Genotypic Variation of Seed Oil Content in Twelve Genotypes of *Citrullus colocynthis* from Morocco

Saïd El Madidi* and Abedlhamid Benmoumou

BVRN Laboratory, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

Received: 11 October 2019, Revised: 22 November 2019, Accepted: 2 January 2020

Abstract

Citrullus colocynthis (L.) Schrad (2n = 22) is a perennial herbaceous species of the family Cucurbitaceae. It has natural tolerance to drought with a deep root system and is widespread in the arid and semi-arid zones of Africa and the Mediterranean region. This plant can be considered as a new potential source of vegetable oil. Twelve accessions of *C. Colocynthis*, collected from different regions of Morocco, were studied for seed and oil yields. The average yields obtained under non-optimal growing conditions are 479 kg/ha for seed yield and 99 kg/ha for oil yield. Significant genotypic variability was observed for seed oil content, seeds yields and oil yields. Heritability and genotypic advance are moderate to high for the characters measured. The exploitation of this variability could be used for the selection of accessions that have good oil yields in limited growing conditions.

Keywords: *Citrullus colocynthis*, seeds yield, oil yield, oil content, oil composition
DOI 10.14456/cast.2020.1

1. Introduction

Citrullus colocynthis (L.) Schrad (2n = 22), is a related species of domesticated watermelon (*Citrullus lanatus var. Lanatus*) [1], it is a perennial herbaceous species of the family Cucurbitaceae that is well adapted to arid zones through its tolerance to drought with a deep root system. It is widespread in the Sahara Desert in Africa and in the Mediterranean region where it grows spontaneously [2, 3]. *Citrullus colocynthis* is widely used for therapeutic purposes in Mediterranean countries and contains many bioactive compounds that are of functional importance. Its fruits are used for the treatment of many diseases, including diabetes, rheumatism, ulcers and cancer [4, 5]. In addition to these therapeutic uses, this plant can potentially be used as a source of biodiesel feedstock [6, 7]. It has also been shown that the seeds of this plant are nutritionally valuable with high protein and mineral content, and the seed oil is edible and has comparable characteristics to most conventional vegetable oils [5, 6, 8]. In recent years, the development of new oil seed crops that can be used as alternatives to conventional plants has generated a lot of interest and *C. colocynthis* is one of the plants that is able to adapt to arid conditions. Thus, its natural variability should be evaluated in order to select the best accessions. Several authors have already shown the existence of a great variability for agro-morphological characters and for the molecular polymorphism [9, 10]. The level of variability and heritability needed to aid selection of cultivars with improving traits and yield is scanty. The present study was undertaken to evaluate the level of genotypic variability and heritability for traits associated with seed yield for selection of high yielded accessions for production of seeds and oil.

*Corresponding author: Tel.: +212 52-822-0957 Fax: +212 52-822-0100
E-mail: s.elmadidi@uiz.ac.ma

2. Materials and Methods

The plant material includes several accessions that are collected from 12 different localities in Morocco (Figure 1). The experimental design was the randomized complete block design with three repetitions. For each plot, 3 plants were randomly selected and 5 fruits per plant were analysed with a total of 45 fruits analyzed for each genotype.The seeds were planted at 75 cm between the plants and 150 cm spacing between the lines. Each plot received a limited amount of water equivalent to 100 mm of irrigation water and no fertilizer or insecticide spraying was applied at any time for the duration of the trial.The measured traits were including fruit weight per plant (FW), seed weight per plant (SW), seed number per plant (SN), weight of 100 seeds per plant (W100) and oil content of seed (OC). For the extraction of the lipid components, the seeds were crushed and then extracted with n-hexane (200 ml) at 40-60°C in a soxhlet apparatus. The total extraction time was 6 h for each repetition. The composition of the oil in fatty acids was determined by Chromatography coupled with Mass Spectrometry [11, 12]. The phenotypic variance in each trial was estimated by:

$\hat{\sigma}_P^2 = \hat{\sigma}_G^2 + \frac{\hat{\sigma}_E^2}{n}$ and the Broad-sense heritability was estimated by: $\hat{\sigma}_G^2 / \hat{\sigma}_P^2$.

$\hat{\sigma}_P^2$ = phenotypic variance, $\hat{\sigma}_G^2$ = genotypic variance, $\hat{\sigma}_E^2$ = environmental variance (error mean square from the analysis of variance) and n = number of replications. The estimate of the genotypic gain was calculated by: $\Delta G_i = \mathrm{i}\ \hat{H}^2\ \hat{\sigma}_P$ where i= selection differential, the value is 1.40 at 20 % selection intensity. The relative genotypic gain ($R\Delta G_i$)was obtained by dividing the genotype gain by the average of the analyzed character. All statistical analyzes were performed using SAS version 9.3 software [13].

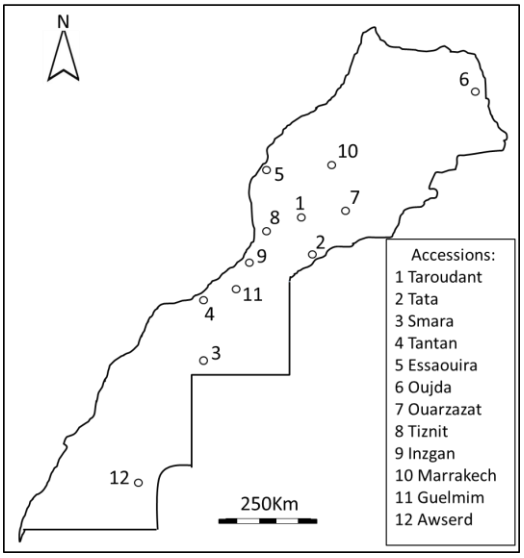

Figure 1. Origin of accessions of *Citrullus colocynthis* (L.) Schrad collected in several localities in Morocco

3. Results and Discussion

The data recorded for morphological and agronomic characteristics showed considerable variability among the accessions (Table 1). The fruits weight, seed number and seed weight per plant varied between 79 to 482, 37 to 682 and 1.94 to 38.25 g, respectively and seed oil content varied between 16.94% to 34.85% of seed weight. The coefficient of variation (CV) estimates ranged from 6.21% to 42.81%. Figure 2 shows the variability observed between the accessions for the seeds weight where accession 11 has the highest average value and accession 8 the lowest value.

Table 1. Descriptive statistics for the measured characters

Characters	Mean	Min	Max	SD	CV (%)
FW (kg/plant)	178.50	79.00	482.00	63.92	35.81
SN	222.15	37.00	682.00	90.68	40.82
SW(g/plant)	11.97	1.94	38.25	5.12	42.81
W100	5.32	0.00	9.40	0.80	15.12
OC (%)	24.75	16.94	34.85	1.78	6.21

FW: fruit weight, SN: seed number, SW: seed weight, W100: weight of 100 seeds, OC: oil content, SD: Standard deviation and CV: Coefficient of variation

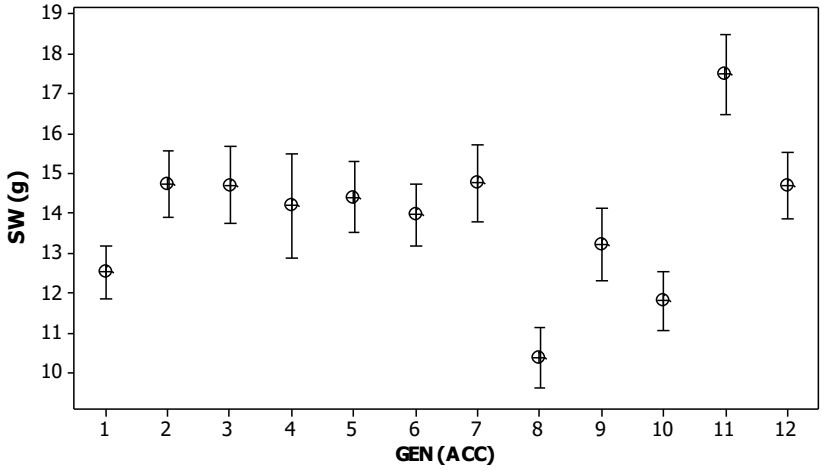

Figure 2. Variation of *C. colocynthis* seeds weight (sw, g) for different accessions (95% CI for the mean)

Heritability in the broad sense refers to the part of the genotype variance in the phenotypic variance of a trait and it is a statistical datum allowing an estimation by genetic factors in the expression of the character [14, 15]. The estimation of heritability is a first step in establishing a genetic evaluation for a given trait and the knowledge of this parameter is particularly important for the objective of selection. The $\hat{H}^2$ estimates ranged from 0.33 to 0.62 and the relative genotypic gain ranged from 10.23 to 24.68% (Table 2). These results indicated that the genotypic component played a relatively important role in the expression of these traits and its significant genetic progress could be obtained by selection based on phenotypic performance.

The comparison of accessions means showed highly significant differences (Table 3). The p-values were respectively equal to 0.0032 and 0.0065 for the seed yield and the oil yield respectively. For seed yield, average values were grouped into 3 homogeneous groups and ranged from 301.95 kg/ha (accession 4) to 673.68 kg/ha (accession 11). For oil yield, the average values were arranged into 4 homogeneous groups and varied between 63.85 kg/ha (accession 10) and 151.69 kg/ha (accession 11).

Table 2. The Broad-sense heritability and relative genotypic gain

Characters	$\hat{H}^2$	$R\Delta G_i$
Fruit weight	0.41	24.68%
Seed number	0.45	12.60%
Seed weight	0.43	10.23%
Weight of 100 seeds	0.33	14.73%
Oil content	0.62	18.23 %

Table 3. Means of seed and oil yields from 12 accessions of *Citrullus colocynthis* (L.) Schrad

Accessions	**Seeds yield (Kg/ha)**	**Oil yield (Kg/ha)**
4	301.95^{a}	64.57^{a}
10	342.95^{a}	63.85^{a}
7	345.37^{a}	67.93^{a}
8	361.82^{a}	77.10^{ab}
3	368.57^{a}	85.48^{abc}
1	465.70^{ab}	95.86^{bc}
5	485.28^{ab}	97.75^{c}
9	493.46^{ab}	114.60^{cd}
6	513.82^{b}	104.20^{cd}
2	629.46^{bc}	127.70^{cd}
12	642.94^{c}	132.10^{d}
11	673.68^{c}	151.69^{d}

Means within columns with different superscript are significantly different ($p < 0.01$) using the Duncan New Multiple Range Test (DMRT).

The fatty acid profiles of the seed oil showed an unsaturated fatty acid content of 74.10 % and the predominant fatty acid was linoleic acid (C18.2) in 60.06 %, followed by oleic acid (C18.1) (14.4%), palmitic acid (C16.0) (11.14%) and stearic acid (C18.0) (8.36%). The oil content and fatty acid compositions of the seed oil found in this study are in agreement with the results observed in other studies which showed a predominance of unsaturated fatty acids [6, 7, 16].

As indicated in Table 3, the analysis of the data revealed important differences between accessions, with seed yields ranging from the lowest value of 220.28 to the highest value of 815.76 kg per hectare and seed oil yield from 45.12 to 171.71 kg/ha (Table 4). The results of these yield observed in semi-arid conditions in this study without any fertilizer and in conditions of limited irrigation, remain relatively low compared to those potentially obtained under optimal growing conditions. Mertia and Gupta [17] reported the production of 4,400 kg of seed and 1,000 to 1,175 L/ha of colocynth vegetable oil in rainfed conditions. With plant density of four plants per m^2, the extrapolated annual seed yield among the accessions of *Citrullus colocynthis* ranged from 0.47 to 14.95 tons/ha with an overall mean of 5.17 tons/ha [18]. In the desert in Pakistan, some accessions of *C. colocynthis* developed an extensive root system despite of receiving only 35-40 mm of rainfall, yield can be as much as 1-1.5 t of seed/ha, but as much as 40-fold more if rainfall is higher [19].

Table 4. Descriptive statistics for the yield attributes

Yield	Mean	Min	Max	SD	CV (%)
Seed yield (kg/ha)	468.75	220.28	815.76	63.92	35.81
Oil yield (kg/ha)	98.57	45.12	171.71	29.81	36.29

Min: Minimum, Max: Maximum, SD: Standard Deviation, CV: Coefficient of Variation

4. Conclusions

All these results showed that the cultivation of *C. colocynthis* could be an important asset for the production of edible oil, especially under challenging conditions in arid and semi-arid lands. High yields can be achieved by finding the best growing conditions for proper production and selecting the best accessions selected for seed and oil yield with good tolerance to different abiotic stresses.

5. Acknowledgements

This work has received financial support from Ibn Zohr University and the valuable assistance of the management and technicians of the Sidi Bibi experimental center for the installation and monitoring of the field experience.

References

[1] Chomicki, G. and Renner, S.S., 2015. Watermelon origin solved with molecular phylogenetics including Linnean material: another example of museomics. *New Phytologist*, 205, 526-532.

[2] Si, Y., Dane, F., Rashotte, A., Kang, K. and Singh, N.K., 2010. Cloning and expression analysis of the Ccrboh gene encoding respiratory burst oxidase in *Citrullus colocynthis* and grafting onto *Citrullus lanatus* (watermelon). *Journal of Experimental Botany*, 61 (6), 1635-1642

[3] Wang, Z., Hu, H., Goertzen, L.R., McElroy, J.S. and Dane, F., 2014. Analysis of the *Citrullus colocynthis* transcriptome during water deficit stress. *PLoS ONE*, 9(8), e104657

[4] Hussain, A.I., Rathore, H.A., Sattar, M.Z.A., Chatha, S.A.S., Sarker, S.D. and Gilani A.H., 2014. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses and nutritional potential. *Journal of Ethnopharmacol*ogy, 155, 54-66.

[5] Marzouk, B., Marzouk, Z., Haloui, E., Fenina, N., Bouraoui, A. and Aouni, M., 2010. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. *Journal of Ethnopharmacology*. 128 (1), 15-19.

[6] Giwa, S., Abdullah, L.C. and Adam, N.M., 2010. Investigating "Egusi" (*Citrullus colocynthis* L.) seed oil as potential biodiesel feedstock. *Energies*, 3(4), 607-618.

[7] Bello, E.I. and Makanju, A., 2011. Performance evaluation of Egusi melon (*Citrullus colocynthis* L.) seeds oil biodiesel. *Journal of Emerging Trends in Engineering and Applied Sciences*, 2(5), 741-745.

[8] Mabaleha, M.B., Mitei, Y.C. and Yeboah, S.O., 2007. A comparative study of the properties of selected melon seed oils as potential candidates for development into commercial edible vegetable oils. *Journal of the American Oil Chemists' Society*, 84 (1), 31-36.

[9] Shahid, M. and Rao, N. K., 2014. Diversity of *Citrullus colocynthis* (L.) Schrad.(Cucurbitaceae) in the United Arab Emirates. *Journal on New Biological Reports*, 3, 145-150.

[10] Avval, S. E., 2017. Assessing polymorphism information content (PIC) using SSR molecular markers on local species of *Citrullus colocynthis*. Case study: Iran, Sistan-Balouchestan province. *Journal of Molecular Biology Research*, *7*(1), 42-49.

[11] Sparkman, O.D., 2005. Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy Robert P. Adams. *Journal of the American Society for Mass Spectrometry*, 16(11), 1902-1903.

[12] Roberts, L. D., McCombie, G., Titman, C. M. and Griffin, J. L., 2008. A matter of fat: an introduction to lipidomic profiling methods. *Journal of Chromatography* B, 871(2), 174-181.

[13] SAS Institute, 2010. *Statistical Analysis Software Version 9.3 User's Manual*., Cary, NC: SAS Institute Inc. USA.

[14] Nyquist, W.E., 1991. Estimation of heritability and prediction of selection response in plant population. *Critical Review in Plant Sciences*, 10, 235-322.

[15] Falconer, D.S. and Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*. 4th ed., London: Benjamin Cummings.

[16] Gurudeeban, S., Satyavani, K. and Ramanathan, T., 2010. Bitter apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. *Asian Journal of Plant Sciences*, 9(7), 394.

[17] Mertia, R.S. and Gupta, I.C., 1994. Cultivate the colocynth in waste lands. *Indian Horticulture*, April-June, 40-41.

[18] Menon, K., Sood, N. and Rao, N. K., 2016. Study of morpho-agronomic diversity and oil content in desert gourd (*Citrullus colocynthis* (L.) Schrad.). *Australian Journal of Crop Science*, 10 (7), 1000.

[19] Mahajan, S.S. and Kumawat, R.N., 2013. Study of seed dormancy in colocynth (*Citrullus colocynthis* L.) with after-ripening of fruits, seed extraction procedures and period of seed storage. *National Academy Science Letters*, 36, 373-378.