Research Article
Determination of Genetic Parameters Associated with Salt Stress Tolerance in Canola Based on SCoT Markers and Protein Pattern Analysis

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Abstract

Background and Objective: Due to the importance of canola crop at the local and international levels in producing vegetable oils with high nutritional value. Therefore, it was necessary to study the impact of salt stress in cultivating this important crop. **Materials and Methods:** Three Egyptian canola cultivars namely, Pactol, Sirw 4 and Sirw 6 were evaluated under the control experiment conditions and two salinity stress treatment using sea water during two growing seasons for knowing the different impacts of salt stress on a selected group of yield and its components traits and some physiological parameters related to salt stress tolerance in this regard. **Results:** The final results confirmed that the three canola cultivars were recorded a high level of salinity tolerance in all studied traits through both growing seasons. Where the canola cultivar Pactol was coming in the first rank in this regard followed by Sirw 6 and then followed by Sirw 4. However, the tolerance degrees were not equal at every level of salt stress compared to the standard experiment. **Conclusion:** All results proved that the second level of saline stress was the safest for growing and sowing canola plants. Further, biochemical and molecular genetic markers using SCoT primers have already succeeded in identifying the genetic evidence responsible for salt stress tolerance in the three canola cultivars by discovering 50 unique bands (32 positive and 18 negative specific markers).

Key words: Canola, genetic parameters, SCoT markers and SDS-PAGE

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity is one of the most dangerous environmental obstacles that impede and destroy plant production and also affect the distribution and abundance of different plant species. Among the main reasons that lead to salinization of the soil is the high rate of evaporation, the lack of water needed for irrigation and soil washing and the accumulation of salts, especially in lands close to sea water[1]. Canola is considering one of the most important oil crops and an important source of vegetable oil extraction after palm oil and soybean oil. Also, canola oil is one of the best vegetable oils when used in human nutrition as the oil contains only 6% of saturated fatty acids and 94% fatty acids, not saturated[2,3]. Besides, canola oil is used in human nutrition in many countries of the world, such as Canada, Europe, America and Japan. For example, canola oil represents 63% of the total vegetable oils used in Canada, while soybean represents 24% and sunflower oil only 4%. In the same context, canola oil is the fifth crop in terms of global trade, preceded by rice, wheat, corn, cotton and then canola besides, it is considered the third export crop in Canada after wheat and barley. Canola is grown as a winter crop in Egypt and varieties without Erosic fatty acid are grown in oil and glycosinolate in the oilcake and from these varieties is the Pactol variety, which is characterized by a high oil content of the seed, with a ratio of up to 49% and tolerates adverse environmental conditions. Also, canola cultivation is very good in the newly reclaimed land in which traditional winter crops are not available. Therefore, the expansion of canola cultivation in the new lands is a national goal to increase the production of healthy vegetable oils in Egypt. Although it tolerates adverse environmental conditions, high soil salinity affects the final yield and other important plant characteristics, chiefly the oil content. Further, the percentage of waste in the final crop when growing canola in areas damaged by high salinity, especially coastal areas near sea water to 40-50%. Therefore, the strategic objective of this investigation is to try to find out the level of saline stress that canola plants can bear without affecting the properties of the final yield and oil or at least the effect is acceptably reduced. This is the genetic improvement of salinity tolerance in canola cultivars that is desired from this study. The following is a quick review of the results of the most important research and studies conducted in this regard. Youssef *et al.*[4] discovered 13 specific markers uniqueness the canola genotype Masrri-L11 besides, 5 unique bands associated with the accession Masrri-L16 across using RAPD-PCR technique. These specific markers are strong evidence that is directly related to their endurance of salt stress. In general, canola varieties, whether sensitive (Symbol) or tolerant to salt stress are not severely affected by salinity stress in the early stages of growth such as seed germination and seedling growth. While the effect is most severe in the two stages of flowering and the final yield. Besides, Canola cultivars are severely affected when the salt stress is at 12 ds m^{-1}. While, the concentration at 3 ds m^{-1} has a positive effect on the metabolic functions in the cell and in this case the sodium concentration is ideal, especially in the germination stage[5]. Athar *et al.*[6] discussed the importance of using both proline and glycine betaine in a growth environment of two types of canola, especially under salinity conditions. They proved that the addition of proline at a rate of 1-5 mm in a saline environment led to the improvement of germination and seedling growth compared to natural conditions. Bybordi[7] studied the impact of different salinity levels on yield and its components traits in canola varieties and revealed that the canola accessions, SLMO46 and Okapi were exhibited better performance in the traits, plant height and heading date under various levels of salinity compared to the control. While, the cultivar Okabi only recorded the highest mean values in the other attributes under study such as the number of seeds/pant and seed yield/plant under the same conditions, respectively. A large number of genes responsible for both water and salt stresses tolerance were isolated using QTLs mapping in canola[8]. These QTLs maps proved that a large portion of these genes has great potential for genetic improvement to salt stress tolerance in brassica genotypes under climate change conditions. Kumar *et al.*[9] detected the fruitful role of microsatellite (SSR) markers associated with molecular breeding for salinity tolerance in brassica and discovered that SSR markers studies may be greatly helpful in detecting and identifying genes related to salt stress tolerance in canola varieties besides, contribute significantly to mapping QTLs in this regard. The fruitful role of salicylic acid for enhancing salinity tolerance in canola accessions and the impact of salt stress on protein contents in roots and shoots were discussed[10]. He confirmed that protein content was increased under salinity conditions compared to the control experiment. Further, salt stress stimulates gene expression to produce specific proteins in canola cultivars that are tolerant only. This mechanism of salt stress tolerance is not present in salt-sensitive cultivars besides, this fact was proven after the emergence of 15 and 12 protein bands in shoots and roots especially after adding salicylic acid. Athar *et al.*[11] detected the importance of glycine betaine in increasing the

efficiency of photosynthesis in canola genotypes under salinity stress conditions through determining chlorophyll fluorescence parameters as salinity tolerance indices. They confirmed that the fluorescence parameters played a fruitful role in comparing and determining among sensitive and tolerance canola for salinity stress accessions in this regard. Despite the description of a large number of canola accessions as being tolerant to salt stress, the plant's growth and their final yields are greatly affected by this dangerous environmental factor. Besides, the genetic engineering used to develop salt-tolerant canola lines, unfortunately, was slowing down in this regard[12]. Asghari *et al.*[13] studied the effect of salinity stress at the seedling stage in canola cultivars by determining the relationship among some morpho-physiological traits using 11 ISSR markers and detected that the ISSR primers produced 45 polymorphic fragments in all canola accessions. Besides, the cluster analysis divided all canola cultivars into 3 clusters. Where the lowest genetic similarity was observed among Zarfam and Jevel cultivars (0.079). While that, the highest similarity was within Quantum and Hyola 60 whit SLMO46 cultivars (0.32). Salt stress led to highly damage in all yield attributes of canola especially in seed yield when the salt stress reaches 20%[14]. Abiotic stresses greatly affect the productivity of canola crop due to their destructive effect on morphological, physiological and biochemical processes, which is reflected in the efficiency of metabolism processes[15]. However, it was noted that good management of water resources and agriculture besides, the use of the application of antioxidants and the essential components have a great role in mitigating the destructive effects of salt and water stress. Seven canola genotypes were evaluated under three salinity levels (0, 7 and 14 ds m^{-1}) to identify some physiological indices associated with salt-stress tolerance[16]. The final results confirmed that the two canola genotypes, LSG2 and LSN showed higher positive data of agronomic traits under both salinity levels. Where they exhibited a lower percent of Na$^+$ content and a higher percent of K$^+$ in their shoots compared to the two cultivars Sarigol and RGS003. This fact indicated that the two canola cultivars, LSG2 and LSN were considered highly salinity tolerance measurements to salinity physiological indices such as, low SSI

and STI. After all that has been listed, it is possible to briefly clarify the goal of this study, which is to study the physiological and molecular genetic aspects obtained by bearing three canola varieties to salt stress. Besides, clarify the genetic response resulting from exposure to salt stress and try to detect and identify genes responsible for enduring salt stress through analysis of water-soluble protein (protein banding pattern) which would make a big leap in genetic improvement to salt stress tolerance in canola.

MATERIALS AND METHODS

Plant materials: This study included 3 Egyptian canola varieties with highly salt-stress tolerance namely, Sirw 4, Sirw 6 and Pactol in Table 1. These three accessions were brought from the Oil Crops Research Department, Field Crops Research Institute-Agricultural Research Center-Egypt. This investigation aimed to study the genetic behaviour responsible for salt-stress tolerance in some canola genotypes through testing some agro-morphological and physiological traits under normal and salinity conditions in this regard Also, it discussed the physiological, biochemical and molecular genetic effects resulting from exposure to salt stress and determining the safest salt-stress level that canola plants can tolerate it with minimal damage to the final output.

Sowing: The three canola accessions were sown under normal and salinity conditions in plastic pots through a randomized complete block design with three replicates for each experiment in the two growing seasons 2018/2019 and 2019/2020. The four experiments were grown in black plastic bags in the greenhouse of the National Research Centre using sandy soil to (prevent the accumulation of salts during irrigation periods with salted water after dilution until harvest). The first experiment (Normal conditions) means (tap water, 0.5 ds m^{-1}). While, salinity experiment included three salt-stress treatments (3.0, 5.5 and 8.0 ds m^{-1}) were induced using diluted Mediterranean seawater. The chemical analysis for the four kinds of water was shown in Table 2. The package of all other recommendations of canola planting followed in both growing seasons.

Table 1: Classification of the three canola cultivars used in this study

Serial No.	Names of genotypes	Origin	Number of from sowing to harvesting	Response to salinity tolerance	Reaction to diseases
1	Sirw 4	Egypt	150	Tolerance	Tolerance
2	Sirw 6	Egypt	146	Tolerance	Tolerance
3	Pactol	Egypt	148	Tolerance	Tolerance

Table 2: Chemical analysis of all water irrigation kinds (tap water and the three salinity levels of sea water)

Salt treatment	Tap water (Ml)	Sea water (Ml)	Sea water (mix %)	EC (ds m^{-1})	CO$_3^-$	HCO$_3^-$	Cl$^-$	SO$_4^-$	Ca^{++}	Mg^{++}	Na$^+$	K$^+$
1:Control	1000	0	0.0	0.5	-	2.98	0.88	0.27	1.44	1.17	1.45	0.19
Salinity level I	941	59.0	5.9	3.0	-	3.14	28.75	0.38	2.79	9.23	18.94	0.71
Salinity level II	891.80	108.20	10.82	5.5	-	3.21	67.22	0.74	5.37	16.84	37.28	1.37
Salinity level III	842.60	157.40	15.74	8.0	-	3.28	92.33	0.83	8.15	25.42	58.17	2.07
Sea water	-	-	-	50.8	-	3.02	502.08	2.15	46.12	144.05	311.04	10.96

EC: Electrical conductivity

Planting method: Growing was done in the half of October in both growing seasons 2018/2019 and 2019/2020 using 30×40 cm black plastic bags field with about 17 kg of tap water washed sand using sufficient amount of seeds in each pot (15 seeds) to be diluted to 5 seeds after germination, that is, after 15 days from sowing and that's for the four experiments (the control and three salt treatments). All experiments were irrigated every 15 days with 2 L pot^{-1} of irrigated solution corresponding to each salinity level (enough for irrigation and leaching) to avoid salt accumulation. The salt stress was applied to start from the sowing irrigation. After fifteen days from sowing, the plants were thinned and only five seedlings carefully left in each pot to grow until maturity.

System of salinization: The Mediterranean Sea water with (50.8 ds m^{-1}) was used for salting experiments after diluting them to the aforementioned rates namely (3.0, 5.5 and 8.0 ds m^{-1}) as follows:

- The first salinity level (3.0 ds m^{-1}): It means that the percentage of sea water used was 5.9%
- The second salinity level (5.5 ds m^{-1}): It means that the percentage of sea water used was 10.82%
- The third salinity level (8.0 ds m^{-1}): It means that the percentage of sea water used was 15.74%

Each treatment was considered as an independent experiment and the standard experiment was irrigated with tap water with (0.5 ds m^{-1}).

Chemical analysis of planting soil: The soil used in sowing for all experiments were sandy soil (92.0% sand, 3.5.0% slit, 0.8% organic mater, 8% clay and 3.5% clay).

Studied traits: Fifty plants were taken at maturity stage from each canola cultivar of each replicate from each experiment (the control and the three saline treatments) in both growing seasons to evaluate some agro-morphological and physiological traits related to salinity tolerance as follows:

- 1000 seed weight (g): It was recorded as the weight of 1000 random filled grains per plant

- Seed number per pod: It was recorded by a counted number of filled seeds per pod
- Pod number per plant: It was recorded by a counted number of pods per plant
- Seed yield/plant: It was recorded as the weight of seed yield of each plant and adjusted to 14% moisture content
- Oil (%): It was measured by Inframatic 8620 Percor
- Oil yield/plant: It was obtained by multiplying seed yield in percentage oil
- Determination of Na$^+$ uptake, K$^+$ uptake and Na/K ratio: Shoots sampling were determined and performed 25 days from salinization by using three salinity levels of diluted sea water at (3000 ppm or 3.0 ds m^{-1}, 5500 ppm or 5.5 ds m^{-1} and 8.000 ppm or 8.0 ds m^{-1}) besides the samples performed from the control experiment and all samples were weighed and dried for three days at 70°C. Finally, samples were grounded and 1 g dried powder from each sample for all studied materials and was taken for Na$^+$ and K$^+$ determination by flame photometer
- Osmotic adjustment: It was determined by the formula as follows[17]:

$$\frac{OP \times RWC}{100} \text{ (normal)} - \frac{OP \times RWC}{100} \text{ (drought)}^{100}$$

where, OP is the osmotic pressure, RWC is the relative water content.

- The proline content was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline/mL C mL toluene)/115.5 µg/µ mole]/[(g sample/5)] = µ moles proline/g of fresh weight material. The results related to proline content are average values of at least 3-4 samples for each species[18] and modified method[19]
- Glycine betaine contents: It was carried out according to the previously described method[20]

At the physiological maturity stage, the yield was harvested when 40-50% of seeds in the main pods and primary branches turned brown and to evaluate the biological and physiological yield, samples were oven-dried at 70°C for 72 hrs.

Statistical analysis: Each treatment was analyzed as a randomized complete blot design with three replicates independently for each year and all calculated data performed from all studied traits for the four experiments in two seasons were analyzed using the SPSS ver. 17 and analysis of variance were detected as recorded[21]. LSD values were calculated[22] as following:

$$LSD = t\ 5\%\ or\ 1\% \times \sqrt{2MSe\,/\,r}$$

where, r is the number of replicates.

Estimation of salinity tolerance indices: All salinity tolerance indices parameters for grain yield/plant trait only were estimated for the three salinity levels[23-29] as follows:

- GYP is meaning the grain yield/plant for the control experiment
- GYS is meaning the grain yield/plant for the salinity experiment:

$$YSI\ is\ meaning\ yield\ stability\ index = \frac{YS}{YP}$$

where, YS is the average of yield under stress and YP is the average of yield under the control experiment

- YI is the meaning yield index (YS for each genotype/mean of YS for all genotypes)
- MP means (Average yield for both trials): YS+YP/2
- STI is the meaning salinity tolerance index (YP×YS/(mean of YP)^2
- GMP = (YP×YS)$^{0.5}$
- YR is the meaning yield reduction (1-YS/YP)
- SSI is the meaning salinity susceptibility index
- DSI = (1-YS/YW)/D
- Yw is the mean yield under control condition
- D is the environmental stress intensity = 1-(mean yield of all genotypes under stress/mean yield of all genotypes under irrigated conditions)

Estimates of genetic parameters: Variance components, heritability in the broad sense, genetic coefficient of variability (GCV %), phenotypic coefficient of variability (PCV %), D^z or the difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %), expected genetic advance besides and genetic advance as percentage of mean were the most important measurements calculated through the two growing seasons in this study as follows:

- The genetic coefficient of variability (GCV %) and phenotypic coefficient of variability (PCV %) was estimated according to the method suggested by[30] as follows:

$$Environmental\ variance\ (\sigma^2 e) = MSe,\ Genotypic\ variance\ (G\ v)$$
$$or\ (\sigma^2 g) = MS_g - MS_e/r$$

$$Phenotypic\ variance\ (Ph\ v)\ or\ (\sigma^2 ph) = (\sigma^2 e)+(\sigma^2 g)\ or\ MS_e+MS_g$$

where, MSe is the mean square of error, MSg is the mean square of genotypes, R is the number of replicates and X is the mean of trait:

$$Genetic\ coefficient\ of\ variability\ \left(GCV\ \%\right) = \frac{\sqrt{GV}}{x} \times 100$$

$$Phenotypic\ coefficient\ of\ variability\ \left(PCV\ \%\right) = \frac{\sqrt{Phv}}{x} \times 100$$

Estimation of heritability in the broad sense: Broad sense heritability (h^2) expressed as the percentage of the ratio of the genotypic variance (g v) to the phenotypic variance (ph v) and was estimated on a genotype mean basis[30,31]:

$$H^2 B = \frac{\sigma^2 g}{\sigma^2 ph} \times 100$$

where, D^z is the difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %) or (PCV %)-(GCV %).

Estimation of genetic advance: The expected genetic advance (GA) and percentage of the mean (GAM) assuming selection of superior 5% of the genotypes was estimated following the methods illustrated[31] as:

$$(GA) = K \times (\sigma^2 g) \times \sqrt{\frac{Phv}{Phv}}$$

where, K is the standardized selection differential at 5% selection intensity (K = 2.068) and the genetic advance as percentage of the mean (GAM) was computed as:

$$GAM\ (\%) = \frac{GA}{X} \times 100$$

Biochemical molecular markers studies
Protein profile analysis using SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed, in Genetics and Cytology Department, National

Research Centre[32,33]. Water-Soluble Proteins (WSP) of the three canola studied varieties were taken from the leaves of these plants. Then protein fractionations were performed exclusively on vertical slab gel (19.8×26.8×0.2 cm) using the electrophoresis apparatus manufactured by Cleaver, UK. The images were captured by the digital camera (Sony, made in Japan) and transferred directly to the computer. The protein bands were analyzed by the Total Lab program to find out the molecular weight of each band and that to compare the presence and absence of the band among varieties. These dates were imported in MVSP (Multi-Variant Statistical Package) to find the similarity matrix and dendrogram (UPGAMA, suing Jaccard's coefficient) which reflect the relationships among the three canola varieties.

DNA isolation and SCoT analysis: Genomic DNA from fresh leaves of the three canola genotypes namely, Sirw 4, Sirw 6 was extracted[34] by the protocol of Biospin plant genomic DNA extraction Kit (Bio basic). Eleven (SCoT) primers SCoT 1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 12 were selected. Amplification reactions were carried out in a total volume of 25 μL, containing 40-100 ng of isolated genomic DNA, 2.5 μL of 10X buffer [100 mM Tris-Cl-pH 8.3, 0.5 M KCl, 0.1% (w/v) gelatin], 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 0.5 μM primer, 0.5 units Taq DNA polymerase. Amplification conditions were as follow, 95°C for 5 min for the initial denaturation step, followed by 35 cycles at 94°C for 1 min for denaturation, a primer annealing at 50°C for 1 min and an extension at 72°C for 2 min, finally, the extension was carried out at 72°C for 7 min. All PCR amplification products were separated on 1.2% agarose gels in TBA 0.5% then stained with ethidium bromide and visualized under UV light. PCR-generated SCoT bands were detected on gels and then scored as absent (0) or present (1), only clear, reproducible bands were scored.

DNA ladder: The marker of the DNA ladder is used for the molecular genetic differentiation among the three canola genotypes used in this investigation by using SCoT primers. This is vital in gel analysis process to obtain the important molecular weights for this comparison and DNA ladder weights as follows, 250, 500, 700, 1000, 1500, 2000, 4000, 5000, 6000, 8000 and 10000 bp.

Gel documentation: Gels were photographed scanned, analyzed using Gel Doc Vilber Lourmat system (Vilber Company, France) to capture the image and to calculate band intensities.

Data handling and cluster analysis (phylogenetic tree): Data was scored for computer analysis based on the presence or absence of the amplified products for each primer. Pairwise components of the three canola cultivars based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients[35]. The similarity coefficients were then used to construct dendrograms, using the un weighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).

RESULTS

Analysis of variance (ANOVA test): Data presented in Table 3 and associated with the analysis of variance test detected highly significant differences among the three canola cultivars namely, Sirw 4, Sirw 6 and Pactol (Table 1) for all studied traits under the four treatments conditions (The control and the three salinity levels) during both growing seasons (2018/2019 and 2019/2020). Coefficient of variance percentage appeared low in pod number/plant trait and the values were (0.86 and 0.91%), (0.92 and 0.90%), (0.99 and 0.82%) and (1.39 and 1.21%) and seed yield/plant trait (0.85 and 0.96%), (1.21 and 1.52%), (0.76 and 0.79%) and (1.67 and 1.45%) for the four treatments in both years, respectively. While, the values were medium as follows, (17.71% and 21.90 %), (26.83 % and 18.39 %), (27.63% and 35.43%) and (30.71% and 25.11%) in 1000 seed-weight trait and (30.71 and 25.11%), (4.10 and 3.09%), (4.36 and 4.40%), (4.61 and 4.86%) and (7.92 and 7.87%) in seed number/pod, (2.35 and 2.43%), (2.40 and 2.66%), (1.28% and 1.95%) and (3.08 and 2.88%) in oil% trait, (2.43 and 2.65%), (2.75 and 2.81%), (4.17 and 3.97%) and (6.17 and 6.12%) in oil yield/plant trait, (2.20 and 2.42%), (1.17 and 1.53%), (2.27 and 2.24%) and (1.38 and 1.74%) in proline content trait and (3.17 and 3.75%), (1.75 and 1.73%), (2.16 and 2.02%) and (1.66 and 1.53%) in glycine betaine content trait for all treatments in both growing seasons, respectively. Further, the values were high in the rest studied traits and the values were (184.87 and 157.45%), (206.15 and 168.50%), (217.73 and 157.28%) and (142.40 and 129.08%) for Na^+ uptake trait, (43.53 and 39.99%), (44.34 and 40.94%), (20.75 and 27.15%) and (21.42 and 22.18%) in K^+ uptake trait, (283.47 and 333.33%), (520.41 and 369.49%), (535.90 and 358.32%) and (402.17 and 345.84%) in Na/K ratio trait and (55.84 and 74.79%), (156.03

Table 3: ANOVA analysis of all studied traits for the three canola accessions under all treatments in the two growing seasons 2018/2019 and 2019/2020

S.O.V	DF	Seasons/Treatments	1000 seed weight (g)				Seed number per pod				Pod number per plant				Seed yield/plant (g)			
			C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Genotypes	2	2018/2019	34.21***	11.67**	18.03**	9.34**	14.67**	20.05**	8.12**	15.22**	154.03**	79.65**	51.28**	42.55**	14.93**	34.99**	27.45**	33.65**
		2019/2020	32.87**	14.22**	22.05**	13.02**	21.96**	13.82**	10.06**	13.79**	121.54**	38.23**	40.08**	18.39**	24.57**	31.68**	18.33**	68.83**
Replicates	2	2018/2019	15.78**	19.04**	30.02**	8.21**	133.021**	93.43**	23.94**	40.08**	49.23**	29.60**	83.23**	34.08**	119.32**	16.28**	10.49**	55.18**
		2019/2020	23.86**	6.21**	12.77**	10.05**	118.34**	100.04**	12.56**	31.15**	14.87**	32.18**	47.56**	32.18**	125.41**	11.04**	35.94**	41.07**
Error	4	2018/2019	0.67	1.24	1.04	0.81	1.43	1.31	1.06	2.03	1.67	1.55	1.39	1.56	0.69	1.14	0.36	1.02
		2019/2020	1.02	0.58	1.70	0.47	0.79	1.29	1.35	1.74	1.81	1.41	0.94	1.15	0.88	1.79	0.39	0.73
CV (%)		2018/2019	17.71	26.83	27.63	30.71	4.10	4.36	4.61	7.92	0.86	0.92	0.99	1.39	0.85	1.21	0.76	1.67
		2019/2020	21.90	18.39	35.43	25.11	3.09	4.40	4.86	7.87	0.91	0.90	0.82	1.21	0.96	1.52	0.79	1.45

S.O.V	DF	Seasons/Treatments	Oil (%)				Oil yield/plant				Na$^+$ uptake				K$^+$ uptake			
			C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Genotypes	2	2018/2019	107.22**	56.32**	42.13**	18.34**	57.28**	98.12**	38.37**	17.28**	6.34**	11.03**	13.08**	4.56**	7.83**	24.11**	14.19**	10.03**
		2019/2020	94.23**	42.22**	29.66**	27.03**	73.02**	104.0**	45.12**	21.45**	7.09**	15.33**	18.59**	10.02**	5.19**	17.35**	33.08**	12.37**
Replicates	2	2018/2019	12.05**	33.19**	14.36**	8.42**	15.48**	61.08**	13.81**	45.93**	25.42**	31.06**	26.19**	38.75**	19.22**	8.39**	29.24**	52.47**
		2019/2020	10.38**	18.59**	31.48**	19.03**	23.08**	44.73**	22.56**	36.41**	17.46**	24.19**	8.49**	21.29**	26.43**	11.07**	36.04**	38.27**
Error	4	2018/2019	1.25	1.06	0.24	0.82	1.28	1.07	1.54	1.19	0.35	0.68	0.96	0.73	1.55	1.62	0.34	0.28
		2019/2020	1.33	1.29	0.55	0.69	1.52	1.12	1.39	1.07	0.27	0.41	0.57	0.62	1.29	1.40	0.57	0.31
CV (%)		2018/2019	2.35	2.40	1.28	3.08	2.43	2.75	4.17	6.17	184.87	206.15	217.73	142.40	43.53	44.34	20.75	21.42
		2019/2020	2.43	2.66	1.95	2.88	2.65	2.81	3.97	6.12	157.45	168.50	157.28	129.08	39.99	40.94	27.15	22.18

S.O.V	DF	Seasons/Treatments	Na/K ratio				Osmotic adjustment				Proline content				Glycine betaine content			
			C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Genotypes	2	2018/2019	12.55**	35.02**	17.75**	14.92**	-	16.28**	11.08**	7.49**	13.74**	15.08**	26.32**	18.92**	74.55**	83.17**	50.11**	28.93**
		2019/2020	35.88**	28.04**	25.34**	7.55**	-	41.05**	15.34**	13.92**	10.08**	23.11**	32.14**	22.08**	69.03**	78.25**	42.27**	16.24**
Replicates	2	2018/2019	107.86**	48.57**	32.77**	18.34**	-	19.36**	33.34**	9.24**	145.07**	103.22**	18.55**	59.65**	73.82**	85.61**	33.07**	45.29**
		2019/2020	55.13**	23.04**	11.09**	10.03**	-	10.23**	85.23**	17.42**	127.34**	96.05**	126.04**	105.49**	64.93**	114.38**	42.09**	51.77**
Error	4	2018/2019	0.11	0.39	0.83	1.06	-	0.14	0.71	0.29	0.39	0.22	1.24	0.69	0.84	0.58	1.46	1.28
		2019/2020	0.16	0.26	0.43	0.79	-	0.29	0.55	0.33	0.45	0.35	1.18	1.09	1.15	0.67	1.35	1.13
CV (%)		2018/2019	283.47	520.41	535.90	402.17	-	55.84	156.03	168.28	2.20	1.17	2.27	1.38	3.17	1.75	2.16	1.66
		2019/2020	333.33	369.49	358.32	345.84	-	74.79	134.83	164.13	2.42	1.53	2.24	1.74	3.75	1.73	2.02	1.53

*Significant at 5% probability level, **Significant at 1% probability level, C: Control, T_1: Treatment one, T_2: Treatment two, T_3: Treatment three

and 134.83%) and (168.28 and 164.13%) for osmotic adjustment trait for all treatments in both growing seasons, respectively.

Mean performance: By looking at results detailed in Table 4, it can notice that the three canola varieties have already achieved great tolerance to salt stress and excelled in all the studied traits in both growing seasons. Where tolerance was excellent under the first and second levels of saline stress compared to the standard experiment. While the third level of saline stress came in third in terms of endurance during both seasons. On this basis, it can be said that the three canola accessions achieved the highest averages under the first level of saline stress (3.0 ds m^{-1}) and the rate of loss due to salt stress in all studied traits was within the limits of 10.0% followed by the second level of saline stress (5.50 ds m^{-1}), which achieved a loss rate of traits up to 20.0% and then followed by the third level of salinity stress (8.0 ds m^{-1}), in which the rate of loss of traits reached to 40.0% in both growing seasons, respectively. In general, it was observed that the three canola cultivars had achieved the highest mean values in all studied traits except the following traits, Na$^+$, Na$^+$/Ka$^+$ ratio and osmotic adjustment where they achieved the lowest averages for those three traits in both growing seasons under the three salt-stress treatments compared to the control. Accordingly, the most important results can be summarized, which is that canola plants bear salty stress significantly until the second level of saline stress (5.50 ds m^{-1}), meaning that this level is the safest level for plants besides, giving a good final output. The mean values for all treatments in both years were (4.62 and 4.61 g), (4.15 and 4.14 g), (3.69 and 3.68 g) and (2.93 and 2.73 g) in 1000 Seed weight trait, (29.15 and 28.68 g), (26.23 and 25.81 g), (23.31 and 23.89 g) and (17.97 and 16.75 g) in Seed number per pod trait and (148.74 and 147.43), (133.87 and 131.81), (118.99 and 117.94) and (89.24 and 88.45) in pod number/plant trait, respectively. In the same context, the values were (97.65 and 97.63 g), (87.88 and 87.86 g), (78.11 and 78.10 g) and (60.18 and 58.57 g) in seed yield/plant trait, (47.51 and 47.44%), (42.75 and 42.69%), (38.0 and 37.95%) and (29.32 and 28.79%) in oil% trait, (46.43 and 46.40 g), (37.61 and 37.57 g), (29.71 and 29.69 g) and (17.67 and 16.90 g) in Oil yield/plant trait and (0.32 and 0.33), (0.40 and 0.38),(0.45 and 0.48) and (0.60 and 0.61) in Na$^+$ uptake for the same treatments in both growing seasons, respectively. Also, the mean values were (2.86 and 2.84), (2.87 and 2.89), (2.81 and 2.78) and (2.47 and 2.51) in K$^+$ uptake, (0.117 and 0.120), (0.120 and 0.138), (0.170 and 0.183) and (0.256 and 0.257) in Na/K ratio trait, (0.67 and 0.72), (0.54 and 0.55) and (0.32 and 0.35) in osmotic adjustment trait,

(28.36 and 27.63), (39.92 and 38.48), (48.93 and 48.45) and (59.87 and 59.91) in proline content and (28.90 and 28.53), (43.47 and 47.28), (55.82 and 57.26) and (67.94 and 69.36) in glycine betaine content trait for the same treatments in both years, respectively.

Data of combined analysis for all studied traits in both seasons viewed in Table 5 confirmed that the canola cultivar Pactol was recorded the highest mean values for the third salinity stress treatment compared to the rest two canola cultivars in the traits, 1000 seed-weight (3.22 g), pod number/plant (95.66), seed yield/plant (62.19 g), oil % (31.32%), oil yield/plant (19.47%), K$^+$ uptake (3.03) and proline content (65.36). While, this cultivar exhibited the lowest results in seed number/pod, Na$^+$ uptake, Na/K$^+$ ratio, osmotic adjustment and glycine betaine content for the same treatments in both seasons. In the same regard, canola cultivar sirw 4 come in the second rank of highest mean values for the same treatment compared to the control experiment in both seasons in the traits, 1000-seed weight (2.90 g), seed number/pod (18.24) and glycine betaine content (71.17). Whatever, Sirw 6 recorded the highest mean values in seed number/pod (18.36) and glycine betaine content (80.40) traits under the same conditions in both growing seasons, respectively. Of course, the saline stress tolerance level was more superior in the first level and followed by the second level compared to the control for all studied traits in the three canola cultivars in the same order of their superiority in the third level of saline stress.

Genetic parameters: Results viewed in Table 6 confirmed that the values of heritability in a broad sense were high in studied traits for all salinity-stress treatments besides, the standard experiment in both growing seasons. For example not limited, where, the values were (94.34 and 91.22%), (73.67 and 88.67%), (84.47 and 79.95%) and (77.95 and 89.89%) in 1000 seed weight trait, (75.51 and 89.92%), (82.64 and 76.37%), (68.91 and 68.23%) and (68.38 and 69.73%) in seed number/pod trait, (96.81 and 95.66%), (94.37 and 89.69%), (92.28 and 93.27%) and (89.75 and 83.30%) in pod number/plant trait, (87.29 and 89.96%), (90.82 and 84.76%), (96.16 and 93.87%) and (91.42 and 96.88%) in seed yield/plant trait, (96.58 and 95.88%), (94.55 and 91.35%), (98.30 and 94.63%) and (87.68 and 92.71%) in oil% and the values were (93.58 and 94.0%), (96.79 and 96.83%), (88.84 and 91.29%) and (81.83 and 86.38%) in oil yield/plant trait for the four treatments in both growing seasons, respectively. While, the values were medium in K$^+$ uptake trait for the control experiment only in both years and their data were (57.41 and 50.19%), respectively. Further, the values of genotypic

Table 4: Mean performance of all studied traits for all treatment of the three canola genotypes in the two growing seasons (2018/2019 and 2019/2020)

Genotypes	Seasons	1000 seed weight (g)				Seed number per plant				Seed yield/plant (g)			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Sirw 4	2018/2019	4.76	4.28	3.81	3.09	29.43	26.48	23.54	19.12	93.78	84.40	75.02	56.26
	2019/2020	4.52	4.06	3.61	2.71	28.95	26.05	24.60	17.37	92.85	83.56	74.28	55.71
Sirw 6	2018/2019	3.79	3.41	3.03	2.27	31.15	28.03	24.92	18.69	96.02	86.41	76.81	62.41
	2019/2020	4.15	3.73	3.32	2.49	30.08	27.07	24.66	18.04	95.87	86.28	76.69	57.52
Pactol	2018/2019	5.32	4.78	4.25	3.45	26.87	24.18	21.49	16.12	103.15	92.83	82.52	61.89
	2019/2020	5.17	4.65	4.13	2.99	27.03	24.32	22.43	14.86	104.18	93.76	83.34	62.50
Mean	2018/2019	4.62	4.15	3.69	2.93	29.15	26.23	23.31	17.97	97.65	87.88	78.11	60.18
	2019/2020	4.61	4.14	3.68	2.73	28.68	25.81	23.89	16.75	97.63	87.86	78.10	58.57
LSD at 5%	2018/2019	1.42	1.93	1.77	1.56	2.08	1.99	1.79	2.47	1.44	1.85	1.04	1.75
LSD at 1%		2.50	3.40	3.11	2.75	3.65	3.50	3.14	4.35	2.54	3.26	1.83	3.08
LSD at 5%	2019/2020	1.75	1.32	2.26	1.19	1.54	1.97	2.02	2.29	1.63	2.32	1.08	1.48
LSD at 1%		3.08	2.32	3.98	2.09	2.71	3.47	3.55	4.03	2.86	4.09	1.91	2.61

Genotypes	Seasons	Oil (%)				Oil yield/plant (g)				Pod number per plant			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Sirw 4	2018/2019	45.86	41.27	36.68	27.51	43.0	34.83	27.51	15.47	139.29	125.36	111.43	83.57
	2019/2020	44.75	40.27	35.80	26.85	41.55	33.64	26.59	14.95	137.60	123.84	110.08	82.56
Sirw 6	2018/2019	47.54	42.78	38.03	28.52	45.64	36.96	29.21	17.79	145.71	131.14	116.56	87.42
	2019/2020	48.03	43.22	38.42	28.81	46.04	37.29	29.46	16.57	147.03	132.86	117.62	88.21
Pactol	2018/2019	49.13	44.21	39.30	31.93	50.67	41.04	32.43	19.76	161.23	145.12	128.98	96.73
	2019/2020	49.54	44.58	39.63	30.71	51.61	41.79	33.02	19.19	157.68	138.75	126.14	94.60
Mean	2018/2019	47.51	42.75	38.0	29.32	46.43	37.61	29.71	17.67	148.74	133.87	118.99	89.24
	2019/2020	47.44	42.69	37.95	28.79	46.40	37.57	29.69	16.90	147.43	131.81	117.94	88.45
LSD at 5%	2018/2019	1.94	1.79	0.85	1.57	1.96	1.80	2.15	1.89	2.24	2.16	2.05	2.17
LSD at 1%		3.41	3.14	1.49	2.76	3.46	3.16	3.79	3.33	3.95	3.80	3.60	3.82
LSD at 5%	2019/2020	2.0	1.97	1.28	1.44	2.14	1.84	2.05	1.80	2.34	2.06	1.68	1.86
LSD at 1%		3.52	3.47	2.26	2.54	3.77	3.23	3.60	3.16	4.11	3.63	2.96	3.27

Genotypes	Seasons	Na/K ratio				Na+ uptake				K+ uptake			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Sirw 4	2018/2019	0.160	0.207	0.237	0.336	0.41	0.52	0.56	0.73	2.56	2.51	2.36	2.17
	2019/2020	0.169	0.204	0.250	0.352	0.43	0.51	0.58	0.75	2.54	2.49	2.32	2.13
Sirw 6	2018/2019	0.118	0.078	0.186	0.280	0.33	0.42	0.48	0.64	2.78	2.69	2.58	2.28
	2019/2020	0.109	0.146	0.203	0.285	0.31	0.40	0.52	0.66	2.82	2.73	2.55	2.31
Pactol	2018/2019	0.073	0.076	0.088	0.152	0.24	0.26	0.31	0.45	3.25	3.42	3.49	2.96
	2019/2020	0.084	0.066	0.097	0.135	0.27	0.23	0.34	0.42	3.18	3.45	3.47	3.11
Mean	2018/2019	0.117	0.120	0.170	0.256	0.32	0.40	0.45	0.60	2.86	2.87	2.81	2.47
	2019/2020	0.120	0.138	0.183	0.257	0.33	0.38	0.48	0.61	2.84	2.89	2.78	2.51
LSD at 5%	2018/2019	0.57	1.08	1.58	1.79	1.02	1.43	1.70	1.48	2.16	2.21	1.01	0.91
LSD at 1%		1.01	1.91	2.78	3.14	1.80	2.52	2.99	2.61	3.80	3.89	1.78	1.61
LSD at 5%	2019/2020	0.69	0.88	1.14	1.54	0.90	1.11	1.31	1.37	1.97	2.05	1.31	0.96
LSD at 1%		1.21	1.55	2.0	2.71	1.58	1.95	2.30	2.40	3.47	3.61	2.30	1.70

Genotypes	Seasons	Osmotic adjustment				Proline content				Glycine betaine content			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Sirw 4	2018/2019	-	0.65	0.54	0.38	25.34	38.12	48.78	55.19	29.38	41.63	55.19	72.23
	2019/2020	-	0.85	0.57	0.44	24.36	39.04	46.55	56.41	31.74	44.52	57.03	70.11
Sirw 6	2018/2019	-	0.88	0.66	0.32	28.09	36.05	44.15	57.88	33.06	49.86	63.54	78.45
	2019/2020	-	0.79	0.64	0.36	28.47	34.13	47.02	59.14	32.28	56.04	64.39	82.36
Pactol	2018/2019	-	0.48	0.42	0.28	31.67	45.61	53.86	66.54	24.27	38.94	48.75	53.14
	2019/2020	-	0.52	0.45	0.25	30.08	42.28	51.78	64.19	21.58	41.29	50.38	55.61
Mean	2018/2019	-	0.67	0.54	0.32	28.36	39.92	48.93	59.87	28.90	43.47	55.82	67.94
	2019/2020	-	0.72	0.55	0.35	27.63	38.48	48.45	59.91	28.53	47.28	57.26	69.36
LSD at 5%	2018/2019	-	0.65	1.46	0.93	1.08	0.81	1.93	1.44	1.59	1.32	2.10	1.96
LSD at 1%		-	1.14	2.57	1.64	1.91	1.43	3.40	2.54	2.80	2.32	3.69	3.46
LSD at 5%	2019/2020	-	0.93	1.28	0.99	1.16	1.02	1.89	1.81	1.86	1.42	2.02	1.85
LSD at 1%		-	1.64	2.26	1.75	2.05	1.80	3.32	3.19	3.27	2.50	3.55	3.25

*Significant at 5% probability level, **Significant at 1% probability level, C: Control, T1: Treatment one, T2: Treatment two, T3: Treatment three

Table 5: Combined analysis of all studied traits in both years for all treatment of the three canola genotypes

Genotypes	1000 seed weight (g)				Seed number per pod				Pod number per plant				Seed yield/plant (g)			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Sirw 4	4.64	4.17	3.71	2.90	18.24	26.26	24.07	18.24	138.44	124.6	110.75	83.06	93.31	83.98	74.65	55.98
Sirw 6	3.97	3.57	3.17	2.38	18.36	27.55	24.79	18.36	146.37	132.0	117.09	87.81	95.94	86.34	76.75	59.96
Pactol	5.24	4.71	4.19	3.22	15.49	24.25	21.96	15.49	159.45	141.93	127.56	95.66	103.66	93.29	82.93	62.19
LSD at 5%	1.58	1.62	2.01	1.37	1.81	1.98	1.90	2.38	2.29	2.11	1.86	2.01	1.53	2.08	1.06	1.61
LSD at 1%	2.79	2.86	3.54	2.42	3.18	3.48	3.34	4.19	4.03	3.71	3.28	3.54	2.70	3.67	1.87	2.84

Genotypes	Oil (%)				Oil yield/plant				Na^+ uptake				K^+ uptake			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Sirw 4	45.30	40.77	36.24	27.18	42.27	34.32	27.05	15.21	0.42	0.51	0.57	0.74	2.55	2.50	2.34	2.15
Sirw 6	47.78	43.0	38.22	28.66	45.84	37.12	29.33	17.18	0.32	0.41	0.50	0.65	2.80	2.71	2.56	2.29
Pactol	49.33	44.39	39.46	31.32	51.14	41.41	32.72	19.47	0.25	0.24	0.32	0.43	3.21	3.43	3.48	3.03
LSD at 5%	1.97	1.88	1.06	1.50	2.71	1.82	2.10	1.84	0.96	1.27	1.50	1.42	2.06	2.13	1.16	0.93
LSD at 1%	3.46	3.30	1.87	2.65	3.61	3.19	3.69	3.24	1.69	2.23	2.64	2.50	3.63	3.75	2.04	1.65

Genotypes	Na/K ratio				Osmotic adjustment				Proline content				Glycine betaine content			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Sirw 4	0.164	0.205	0.243	0.344	-	0.75	0.55	0.41	24.85	38.58	47.66	55.80	30.56	43.07	56.11	71.17
Sirw 6	0.11	0.112	0.194	0.282	-	0.83	0.65	0.34	28.28	35.09	45.58	58.51	32.67	52.95	63.96	80.40
Pactol	0.078	0.071	0.092	0.143	-	0.50	0.43	0.26	30.87	43.94	52.82	65.36	22.92	40.11	49.56	54.37
LSD at 5%	0.63	0.98	1.36	1.66	-	0.79	1.37	0.96	1.49	1.12	2.66	1.99	2.19	1.82	2.89	2.71
LSD at 1%	1.11	1.73	2.39	2.92	-	1.39	2.41	1.69	1.60	1.41	2.60	2.50	2.56	1.96	2.78	2.55

*Significant at 5% probability level, **Significant at 1% probability level, C: Control, T_1: Treatment one, T_2: Treatment two, T_3: Treatment three

Table 6: Estimates of some genetic parameters for all studied traits in canola genotypes for all treatments in both growing seasons

Genetic parameters	Seasons	1000 seed weight (g)				Seed number per pod				Seed yield/plant (g)				Pod number per plant			
		C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Mean	2018/2019	4.62	4.15	3.69	2.93	29.15	26.23	23.31	17.97	97.65	87.88	78.11	60.18	148.74	133.87	118.99	89.24
	2019/2020	4.61	4.14	3.68	2.73	28.68	25.81	23.89	16.75	97.63	87.86	78.10	58.57	147.43	131.81	117.94	88.45
Genotypic variance	2018/2019	11.18	3.47	5.66	2.84	4.41	6.24	2.35	4.39	4.74	11.28	9.03	10.87	50.78	26.03	16.63	13.66
	2019/2020	10.61	4.54	6.78	4.18	7.05	4.17	2.90	4.01	7.89	9.96	5.98	22.70	39.91	12.27	13.04	5.74
Environmental variance	2018/2019	0.67	1.24	1.04	0.81	1.43	1.31	1.06	2.03	0.69	1.14	0.36	1.02	1.67	1.55	1.39	1.56
	2019/2020	1.02	0.58	1.70	0.47	0.79	1.29	1.35	1.74	0.88	1.79	0.39	0.73	1.81	1.41	0.94	1.15
Phenotypic variance	2018/2019	11.85	4.71	6.70	3.65	5.84	7.55	3.41	6.42	5.43	12.42	9.39	11.89	52.45	27.58	18.02	15.22
	2019/2020	11.63	5.12	8.48	4.65	7.84	5.46	4.25	5.75	8.77	11.75	6.37	23.43	41.72	13.68	13.98	6.89
Heritability in broad sense	2018/2019	94.34	73.67	84.47	77.80	75.51	82.64	68.91	68.38	87.29	90.82	96.16	91.42	96.81	94.37	92.28	89.75
	2019/2020	91.22	88.67	79.95	89.89	89.92	76.37	68.23	69.73	89.96	84.76	93.87	96.88	95.66	89.69	93.27	83.30
GCV (%)	2018/2019	72.37	44.88	64.47	57.51	7.20	9.52	6.57	11.65	2.22	3.82	3.84	5.47	4.79	3.81	3.42	4.14
	2019/2020	70.65	51.46	70.75	74.89	9.25	7.91	7.12	11.95	2.87	3.59	3.13	8.13	4.28	2.65	3.06	2.70
PCV (%)	2018/2019	74.51	52.29	70.14	65.20	8.29	10.47	7.92	14.10	2.38	4.01	3.92	5.72	4.87	3.92	3.56	4.37
	2019/2020	73.97	54.65	79.13	78.98	9.76	9.05	8.62	14.31	3.03	3.90	3.23	8.26	4.52	2.80	3.17	2.96
D^z	2018/2019	2.14	7.41	5.67	7.69	1.09	0.95	1.35	2.45	0.16	0.19	0.08	0.25	0.07	0.11	0.14	0.16
	2019/2020	3.32	3.19	8.38	4.09	0.51	1.14	1.50	2.36	0.16	0.31	0.10	0.13	0.10	0.15	0.11	0.23
GA (or expected genetic advance)	2018/2019	6.71	3.30	4.52	3.07	3.77	4.69	2.63	3.58	4.20	6.61	6.09	6.51	14.50	10.25	8.10	7.24
	2019/2020	6.43	4.14	4.81	4.0	5.20	3.69	2.90	3.45	5.50	6.00	4.89	9.69	12.77	6.86	7.21	4.52
GAM (or genetic advance as percentage of mean) (%)	2018/2019	145.23	79.51	122.49	104.77	12.93	17.88	11.28	19.92	4.30	7.52	7.79	10.81	9.75	7.66	6.80	8.11
	2019/2020	139.47	100.0	130.70	146.52	18.13	14.29	12.13	20.59	5.63	6.82	6.26	16.54	8.66	5.20	6.11	5.11

Table 6: Continued

Genetic parameters	Seasons	Oil (%)				Oil yield/plant				Na⁺ uptake				K⁺ uptake			
		C	T₁	T₂	T₃	C	T₁	T₂	T₃	C	T₁	T₂	T₃	C	T₁	T₂	T₃
Mean	2018/2019	47.51	42.75	38.0	29.32	46.43	37.61	29.71	17.67	0.32	0.40	0.45	0.60	2.86	2.87	2.81	2.47
	2019/2020	47.44	42.69	37.95	28.79	46.40	37.57	29.69	16.90	0.33	0.38	0.48	0.61	2.84	2.89	2.78	2.51
Genotypic variance	2018/2019	35.32	18.42	13.96	5.84	18.66	32.35	12.27	5.36	1.99	3.45	4.04	1.27	2.09	7.49	4.61	3.25
	2019/2020	30.96	13.64	9.70	8.78	23.83	34.29	14.57	6.79	2.27	4.97	6.0	3.13	1.30	5.31	10.83	4.02
Environmental variance	2018/2019	1.25	1.06	0.24	0.82	1.28	1.07	1.54	1.19	0.35	0.68	0.96	0.73	1.55	1.62	0.34	0.28
	2019/2020	1.33	1.29	0.55	0.69	1.52	1.12	1.39	1.07	0.27	0.41	0.57	0.62	1.29	1.40	0.57	0.31
Phenotypic variance	2018/2019	36.57	19.48	14.20	6.66	19.94	33.42	13.81	6.55	2.34	4.13	5.0	2.0	3.64	9.11	4.95	3.53
	2019/2020	32.29	14.93	10.25	9.47	25.35	35.41	15.96	7.86	2.54	5.38	6.57	3.75	2.59	6.71	11.40	4.33
Heritability in broad sense	2018/2019	96.58	94.55	98.30	87.68	93.58	96.79	88.84	81.83	85.04	83.53	80.80	63.50	57.41	82.21	93.13	92.06
	2019/2020	95.88	91.35	94.63	92.71	94.0	96.83	91.29	86.38	89.37	92.37	91.32	83.46	50.19	79.13	95.0	92.84
GCV (%)	2018/2019	12.50	10.03	9.83	8.24	9.30	15.12	11.79	13.10	440.83	464.35	446.66	187.82	50.54	95.35	76.4	72.98
	2019/2020	11.72	8.65	8.20	10.29	10.52	15.58	12.85	15.41	456.56	586.67	510.31	290.02	40.14	79.73	118.37	79.88
PCV (%)	2018/2019	12.72	10.32	9.91	8.80	9.61	15.37	12.50	14.48	478.03	508.06	496.90	235.70	66.70	105.16	79.17	76.06
	2019/2020	11.97	9.05	8.43	10.68	10.85	15.83	13.45	16.58	482.95	610.39	534.0	317.45	56.66	89.63	121.45	82.90
D^z	2018/2019	0.22	0.29	0.08	0.56	0.31	0.25	0.71	1.38	37.20	43.71	50.24	47.88	16.16	9.81	2.77	3.08
	2019/2020	0.25	0.40	0.23	0.39	0.33	0.25	0.60	1.17	26.39	23.72	23.69	27.43	16.52	9.90	3.08	3.02
GA or (expected genetic advance)	2018/2019	12.07	8.63	7.66	4.67	8.64	11.57	6.82	4.33	2.69	3.51	3.73	1.85	2.26	5.13	4.28	3.57
	2019/2020	11.26	7.30	6.26	5.90	9.78	11.91	7.54	5.0	2.94	4.43	4.84	3.34	1.67	4.23	6.63	3.99
GAM or (genetic advance as percentage of mean) (%)	2018/2019	25.40	20.18	20.15	15.92	18.60	30.76	22.95	24.50	840.62	877.50	828.88	308.33	79.02	178.74	152.31	144.53
Proline content	2019/2020	23.73	17.10	16.49	20.49	21.07	31.70	25.39	29.58	890.90	1165.78	1008.33	547.54	58.80	146.36	238.48	158.96

Genetic parameters	Seasons	Glycine betaine content				Proline content				Osmotic adjustment				Na/K ratio			
		C	T₁	T₂	T₃	C	T₁	T₂	T₃	C	T₁	T₂	T₃	C	T₁	T₂	T₃
Mean	2018/2019	0.117	0.120	0.170	0.256	-	0.67	0.54	0.32	28.36	39.92	48.93	59.87	28.90	43.47	55.82	67.94
	2019/2020	0.12	0.138	0.183	0.257	-	0.72	0.55	0.35	27.63	38.48	48.45	59.91	28.53	47.28	57.26	69.36
Genotypic variance	2018/2019	4.14	11.54	5.47	4.62	-	5.38	3.45	2.40	4.45	4.95	8.36	6.07	24.57	27.53	16.21	9.21
	2019/2020	11.90	9.26	8.30	2.25	-	13.58	14.79	4.53	3.21	7.58	10.32	6.99	22.62	25.86	13.64	5.03
Environmental variance	2018/2019	0.11	0.39	0.83	1.06	-	0.14	0.71	0.29	0.39	0.22	1.24	0.69	0.84	0.58	1.46	1.28
	2019/2020	0.16	0.26	0.43	0.79	-	0.29	0.55	0.33	0.45	0.35	1.18	1.09	1.15	0.67	1.35	1.13
Phenotypic variance	2018/2019	4.25	11.93	6.30	5.68	-	5.52	4.16	2.69	4.84	5.17	9.60	6.76	25.41	28.11	17.67	10.49
	2019/2020	12.06	9.52	8.73	3.04	-	13.87	15.34	4.86	3.66	7.93	11.50	8.08	23.77	26.53	14.99	6.16
Heritability in broad sense	2018/2019	97.41	96.73	86.82	81.33	-	97.46	82.93	89.21	91.94	95.74	87.08	89.79	96.69	97.93	91.73	87.79
	2019/2020	98.67	97.26	95.07	74.01	-	97.90	96.41	93.20	87.70	95.58	89.73	86.50	95.16	97.47	90.99	81.65
GCV (%)	2018/2019	1739.05	2830.88	1375.76	839.61	-	346.19	343.96	484.12	7.43	5.57	5.90	4.11	17.15	12.07	7.21	4.46
	2019/2020	2874.69	2205.09	1574.30	583.65	-	511.82	699.23	608.10	6.48	7.15	6.63	4.41	16.67	10.75	6.44	3.23
PCV (%)	2018/2019	1762.01	2878.31	1476.45	930.96	-	350.66	377.70	512.53	7.75	5.69	6.33	4.34	17.44	12.19	7.53	4.76
	2019/2020	2893.95	2235.83	1614.56	678.42	-	517.25	712.11	629.86	6.92	7.31	6.99	4.74	17.08	10.89	6.76	3.57
D^z	2018/2019	22.96	47.43	100.69	91.35	-	4.47	33.74	28.41	0.32	0.12	0.43	0.23	0.29	0.12	0.32	0.30
	2019/2020	19.26	30.74	40.26	94.77	-	5.43	12.88	21.76	0.44	0.16	0.36	0.33	0.41	0.14	0.32	0.34
GA or (expected genetic advance)	2018/2019	4.15	6.90	4.50	4.0	-	4.73	3.49	3.02	4.18	4.50	5.57	4.82	10.07	10.73	7.97	5.88
	2019/2020	7.08	6.20	5.80	2.66	-	7.54	7.80	4.24	3.46	5.56	6.29	5.08	9.59	10.38	7.28	4.19
GAM or (genetic advance as percentage of mean) (%)	2018/2019	3547.0	5750.0	2647.05	1562.50	-	705.97	646.29	943.75	14.73	11.27	11.38	8.05	34.84	24.68	14.27	8.65
	2019/2020	5900.0	4492.75	3169.39	1035.01	-	1047.22	1418.18	1211.42	12.52	14.44	12.98	8.47	33.61	21.95	12.71	6.04

GCV (%): Genotypic coefficient of variance percentage, PCV (%): Phenotypic coefficient of variance percentage, D^z: Difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %), C: Control, T₁: Treatment one, T₂: Treatment two, T₃: Treatment three

variance were higher than the values of environmental variance in all traits under testing of all experiments for the two growing seasons. Therefore, the biggest part of the phenotypic variance was the genetic variance. Also, the PCV% were higher than its peers of GCV% in all studied traits of all experiments for the two growing seasons. The differences among phenotypic and genotypic coefficient of variation (D^Z) were low in all studied traits under all conditions in both growing seasons except the traits, 1000-seed weight for the second and third salinity-stress levels, Na^+ uptake for all treatments, K^+ uptake for the control and salinity-stress level one, Na/K ratio for all experiments and osmotic adjustment for the second and third salt-stress levels in both years where they were high, respectively. Data assessment of expected genetic advance (GA) based on 5% selection exhibited different results for all attributes under studying in both growing seasons. Where the values ranged from low to medium and were good until the second level of saline-stress in most studied traits during the two cultivation seasons. For example but not limited, the values were (4.20 and 5.50), (6.61 and 6.0), (6.09 and 3.32) and (6.51 and 9.69) for the four treatments in seed yield/plant and (8.64 and 9.78), (11.57 and 11.91), (6.82 and 7.54) and (4.33 and 5.0) for the four treatments in oil yield/plant in both growing seasons, respectively. Concerning GAM%, all studied traits recorded high values of this genetic parameter for the four treatment in both years except, the traits pod number/plant and seed yield/plant for all treatments and glycine betaine content for the third salt-stress level where they exhibited low results under the same conditions in both growing seasons in this regard.

Salinity tolerance indices parameters: Results shown in Table 7 detected that the three canola genotypes, Sirw 4, Sirw 6 and Pactol exhibited mean values for YSI parameter ranged from 0.59-0.90 for the three salinity levels in both growing seasons. Further, the three canola verities recorded the highest mean values for MP and GMP parameters in both growing seasons for the three salinity levels. However, the values were higher in the first level, followed by the second level and then followed by the third level. Where, the mean values of MP parameter were (89.09, 91.21 and 97.99 g) and (88.20, 91.07 and 98.97 g) for the first salinity level, (84.40, 86.41 and 92.83 g) and (83.56, 86.28 and 93.76 g) for the second salinity level and were (75.02, 79.21 and 82.52 g) and (74.28, 76.69 and 83.34 g) for the third salinity level for the three canola cultivars in both years, respectively. For YI parameter, the two canola cultivars Sirw 4 and Sirw 6 were exhibited mean values lower than one in both growing seasons for the three salinity-stress treatments and the mean

values were (0.96 and 0.98) and (0.95 and 0.98) for the first and second salt level, respectively. While, the canola cultivar sirw 4 only were recorded mean values lower than one (0.93) for YI parameter of the third salinity level in the first season and exhibited (0.95 and 0.98) for the second season of the same salinity level, respectively. But, the canola cultivar Pactol only was exhibited mean values higher than one of (YI) parameter where the values were (1.05, 1.05 and 1.02) for the first season of all salinity treatments and were (1.06) for the same treatments in the second year, respectively. The three canola cultivars were recorded the lowest data of YR parameter in both growing seasons and this decreasing reached below the limit in the first saline stress level, followed by the second level and then followed by the third level, respectively. Concerning SSI parameter, the three canola genotypes in the first salinity level in the second season only besides, Sirw 6 cultivar for the third salinity level in the first growing season was exhibited mean values lower than the unity, respectively.

Biochemical molecular markers studies
Protein profile analysis using SDS-PAGE: Table 8 and Fig. 1 showed a comparative protein expression extracted from 3 canola cultivars (Sirw 4, Sirw 6 and Pactol) under normal and three levels of salt stress conditions. Comparing the protein profiles using SDS-PAGE showed that salt treatment did not induce significant changes in the protein pattern. It was found a total number of 7 bands with different MWs ranged from 8-70 KDa can be detected under normal and salt stress conditions. Bands with MWs 70, 35, 30, 12 and 8 KDa have appeared with a slight difference in their intensities under both normal and salt stress conditions for all cultivars. In contrast, bands with MWs18 and 55 KDa were disappeared in all cultivars except the canola cultivar Sirw 6 which was exhibited these faint bands under control and the first 2 levels of salt stress conditions.

Molecular description using SCoT primers:
SCoT analysis profile: The eleven SCoT primers namely; SCoT-1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 12 exhibited a total of 131fragments, 81 of them were monomorphic, while that, 50 bands were polymorphic with 38.16% (polymorphism) included 32 unique bands or positive specific markers as shown in Fig. 2 and Table 9. The average number of polymorphic SCoT markers was 4.54 bands for each primer, the number of fragments ranged from 9-20 and a molecular size ranging from 150-1600 bp, respectively. Also, the highest number of total bands were observed in primers; SCoT-5 (22), followed by SCoT-4 (14), followed by SCoT-1, 2, 6, 7 and 9 where they recorded 12 fragments for all of them are equal,

Table 7: Estimation of salinity tolerance indices for the three canola genotypes especially for seed yield/plant trait under normal and three levels of salinity during the two growing season

Salinity tolerance indices (3.0 ds m⁻¹)

Season 2018/2019

Genotypes	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sirw 4	93.78	84.40	0.89	0.96	89.09	0.83	88.96	0.11	1.1
Sirw 6	96.02	86.41	0.89	0.98	91.21	0.87	91.08	0.11	1.0
Pactol	103.15	92.83	0.90	1.05	97.99	1.0	97.85	0.10	1.0

Season 2019/2020

Genotypes	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sirw 4	92.85	83.56	0.89	0.95	88.20	0.81	88.08	0.11	0.12
Sirw 6	95.87	86.28	0.90	0.98	91.07	0.86	90.94	0.10	0.11
Pactol	104.18	93.76	0.89	1.06	98.97	1.02	98.83	0.11	0.12

Salinity tolerance indices (5.5 ds m⁻¹)

Season 2018/2019

Genotypes	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sirw 4	93.78	75.02	0.79	0.96	84.40	0.73	83.87	0.21	1.05
Sirw 6	96.02	76.81	0.79	0.98	86.41	0.77	85.87	0.21	1.05
Pactol	103.15	82.52	0.80	1.05	92.83	0.89	92.26	0.20	1.0

Season 2019/2020

Genotypes	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sirw 4	92.85	74.28	0.80	0.95	83.56	0.72	83.04	0.20	1.0
Sirw 6	95.87	76.69	0.79	0.98	86.28	0.77	85.74	0.21	1.05
Pactol	104.18	83.34	0.79	1.06	93.76	0.91	93.17	0.21	1.05

Salinity tolerance indices (8.0 ds m⁻¹)

Season 2018/2019

Genotypes	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sirw 4	93.78	56.26	0.59	0.93	75.02	0.55	72.63	0.41	1.07
Sirw 6	96.02	62.41	0.64	1.03	79.21	0.62	77.41	0.36	0.94
Pactol	103.15	61.89	0.60	1.02	82.52	0.66	79.89	0.40	1.05

Season 2019/2020

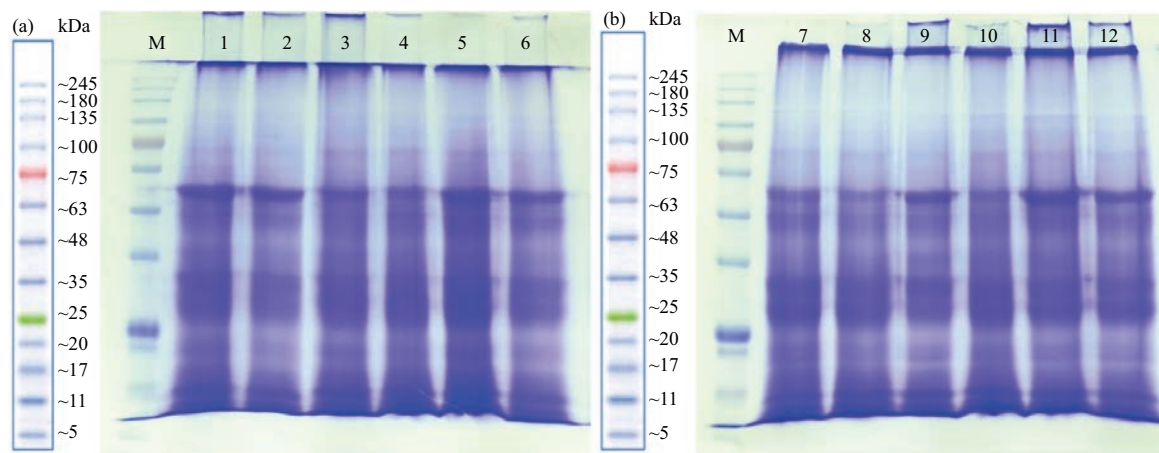| Genotypes	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sirw 4	92.85	55.71	0.60	0.95	74.28	0.54	71.92	0.40	1.0
Sirw 6	95.87	57.52	0.59	0.98	76.69	0.57	74.25	0.41	1.02
Pactol	104.18	62.50	0.59	1.06	83.34	0.68	80.69	0.41	1.02

Fig. 1: SDS-PAGE of protein extracted from Sirw 4, Sirw 6 and Pactol canola cultivars under control and three levels of salt stress conditions

M: Marker, Lane 1: Sirw 4 (control), Lane 2: Sirw 4 (1st salinity level), Lane 3: Sirw 4 (2nd salinity level), Lane 4: Sirw 4 (3rd salinity level), Lane 5: Sirw 6 (control), Lanes 6: Sirw 6 (1st salinity level), Lane 7: Sirw 6 (2nd salinity level), Lane 8: Sirw 6 (3rd salinity level), Lane 9: Pactol (control), Lane 10: Pactol (1st salinity level), Lane 11: Pactol (2nd salinity level) and Lanes 12: Pactol (3rd salinity level), respectively

Table 8: Electrophoretic pattern of protein extracted from 3 canola cultivars (Sirw 4, Sirw 6 and Pactol) under control and three levels of salt stress

Band No.	MW	1	2	3	4	5	6	7	8	9	10	11	12
1	70	++++	+++	++	++	++++	+++	++	+	+++	+	++++	+++
2	55	-	-	-	-	++	+	+	-	-	-	+	-
3	35	++++	+++	++++	++++	++++	+++	+++	+++	++	+++	++	++
4	30	++++	+++	++++	++++	++++	+++	+++	+++	++	+++	+++	+++
5	18	-	-	-	-	+	+	+	-	-	-	-	-
6	12	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
7	8	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Total		5	5	5	5	7	6	6	5	5	5	5	5

+: Very faint, ++: Faint, +++: Very dark, -: Absence of bands

Table 9: Band variation and polymorphism percentage for the three canola genotypes using SCoT primers

No.	ISSR primers	Total bands	Monomorphic bands	Polymorphic bands	Unique band	Polymorphism (%)	Range size (bp)	Sequence
1	SCoT-1	12	5	7	6	58.33	150-1200	5'-ACGACATGGCGACCACGC-3'
2	SCoT-2	12	7	5	2	41.66	150-1000	5'-ACCATGGCTACCACCGGC-3'
3	SCoT-3	9	5	4	2	44.44	210-1100	5'-ACGACATGGCGACCCACA-3'
4	SCoT-4	14	9	5	5	35.71	170-1300	5'-ACCATGGCTACCACCGCA-3'
5	SCoT-5	20	11	9	7	45.0	200-1600	5'-CAATGGCTACCACTAGCG-3'
6	SCoT-6	12	9	3	2	25.0	200-1100	5'-CAATGGCTACCACTACAG-3'
7	SCoT-7	12	9	3	1	25.0	300-1600	5'-ACAATGGCTACCACTGAC-3'
8	SCoT-9	12	7	5	1	41.66	200-1500	5'-ACAATGGCTACCACTGCC-3'
9	SCoT-10	9	6	3	2	33.33	200-1000	5'-ACAATGGCTACCACCAGC-3'
10	SCoT-11	9	7	2	2	22.22	250-900	5'-ACAATGGCTACCACTACC-3'
11	SCoT-12	10	6	4	2	40.0	200-800	5'-CAACAATGGCTACCACCG-3'
Total		131	81	50	32	38.16	150-1600	

followed by SCoT-12 (10) and then followed by the SCoT primers number 3, 10 and 11 where they produced 9 fragments for all of them are equal, respectively. Further, the highest number of polymorphic bands appeared in primers SCoT-5 and 1 (9 and 7) and the same two primers were generated the biggest number of unique bands (7 and 6), respectively. While, primer SCoT-11 recorded the lowest number of polymorphic fragments (2) and the two primers SCoT-7 and 9 also were produced the lowest number of unique bands (1), respectively. In addition, the highest polymorphism percentage was appeared in primers; SCoT-1 (58.33%), followed by SCOT-5 (45.0%), followed by SCoT-3 (44.44%), followed by SCoT-2 and 9 (41.66%) and followed by the primer SCoT-12 (40.0%), respectively. While that, the
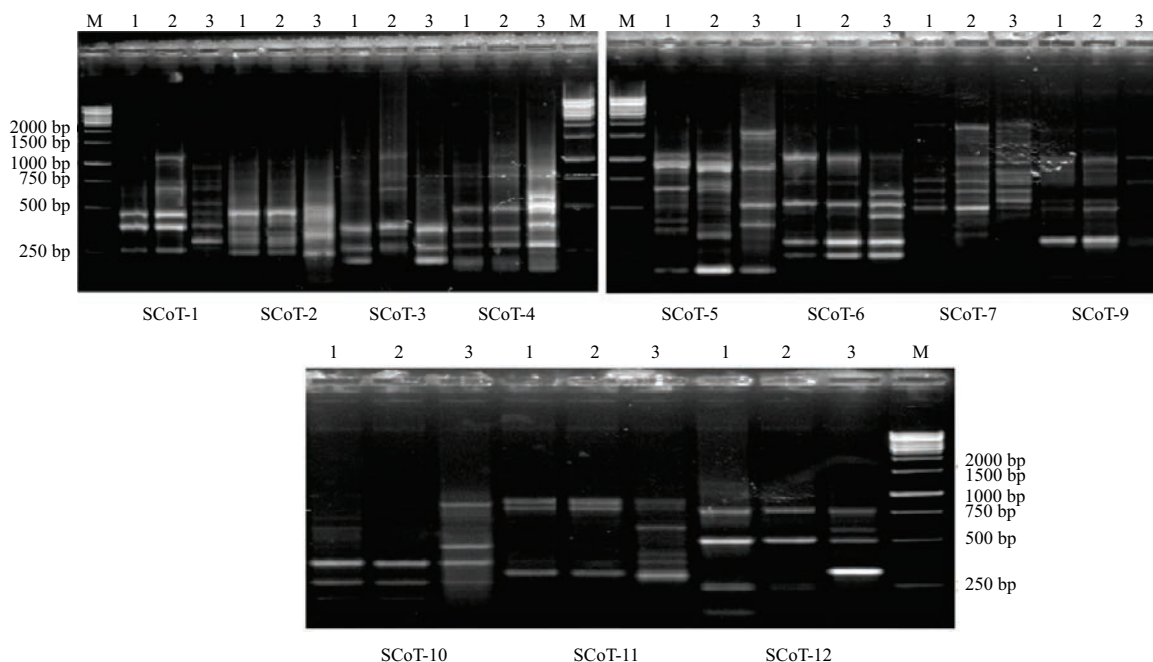
Fig. 2: SCoT profiles produced with eleven primers, M ladder 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, 5000, 6000, 8000 and 10000 bp for the marker of the three canola cultivars 1(Sirw 4), 2(Sirw 6) and 3(Pactol)

Table 10: Total bands obtained from the eleven SCoT primers of the three canola varieties and all amplified fragments for each genotype

Genotypes	SCoT primers											
	SCoT -1	SCoT -2	SCoT -3	SCoT -4	SCoT -5	SCoT -6	SCoT -7	SCoT -9	SCoT -10	SCoT -11	SCoT -12	Total
Sirw 4	7	10	7	9	14	10	9	11	7	7	8	99
Sirw 6	7	10	8	10	17	10	11	11	7	7	8	106
Pactol	9	9	6	13	13	11	12	8	8	9	8	106
Total bands	23	29	21	32	44	31	32	30	22	23	24	311

lowest polymorphism percentage was observed in primer SCoT-11 (22.22%) in this regard. Results presented in Table 10 revealed that the two canola genotypes; Sirw 6 and Pactol exhibited the highest number of bands (106) for both of them and were coming in the first rank then followed by Sirw 4 (99) which coming in the second rank of this context, respectively. Besides, SCoT primers; 4, 5, 6, 7 and 9 recorded the highest number of fragments (32, 44, 31, 32 and 30) for each one of them in all canola cultivars. While, the two SCoT primers; 3 and 10 showed the lowest number of fragments (21 and 22) for both of them, respectively. Besides, the rest SCoT primers were generated the different number of amplified fragments.

Data presented in Table 11 succeeded in finding the molecular genetic differences between the three canola cultivars which identified specific markers for each variety as a classification basis for these genotypes. Besides, these bands can be considered as molecular genetic markers for salt stress tolerance in these three canola accessions in this regard.

Below is a detailed presentation of these special markers as follow. The primer SCoT-1 exhibited 7 markers as follow, one negative with size 1000 bp for pactol and six positive specific markers (four of them with sizes 950, 550, 320 and 230 bp for pactol, one positive for sirw 4 with size 200 bp and one positive marker with size 150 bp for sirw 6), respectively. Five markers were generated by primer SCoT-2 for canola cultivar pactol as follow, two positive with sizes 520 and 480 bp and the three negative markers with sizes 500, 300 and 280 bp, respectively. For primer SCoT-3, four specific markers were generated in this regard, three positive markers for sirw 6 with sizes, 210, 650 and 800 bp and one negative marker for pactol with the size of 370 bp, respectively. Concerning primer SCsoT-4, five positive markers were observed by this primer one marker with size 1300 bp for sirw 6 and four markers with sizes of 230, 400, 480 and 630 bp were obtained for pactol, respectively. The primer SCoT-5 exhibited nine specific markers as follow, one positive marker with the size of 1400 bp

Table 11: Mapping of positive (P) and negative (N) specific markers for the three canola genotypes using 11 SCoT primers

SCoT primers	MS (bp)	Sirw 4	Sirw 6	Pactol	(P or N) marker
SCoT-1	1000	+	+	-	N (Pactol)
	950	-	-	+	P (Pactol)
	550	-	-	+	P (Pactol)
	320	-	-	+	P (Pactol)
	230	-	-	+	P (Pactol)
	200	+	-	-	P (Sirw 4)
	150	-	+	-	P (Sirw 6)
SCoT-2	520	-	-	+	P (Pactol)
	500	+	+	-	N (Pactol)
	480	-	-	+	P (Pactol)
	300	+	+	-	N (Pactol)
	280	+	+	-	N (Pactol)
SCoT-3	800	-	+	-	P (Sirw 6)
	650	-	+	-	P (Sirw 6)
	370	+	+	-	N (Pactol)
	210	-	+	-	P (Sirw 6)
SCoT-4	1300	-	+	-	P (Sirw 6)
	630	-	-	+	P (Pactol)
	480	-	-	+	P (Pactol)
	400	-	-	+	P (Pactol)
	230	-	-	+	P (Pactol)
SCoT-5	1400	-	-	+	P (Pactol)
	700	-	+	-	P (Sirw 6)
	680	-	+	-	P (Sirw 6)
	590	+	-	-	P (Sirw 4)
	450	+	+	-	N (Pactol)
	400	+	+	-	N (Pactol)
	300	-	+	-	P (Sirw 6)
	260	-	+	+	N (Sirw 4)
	240	+	-	-	P (Sirw 4)
SCoT-6	700	+	+	-	N (Pactol)
	630	-	-	+	P (Pactol)
	430	-	-	+	P (Pactol)
SCoT-7	800	-	-	+	P (Pactol)
	650	-	+	+	N (Sirw 4)
	400	-	+	+	N (Sirw 4)
SCoT-9	1500	+	+	-	N (Pactol)
	850	+	+	-	N (Pactol)
	500	+	+	-	N (Pactol)
	490	-	-	+	P (Pactol)
	200	+	+	-	N (Pactol)
SCoT-10	900	-	-	+	P (Pactol)
	450	-	-	+	P (Pactol)
	200	+	+	-	N (Pactol)
SCoT-11	630	-	-	+	P (Pactol)
	350	-	-	+	P (Pactol)
SCoT-12	600	-	-	+	P (Pactol)
	300	-	-	+	P (Pactol)
	250	+	+	-	N (Pactol)
	200	+	+	-	N (Pactol)
Range	1500-150				
Total		18	26	24	32 (positive)+18 (negative) markers

Table 12: Genetic similarity % in the three canola genotypes using SCoT primers

Genetic similarity	Sirw 4	Sirw 6	Pactol
Sirw 4	1.0		
Sirw 6	0.930	1.0	
Pactol	0.81	0.80	1.0
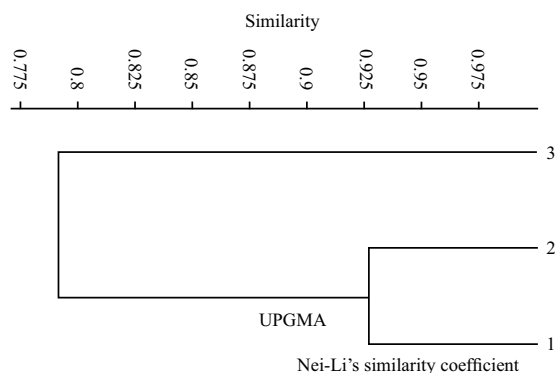
Fig. 3: Dendrogram representing the genetic relationship among the three canola genotypes using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from SCoT markers, 1(Sirw 4), 2(Sirw 6) and 3(Pactol)

for pactol, two positive with sizes, 240 and 590 bp for sirw 4, three positions for sirw 6 with sizes of 300, 680 and 700 bp, one negative marker for sirw 4 with size 240 bp and two negative markers with sizes of 400 and 450 bp for pactol, respectively. Three specific markers were generated by primer SCoT-6 for the canola cultivar pactol as follow, one negative with the size of 700 bp and two positives with sizes of 430 and 630 bp. While primer SCoT-7 recorded three markers one positive with size 800 bp for pactol and two negatives with sizes 400 and 650 bp for sirw 4. The primer SCoT-9 exhibited five specific markers for the canola cultivar pactol one positive with the size of 490 bp and four negative with sizes of 200, 500, 850 and 1500 bp, respectively. In the same context, primer SCoT-10 produced two positive markers with sizes 450 and 900 bp and one negative with size 200 bp for pactol only. Also, two positive specific markers with sizes of 350 and 630 bp were generated by primer SCoT-11 for the canola cultivar pactol. In the same track, the primer SCoT-12 showed four markers only for the canola cultivar pactol as following two positive with sizes of 300 and 600 bp and two negatives with sizes of 200 and 250 bp, respectively.

Proximity matrix analysis (genetic similarity): Results showed in Table 12 exhibited (3) pairwise comparisons to debate the genetic relationships among the three canola cultivars detected in terms of genetic similarity. The genetic similarity values ranged from (0.930-0.800) with an average of (0.865). Where the highest rank of genetic similarity (0.930) was obtained between (Sirw 4 and Sirw 6). While that, the lowest rank of similarity was (0.80) was observed among (Sirw 4 and Pactol), respectively. Also, the only rest genetic similarity value was appeared high (0.810) between (Sirw 6 and Pactol).

Cluster analysis: Data of cluster analysis or phylogenetic tree viewed in Fig. 3 divided the three canola genotypes into two main clusters. Where the cluster I included genotypes, 1 (Sirw 4) and 2 (Sirw 6). While cluster II included genotype 3 (Pactol) only.

DISCUSSION

Results shown in Table 3 showed the weakness extent of environmental variation in inheriting in all traits under studying for all treatments during the two growing seasons. This strongly reflects the great genetic stability of the three canola genotypes. Also, it confirms that they were very different from each other and this variation enriches the plant breeding process to salt-stress tolerance. Besides, these results confirmed that these three canola accessions possessed genes and hereditary factors that were responsible for enduring salt stress and that they are used in the national program to promote the Egyptian canola crop to tolerate environmental and biological stresses would be fruitful[11,16,36,37]. There is no doubt that the current study sheds light on important scientific aspects related to salinity tolerance in canola plants. As it dealt with the most important biological and physiological aspects that plants exhibit in the face of high salt stress, dealing with a large number of yield and its components and physiological attributes that discuss this point specifically in this context, Table 4. Also, this study used three canola varieties known for their high fame in tolerating salt stress namely, Sirw 4, Sirw 6 and Pactol but the new thing is to test a large number of important traits previously mentioned besides, knowing the reaction of each variety separately to this dangerous environmental factor during using three levels of sea water compared to the control within two years besides, with data collected through a combined statistical analysis, Table 5. Among the most important scientific facts observed in this regard is that these three canola varieties were able to reduce the loss in all studied traits as well as, reducing Na+ uptake, Na+/K+ ratio in parallel with high calcium absorption rate under salt-stress treatments compared to the control. Where the loss rate was 10.0% for the first dose of saline stress, 20.0% for the second dose and 40.0% for the third dose, respectively. Also, these genotypes were able to reduce the rate of osmotic adjustment and o succeeded in increasing the content of proline and glycine betaine under salt stress treatments compared to the control as their percentage in the third level of saline stress was much higher than the standard experiment. These results are in agreement with results of the study conducted previously[16] in the fact that controlling the entry of sodium in small quantities

through the cavities or the sodium pump and at the same time allowing the entry of calcium in large quantities is considering one of the most important mechanisms carried out to salt-stress tolerance by plants especially canola. Also, modifying osmosis by reducing it to the minimum levels to prevent the exit of water from the cells and the occurrence of leaves drying during exposure to high levels of salt stress is one of the most important physiological mechanisms for controlling the high limit of osmotic pressure which causes loss of a large water amount is also considered one of the most biological and physiological defences enjoyed tolerated plants and this fact was confirmed[38] in canola and Cakile maritima[39], emphasized the importance of low osmotic pressure in maintaining an ideal level of osmosis required to water stress tolerance in some barley genotypes by controlling the entry and exit of water necessary for vital processes besides, maintaining a large amount of it. In the same manner, it was noticed that the three canola varieties endured the salt stress of the three salt concentrations of seawater compared to the control in both growing seasons and the degree of tolerance differed at each salt level. Where, the level of endurance was 90.0% under the first level of salinity stress, 80.0% for the second level and 60.0% for the third level of salt stress compared with the standard experiment. Accordingly, it is evident that the canola varieties are highly tolerant and gave highly yielding of seeds, oil and the rest of the other traits under the second saline level, where the loss rate is about 20.0%. So, it can be said that canola plants tolerate even 5.5 ds m^{-1} or 5500 ppm. Therefore, it is advised to cultivate it in newly reclaimed lands, provided that it does not exceed the salinity level of 5.5 ds m^{-1} because this is the safest level for cultivating and give a good level of final yield. Accordingly, these three types of canola are fertile genetic materials for conducting a genetic improvement program to transfer the salinity-tolerant trait to the rest of the other lines, which are still under experimenting and testing using various biotechnological and genetic engineering methods. Data presented in Table 6 and related to heritability in a broad sense confirmed that the genetic variation was very important for controlling and inheriting the previously studied traits under all conditions in both seasons. While the previous results showed also diminishing the role of environmental variation in the process of influencing and controlling the previous traits under the four treatments for the two years[37]. Moreover, the values of heritability reflect the fruitful role of additive and additive X additive gene action in inheriting the important quantitative traits such as seed yield/plant and oil yield/plant. Besides, it also participates in the identification of important genetic values for some physiological attributes

associated with salt-stress tolerance in canola plants, such as osmotic adjustment and each of both proline and glycine betaine contents and their main role in enriching the process of salt stress tolerance. These results are to be proven because they are of interest to plant breeders in this context[37]. Also, data showed in (Table 6) noted that the PCV (%) values were higher than their counterparts in GCV (%) in all studied traits for the four experiments (the control and the three salinity-stress treatments) in both growing seasons. This fact revealed that the genetic improvement of these traits was not dependent on the genotype only but also the environment and the interaction between environmental X genotype. Thus, selection processes for salinity tolerance traits especially in the previously studied traits through phenotype could be very important in this investigation[37]. Data obtained from (GA) and (GAM)% parameters for all attributes under testing for all experiments in both growing seasons indicated that additive and non-additive types of gene action were played a functional role in controlling the previous traits for salt-stress tolerance in canola plants. Thus, the simple selection process for the low level of Na^+ uptake, Na^+/K ratio and osmotic adjustment besides, high level of proline and glycine betaine contents would be fruitful when the selection process is made based on individual plants[37]. Results of salinity tolerance indices confirmed that the three canola genotypes exhibited highly salinity tolerance in the three salinity-stress doses in both growing seasons because they were able to reduce the final loss rate in the final yield under salt-stress levels compared to the control experiment, Table 7. Also, results revealed that the high endurance rates reached their peak below the first saline stress level, followed by the second level and the third level of saline stress came in the last place in terms of tolerance degrees. Further, the cultivars Sirw 4 and 6 showed high tolerance in some parameters in both years. While Pactol genotype was recorded the highest rank of salinity tolerance in the rest of salinity tolerance indices parameters. In the end, it can be summarized the fact that the first level of saline stress was very suitable for the three canola varieties as the loss rate in the final yield did not exceed 10.0%. While the final yield loss reached 20.0% under the second saline stress level and this is a reasonable and very acceptable percentage in this regard. Although canola varieties were tolerated under the third level of saline stress conditions, the final rate of loss in yield reached 40.0% and this is a loss rate that cannot be accepted in any case. Therefore, the second level of salt stress was the safest for plants which proved that canola varieties tolerate even 5500 ppm or (5.5 ds m^{-1}) of seawater and give 80.0% of the final seed yield[15]. Plants tend to cope with the effects of salt stress by changing their gene

expression and protein accumulation. To identify proteins involved in salt stress response in canola, the SDS-PAGE method was used. In this study, protein bands with MW about 18 and 55 kDa were severely affected by salinity and were not expressed except for Sirw 6 canola cultivar under control and the first 2 levels of salt stress conditions, Table 8 and Fig. 1. These results are consistent with another study[40] who found that gene expression pattern is changed upon exposure to high temperature. Protein profile also showed that for some bands difference between control and salt stress levels were about the presence or absence of bands and for some bands, the difference was in the intensity of their expression. Many studies have been reported the appearance of new protein bands or the absence of others under salinity stress[41,42]. The intensity of the band's expression is an important indicator for salinity stress which has been reported in many researches[43,44]. The present results are similar to previous study[45] who analyzed 24 rice genotypes under drought stress conditions for proteins profile and who studied the changes in leaf protein pattern for 12 maize genotypes under drought stress conditions[46]. These findings may help to explain the salt tolerance mechanisms and to produce salt-tolerant canola plants.

Molecular genetic markers using 11 SCoT primers succeeded in finding the genetic differences and accurate comparison between the three canola varieties at the molecular level. These primers generated 131 fragments including 81 monomorphic bands, 50 polymorphic amplicons included 32 unique or positive specific markers besides, 18 negatives as the classification basis of the three canola varieties for salt stress tolerance, Table 9 and 11 and Fig. 2. Further, the primers, SCoT-1, 3 and 5 exhibited highly rank of polymorphism (%) as follow, 58.33, 44.44 and 45% which confirmed that these primers considered the best genetic method is not only to compare among the three canola varieties but also to find out the genetic causes of salt stress tolerance in the canola plant. In the same track, the primers SCoT-4, 5, 6, 7 and 9 generated the highest number of bands for the three canola verities namely, Sirw 4, Sirw 6 and Pactol and the values were (32, 44, 31, 32 and 30) indicated that these primers have already succeeded in determining the total number of amplicons for each variety and this also proves that the decision to choose it was correct, Table 10[37,47-50]. Moreover, the importance of results presented in Table 11 is summarized in the classification and molecular genetic identification of each canola variety separately and linking it to the mechanism of its salt stress tolerance through generating positive and negative specific markers and their molecular weights determined by SCoT primers. This will lead to a quantum leap

in the field of salt stress tolerance in canola crop by taking advantage of these tolerant genotypes and transferring tolerance genes to other varieties and lines that are more sensitive to unfavourable environmental conditions, especially salt stress. This will be achieved through both plant breeding and biotechnology programs. The thing that will support the success of this step is that the three canola genotypes were already closely related genetically to each other as the genetic similarity was 93.0% among Sirw 4 and Sirw 6 and, 80.0% between sirw 4 and Pactol and 81.0% among Sirw 6 and Pactol, Table 12 and Fig. 3[37,47-50].

CONCLUSION

The present study succeeded in discussing the salt stress tolerance in three Egyptian canola varieties using three dilute levels of Mediterranean Sea water namely, (3.0, 5.5 and 8.0 ds m^{-1}) besides, the control treatment in both growing seasons. Yield and its components and some physiological traits related to salinity tolerance were the most important measurements evaluated for the control treatment and the other salinity stress experiments. Further, salinity tolerance indices test were conducted in seed yield/plant trait of the three canola cultivars under the three salt stress treatment conditions compared with the control experiment. Molecular genetic markers through using 11 SCoT primers were used to compare among the three canola cultivars and determine the genetic evidence at the molecular level responsible for the canola's tolerance to salt stress. Also, biochemical genetic studies through protein profile analysis (SDS-PAGE) was used to know the different biochemical effects of salt stress on protein content in the three canola cultivars. Results detected that the canola cultivar Pactol was recorded the first rank of salinity tolerance followed by Sirw 6 and then followed by Sirw 4 under all salt stress levels especially the first and second level of salinity stress for all studied traits. Where, the second salinity level (5.5 ds m^{-1}) was considered the safest limit for growing canola plants and producing good yield with a loss not exceeding 20%. But, it is not recommended to grow canola under the third salinity level (8.0 ds m^{-1}) where the percentage of loss in all studied traits, especially seed yield/plant reached 40%.

SIGNIFICANCE STATEMENT

This investigation discovered that canola plants can tolerate salt stress up to 5.5 ds m^{-1}. This fact has been found out after estimating the number of yield and its components traits besides, some physiological attributes related to salt stress tolerance under the three levels of seawater compared to the standard experiment through two growing seasons. Based on these results, it can be concluded that canola plants can be cultivated with great density in new and reclaimed lands and tolerate salt stress up to 5.5 ds m^{-1} with good yield. Also, this study will help the researchers to uncover the critical areas in determining the safest limits of canola growth in environments and lands affected by salinity while ensuring high productivity. Accordingly, a new theory can be reached that will determine the biochemical and molecular genetic markers responsible for salinity tolerance in canola plants under Egyptian conditions.

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