Evaluation of the variability of *citrullus colocynthis* (l) schrad as potential biodiesel feedstock: oil content, oil yield and the fatty acid composition

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Abstract. *Citrullus colocynthis* has been garnering interest in recent times as a potential biodiesel feed stockcrop due to its high seed oil content and its natural adaptation to drought The variability seed oil content (OC), Oil yield (kg/ha) (OY) and fatty acid composition were investigated for 12 accessions collected in different localities in Morocco. Analysis of the data revealed high variability among the accessions, with seed oil content ranging between 17.1 and 24.3 % of seed weight and Oil yield from 35.3 to 172.7 kg/ha. The predominant fatty acid of the seed oil was linoleic acid, C18:2, ranging from 67.0 to 73.0 % of total fatty acids and the average values of unsaturated fatty acids vary between 75.25 and 81.94 %.The wide range of variations found in this study for the traits measured offers the possibility of genetic improvement to develop cultivars for biodiesel production compatible with sustainable and ecological agriculture.

1 Introduction

The increasing awareness of the depletion and shortage of fossil fuel and many global environmental considerations led to the demand of renewable and friendly energy. *Citrullus colocynthis* (L.) Schrad (2n = 22), closely related to domesticated watermelon (*Citrullus lanatus* var. lanatus) [1], is a very drought-tolerant perennial herbaceous species in the Cucurbitaceae family. It is a wild native plant growing in arid areas, widely distributed in the Sahara-Arabian desert in Africa and the Mediterranean region [2, 3]. 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. This plant has a natural adaptation to drought and several studies have reported that this plant has a great potential for adaptation to drought with a tolerance to water deficit [4, 5, 6]. C. colocynthis being an exceptionally hardy plant with a potential for use as biodiesel feedstock [7, 8]. The plant was also shown to be rich in nutritional value with high protein contents and important minerals as well as edible quality of seed oil [9]. The main objective of this study is the assessment of the variability of 12 accessions of genetic material of Citrullus colocynthis (l) schrad for oil content, oil yield and fatty acid composition of seeds in the perspective that this plant has several arguments to be considered as a candidate potential for biodiesel production.

2. Materials and Methods

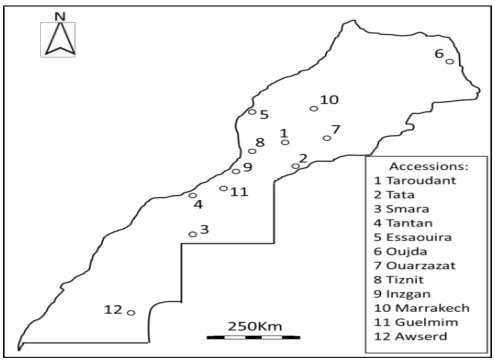

Fig. 1. Origin of accessions of Citrullus colocynthis (L.) Schrad collected in several localities in Morocco

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12 accessions of *C. colocynthis* collected from different regions from Morocco (Fig. 1). The Accessions were compared over two consecutive seasons in field trials presented in a randomized full block plan with three replicates. The measured traits were including seed oil content (OC) and the Oil yield (kg/ha) (OY). Seed oil content is extracted from 400 g of seeds from each plot with three replicates per accession. 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 hours for each repetition. The composition of the oil in fatty acids was determined by Chromatography coupled with Mass Spectrometry. Statistics analysis was carried out using computer software SAS version 9.3 (SAS Institute Inc. 2010)

3 Results

The oil yield varies between 35.27 and 171.70 kg/hectare while the seed oil content (OC) shows the lowest CV and the minimum and maximum are respectively 17.1 and 24.3 % (Table 1).

Table 1. Descriptive statistics of OC and OY

	Mean	Min	Max	SD	CV	SE
OC (%)	21.40	17.100	24.29	1.63	7.65	0.19
OY (Kg/ha)	89.84	35.268	171.70	34.99	38.95	4.12

OC : seed oil content, OY : Oil yield (kg/ha), Min : Minimum, Max : Maximum, SD : standard deviation: CV : coefficient of variation and SE : standard error.

The comparison of accessions means showed highly significant differences (Table 2). For the OC, average values are grouped into 5 homogeneous groups and ranged from 19.25±1.65 (accession 6) to 22.96±0.67 (accession 11). For oil yield, the average values are arranged also in 5 homogeneous groups and vary between 61.21±13.31 kg/ha (accession 10) and 146.97±30.08 kg/ha (accession 11).

Table 2. Variability of OC and OY according to the accessions

A	A 1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
OC	21.02 ±0.83 bc	21.04 ±1.4 bc	23.19 ±0.92 e	21.97 ±0.88 cde	21.09 ±1.52 bc	19.25 ±1.65 a	20.02 ±0.84 ab	21.78 ±1.05 cd	22.93 ±1.03 cde	20.12 ±1.73 ab	22.96 ±0.67 de	21.53 ±1.45 c
OY	84.88 ±19.8 2 abc	123.11 ±9.07 de	80.43 ±19.7 6 ab	63.43 ±10.8 7 ab	78.2± 23.86 ab	87.56 ±4.56 abc	73.97 ±20.7 9 ab	66±10 .75 ab	96.25 ±43.1 8 bcd	61.21 ±13.3 1 a	146.97 ±30.0 8 e	116.11 ±7.51 cde

OC : seed oil content, OY : Oil yield (kg/ha). a,b,c,d : means within columns with different superscript are significantly different ($p < 0,01$)

The analysis of the composition of the oils of *Citrullus colocynthis* by gas chromatography shows that the oil of the colocynth, taking into account all the accessions, is mainly composed of fatty acids: linoleic (C18: 2), acid oleic (C18: 1), stearic acid (C18: 0) and palmitic acid (C16: 0) with 66%, 13%, 10% and 10% respectively, the content of other fatty acids is less than 1% (Fig. 2).

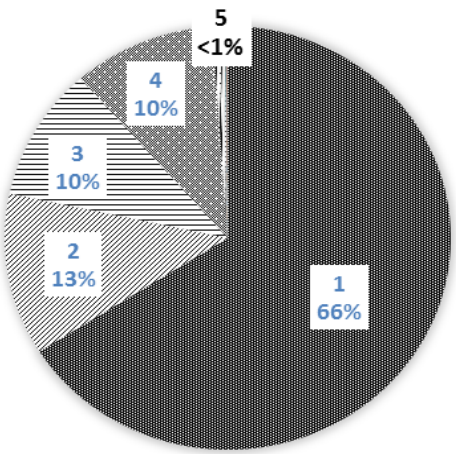

Fig. 2. Average fatty acid composition of seeds of *Citrullus colocynthis* : 1 : (C18:2), 2 : (C18:1), 3 : (C18:0), 4 : (C16:0) and 5 : others

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Table 3. Variability between the accessions depending on the most important fatty acids

	C16.0	C18.0	C18.1	C18.2	UFA	UFA /SFA
A1	11.72 f	12.37 d	13.87 bc	60.57 a	75.25 a	3.05 a
A2	9.46 abcd	11.30 bcd	11.81 ab	65.98 abc	78.61 abc	3.685 abc
A3	8.82 ab	9.88 abcd	11.48 ab	68.29 c	80.65 bc	4.22 cd
A4	9.82 bcde	9.37 abc	12.84 abc	66.58 bc	80.20 bc	4.07 bcd
A5	10.84 cdef	11.43 bcd	15.20 c	61.16 ab	77.07 ab	3.36 ab
A6	9.51 abcd	9.49 abc	11.13 ab	68.30 c	80.24 bc	4.18 bcd
A7	9.15 abc	9.53 abc	13.17 abc	66.74 bc	80.67 bc	4.19 bcd
A8	11.16 def	9.68 abc	11.28 ab	66.43 bc	78.50 abc	3.69 abc
A9	8.04 a	9.43 abc	11.50 ab	69.87 c	81.94 c	4.54 d
A10	11.96 f	8.89 ab	11.77 ab	65.85 abc	78.47 abc	3.67 abc
A11	8.79 ab	8.57 a	10.45 a	70.86 c	82 c	4.56 d
A12	11.33 ef	12.01 cd	13.39 bc	61.63 ab	75.95 a	3.19 a

a,b,c,d : Means within columns with different superscript are significantly different ($p < 0,05$)

The varability analysis of the fatty acid composition showed that the degree of unsaturation was over 75% and ranging from 75.25 to 82 %. Linoleic acid was found to be the dominant fatty acid, ranging from 67.0 to 73.0 %, followed by oleic, ranging from 10.45 to 15.20 %, stearic acide (8.57 - 12.37 %) and palmitic acide (8.04 - 11.72 %,) (Table 3). The statistical analysis shows significant differences (p-value <0.05) between the accessions studied for all the fatty acids analysed. Table 3 presents the results of the comparison between the accessions for the most dominant fatty acids. For the linoleic fatty acid (C18.2), the mean values are separated into 4 groups with the lowest value observed in accession 1 (60.57) while the highest value is obtained for accession 11 (70.86). These results also show that the average values of unsaturated fatty acids (UFA) vary between 75.25% (accession 1) and 81.94 (accession 9). Regarding the calculated ratio between unsaturated and saturated fatty acids (UFA / SFA), it reaches 4.5 for the accessions A9 and A11, the lowest values of this ratio are recorded for accessions A1 and A12 with 3.04 and 3.18 respectively.

4 Conclusions and Discussion

The analysis of the average fatty acid composition shows that the major acids are linoleic acid followed by oleic acid (C18: 1), stearic acid (C18: 0) and palmitic acid (C16: 0) with 66, 13, 10 and 10% respectively. These fatty acid composition are in agreement with the results of other studies [7, 10, 11, 12].

The statistical analysis of the fatty acid contents of the oils of the different accessions reveals significant differences between the studied accessions. The proportions of linoleic acid (C18: 2) vary between 60.57% and 70.86%, the highest value was recorded in accession A11 with a percentage of 70.86%. This variability may be due to the influence of several factors. A study related to variations in the content of linoleic acid, the authors report that the synthesis of this fatty acid is influenced by temperature and it is synthesized in large quantities at lower temperatures due to the sensitivity of an enzyme involved in its synthesis [13]. The examination of the ratio between unsaturated and saturated fatty acids shows that the maximum value is 4.5 observed in accessions A9 and A11, on the other hand the minimum value which is 3.04 is recorded in A1. Ratios similar to those in this study have been reported by Nehdi et al. [12] and by Milovanovic & Picuric [11].

The oil yield values obtained in this study under semi-arid conditions without any fertilizer, under conditions of limited irrigation and with a low density (1 plant per m^2), remain relatively low compared to those potentially obtained under optimal growing conditions and optimal planting density. The oil yield values obtained in this study under semi-arid conditions without any fertilizer and under conditions of limited irrigation, remain relatively low compared to those potentially obtained under optimal growing conditions. The authors of a study conducted on the production of *Citrullus colocynthis*, reported that production can reach 1000 to 1175 L / ha of colocynth vegetable oil in rainy conditions [14]. With plant density of four plants per m^2, the extrapolated annual oil yield among the accessions of *Citrullus colocynthis* ranged from 0.07 and 3.44 tons / ha with an overall mean of 0.98 ton / ha [15]. The evaluation of seed and oil yields of 28 accessions grown under suitable irrigation and fertilization conditions allowed an estimation of oil yield ranging from 250 to 400 L / ha based on seed yield [16]. All the results of this study and also those of similar studies, allow us to think that this plant has a great potential to be used for grain production for oil extraction, especially under difficult conditions on arid and semi-arid lands and the exploitation of the natural variability could be used for the selection of accessions that have good oil yields in limited growing conditions and in

marginal lands especially taking into account that the heritability and genotypics advances estimates for traits involved in seed and oil yield are moderate to high [17].

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