reflecting the close metabolic relationship between these unsaturated fatty acids due to the activity of fatty acid desaturase enzymes during lipid biosynthesis (Bourhia *et al*. 2021; Thamer and Thamer 2023). Total phenolic content was positively correlated with total carotenoids concentration (r = 0.52), suggesting coordinated antioxidant accumulation in accessions adapted to arid environmental conditions.

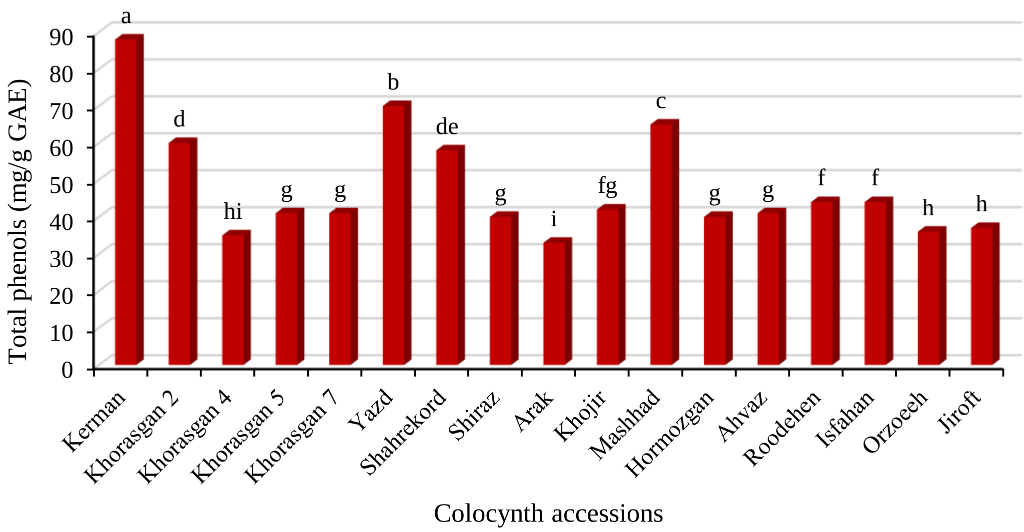

Figure 8. Total phenols in *Citrullus colocynthis* accessions. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

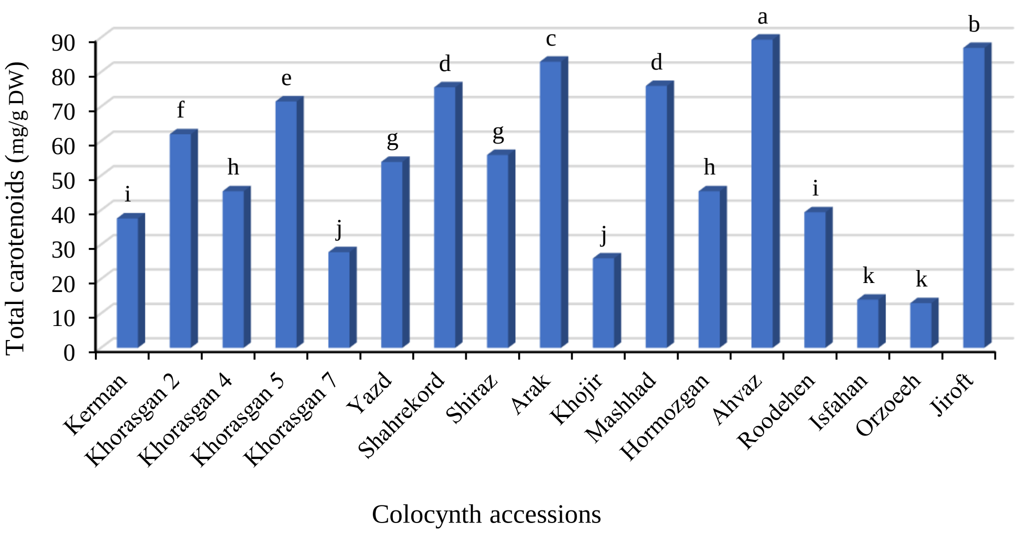

Figure 9. Total carotenoids in *Citrullus colocynthis* accessions. Different letters indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

Table 2. Pearson correlation coefficients among selected morphological and phytochemical traits of *Citrullus colocynthis* accessions.

Trait	FN	FW	FY	CMD	SW	LA	OA	Ph	Car
Fruit number (FN)	1								
Fruit weight (FW)	0.31	1							
Fruit yield per plant (FY)	0.85**	0.58**	1						
Central mesocarp diameter (CMD)	0.12	0.69**	0.42*	1					
Seed weight (SW)	-0.18	0.21	0.09	0.17	1				
Linoleic acid (LA)	0.24	-0.11	0.19	0.08	-0.06	1			
Oleic acid (OA)	-0.09	0.14	-0.12	-0.16	0.05	-0.93**	1		
Total phenols (Ph)	0.28	-0.17	0.11	0.09	-0.22	0.44*	-0.39	1	
Total carotenoids (Car)	0.17	0.26	0.21	0.13	-0.15	0.31	-0.18	0.52*	1

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

Principal component analysis

PCA revealed that the first three principal components explained 81.21% of the total variation among the evaluated *C. colocynthis* accessions, indicating a high level of efficiency in summarizing the phenotypic diversity of the germplasm (Table 5). The first principal component (PC1), accounting for 43.81% of the total variance, was primarily associated with fruit-related morphological traits, including fruit weight, fruit yield, fruit number, central mesocarp diameter, and pericarp diameter. This component, therefore, represented the overall fruit productivity and fruit size potential of the accessions. The second principal component (PC2), which explained 24.29% of the total variance, was mainly influenced by phytochemical and seed-related traits, particularly linoleic acid, total phenolic content, total carotenoid concentration, seed length, and seed weight. Positive loadings of linoleic acid and phenolic compounds on PC2 suggest that this axis reflects the medicinal and nutritional quality of the accessions. In contrast, oleic acid exhibited a high negative loading on PC2, indicating an inverse relationship between oleic and linoleic acid accumulation, which was also indicated by their strong negative correlation coefficient. The third principal component (PC3) contributed 13.11% of the total variance and was moderately associated with total carotenoids and pericarp diameter, suggesting a secondary contribution of antioxidant-related traits and fruit structural characteristics to accession differentiation. Overall, the PCA results demonstrated substantial morphological and phytochemical divergence among Iranian colocynth accessions and confirmed the usefulness of combined agronomic and biochemical traits for germplasm characterization and selection in breeding programs.

Table 5. Coefficients of the three principal components for selected morphological and phytochemical traits in *Citrullus colocynthis* accessions.

Traits	**PC1**	**PC2**	**PC3**
Fruit number	0.71	-0.18	0.34
Fruit weight	0.91	0.06	-0.09
Fruit yield per plant	0.88	-0.11	0.21
Central mesocarp diameter	0.84	0.14	-0.08
Pericarp diameter	0.63	0.29	0.41
Seed weight	0.36	0.67	0.22
Seed length	0.18	0.81	-0.16
Linoleic acid	-0.22	0.83	-0.11
Oleic acid	0.09	-0.79	0.18
Total phenols	-0.14	0.76	0.31
Total carotenoids	0.27	0.69	0.42
Eigenvalue	4.82	2.67	1.44
Variance (%)	43.81	24.29	13.11
Cumulative variance (%)	43.81	68.10	81.21

Cluster analysis

The heatmap of the hierarchical clustering based on morphological and phytochemical traits of the Iranian *C. colocynthis* accessions is shown in Figure 10. The dendrogram classified the accessions into three major groups at a genetic distance of approximately 25 units. Accessions such as Orzoeeh, Jiroft, and Khorasgan 5 which were grouped in the first cluster, were mainly characterized by a higher fruit number, fruit yield, and linoleic acid content, indicating their superior agronomic and oil-quality potential. In contrast, accessions in the second group, such as Kerman and Yazd, showed higher total phenolic content, suggesting their stronger antioxidant capacity and possible adaptation to environmental stress conditions. Accessions in the third cluster exhibited higher levels of antioxidant-related compounds, indicating their potential value for pharmaceutical and nutraceutical applications. Overall, this clustering pattern indicates substantial divergence among the evaluated germplasm, which is likely associated with adaptation to different ecological and climatic conditions across various regions of Iran. Previous studies have also demonstrated that morphological and phytochemical markers are reliable tools for assessing genetic diversity in medicinal and oilseed plants (Mohammadi and Prasanna 2003).

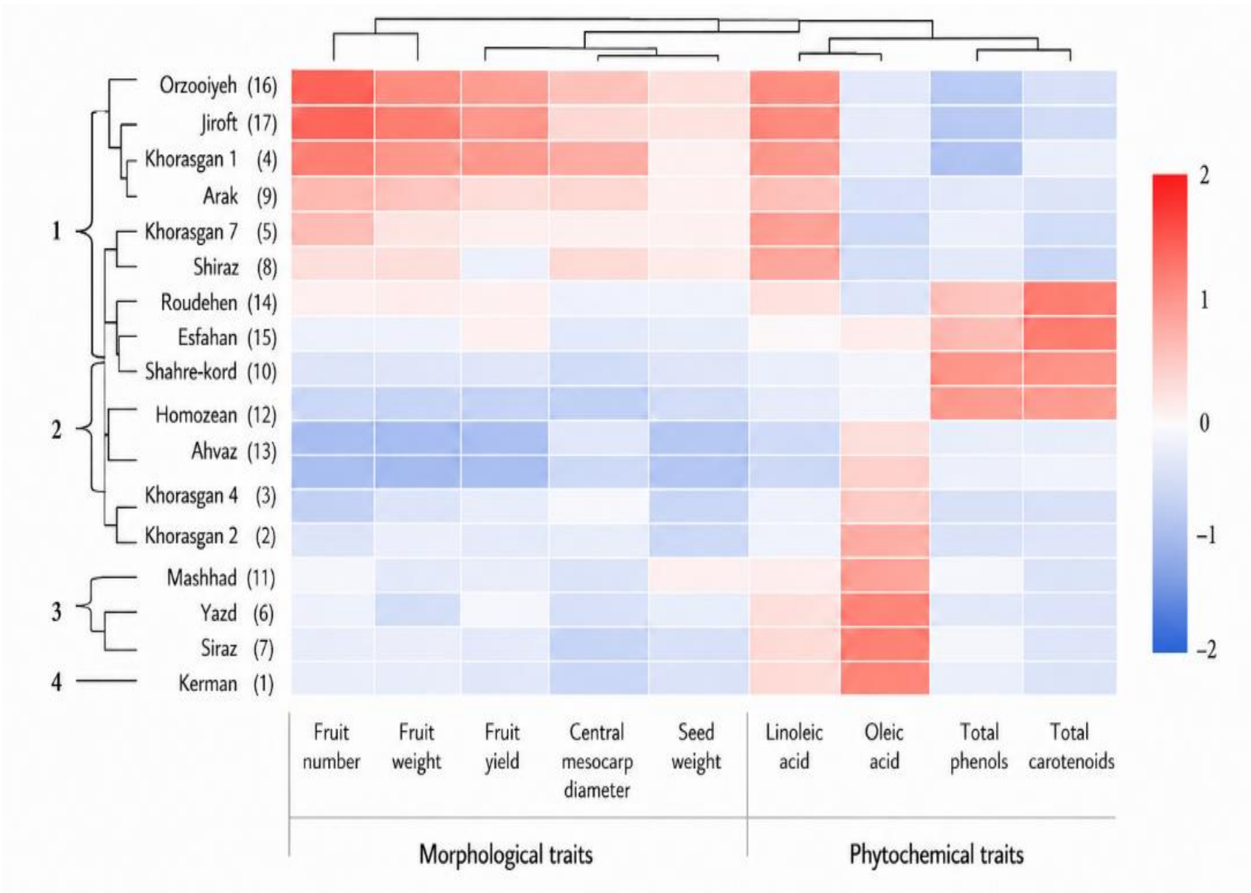

Figure 10. Heatmap of the hierarchical clustering of 17 *Citrullus colocynthis* accessions based on morphological and phytochemical traits using Ward's clustering method and Euclidean distance. The cophenetic correlation coefficient was 0.86 (Sneath and Sokal 1973). Red and blue colors indicate higher and lower values, respectively.

The present findings demonstrate that Iranian *C. colocynthis* germplasm represents a valuable genetic resource for breeding programs aimed at improving fruit yield, seed oil quality, and antioxidant properties under arid and semi-arid environmental conditions.

Conclusion

The present study revealed substantial morphological and phytochemical diversity among Iranian *C. colocynthis* accessions, indicating the existence of valuable genetic variation within this native germplasm. Significant differences among accessions in fruit yield, number of fruits, fruit weight, seed traits, fatty acid composition, phenolic compounds, and carotenoid content demonstrated strong potential for the identification of superior genotypes with agronomic and medicinal importance.

Accessions such as Jiroft, Khorasgan 5, Arak, and Orzoeeh showed superior performance for fruit yield, fruit number, and linoleic acid content. The high proportion of unsaturated fatty acids, particularly linoleic acid, further emphasized the nutritional and pharmaceutical value of the Iranian colocynth germplasm. In addition, Kerman and Yazd showed higher total phenolic content, and Ahvaz, Jiroft, and Arak exhibited higher total carotenoid concentrations.

Multivariate analyses, including PCA and cluster analysis, further confirmed the existence of considerable genetic divergence among the colocynth accessions and highlighted the usefulness of combined morphological and phytochemical traits for germplasm characterization and breeding programs.

Future studies should incorporate molecular and metabolomics analyses, together with multi-environment evaluations, to better identify stress-tolerant and high-value medicinal colocynth genotypes suitable for sustainable cultivation in arid and semi-arid regions.

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Ethical Considerations

The authors confirm that this study is original and that the data presented are authentic, avoiding any form of fabrication or falsification.

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this work.

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