



## Morphological, Biochemical and Barcoding Analysis of Different Egyptian Jew's mallow (*Corchorus olerius* L.) Landraces

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**ABSTRACT:** Jew's mallow is used as a food source, medicinal plant and natural antioxidant agent as well as biofuel source. Its numerous beneficial properties made it a valuable crop worldwide. This study was carried out to investigate the morphology, biochemical genetic analysis and DNA barcoding for three different Jew's mallow (*Corchorus olerius*) Egyptian landraces. They were collected from different location within Egypt namely, Fallahy (North), Seady (South) and Swain (West). Results revealed that, Siwan landrace had higher values for plant height, leaves number/plant, branches number/plant and fresh weight followed by Seady, then Fallahy. Biochemical values generally showed no significant differences between most of the measured elements. Mineral composition data showed numerical increase in the Siwan landrace in some minerals than others including Boron, Sodium, Magnesium, Aluminum, Calcium, Titanium, Chromium and Zinc, while Seady landrace recorded the highest values for Cobalt, Nickel, Copper, Indium and Bismuth. PCR was performed for the landraces along with two universal primers (*RbcL-MatK*). *RbcL* gene was amplified at 800-900 bp, while *MatK* gene was at 900 bp. Data was uploaded on DNAMAN program and partial sequences of *RbcL* and *MatK* genes were submitted to DDBJ Gene Bank with accession numbers of LC732565, LC732566 and LC732567 for *RbcL* gene and LC732049, LC732050 and LC732051 for *MatK* gene. This study showed that, the Siwan landraces was more unique from the other two landraces; furthermore, DNA barcoding using *RbcL* and *MatK* genes is an effective and sufficient tool in identifying different *C. olerius* landraces in Egypt.

**Keywords:** *Corchorus olerius*, morphology, molecular, barcoding, *RbcL*, *MatK*.

### INTRODUCTION

Jew's mallow, (*Corchorus olerius* L.) commonly recognized as Melokhya in Arabic, belongs to *Malvaceae* family. It is a tropical green leafy summer vegetable, traditionally cooked in Middle East, Asia and different parts of Africa (**El-Haddad et al., 2020**). Culinary leaves taste very delicious among Egyptians, juicy leaves soften quickly when cooked, resulting in a sticky, slimy broth that can be eaten with starchy foods (**Adediran et al., 2015**). 100 g of edible leaves of *C. olerius* contains, approximately 85g water, 5g protein, 0.7g oil, 5g carbohydrates, 4g dietary fiber, 258g Mg and Ca, 6mg iron, 3000IU vitamin A, 1.5mg nicotinamide, 75mg ascorbic acid (**Aluko et al., 2014**). It is well-thought-out to be the major source of natural fibers worldwide (**El-Haddad et al. 2020**). *C. olerius* L. was described as a medicinal plant. Leaves can be used as laxatives either in a juice form, or fried for skin care in creams and as a therapeutic agent for a wide range of health disorders. Its fresh leaves are known to be high in beta-carotene for sharp eyesight, iron for rich red blood cells, calcium for

healthy bones and teeth, and vitamin C for smooth skin, strong immunity system and rapid wound healing (**Islam, 2013**). Moreover, it was found that, plant extraction has antitumor-promoting activity using an immune- blotting analysis (**Furumoto et al., 2002**). Its high nutritional value and potential health benefits make it a valuable addition to the Egyptian diet (**El-Haddad et al., 2020**).

*C. olerius* has high morphological diversity based on different measurable traits on wide range of cultivated areas including Nigeria, Senegal, Egypt, Pakistan, beside other countries around the world (**Adebo et al., 2015**). Over the years, crop improvement programs were directed to select high-quality and fine-fiber varieties. However, growing Jew's mallow, as a vegetable, is rare in breeding programs (**Adjatin et al., 2019**). In Egypt, Seady and Fallahy Jute Mallow (Molekhya) landraces are the most famous one, while Siwan landrace, is not well known according to its geographical isolated habitat in Siwa Oasis although it's the most valuable among them. Morphological description, molecular analysis and

genetic barcoding using two plastid genes *RbcL* and *MatK* can differentiate them from one another according to the different cultivation locations (Hollingsworth *et al.*, 2009). The *matK* gene sequence is more challenging to be amplified and sequenced than *RbcL* as; it is longer than *RbcL* by 300bp, and more delicate to be degraded, that's why, sequence reaction quality which has mononucleotide repeats is easily affected (Cohen, 2011). Therefore, this investigation was carried out to study the morphological description along with biochemical genetic analysis and the molecular barcoding of three different Egyptian landraces of Jew's mallow, (*C. olerius*) cultivated in Egypt.

## 2. MATERIALS AND METHODS

The current work took place at the Agriculture Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, with collaboration with the Medicinal and Aromatic Plants Research Department, Horticultural Research Institute, Agricultural Research Center, Alexandria, Egypt. Three landraces of Jew's Mallow (*C. olerius* L.) namely: Fallahy, Seady and Siwan were used during the current study. The plant material (seeds) were collected and selected from different locations at Northern, Southern and Western Egypt, respectively. Seeds were cultivated at Faculty of Agriculture Farm, Alexandria University, during the cultivation seasons 2021 and 2022.

### 2.1. Morphological Studies:

Random sample of 20 plants were selected for the morphological measurements of the three landraces under study. Morphological studies included; plant height (cm), number of leaves/plant, number of branches/plant as well as fresh weight (g), dry weight (g) and moisture content. The field experiment design was a randomized complete blocks with three replicates for each landrace. Seeds of each Jew's Mallow landrace were sown in rows approximately 40cm apart. Each experimental unit consisted of 10 rows, 3.5m along and 0.20m a part. The sowing date was the 24<sup>th</sup> June on 2021 summer season.

### 2.2. Biochemical Assay:

Chlorophyll contents (A and B) were measured according to Barros *et al.* (2011) with

some modifications. Also, total phenolic compounds, total flavonoids were extracted using methanol as described by the previous author. Proline content was determined following Bates *et al.* (1973) procedure, using Aqueous Sulfosalicylic Acid (3%), Acid Ninhydrin; Ninhydrin (1.25 g), glacial acetic acid (30 ml) and 6M phosphoric acid (20 ml). Total dietary fiber was estimated by non-enzymatic gravimetric technique of Li and Cardozo (1994). The soluble and insoluble dietary fibers were determined according to Asp *et al.* (1983). Total soluble solids were directly measured using a hand refractometer (Model: ATAGO, Tokyo, Japan). Vitamin C content was estimated using the 2, 6-dichlorophenol indophenol method as illustrated by AOAC (1975). Quantification of total protein content, Bovine serum albumin (BSA) was used as standard reagent for the standard curve preparation which helped in estimating the total protein content (Sarkar *et al.*, 2020). Plant leaves content of actual nitrogen (N) was calculated using the following equation.

$$N_{total} = \frac{v_s - v_b}{m_d} \times NH_2 SO_4 \times 0.014 \text{ meq N} \times 100$$

Where  $v_s$  is the sample consumed volume,  $v_b$  is the control treatment consumed volume,  $NH_2SO_4$  is the normality of sulphuric acid at 0.014, meq is the dry weight sample. Mineral composition and determination were done using the atomic absorption spectrophotometer with the dry burning method (Kacar and Inal, 2008).

### 2.3. Molecular Studies

Isolation of the genomic DNA was done from seeds of the three landraces under study according to Irfan *et al.* (2013), with some modifications. DNA extraction as well as purification was achieved using CTAB (Cetyl-tetramethyl ammonium bromide) method (Muray and Thompson, 1980). PCR amplification was performed using biosystems® proflex™ PCR system. The PCR amplification conditions were optimized for all reactions by using extracted DNA in a total volume reaction of 25  $\mu$ l as; Master Mix (12.50  $\mu$ l) and primers (1.25  $\mu$ l of each) (Table 1) (forward and reverse), DNA template (2  $\mu$ l) and de-ionized water (up to 25  $\mu$ l) (Erlich, 1989).

**Table (1): Primer sequence, Tm value and GC (%).**

| Name        | Primer sequence (5`-3`)        | Tm   | GC (%) |
|-------------|--------------------------------|------|--------|
| <i>RbcL</i> | F: AGACCTWTTTGAAGAATTCWGT      | 60.1 | 31.8   |
|             | R: TCGGTYAGAGCRGGCATRTGCCA     | 64.7 | 52.1   |
| <i>MatK</i> | F: CGTACAGTACTTTTGTGTTTACGAG   | 53.9 | 40.0   |
|             | R: ACCCAGTCCATCTGGAAATCTTGGTTC | 60.4 | 48.0   |

PCR amplification conditions for *RbcL* gene were 30 cycles as follows: 94 °C / 5 minutes (1 cycle); 94 °C / 45 sec, 50 °C / 45 sec, 72 °C / 45 sec (30 cycles); 72 °C / 7 minutes (1 cycle); 4 °C (24 hrs) as described by **Fay et al. (1997)**. While PCR amplification conditions for *MatK* gene were 45 cycles as follows: 94 °C / 5 minutes (1 cycle); 94 °C / 30 sec, 45 °C / 30sec, 72 °C / 30 sec; 72 °C / 7 minutes (1 cycle); 4 °C (24 hrs). Bands of the previously mentioned genes were eluted using an elution kit (@iNtRON Biotechnology, Inc. Korea) as described by **Bjourson et al. (1992)**. Sequence analysis includes all *Malvaceae* family plant species were available on GenBank to find interspecific and intergeneric variation. *C. olitorius* species sequences for *RbcL* and *MatK* were obtained in Fasta format from NCBI. Several sequence alignments of the *RbcL* and *MatK* genes were performed using the PROMALS server (**Pei and Grishin, 2007**), Clustal Omega server (**Sievers et al., 2011**), the BIOEDIT software (**Hall, 2001**), Clustal X and MEGA - x (**Stecher et al., 2020**). Those are offline-software which performed optimum alignment for sequence to extract the conserved regions. Evolutionary history was derived following the neighbor-joining method (**Tamura et al., 2004**). Mafft server (**Katoh et al., 2015**) Clustal Omega server and MEGA-x software (www.megasoftware.net) were used for the phylogenetic tree construction.

### 3. RESULTS AND DISCUSSION

Three landraces of Jew's Mallow (Fallahy, Seady and Siwan) seeds were collected and selected from Egyptian different locations to

study their morphological, biochemical and molecular barcoding variations.

#### 3.1. Morphological studies:

No variations were observed in seed germination among the three Jute Mallow landraces under study. Results indicated that the germination percentage ranged from 98 to 100%. Morphological measurements are shown in **Table (2)**. Siwan landrace highest mean value was recorded for plant height (50.00 cm) compared with the lowest value in Fallahy (38.67 cm), while Seady recorded (46.00 cm). As for no. of leaves/plant, no. of branches/plants as well as fresh weight, Siwan landrace showed the highest values followed by Seady, then Fallahy with no significant difference among them as presented in **Table (2)**. Those results were in agreement with previous studies which stated that, morphological differences among the landraces very important and of useful significance. **Helaly et al. (2017)** reported that, wild landrace can be significantly superior in most morphological parameters compared to the domesticated variety. Also, current results agreed with morphological studies on Jew's mallow by **Sajib et al. (2008)** who described *C. Olitorius* and *C. Capsularis* morphologically. **Adebo et al. (2015)** who reported that, *C. Olitorius* has high morphological diversity based on the different weather conditions in Egypt. Furthermore, current findings agreed with **Abd-Allah et al. (2006)** who reported that, six genotypes of Jew's mallow were obviously different in their morphological traits as they were cultivated at different regions of Egypt.

**Table (2): Morphological characters of the three tested Jew's mellow.**

|         | Plant height (cm)          | No. of leaves /plant       | No. of branches /plant    | Fresh weight (g)          | Dry weight (g)             | Moisture content (%) |
|---------|----------------------------|----------------------------|---------------------------|---------------------------|----------------------------|----------------------|
| Fallahy | 38.67 <sup>b</sup> ± 0.88  | 43.00 <sup>a</sup> ± 10.21 | 10.67 <sup>a</sup> ± 1.20 | 20.33 <sup>a</sup> ± 3.18 | 18.679 <sup>a</sup> ± 1.30 | 81.321               |
| Seady   | 46.00 <sup>ab</sup> ± 2.62 | 49.67 <sup>a</sup> ± 7.31  | 12.67 <sup>a</sup> ± 0.72 | 22.67 <sup>a</sup> ± 2.03 | 19.637 <sup>a</sup> ± 2.67 | 80.363               |
| Siwan   | 50.00 <sup>a</sup> ± 3.61  | 57.33 <sup>a</sup> ± 3.71  | 14.00 <sup>a</sup> ± 2.65 | 23.33 <sup>a</sup> ± 4.41 | 20.52 <sup>a</sup> ± 1.19  | 79.480               |

\* Mean of 20 randomly selected plants ± Standard error (SE)

#### 2. Biochemical Assay:

Biochemical values of the tested Jew's mallow landraces were recorded in **Table (3)** as mean of three replicates ± standard error. Generally there were no significant differences between most of the measured elements; however there were slit increase among some of them. Chlorophyll A content was high in Seady (8.326),

followed by Siwan landrace (8.026) and finally, Fallahy (7.885), while Chlorophyll B and Chlorophyll A+B, values were similar and ranged from 3.182 to 3.268 and 11.153 to 11.526, respectively. Fiber content in each gram was 2.187, 2.054 and 1.642 for Siwan, Seady, and Fallahy respectively. For protein, Siwan recorded the highest value (13.586) and was superior on Fallahy

(12.489) and Seady (12.241) landraces. Biochemical assay values of *C. olitorius* followed the same trend as mentioned by **Ahmed and Nizam (2008)** who reported that, *Corchorus spp.* contain a considerable amount of vitamins, calcium and carotene. This was also supported by **Nemb et al. (2011)** who stated that, *C. olitorius* leaves contain protein, carbohydrates, fiber, Ca, Fe, vitamin A and ascorbic acid. **Mahmood et al., (2021)** reported that, Jew's mallow contains a variety of flavonoids with the highest

concentration found in the leaves of the plant; and that, flavonoids concentration and distribution were influenced by factors such as light intensity, temperature, and plant maturity. **Ismail et al. (2020)** added that Jew's mallow was found to have beneficial properties like high antioxidant activity, which is attributed to phytochemicals existence like flavonoids, phenolic acids, and carotenoids, also, can be used in food preservation due to its elevated level of antioxidants.

**Table (3): Biochemical means of the three tested Jew's mallow.**

|                             | Fallahy       | Seady         | Siwan         | L.S.D  |
|-----------------------------|---------------|---------------|---------------|--------|
| <b>Chlorophyll A</b>        | 7.885 ± 2.35  | 8.326 ± 1.41  | 8.026 ± 1.58  | 2.558  |
| <b>Chlorophyll B</b>        | 3.268 ± 0.63  | 3.2 ± 0.08    | 3.182 ± 0.51  | 0.879  |
| <b>Chlorophyll A+B</b>      | 11.153 ± 2.98 | 11.526 ± 1.48 | 11.207 ± 2.09 | 3.335  |
| <b>Phenol</b>               | 24.303 ± 8.18 | 17.318 ± 5.23 | 30.181 ± 5.33 | 15.520 |
| <b>Flavonid</b>             | 3.393 ± 0.20  | 4.662 ± 1.81  | 3.048 ± 0.62  | 2.753  |
| <b>Proline</b>              | 15.18 ± 0.38  | 13.365 ± 2.75 | 12.639 ± 1.73 | 5.094  |
| <b>Fiber / g</b>            | 1.642 ± 0.13  | 2.054 ± 0.36  | 2.187 ± 0.29  | 0.750  |
| <b>Total soluble solids</b> | 9.566 ± 0.35  | 10.133 ± 0.12 | 10.333 ± 0.15 | 0.537  |
| <b>Vitamin C</b>            | 32.354 ± 0.83 | 32.152 ± 0.35 | 31.824 ± 2.03 | 3.352  |
| <b>Protein</b>              | 12.488 ± 1.03 | 12.241 ± 1.14 | 13.586 ± 0.65 | 1.885  |
| <b>Nitrogen (%)</b>         | 1.998 ± 0.16  | 1.958 ± 0.18  | 2.173 ± 0.10  | 0.302  |
| <b>Carotenoide</b>          | 1.970 ± 0.38  | 2.086 ± 0.25  | 2.160 ± 0.47  | 0.567  |
| <b>T- Carbonayl (%)</b>     | 11.368 ± 1.57 | 11.486 ± 0.90 | 11.562 ± 0.18 | 2.616  |
| <b>Phosphorus (%)</b>       | 0.693 ± 0.09  | 0.636 ± 0.05  | 0.886 ± 0.05  | 0.169  |
| <b>Potassium (%)</b>        | 1.333 ± 0.11  | 1.307 ± 0.12  | 1.451 ± 0.07  | 0.202  |

\*Values are presented as means of three replicates ± (Standard Error)

Mineral composition results of the *C. olitorius* landraces under study were expressed in ppm (milligrams/kg) as illustrated in Table (4). Data showed numerical increase in the Siwan landrace for some minerals than others including Boron, Sodium, Magnesium, Aluminum, Calcium, Titanium, Chromium and Zinc which recorded; 9.2, 6918.6, 808.1, 194.9, 232.9, 40.9, 5.9 and 18.6 ppm, respectively. While Fallahy landrace recorded the lowest values for Sodium, Aluminum, Calcium, Titanium, Iron and Zinc which recorded; 2630.2, 157.2, 181.6, 27.5, 10.7 and 12.4 ppm

respectively, whereas Seady landrace recorded the highest values for Cobalt, Nickel, Copper, Indium and Bismuth, were 1.4, 4.4, 12.3, 1.2 and 2.3 ppm and the lowest values were detected in Seady for Magnesium, Potassium, and Barium (631.5, 5677.9 and 2.5 ppm, respectively). Current findings were in agreement with **Helaly et al. (2017)** who described Jute Mallow plants as a medicinal plant in the Middle East, as they contains phenolic compounds, polysaccharides, carotenoids, minerals, sugars, proteins and multi-vitamins.

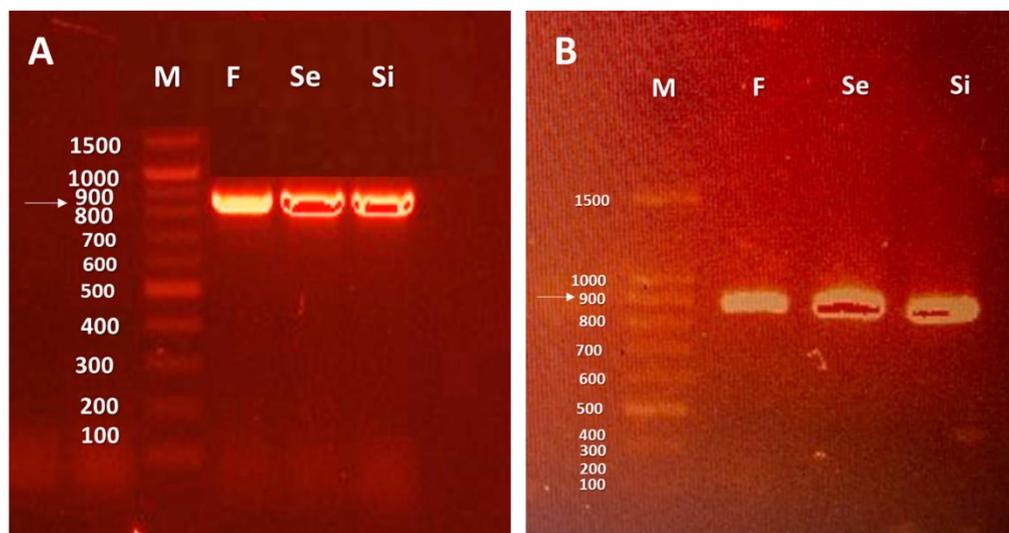
**Table (4): Minerals composition of the three tested Jew's mallow.**

| Elements  | Fallahy  | Seady    | Siwan    |
|-----------|----------|----------|----------|
| Lithium   | 0.009    | 0.019    | 0.133    |
| Boron     | 0.193    | 0.227    | 9.235    |
| Fluorine  | 4.006    | 4.149    | 4.229    |
| Sodium    | 2630.226 | 3232.364 | 6918.628 |
| Magnesium | 679.764  | 631.496  | 808.091  |
| Aluminum  | 157.190  | 182.337  | 194.946  |
| Potassium | 6233.749 | 5677.914 | 5926.804 |
| Calcium   | 181.603  | 184.026  | 232.909  |
| Titanium  | 27.491   | 32.901   | 40.905   |
| Chromium  | 4.589    | 4.989    | 5.931    |
| Manganese | 58.345   | 62.427   | 58.674   |
| Iron      | 10.695   | 12.392   | 12.860   |
| Cobalt    | 0.964    | 1.425    | 0.812    |
| Nickel    | 2.608    | 4.355    | 2.251    |
| Copper    | 7.845    | 12.316   | 7.372    |
| Zinc      | 12.374   | 16.546   | 18.613   |
| Gallium   | 0.543    | 0.452    | 0.320    |
| Strontium | 230.260  | 108.389  | 204.655  |
| Argentum  | 2.117    | 0.742    | 0.755    |
| Cadmium   | 0.177    | 0.177    | 0.074    |
| Indium    | 0.843    | 1.170    | 0.436    |
| Iodine    | 1.792    | 1.402    | 1.122    |
| Barium    | 12.946   | 2.545    | 6.036    |
| Bismuth   | 1.745    | 2.288    | 0.201    |

**3.3. Molecular studies:**

Extracted DNA concentration was measured (400 to 600 ug/ml) and determined using a Nano drop spectrophotometer (Thermo Scientific™ NanoDrop™ One/OneC Microvolume UV-Vis). PCR was performed for the two universal primers (*RbcL-MatK*), using PCR program as mentioned before.

Electrophoresis of the *RbcL* amplified gene took place as illustrated in **Figure (1-A)**. Gene amplified fragments were located between 800-900 bp. This was performed using the retrieved DNA directly. *MatK* amplified gene is presented in **Figure (1-B)** for the three landraces of Jew's mallow genomic DNA samples. Gene was located at 900 bp.



**Figure (1)** Agarose electrophoresis of (A) *RbcL* gene and (B) *MatK* gene, in three landraces of *C. olerarius* as follow: Fallahy (F), Seady (Se) and Siwan (Si). (M) DNA ladder (100 – 1500 bp).

### 3.3.1. Single Nucleotide Polymorphism (SNP) Identification and Frequencies:

Nucleotides were identified using DNAMAN® software (Lyon BioSoft, Quebec, Canada). Sequences were added in DDBJ and the accession number has been obtained as demonstrated in **Table (5)**. Fasta format files were uploaded on DNAMAN program and partial sequences

of *RbcL* and *MatK* genes obtained from the three landraces of *C. olitorius* were compared with each other. The three sequences were submitted in DDBJ Gene Bank with accession numbers of **LC732565**, **LC732566** and **LC732567** for *RbcL* gene and **LC732049**, **LC732050** and **LC732051** for *MatK* gene.

**Table (5): Authors and Accession numbers for *RbcL* and *MatK* gene recorded for *Corchorus olitorius* landraces under study.**

| Name of Author (S)   | Research Title   | Journal  | Published Date |
|--|--|--|----------------|
| <i>RbcL</i> gene   |  |  |                |
| Elwakeel, H.M.F.,<br>Abdelsalam, N.R.,<br>Zaitoun, A.F., Gomaa,<br>S.E., Mohamed, B.G.<br>and Abdullah, M.I. | <i>Corchorus olitorius</i> F_BM01 chloroplast <i>rbcL</i> gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds  | Gene bank: LC732565<br><a href="https://www.ncbi.nlm.nih.gov/nuccore/LC732565.1">https://www.ncbi.nlm.nih.gov/nuccore/LC732565.1</a> | 2022           |
|  | <i>Corchorus olitorius</i> S_BM02 chloroplast <i>rbcL</i> gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds  | Gene bank: LC732566<br><a href="https://www.ncbi.nlm.nih.gov/nuccore/LC732566.1">https://www.ncbi.nlm.nih.gov/nuccore/LC732566.1</a> | 2022           |
|  | <i>Corchorus olitorius</i> Si_BM03 chloroplast <i>rbcL</i> gene for ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds | Gene bank: LC732567<br><a href="https://www.ncbi.nlm.nih.gov/nuccore/LC732567.1">https://www.ncbi.nlm.nih.gov/nuccore/LC732567.1</a> | 2022           |
| <i>MatK</i> gene   |  |  |                |
| Elwakeel, H.M.F.,<br>Abdelsalam, N.R.,<br>Zaitoun, A.F., Gomaa,<br>S.E., Mohamed, B.G.<br>and Abdullah, M.I. | <i>Corchorus olitorius</i> F01 chloroplast <i>matK</i> gene for maturase K, partial cds  | Gene bank: LC732049<br><a href="https://www.ncbi.nlm.nih.gov/nuccore/LC732049.1">https://www.ncbi.nlm.nih.gov/nuccore/LC732049.1</a> | 2022           |
|  | <i>Corchorus olitorius</i> S01 chloroplast <i>matK</i> gene for maturase K, partial cds  | Gene bank: LC732050<br><a href="https://www.ncbi.nlm.nih.gov/nuccore/LC732050">https://www.ncbi.nlm.nih.gov/nuccore/LC732050</a>     | 2022           |
|  | <i>Corchorus olitorius</i> Si01 chloroplast <i>matK</i> gene for maturase K, partial sequence  | Gene bank: LC732051<br><a href="https://www.ncbi.nlm.nih.gov/nuccore/LC732051">https://www.ncbi.nlm.nih.gov/nuccore/LC732051</a>     | 2022           |

#### 3.3.1.1. Barcoding analysis of *RbcL*:

Sequences were compared to the three landraces of *Corchorus olitorius*, the highest SNP's frequency was found in LC732567. Data in **Table (6)** illustrated the amino acid composition of *RbcL* gene sequence for the three landraces under study grown in Egypt by bioedit sequence alignment editor version 7.2.5 (12/11/2013). The highest Alanine value was recorded in Seady landrace (30.02%), followed by both the other landraces 26.96 and 26.89 as presented in **Table (6)**. A+T as well as C+G value was the highest at Fallahy

and Siwan landraces. Phylogenetic analysis data for chloroplast *RbcL* gene of the three landraces for Jew's Mallow grown in Egypt is shown in **Table (7)**. Data showed high similarity percentage and phylogenetic similarity based on *RbcL* gene plant sequencing. Similarity percentage ranged from 99.51 to 99.88 for all the studies landraces with others in gene bank. *C. olitorius* Seady collected sample showed the highest similarity with *C. olitorius* (99.88%) with the other matching as presented in **Table (7)**. Data in **Table (8)** showed the nucleotides

content in the studied landraces. Average nucleotides number was 1034 nucleotides using *RbcL* gene. GC content and AT content in *C. olitorius* Fallahy was identical with *C. olitorius* Seady. GC content represent 42.46% while AT content represent 57.54% in the total number of nucleotides (**Table 8**). *C. olitorius* Siwan, nucleotides composition was A: 28.43%, C: 20.02%, G: 22.82%, T: 28.72% while GC content was 42.84% and AT content was 57.16% in the total number of nucleotides (1034). *RbcL* phylogenetic tree was divided into two main clusters with *C. olitorius* Fallahy and *C. olitorius* Siwan at the same branch, while *C. olitorius* Seady was in a separate branch, as shown in **Figure (2)**.

**Table (6): Amino acid composition of *RbcL* sequences of *C. olitorius* landraces grown in Egypt and it's similar in NCBI.**

| Sample  | Alanine (%) | Cysteine (%) | Glycine (%) | Threonine (%) | A+T   | C+G   |
|---|-------------|--------------|-------------|---------------|-------|-------|
| <i>Corchorus olitorius</i> Fallahy  | 26.96       | 19.11        | 24.30       | 29.62         | 56.58 | 43.41 |
| <i>Corchorus olitorius</i> Seady  | 30.02       | 23.70        | 18.86       | 27.30         | 57.32 | 42.56 |
| <i>Corchorus olitorius</i> Siwan  | 26.89       | 19.07        | 24.49       | 29.55         | 56.44 | 43.56 |
| NC_044468.1:59352-60158 <i>Corchorus olitorius chloroplast complete genome</i>  | 27.26       | 18.84        | 23.67       | 30.24         | 57.5  | 42.51 |
| MF135415.1:255-1061 <i>Corchorus olitorius voucher Li ZY Fu LZ Xu SZ Fu JG 13408 ribulose-15-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene partial cds chloroplast</i> | 27.51       | 18.71        | 23.54       | 30.24         | 57.75 | 42.25 |
| NC_044467.1:58650-59456 <i>Corchorus capsularis chloroplast complete genome</i>   | 27.51       | 18.59        | 23.54       | 30.36         | 57.87 | 42.13 |
| MH767740.1:179-985 <i>Corchorus aestuans voucher LSC49 ribulose-15-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene partial cds chloroplast</i>                           | 30.48       | 23.54        | 18.59       | 27.39         | 57.87 | 42.13 |
| NC_044467.1:58693-59482 <i>Corchorus capsularis chloroplast complete genome</i>   | 27.22       | 18.86        | 24.18       | 29.75         | 56.97 | 43.04 |
| MH767740.1:153-942 <i>Corchorus aestuans voucher LSC49 ribulose-15-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene partial cds chloroplast</i>                           | 29.87       | 24.18        | 18.86       | 27.09         | 56.96 | 43.04 |
| KT894204.1:1951706-1952496 <i>Corchorus capsularis mitochondrion complete genome</i>  | 29.96       | 24.02        | 18.71       | 27.31         | 57.27 | 42.73 |
| KT894204.1:341972-342114 <i>Corchorus capsularis mitochondrion complete genome</i>  | 28.67       | 21.68        | 16.78       | 32.87         | 61.54 | 38.46 |

**Table (7): *RbcL* gene sequences similarities for *C. olitorius* landraces under study.**

| Sample ID | Morphological identification | BLAST search match  | BLAST similarity (%) | Phylogenetic affinity       |
|-----------|------------------------------|---------------------|----------------------|-----------------------------|
| 1         | <i>C. olitorius</i> Fallahy  | <i>C. olitorius</i> | 99.51%               | <i>C. olitorius</i> Fallahy |
| 2         | <i>C. olitorius</i> Seady    | <i>C. olitorius</i> | 99.88%               | <i>C. olitorius</i> Seady   |
| 3         | <i>C. olitorius</i> Siwan    | <i>C. olitorius</i> | 99.74                | <i>C. olitorius</i> Siwan   |

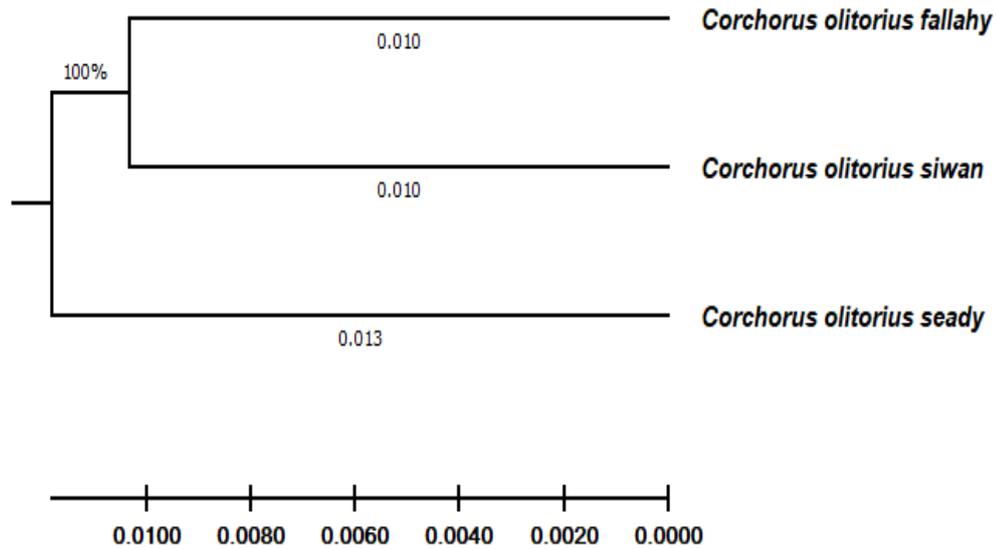


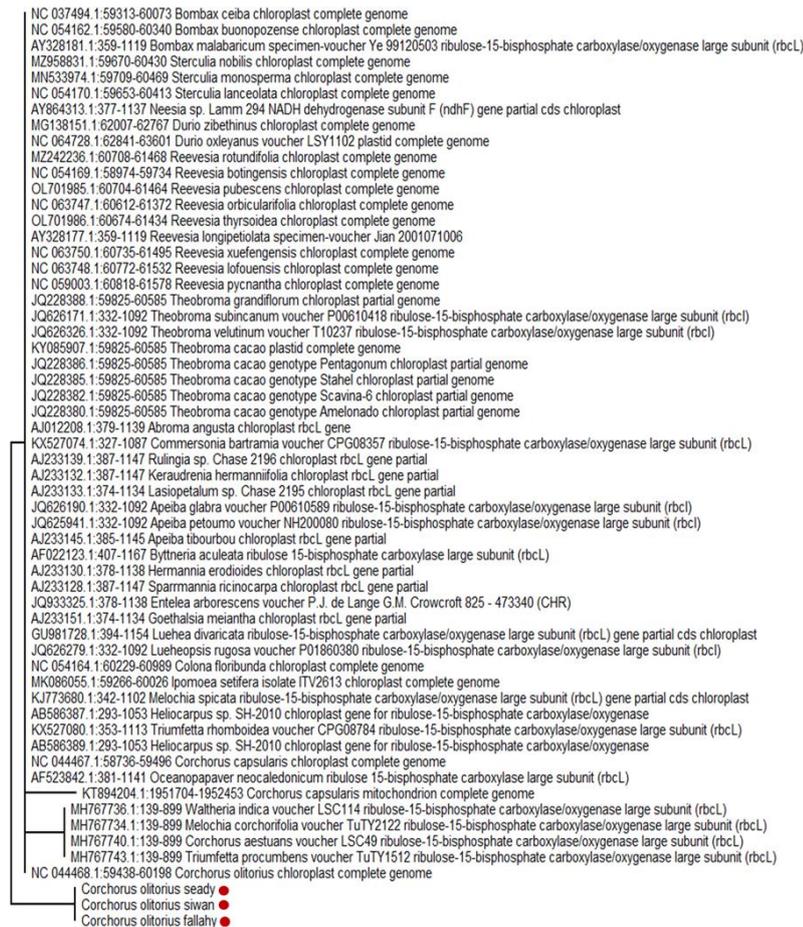
Figure (2): Phylogenetic tree of the three *C. olitorius* landraces under study using *RbcL* gene.

Table (8): Nucleotides content of *RbcL* sequences of *C. olitorius* landraces grown in Egypt.

| Sample                             | A   | C   | G   | T   | G+C | A+T | Total |
|------------------------------------|-----|-----|-----|-----|-----|-----|-------|
| <i>Corchorus olitorius Fallahy</i> | 295 | 205 | 234 | 300 | 439 | 595 | 1034  |
| <i>Corchorus olitorius Seady</i>   | 301 | 204 | 235 | 294 | 439 | 595 | 1034  |
| <i>Corchorus olitorius Siwan</i>   | 294 | 207 | 236 | 297 | 443 | 591 | 1034  |

The phylogenetic analysis of the three *C. olitorius* landraces using *RbcL* genes sequencing were recorded in **Figure (3)**, dendrogram was divided into two main branches, one of them had

the three landraces under study, *C. olitorius* Fallahy, *C. olitorius* Siwan and *C. olitorius* Seady in the same cluster.



**Figure (3): Phylogenetic tree of the three *C. oltorius* landraces grown in Egypt using *RbcL* gene.**

Barcoding analysis of *MatK* gene compared with the three landraces of *C. oltorius* revealed that, the highest SNP's frequency was found in LC732051. There were no base substitutions between all sequences aligned according to data in **Table (5)**. There were three deletions found in nucleotide 257, 568, 569 and 577 in *C. oltorius* Siwan landrace. Results in **Table (9)** represented amino acid composition of *MatK* sequences of the three landraces of *C. oltorius* under study as compared with genes in NCBI by BioEdit sequence Alignment Editor Version 7.2.5(12/11/2013). Cysteine and Glycerin usually ranged between 33.66 and 34.64, meanwhile Alanine and Threonine ranged between 65.36 and 66.34. Data in **Table (10)** showed similarity percentage calculated by BLAST and the phylogenetic similarity based on *MatK* gene plant sequencing. There was high similarity percentages ranged from 99.62 to 100 for the landraces under study compared with others in the gene bank. *C. oltorius* Seady collected sample was identical to *C. oltorius* of the database with 100%, while *C. oltorius* Fallahy showed high similarity of 99.62%, as shown in **Table (10)**.

Data in **Table (11)** presented the nucleotides content of the landraces under study

and the total number of nucleotides. Nucleotides average number was 775 nucleotides using *MatK* gene. The nucleotide composition percentages of the landrace *C. oltorius* Fallahy were A= 29.81%, C= 16.90%, G= 16.39% and T= 36.90%, while the G+C= 33.29% and the A+T= 66.71%. It's clear that the G+C content and A+T content in Seady was identical with Siwan as G+C= 33.81% and A+T= 66.19% in a total number of 775 nucleotides, as shown in **Table (11)**. The phylogenetic tree was divided into two main clusters; one of them contains Seady and Siwan as shown in **Figure (4)**. This phylogenetic differentiation might be due to the difference in the environmental conditions which lead to natural selection which agreed with previous studies supporting that, DNA polymorphisms is a powerful approach for understanding the evolutionary of different landraces and determine how genomic regions is functional (**Begun et al., 2007**). Amino acids amount of change is nowadays easily to be determined to be used in adaptation studies, the locations of these changes in the protein's three-dimensional structure and mechanisms by which substitutions influence stability and kinetic properties (**Somero, 2010**). It was also reported that DNA barcoding projects

begin with a tissue sample from which all subsequent genetic processing takes place. “Barcode” in a Gene bank is achieved by DNA barcode records submission with specific metadata elements associated with the specimen source (Kress and Erickson, 2012)

**Table (9) Amino acid composition of *MatK* sequences of three landraces of *Corchorus olitorius* grown in Egypt.**

| Sample  | Alanine (%) | Cysteine (%) | Glycine (%) | Threonine (%) | A+T   | C+G   |
|---|-------------|--------------|-------------|---------------|-------|-------|
| LC732049.1 <i>Corchorus olitorius</i> F01 chloroplast <i>matK</i> gene for maturase K, partial cds. (Fallahy)                   | 30.12       | 16.91        | 16.91       | 36.05         | 66.17 | 33.82 |
| LC732050.1 <i>Corchorus olitorius</i> S01 chloroplast <i>matK</i> gene for maturase K, partial cds (Seady)                      | 28.89       | 17.11        | 16.55       | 37.45         | 66.34 | 33.66 |
| LC732051.1 <i>Corchorus olitorius</i> Si01 chloroplast <i>matK</i> gene for maturase K, partial sequence (Siwan)                | 31.01       | 16.74        | 17.21       | 35.04         | 66.05 | 33.95 |
| NC_044468.1:2293-2965 <i>Corchorus olitorius</i> chloroplast, complete genome   | 35.96       | 16.94        | 16.94       | 30.16         | 66.12 | 33.88 |
| KJ012546.1:88-722 <i>Corchorus hirsutus</i> voucher 0132455899 maturase K ( <i>matK</i> ) gene, partial cds; chloroplast        | 28.98       | 17.32        | 17.32       | 36.38         | 65.36 | 34.64 |
| MF694843.1:126-759 <i>Corchorus depressus</i> voucher KMC019 maturase K ( <i>matK</i> ) gene, partial cds; chloroplast          | 29.02       | 17.19        | 17.35       | 36.44         | 65.46 | 34.54 |
| KJ012546.1:98-701 <i>Corchorus hirsutus</i> voucher 0132455899 maturase K ( <i>matK</i> ) gene, partial cds; chloroplast        | 29.80       | 17.22        | 17.38       | 35.60         | 65.4  | 34.6  |
| MF694843.1:136-739 <i>Corchorus depressus</i> voucher KMC019 maturase K ( <i>matK</i> ) gene, partial cds; chloroplast          | 29.80       | 17.05        | 17.55       | 35.60         | 65.4  | 34.6  |
| MK290486.1:101-704 <i>Corchorus trilocularis</i> isolate UHURU1518-15 maturase K ( <i>matK</i> ) gene, partial cds; chloroplast | 29.80       | 17.05        | 17.55       | 35.60         | 65.4  | 34.6  |

**Table (10): *MatK* gene sequences similarities for *C. olitorius* landraces under study.**

| Sample ID | Morphological identification | BLAST search match  | BLAST similarity (%) | Phylogenetic similarity |
|-----------|------------------------------|---------------------|----------------------|-------------------------|
| 1         | <i>C. olitorius</i> Fallahy  | <i>C. olitorius</i> | 99.62%               | Fallahy                 |
| 2         | <i>C. olitorius</i> Seady    | <i>C. olitorius</i> | 100%                 | Seady                   |
| 3         | <i>C. olitorius</i> Siwan    | <i>C. olitorius</i> | 99.87%               | Siwan                   |

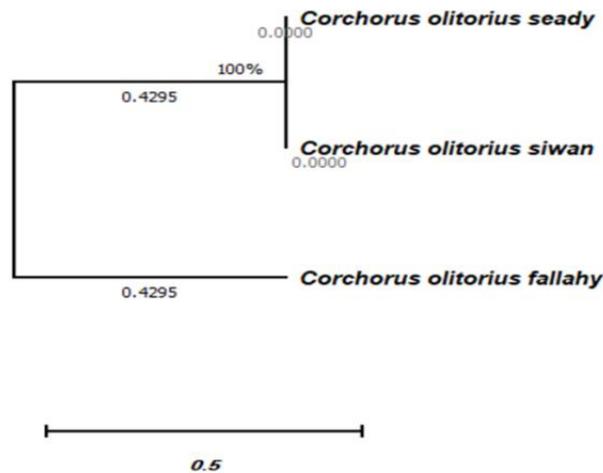


Figure (4): Phylogenetic tree of the three *C. olitorius* landraces under study using *MatK* gene

Table (11) Nucleotides composition of *Matk* sequences using BioEdit sequence Alignment Editor.

| Sample                      | A (%) | C (%) | G (%) | T (%) | G+C content | A+T content |
|-----------------------------|-------|-------|-------|-------|-------------|-------------|
| <i>C. olitorius</i> Fallahy | 29.81 | 16.90 | 16.39 | 36.90 | 33.29       | 66.71       |
| <i>C. olitorius</i> Seady   | 36.90 | 16.90 | 16.90 | 29.29 | 33.81       | 66.19       |
| <i>C. olitorius</i> Siwan   | 36.90 | 16.90 | 16.90 | 29.29 | 33.81       | 66.19       |

Phylogenetic tree (Figure 4) was designed with a scale, at which a branch lengths was measured with number of substitutions /site. According to the previous data of *MatK* genes and using the three landraces of *C. olitorius*, the cluster analysis was divided into two branches; Fallahy was in separated branch and both Siwan and Seady in a separated branch. The phylogenetic analysis of

the three *C. olitorius* landraces using *MatK* genes sequencing are presented in Figure (5). Dendrogram in Figure (5) was divided into two main branches, one of them had the three landraces under study, *C. olitorius* Falahy and *C. olitorius* Siwan were in the same cluster, while *Corchorus olitorius* Seady followed them.

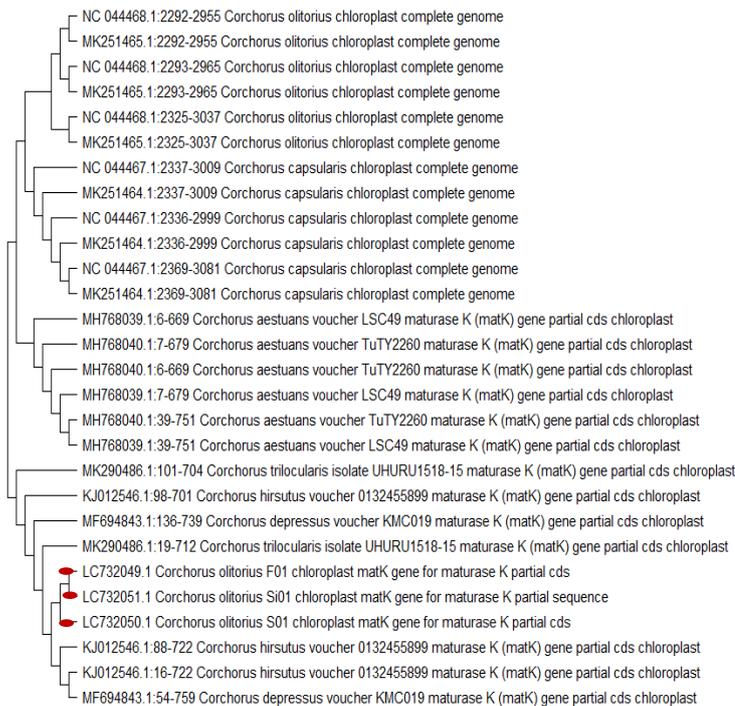


Figure (5) Phylogenetic tree of *C. olitorius* landraces under study with genes in NCBI using *MatK* gene

Current study declared that *RbcL* and *MatK* genes are effective tools to be used in barcoding to relate plants, as well as the difference between species and individuals as supported by **Hebert *et al.*, (2004)**, who reported that, Taxonomists are highly interested in DNA barcoding, it is widely used to support ownership or intellectual property rights. The same authors defined DNA barcode, as one or more short genetic sequences obtained from a genomic uniformed portion (standard) to identify species. Similarly, **Cowan *et al.* (2006)** mentioned that barcoding allows scientists to identify and recover information about known species quickly and accurately. **Cohen (2011)** mentioned that *RbcL* and *MatK*, were identified as core barcode loci. However, *RbcL* gene is much easier to amplify and sequence than *MatK* for several reasons. In addition, studying the biochemical differences among different landraces can lead to identifying the most valuable one, containing the beneficial active ingredient and the most delicious as an edible one (**Islam, 2013**). He also added, barcoding is a characterizing method for species involving a small sequence from a specific and agreed-upon position at the organism genome. **Kress *et al.* (2005)** stated that *RbcL* is commonly used in phylogenetic investigations with wide range on the Gene bank ( $\leq 50000$ ). This gene is easy to be amplified, sequenced, and aligned at most plants, as it has a barcoding region specified for the species, genus and the families they belong to. However, *RbcL* genes loci are hard to change and have the lowest plastid genes divergence in flowering plants. **Mildenhall (2006)**; reported that using DNA barcoding to maintain food hygiene and labeling licensing for identity confirmation (**Galimberti *et al.*, 2012** and **Huxley-Jones *et al.* 2012**); as well as ecology and environmental studies (**Valentini *et al.*, 2009**). The *MatK* gene, also known as Megakaryocyte-associated tyrosine kinase, is a member of the non-receptor tyrosine kinase family. This gene is found on chromosome 10 in humans and is expressed in various tissues, including the bone marrow, spleen, and thymus. (**El-Rokiek *et al.*, 2016**). The *RbcL* gene, known as ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, is a significant gene involved in photosynthesis in plants (**Alqurashi and Awad, 2011**). Previous investigations found that Jew's mallow contains a significant amount of *RbcL* and *MatK* genes expression in both leaves and stems. It was also found that, those genes expression were high in the early stages of growth and decreased as the plant matured. Furthermore, researchers observed that their expression was influenced by environmental conditions. It was found also that; gene expression increases under low-temperature conditions and decrease under high-temperature conditions.

That's to say, Jew's mallow is a valuable base for *RbcL* and *MatK* genes, as they are influenced by environmental factors. (**Alqurashi and Awad, 2011** and **El-Rokiek *et al.*, 2016**).

#### 4. CONCLUSION

Based on the morphological and molecular studies it was concluded that Siwian landraces was more unique from the other two landraces, also DNA barcoding using *Matk* and *Rbcl* genes is an effective and sufficient tool in identifying different *Corchorus olitorius* landraces in Egypt.

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## المخلص العربي

## الترميز الوراثي والتحليل البيوكيميائي والمورفولوجي للسلاسل البلدية المصرية لنبات الملوخية

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1 قسم النبات الزراعي - كلية الزراعة ساها باشا- جامعة الإسكندرية- جمهورية مصر العربية

2 قسم بحوث النباتات الطبية والعطرية - معهد بحوث البساتين - مركز البحوث الزراعية- جمهورية مصر العربية

الملوخية أو الجوت واحدة من أهم النباتات التي لها العديد من الإستخدامات المختلفة على مستوى العالم، حيث تنتمي الملوخية إلى العائلة الخبازية وتضم حوالي 40 إلى 100 صنف أو أكثر حيث تتفاوت أطوال سيقانها وتزرع من أجل أوراقها الغضة. تمت هذه الدراسة في قسم النبات الزراعي، كلية الزراعة (ساها باشا)، جامعة الإسكندرية بالتعاون مع قسم النباتات الطبية والعطرية، معهد بحوث البساتين، مركز البحوث الزراعية، جمهورية مصر العربية للتحقيق في الوصف المورفولوجي وترميز الحمض النووي والتحليل الجيني الكيميائي الحيوي لثلاثة أنواع مختلفة من الملوخية المزروعة في مصر. سجلت السلالة السيوي أختلاف معنوي عن السلالات الأخرى في صفة طول النبات. كما أظهرت السلالة السيوي أعلى قيم يليها السلالة الصعيدي ثم السلالة الفلاحي في صفات عدد الأوراق/نبات وعدد الأفرع/نبات والوزن الغض مع عدم وجود فروقات معنوية بينهم. وقد أظهرت القيم البيوكيميائية بشكل عام عدم وجود فرق معنوي بين معظم العناصر التي تم تقديرها. كما أظهرت نتائج المحتوي المعدني زيادة عددية في السلالة السيوي لبعض المعادن عن غيرها بما في ذلك البورون والصوديوم والمغنيسيوم والألمونيوم والكالسيوم والتيتانيوم والكروم والزنك، بينما سجلت السلالة الصعيدي أعلى قيم للكوبالت والنيكل والنحاس والإنديوم. تم إجراء تفاعل البلمرة المتسلسل (PCR) للأثنين من البادئات المتعارف عليها (*RbcL-MatK*)، حيث يقع الوزن الجزيئي للقطعة الجينية المتميزة لجين الـ *RbcL* بين 800-900 قاعدة، بينما كانت الوزن الجزيئي 900 قاعدة للجين *MatK*. تم إستخدام برنامج DNAMAN للمقارنة الجزيئية لجينات *RbcL* و *MatK* التي تم الحصول عليها من السلالات البلدية من *C. olitorius* ومقارنة بعضها البعض. وتم تسجيل تسلسل الجينات الثلاثة في DDBJ Gene Bank بالرقم التعريفي التالي LC732565 و LC732566 و LC732567 لجين *RbcL* والرقم التعريفي التالي LC732049 و LC732050 و LC732051 لجين *MatK*. وخلصت الدراسة إلى أن السلالة المحلية السيوية كانت أكثر تميزاً عن السلالتين الأخرين. علاوة على ذلك، يعد إستخدام جينات *RbcL* و *MatK* في تعريف السلالات وتمييزها أداة فعالة وكافية في تحديد سلالات مختلفة من *C. olitorius* في جمهورية مصر العربية.