

# MORPHOLOGICAL AND MOLECULAR MARKER SCREENING FOR DROUGHT TOLERANCE IN EGYPTIAN JEW'S MALLOW (*CORCHORUS OLITORIUS* L.) LANDRACES

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## Abstract

Drought is the most important abiotic stress that severely affects the growth and yield of crops. The current study aimed to evaluate seven Jew's Mallow landraces (collected from different locations of Egypt) for both their response to water deficit and the level of genetic diversity among them. The water regime conditions that based on either 95% or 60% field capacity (FC) were applied after 21 days from the sowing date. Some morphological traits were measured, and the genetic diversity was evaluated by using random amplified polymorphic DNA (RAPD) marker. The results showed that the landraces differed in their responses to water deficit. All the studied agro-morphological traits were reduced under the stress condition, as well as chlorophyll content was significantly reduced. Furthermore, all the landraces accumulated the highest value (4.29) of proline at 60% FC. According to some drought indices, L6 was the most tolerant for drought stress, followed by L4, L7 and L5, while L1 was the lowest one. On the other hand, a moderate level of polymorphism (58.6%) was detected and a highly range of similarity relationships (0.61 to 0.84) were observed. Furthermore, the RAPD dendrogram was able to discriminate among the genotypes according to both their geographical and their drought tolerance capacity. The superiority of some landraces under drought stress conditions especially L6 suggested that landraces could be a beneficial tool for Jew's Mallow improvement breeding programs.

Keywords: drought, ecotypes, molokhiya, RAPD marker, leafy vegetables

## INTRODUCTION

Molokhiya or Jew's Mallow (*Corchorus olitorius* L.) belongs to the genus of about 40–100 species in the family *Tiliaceae*. It is the most important and popular summer leafy vegetable in Africa, Asia and Middle East countries, especially in Egypt and Sudan. It is eaten as a fresh vegetable soup. Even though the dried leaves could be used as well, and millions of households in developing countries depend on the Jew's Mallow as a source for food. However, these

leaves are a rich source of iron, protein, calcium minerals, thiamin, riboflavin, niacin and dietary fiber. Also, it contains several phenolic antioxidative compounds, polysaccharides, carotenoids and folic acid (Fawusi *et al.*, 1984; Abou Hadid *et al.*, 1994; Ghoneim and El-Araby, 2003; Abd-Allah, 2006; Abd-Allah and Nasr, 2006; Abd-Allah *et al.*, 2010; Helaly *et al.*, 2016; Ufoegbune *et al.*, 2016). In Egypt, the total cultivated area in 2018 was 887.8 hectares<sup>1</sup> with a total production of 2,172 tons and an

average yield of 0.409 tons per hectare<sup>-1</sup> (FAO, 2018). Water deficit is a major problem that causes a huge morphological, physiological, biochemical, and molecular changes in plants lead to many harmful effects on growth, yield and quality of plants, especially herbaceous plants (Ahmed *et al.*, 1998; Palve and Sinha, 2005; Helaly *et al.*, 2016; Bashandy and El-Shaieny, 2016; Wang *et al.*, 2016; El-Shaieny, 2017 a and b).

The water percentage in leafy vegetables was ranged from 80 to 95%, it is containing a lot of water quantities. In addition, their yield and quality suffer very quickly from drought. Leafy vegetables are very sensitive to water deficiency, and its yield and quality were significantly decreased in such environments. In case of the shortages occurs early in the crop's development, maturity may be delayed, and yields are often reduced. Also, when it occurs later in the growing season, quality is often reduced even though total yields are not affected (Ufoegbune *et al.*, 2016). The world population, climate changes and pollution of natural resources of water were increased rapidly, caused limitation of water availability (Boamah *et al.*, 2011). Furthermore, the selection of favorable genotypes with desirable traits needs to detect and check the genetic diversity and relationships among them. Molecular markers are effective and rapid method to characterize genetic diversity (Jarvis *et al.*, 2000). Random amplified polymorphic DNA (RAPD) one of many types of DNA molecular markers that have been widely used to detect genetic variations (Williams *et al.*, 1990). RAPD marker has been successfully performed in different plant species (Vierling and Nguyen, 1992; Menkir *et al.*, 1997; Rocha *et al.*, 2010; Kumar *et al.*, 2017; Moolendra *et al.*, 2018), as well as it has been applied in Jew's Mallow (Roy *et al.*, 2006; Haque *et al.*, 2007; Heikal *et al.*, 2015; Youssef *et al.*, 2019). Therefore, the present work was designed to study the Jew's Mallow landraces response, to different levels of soil moisture stress, to select the superior landraces that produce the greatest growth and yield under

I: List of 7 Jew's Mallow landraces used in this study

No	Name	Original place of collection
1	L1	Alexandria, Egypt
2	L2	Balat, New Valley, Egypt
3	L3	Eldakhla, New Valley, Egypt
4	L4	Esna, Luxor, Egypt
5	L5	Eddfo, Aswan, Egypt
6	L6	El-Monshaa, Sohag, Egypt
7	L7	Dandara, Qena, Egypt

\* L1 – L7 landraces

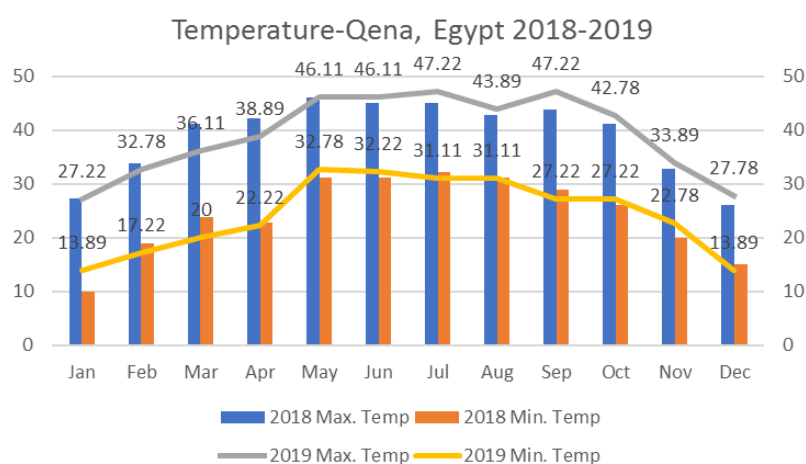
unfavorable conditions. Furthermore, to detect and test the level of genetic diversity and the genetic relationships among all these collected landraces

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

Seven Jew's Mallow (*Corchorus olitorius* L.) landraces namely, L1, L2, L3, L4, L5, L6 and L7 were collected from different locations of Egypt and were used in the current study (Tab. I). The morphological screening experiment was performed in two summer seasons of 2018 and 2019, at the Agricultural Experimental Farm, Faculty of Agriculture, South Valley University, Qena Governorate, Egypt, the site is at (latitude 26° 11' 22.2" N to Longitude 32° 44' 25.5" E), and 81 m above sea level. Monthly maximum and minimum temperature degree of Qena region, Egypt were listed in Fig. 1.

Seeds of the seven landraces were sown, on 25<sup>th</sup> and 26<sup>th</sup> March 2018 and 2019, respectively. The seeds were sown in rows about 20 cm apart and then irrigated. Plants were thinned to 5 or 20 cm between plants for fresh leafy yield or seed yield, respectively. Each experimental unit consisted of 10 rows, each of 4 m in length and 0.20 m apart, i.e.,



1: Monthly temperature degree maximum and minimum of Qena, Egypt

the unit area was 8 m<sup>2</sup>. Three cuttings were taken from each genotype for fresh leafy yield. The first cut was at 40 days after sowing, while the second and the third cuts were taken 25 days intervals. Differential water-stress regimes were imposed at 3 weeks after emergence. The plants were irrigated every 5 days (95% FC field capacity) as a control, and stress treatment was irrigated every 10 days (60% FC). The total amounts of applied irrigation throughout both seasons were calculated according to the concept of equivalent water depth. It equaled to 13000 m<sup>3</sup> hectare<sup>-1</sup> for the control (irrigation at 95% FC) and 8300 m<sup>3</sup> hectare<sup>-1</sup> for the stress (irrigation at 60% FC). The furrows were small, parallel channels, made to carry water to irrigate the plants by surface irrigation.

The furrows are small, parallel channels, made to carry water to irrigate the plants by surface irrigation. The irrigation treatments were separated by five meters to avoid horizontal soil water movement. The recommended that agronomic practices were followed to obtain optimum yield.

### Morphological Phenotypic Measurements

#### Fresh Foliage Yield and Its Components

In each cut, vegetative measurements were recorded as a mean of 20 randomly taken plants per entry. These characters were: stem length (SL) (cm), plant weight (PW) (g), leaves weight per plant (LW/P) (g), plant height (PH) (cm), and the number of leaves/plant (No. L/P). Total fresh foliage yield (TFY) was recorded in Kg per plot as the total weight of plants for each cutting each entry and the total yield in kg per m<sup>2</sup> was calculated for the three cuts taken from each entry.

#### Seed Yield and Its Components

At the end each of the season, the following traits were recorded as an average of 20 randomly taken plants; total seed yield/plant (SY/P) (g/plant), number of pods/plant (NP/P), number of seeds/pod (NS/P) and weight of 1000 seeds (W1000 S) (g).

### Physiological Traits

#### Relative Amount of Chlorophyll Content (RCC)

The relative amount of chlorophyll content was determined by chlorophyll Meter (Minolta SPAD-502 meter, Tokyo, Japan). Fifteen readings per plant (five leaves per plant) were taken from the widest portion of the leaf lamina, to avoid major veins. The samples were selected with concerning the heterogeneities within each sample point. The selected leaves were clean, dry, green and free of signs of disease or damage. Then the values were averaged as SPAD units.

#### Proline Content Determination (PROC)

Free proline was determined according to Bates *et al.* (1973). Samples (100 mg) were homogenized in 10 ml of 3% aqueous sulfosalicylic acid for 10 min, followed by filtration. Two milliliters of the filtrate were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin and then warmed in a water bath for 1 h at 90 °C. After cooling, the developed color was extracted in 4 ml toluene and measured by a photometric method using T80 UV-VIS Spectrophotometer at 520 nm against toluene. The content of proline was determined from the constructed calibration curve constructed using standard solutions of L-proline at concentrations ranging from 0.01 to 1 mM. The level of free proline in control was taken at 100%.

#### Drought Indices

Some drought indices were calculated using the equations according to Fernandez (1992) for stress tolerance index (STI), Fischer and Maurer (1978) for stress susceptibility index (SSI) and Rosielle and Hamblin (1981) for tolerance (TOL) and geometric mean productivity (GMP).

#### Statistical Analysis

All obtained data were statistically analyzed with the technique of analysis of variance (ANOVA) by using SAS computer software package SAS 9.1 program software, (SAS, 2004). Also, the Least Significant of Difference (LSD) method was used to test the differences between treatment means at a 5% level of probability as described by (Gomez and Gomez, 1984).

#### Molecular Characterization

The molecular study was done at the Department of Genetics, Faculty of Agriculture, New Valley University, Egypt.

#### DNA Extraction

Fresh young leaves were collected from 40 selected randomly seedlings for each genotype. The leaves were ground under liquid nitrogen to a fine powder and then the genomic DNA was extracted using DNA isolation kit (Favorgenv Biotech Corp. Cat. No. FAPGK001) as described in the manufacturer manual. DNA quality and quantity were performed by spectrophotometer, and then were diluted to a final concentration of 50 ng/μL.

#### PCR Analysis

Twelve RAPD primers were used; they were purchased from EZ Biolab, USA. PCR reactions were performed in 25-μL reactions containing 2.5 μL of PCR buffer (10X) containing MgCl<sub>2</sub> (15 mM), 1.5 μL of 10 mM dNTPs, 2 μL of 10 μM of each primer, 0.2 μL of Taq DNA polymerase (5 U/μL) and 2 μL (50 ng) of genomic DNA. PCR amplifications were performed in a Thermal Cycler (Labocon, U.K.). The instructions of

the used program as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, and extension at 72 °C for 3 min and a final extension at 72 °C for 7 min. PCR products were separated on 1.5% agarose gel using 1×TBE (Tris-Borate-EDTA) running buffer at 5 V/cm and then, the gels were stained with ethidium bromide (0.5 µg/ml) and visualized under UV light.

### Data Analysis

The detected bands were scored as 1 (present) and 0 (absent). Cluster analysis was carried out using the unweighted pair *group* method with arithmetical average (UPGMA) and dendrograms were constructed based on Jaccard's genetic similarity index (1908). Statistical analysis was performed using the software MEGA program. Resolving power ( $R_p$ ) of each primer was calculated using the formula:  $R_p = \sum I_b$  (Band informativeness) according to of Jaccard's genetic similarity index (1908). Whereas,  $I_b$  was calculated by the formula of  $I_b = 1 - (2 \times |0.5 - p|)$ , where  $p$  is the percentage of

genotypes containing the band. PIC was calculated according to  $1 - p^2 - q^2$ , where  $p$  is frequency of present band and  $q$  is the frequency of the absent band.

## RESULTS AND DISCUSSION

### Performance of Jew's Mallow Landraces

#### Vegetative Growth Evaluation

According to the analysis of variance, results in Tab. II shows significant differences among soil moisture stress conditions, the landraces and their interactions for all the measured traits. Concerning the TFY, under the favorable condition, its value ranged from 5.97 to 8.37 kg for L1 and L6, respectively. In contrast, these values were reduced under the stress condition and ranged from 3.60 to 6.95 kg for also L1 and L6, respectively Tab. II. Clearly that the L1 was the most sensitive to drought stress and it had a reduction of 39.7%, while L6 was the most tolerant landrace and had

II: Means performance and mean squares of growth traits of 7 Jew's Mallow landraces under water deficit, calculated from the combined data over two seasons, 2018 and 2019

Landraces	FC %	TFY (Kg)	PW (g)	LW/P (g)	PH (cm)	No. L/P	SL (cm)
L1	FC 95	5.97 fg	33.35 e	12.34 d	57.83 a	14.00 d	43.34 e
	FC 60	3.60 j	20.00 k	7.55 h	35.47 h	8.95 k	25.74 j
L2	FC 95	6.54 de	27.38 i	17.30 a	44.20 e	12.52 f	23.56 k
	FC 60	4.32 i	20.05 k	11.33 e	29.16 j	8.00 l	15.97 m
L3	FC 95	6.02 fg	30.04 h	17.03 a	33.81 i	14.53 c	27.11 i
	FC 60	4.19 i	20.10 k	11.25 e	22.50 k	9.10jk	19.22 l
L4	FC 95	7.81 b	41.08 c	12.53 d	39.88 g	12.00 h	47.67 d
	FC 60	6.25 ef	32.00 f	10.08 f	34.67 hi	9.27 j	38.17 g
L5	FC 95	6.58 d	49.96 b	11.43 e	52.33 c	15.00 b	39.51 f
	FC 60	5.00 h	37.00 d	8.59 g	40.00 g	11.05 i	29.58 h
L6	FC 95	8.37 a	67.99 a	14.45 c	48.79 d	14.96 b	60.36 a
	FC 60	6.95 c	57.00 b	12.61 d	42.90 f	12.25 g	54.51 c
L7	FC 95	7.62 b	31.06 g	15.98 b	54.54 b	17.96 a	55.41 b
	FC 60	5.85 g	24.08 j	12.53 d	42.55 f	13.00 e	43.68 e
LSD 05		0.2963	0.2095	0.4512	1.052	0.2364	0.4377
Mean Squares							
SOV	d.f						
Landraces	6	6.950**	1022.350**	24.315**	335.854**	20.211**	1109.287**
Field Capacity	1	34.90**	1392.992**	157.517**	1517.164**	184.716**	1053.109**
L × FC	6	0.189**	18.760**	4.080**	50.183**	1.838**	22.109**
Error	28	0.0222	0.0105	0.051	0.277	0.0145	0.0477

The different letters represent statistically significant differences between treatments ( $p < 0.05$ ).

\*FC (Field Capacity), (TFY) total fresh foliage yield, (PW) plant weight, (LW/P) leaves weight, (PH) plant height, (No. L/P) the number of leaves/plant, and (SL) stem length.

a reduction of 17.0%. Moreover, the plant weights of all the landraces were significantly affected by soil moisture stress Tab. II. L6 produced the heaviest plants under both normal and stress conditions (67.99 and 57 g), respectively. on the contrary, L1 gave the lowest value (20.0 g) of the plant weights under stress conditions. For leaves weight per plant trait, all the landraces were significantly affected by soil moisture stress conditions. The most reduction of leaves weight was observed by L1 (38.8%), but L6 had the highest value (12.61 g) of leaves weight under the stress condition. Furthermore, the plant height significantly varied among all the landraces under both the normal and stressed conditions, L1 had the most reduction. All the landraces were significantly affected by the stress conditions, the best tolerant landrace observed was L6 with reduction (5.9 cm, 12.1%). On the other hand, the leaves number per plant ranged from 17.96/lp for the L7 to 12.0 for the L4 under normal conditions. Soil moisture stress decreased the number of leaves per plant for all landraces, the L7, L6 and L5 showed the highest value (13.0, 12.3 and 11.1, respectively), while the lowest number was given by the L2 and L1. Moreover, L1 and L2 had the highest reduction value (36.1%). Furthermore, the stem length trait was measured and L1 recorded the most reduction (40%) in both favorable and unfavorable conditions. As well, L6 had the highest value (60.36 and 54.51) under both favorable and unfavorable conditions, respectively. The obtained results indicated the growth of *C. olitorius* landraces were significantly affected by soil moisture stress, for all studied traits. The results are in harmony with findings by (Fawusi, *et al.* 1984; Ayodele and Fawusi, 1989 and 1990; Prodhan *et al.*, 2001; Shiwachi *et al.*, 2008; Fasinmirin and Olufayo, 2009; Ghosh *et al.*, 2013 and Dhar *et al.*, 2018). They reported that *C. olitorius* plants which grown under drought stress were found to be shorter than plants that received full irrigation. Also, they found that the stem length, total fresh foliage yield, plant weight, leaves weight and number of leaves per plant were decreased significantly under water deficit conditions. These results may be due to that *C. olitorius* landraces responded diversely to different environments, suggesting the screening of cultivars under different environments are viewed as the most effective method for obtaining tolerant genotypes (El-Shaieny, 2017). In addition to, inhibition of cell division or cell enlargement and plant wilting grown under stress conditions (Raza *et al.*, 2014; Khan *et al.*, 2015; El-Shaieny, 2017; Yakoub *et al.*, 2016) study the effects of different water supply treatments (100%, 70% and 40% of the field water capacity) on agronomic and physiological parameters of *C. olitorius* plants, they reported that significant differences were obtained at  $P < 0.05$ , in all studied characters under acute and severe soil moisture stress as compared with control treatment.

### Seed Yield and Its Components

Seed yield and its attributes were significantly affected by soil moisture stress in all the landraces Tab. III. L1 was the most susceptible to soil moisture stress that had the lowest seed yield value (3.96 g), but L6 was the best one that produced the highest value of seed yield (5.72 g). On the other hand, L6 had the best performance of the number of pods per plant (60.33 and 54.33) under both unstressed and stressed conditions, respectively. In contrast, L1 and L2 recorded the most reduction (37.9% and 32.6%), respectively. For the number of seeds per pod, L1 landrace showed the highest reduction (31.6%), while the lowest reduction was 9.9% in L6. About the weight of 1000 seeds per plant, the values ranged from 1.21 g to 1.91 g for L2 and L1, respectively. Moreover, W1000S was significantly affected by soil moisture stress, the highest reduction value (11.3%) was recorded by L1, while the L5, L2 and L6 landraces had the lowest reduction values (0.6, 0.8 and 1.0%), respectively. The seeds yield per plant traits were reduced significantly for all studied traits under unfavorable condition (60% FC). These results indicate that water deficit decreased vegetative growth parameters, due to its harmful role in the photosynthesis processes, this led to a decrease in growth and development of plants, as a result of energy impairment production in the plant cell. Similar results were detected in tomatoes (Pervez *et al.*, 2009; Celebi, 2014; Khan *et al.*, 2015) cowpea (Farouk *et al.*, 2011; Hussein *et al.*, 2014; Hussein and Abd El-Hady, 2015; El-Shaieny, 2017) and wheat (Al Ameen, 2012; Kheiralla and Ismail, 1995).

### Physiological Traits

#### Relative Amount of Chlorophyll and Proline Contents

For further screening of drought-tolerant landraces, we measured the relative amount of chlorophyll and proline under a normal and stressed condition. Concerning the relative amount of chlorophyll content, the results in Tab. III showed that the highest value (84.86) was detected in L2 at normal condition, but at abnormal conditions, both L2 and L6 shared the highest value. For proline content, water deficit stress caused a significant increase in proline amount in all landraces. The highest accumulation of proline was 4.29 in L6 Tab. III. Clearly, the relative amount of chlorophyll content decreased with the increasing drought stress levels. While severe and moderate water deficit increased proline content in the leaves, that may be due to the ability of plants to maintain normal physiological functions and to adapt to unfavorable environments.

III: Means performance and mean squares of yield components and chlorophyll and proline content of 7 Jew's Mallow landraces under water deficit, calculated from the combined data over two seasons, 2018 and 2019

Landraces	FC %	SY/P (g)	No. P/P	NS/P	W1000S (g)	RCC	PROC
L1	FC 95	5.33 d	45.08 e	192.43 b	1.60 i	78.48 b	2.55 d
	FC 60	3.96 g	28.00 h	131.67 k	1.42 j	59.46 j	4.16 a
L2	FC 95	6.44 b	35.33 g	137.67 j	1.21 m	84.86 a	2.16 e
	FC 60	4.50 f	23.83 i	100.33 m	1.20 n	72.63 f	4.19 a
L3	FC 95	6.20 b	35.00 g	179.67 e	1.78 c	75.71 d	2.17 e
	FC 60	4.57 f	24.00 i	124.67 l	1.76 d	60.88 i	3.93 b
L4	FC 95	6.43 b	55.33 b	174.67 f	1.63 g	71.61 fg	1.94 f
	FC 60	4.52 f	49.33 d	140.33 i	1.61 h	61.33 i	3.79 bc
L5	FC 95	6.36 b	54.67 bc	196.67 a	1.70 e	73.84 e	1.90 f
	FC 60	4.98 e	45.00 e	139.00ij	1.69 f	60.87 i	3.70 c
L6	FC 95	6.98 a	60.33 a	189.33 c	1.91 a	71.35 g	1.37 g
	FC 60	5.72 c	54.33 c	170.67 g	1.89 b	65.42 h	4.29 a
L7	FC 95	5.80 c	40.33 f	184.67 d	1.41 k	77.09 c	1.87 f
	FC 60	4.65 f	34.67 g	151.65 h	1.39 l	65.22 h	3.65 c
LSD 05		0.3222	0.8890	2.666	0.004467	1.076	0.1998
Mean Squares							
SOV	d.f						
Landraces	6	1.230**	763.447**	2255.625**	0.332**	103.840**	0.300**
Field Capacity	1	24.335**	959.537**	18873.936**	0.0168**	1626.406**	40.535**
E × FC	6	0.475**	25.739**	367.780**	0.0058**	24.172**	0.290**
Error	28	0.0257	0.198	1.781	0.00003	0.291	0.00978

The different letters represent statistically significant differences between treatments ( $p < 0.05$ ).

\*Field capacity (FC), (SY/P) seed yield per plant, (No. P/P) number of pod per plant, (NS/P) number of seeds per pod, (W1000S) weight of 1000 seeds, (RCC) relative amount of chlorophyll content, and (PROC) proline content.

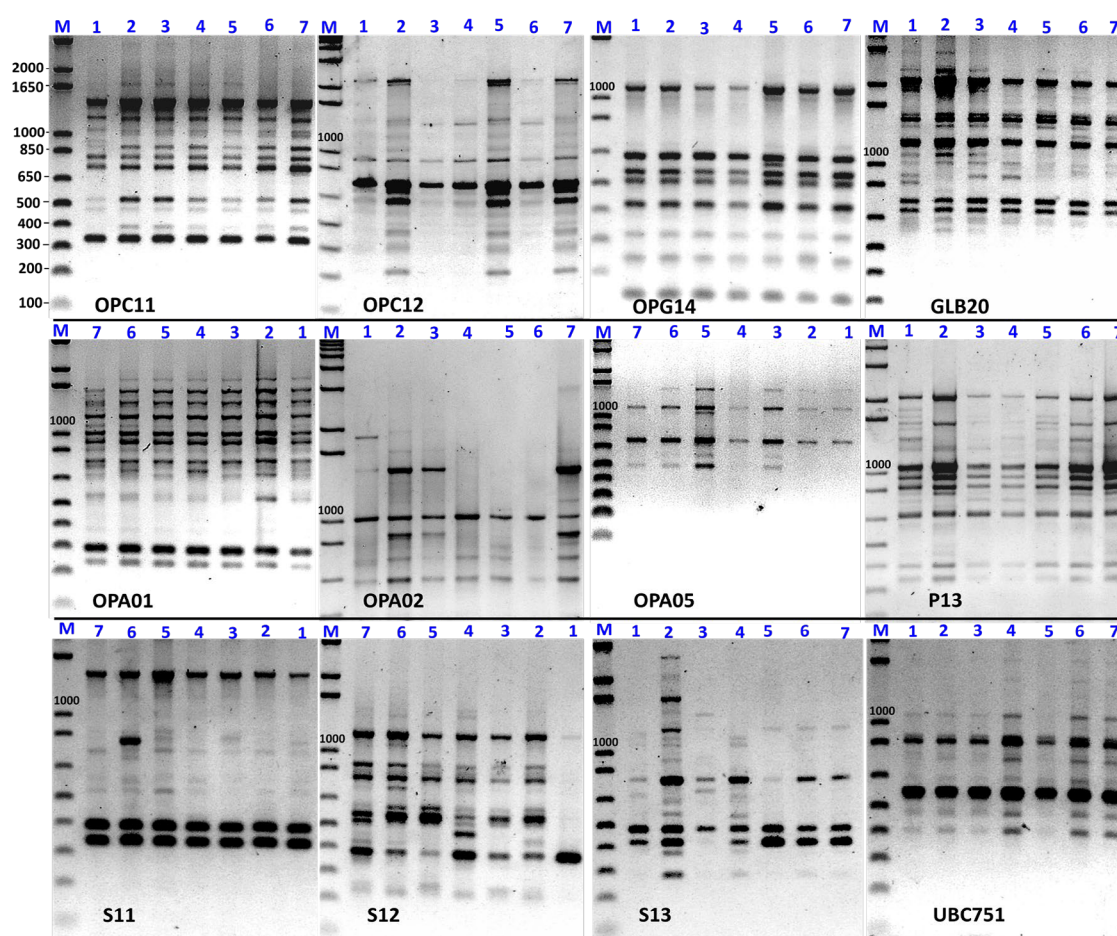
### Drought Tolerance Parameters

Furthermore, to identify the most drought stress tolerant landraces, some drought tolerance indices were calculated based on their grain yield under non-stress and stress conditions Tab. IV. L4 and L6 were the most tolerant genotypes, they had the highest value of stress tolerance index (STI) (1.00 and 1.09) and geometric mean productivity (GM) (6.99 and 7.63), respectively. Furthermore, they had the least values of stress susceptibility index (SSI) (0.768 and 0.653), respectively. Landraces with low SSI values ( $< 1$ ) can be considered to be drought-tolerant, due to the exhibited smaller yield reductions under water stress compared with well-watered conditions than the mean yield of all landraces. SSI and STI could able to differentiate the tolerant genotypes under drought stress conditions, which are fitted with many previous studies (Andarab, 2013; Anwaar *et al.*, 2020; Sánchez-Reinoso *et al.*, 2020).

### Molecular Analysis

#### C- RAPD Marker Analysis

To study genetic diversity and relationship among the studied seven genotypes, RAPD molecular marker was performed. Twelve primers were used Fig. 2 and they detected 140 bands with size varied from 135 to 2440 bp. Only 82 bands out of them were polymorphic, with a range of 20 to 84.62% polymorphism Tab. V. The lowest polymorphism was given by OPG14, while S12 primer gave the highest polymorphism (84.62). Furthermore, the polymorphism average was 58.6%. Indeed, this moderate level of polymorphism indicating the presence of moderate variability among evaluated landraces. Many researchers have been used RAPD markers to assess the genetic diversity in Jew's Mallow. In the current study, the detected polymorphism rate was slightly higher than the finding of Heikal *et al.* (2015), (28.3%) and Youssef *et al.* (2019) (44.44%). Moreover, the estimated OPC12 value ranged from 0.07 to 0.33 with an average of 0.21. The lowest value was



2: RAPD pattern of 7 Jew's Mallow landraces was produced by twelve primers. 1, L1; 2, L2; 3, L3; 4, L4; 5, L5; 6, L6; 7, L7. M, kbp DNA marker.

IV: Mean comparison between Jew's Mallow landraces for total fresh foliage yield (kg), under favorable and unfavorable conditions

Landraces	$Y_p$	$Y_s$	STI	SSI	TOL	GM	RDY	Class
L1	5.97	3.60	0.66	1.527	2.37	4.64	0.397	More sensitive
L2	6.54	4.32	0.76	1.306	2.22	5.32	0.339	Moderate sensitive
L3	6.02	4.19	0.72	1.169	1.83	5.02	0.304	Moderate sensitive
L4	7.81	6.25	1.00	0.768	1.56	6.99	0.200	High Tolerant
L5	6.58	5.00	0.82	0.924	1.58	5.74	0.240	Moderate Tolerant
L6	8.37	6.95	1.09	0.653	1.42	7.63	0.170	High Tolerant
L7	7.62	5.85	1.09	0.893	1.77	6.68	0.232	Moderate Tolerant
Mean	6.99	5.17						

(STI) Stress tolerance index, (SSI) stress susceptibility index, (TOL) tolerance index, (GMP) geometric mean productivity, (RDY) yield reduction

for the OPG14 primer, but the highest value was recorded by the OPC12 primer. Furthermore, the estimated  $R_p$  value ranged from 0.86 for OPG14 primer to 6.58 for the OPC12 primer. However, the OPC12 primer had the highest value for both PIC and  $R_p\%$  that makes it the most informative used primer. On the other hand, the cluster analysis of genetic similarity matrices, according to Jaccard's

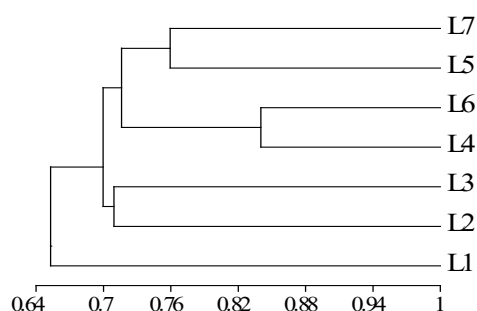
similarity coefficient Tab. VI revealed that, high similarity (0.61 to 0.84) was detected among all the seven genotypes. The highest similarity (0.84) was between L4 and L6 landraces, while L1 and L7 showed the lowest similarity (0.61). Moreover, the constructed dendrogram by UPGMA classified the seven landraces into two main clusters Fig. 3. The first one contained only the L1 landrace, while the

## V: Polymorphism obtained by twelve RAPD primers in 7 Jew's Mallow landraces

Primers	Primer sequence 5' → 3'	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %	PIC	RP
OPC11	AAAGCTGCGG	330–1380	13	9	4	30.77	0.09	1.42
OPC12	TGTCATCCCC	135–1125	12	3	9	75	0.33	6.58
OPG14	GGATGAGACC	135–1083	10	8	2	20	0.07	0.86
GLB20	GCACCCTTAC	690–2440	14	8	6	42.86	0.16	3.42
OPA01	CAGGCCCTTC	210–1760	13	10	3	23.08	0.08	1.44
OPA02	TGCCGAGCTG	645–1885	9	2	7	77.78	0.27	3.7
OPA05	AGGGGTCTTG	475–1455	6	2	4	66.67	0.25	2.02
P13	GGAGTGCCTC	400–2070	15	4	11	73.33	0.25	5.42
S11	GTA GAC CCGT	320–1400	10	4	6	60	0.19	2.84
S12	CCT TGA CGCA	185–1120	13	2	11	84.62	0.29	5.7
S13	TTC CCC CGCT	280–1640	16	3	13	81.25	0.26	5.72
UBC751	CCC ACC ACA C	395–1046	9	3	6	66.67	0.23	3.14
Mean			11.67	4.83	6.83	58.50	0.21	3.52
Total			140	58	82			

## VI: The similarity indices among the 7 Jew's Mallow landraces based on RAPD marker

Landraces	L1	L2	L3	L4	L5	L6	L7
L1	1.00						
L2	0.64	1.00					
L3	0.71	0.71	1.00				
L4	0.66	0.68	0.69	1.00			
L5	0.64	0.68	0.70	0.67	1.00		
L6	0.66	0.68	0.72	0.84	0.72	1.00	
L7	0.61	0.74	0.72	0.72	0.76	0.77	1.00



3: The dendrogram of genetic distances among the 7 Jew's Mallow landraces using UPGMA cluster analysis of Jaccard's similarity coefficient based on RAPD marker

second one contained all the collected landraces from Upper Egypt and divided into two sub-clusters, one of them contained L2 and L3 landraces, but the second one divided into two groups, one of them gathered both L4 and L6 landraces, but the second one contained L5 and L7. The constructed dendrogram could able to separate the landraces according to their geographic location, whereas the landraces which were collected from Alexandria were located in one cluster, and the rest of landraces were collected from Upper Egypt grouped in the other cluster. Classifications of these landraces are consistent with the results of Heikal *et al.* (2015). The Upper Egypt landraces are genetically close similar, this may be because they evolved from the same ancestral origin.



## CONCLUSION

Landraces are a reliable source for the detection of genetic variability. Thus, the present study aimed to evaluate seven landraces that were collected from different locations across Egypt. They were evaluated according to their morphology and molecular diversity under drought stress. All the studied traits significantly varied among all the evaluated landraces whether under normal or stressful conditions. Among them, both landraces L6 and L4 revealed superior performance under soil moisture stress. On the other hand, the RAPD marker had a powerful ability to distinguish the genetic diversity among the collected landraces. The similarity among them was high. Furthermore, the dendrogram was able to classify all the landraces according to their geographic region and their drought tolerance ability. However, the superiority of the L7 landrace under drought stress suggested that landraces could be used through the genetic improvement breeding program of Jew's Mallow.

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