J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 10 (2): 29 - 37, 2019

Chemical and Molecular Comparative Study on Different Genotypes of Pepper *(Capsicum annuum*, L.)

Heba S. A. Taha[1] ; T. F. Taha[2] and M. H. Arisha[3]
[1]**Genetics Department, Faculty of Agriculture, Zagazig University, Egypt**
[2]**Biochemistry Department, Faculty of Agriculture, Zagazig University, Egypt**
[3]**Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt**

ABSTRACT

Chilli or pepper (*Capsicum annuum*, L.) is an imperative commercial crop developed solely in tropical and temperate zones of the world. Various pepper lines were developed from two different regions; China and Egypt have been studied for their diversity of total phenols, flavonoid content and antioxidants activity as well as its molecular genetic diversity using two different fingerprinting systems (RAPD and SCoT primers). The results showed that ethanolic extract (70%) of ILICA-256 and WAXY genotypes were contained a high level of total phenols (53.78 and 36.18 mg/g), respectively while ethanolic extract (70%) of genotypes ZUN-LA, P-70, ILICA-256, and WAXY were contained total flavonoids as follow, 0.43, 0.41, 0.37 and 0.36 mg/g, respectively and showed that highest value was recorded with genotype ZUN-LA. Antioxidant activity showed that P-70, ILICA-256, B-6, WAXY, and Z-1 extracts have a higher antioxidant activity against DPPH (21.49, 21.49, 18.86, 17.54 and 16.67%, respectively than that observed in R-15 and ZUN-LA as follow 12.72% and 13.77%, ferric reducing power results of the six lines ILICA-256, B-6, WAXY, P-70, Z-1 and ZUN-LA extracts had high mean values of FRAP with values of 1.136, 1.072, 1.00, 0.99, 0.988 and 0.83, respectively than R-15 0.75 compared with TBHQ and quercetin. In molecular study six RAPD primers and five SCoT primers were used. RAPD-PCR analysis showed that studied lines are polymorphic in 47.61% while, SCoT markers scored polymorphism as 84.21%. Lines B-6 and ILICA-256 couldn't be distinguished with any specific markers while R-15 line was the most distinctive line that it characterized by the presence of five unique positive bands. P-70 was expressed by the presence of three positive unique bands and the absence of one band. ZUN-LA, Z-1, and WAXY were distinguished by one positive unique band for each. From pooled RAPD-SCOT data; the most two genetically related lines were ILICA-256 and WAXY while the most diverse line was R-15.

Keywords: Pepper, Polyphenols, RAPD and SCoT

INTRODUCTION

Pepper (*Capsicum annuum*, L.) is a high-value vegetable economically familiar crop. It is an important individual in the family *Solanaceae*. It is used in many forms as either row, dried, pickled or as spices (Ashrafi *et al*., 2012).

Oxidative stress of free radicals causes obsessive conditions, for example, cardiovascular infection, disease, diabetes and arthritis (Vera-Ramirez *et al*., 2011) Antioxidant agents can fill in as cautious variables against free radicals in the human body (Wojtunik-Kulesza *et al*., 2016). Phenolic components have been related to the medical advantages got from devouring elevated amounts of foods grown from the ground (Parr and Bolwell, 2000). Phenolic components are optional intermediate metabolites that are subordinates of the pentose phosphate, shikimate, phenylpropanoid pathways in plants (Randhir *et al*., 2004). Phenols are generally appropriated in the plant kingdom and show an extensive variety of contrasting natural impacts, for example, antioxidant, anti-inflammatory, anti-allergic and anti-carcinogenic agents. A significant number of these capacities have been credited to the cancer prevention agent action of phenols which phenolic cell reinforcements act principally by giving a hydrogen iota to a free radical, i.e. scavenging of free radicals (Manach *et al*., 2005). Flavonoids and phenolic components are critical segments of pepper, the two substances have demonstrated their capacity to expel (or deactivate) free radicals, over having the capacity to shield lipids and nutrients C from being devastated in the oxidative procedure (Frozza *et al*., 2013).

Pepper beside its antioxidant agents has many benefits for humans health, it is rich in many vitamins i.e. C, E, B5, and A (Howard *et al*., 2000) and some important minerals such as potassium, magnesium, iron, calcium, phosphorus and β-carotene. Pepper extracts also have antimicrobial activity (Salam, 2015). It also has an antioxidant activity which qualifies it to be used in the protection against many diseases such as cancer, rheumatism, stiff joints, and bronchitis and chest colds with a cough and headache, arthritis, heart arrhythmias and in stomachic (Pawar *et al*., 2011 and Mateos *et al* 2013).

Agricultural crop development programs are based mainly on genetic variation between different structures. Germplasm demonstration is important to describe the relationship between conservation and employment of plant genetic resources (Thul *et al*., 2012). Therefore, genetic variation is a valuable and necessary source of effective breeding and the induction of new varieties (Maric *et al*., 2004). Evaluation of genetic diversity based on morphological variations which are often obstacle as morphological traits where it highly environmental sensitive (Hossain *et al*., 2014). Therefore, it was an urgent need for more distinguishable tools to evaluate the genetic diversity.

Evolution in molecular biology techniques contributed to developing many DNA markers that can measure the genetic polymorphism at the molecular level that not affected either by the stage of growth, season or by the method of agriculture (Kwon *et al*., 2002). Different molecular PCR-based techniques were used to evaluate genetic diversity in pepper genotypes; (RAPD) random amplified polymorphic DNA (Renganathan *et al*., 2017), (ISSR) inter-simple sequence repeats (Tsonev *et al*., 2017), (SSR) simple sequence repeats (Buso *et al*., 2016), and (SCoT) start codon targeted polymorphism (Tsaballa *et al*., 2015)

RAPD-PCR based technique relies on the rule of the amplification of genomic DNA using one primer on random nucleotide sequence without any need of former information about the genome. Its dominant marker (Williams *et al*., 1990) could be used successfully to assess genetic variability and polymorphism in plant genome (Tiwari *et al*., 2016). RAPD primers have been intensively used to analyze genetic distances among many crop plants

as citrus lines (Sayed, 2016), aloe Species (Chandra and Choudhary, 2014) and wheat (Eid, 2019).

SCoT-PCR based technique is dependable for its benefits such as adequacy, useful, low cost and dominant as well as RAPD. This technique is designed to target the sequences embracing the ATG translation start codon, elucidating the connection between functional genes and their related traits (Rathore *et al*., 2014), also SCoT has used in abundance to estimate genetic diversity and relationships in mango (Luo *et al*., 2011), Cicer species (Amirmoradi *et al*., 2012), date palm and Maize (Al-Qurainy *et al*., 2015, Vivodík *et al*., 2016).

This study was conducted to estimate and analyze diversity in some *(Capsicum annuum*, L.) genotypes at two levels: 1) Biochemical level: using total phenols, flavonoids content and free radical scavenging activity DPPH assay, 2) Molecular genetic level: using two molecular passed PCR markers (RAPD and SCoT). Also to compare the efficiency of the two primers in estimating genetic diversity.

MATERIALS AND METHODS

This study was executed in molecular and biochemistry laboratories in Genetics and Biochemistry Departments, Faculty of Agriculture, Zagazig University.

Seven different pepper lines: R-15, P-70, ZUN-LA, Z-1, B-6, ILICA-256 and WAXY were used. Seeds were obtained from: Pepper research group, Collage of Horticulture, Northwest A&F University, Shaanxi, China except the two lines ILICA-256 and WAXY were obtained from Agriculture research center, Giza, Egypt. All of these lines were obtained as lines grown for 6 generations using self-pollination.

Each genotype seeds were planted in four lines; each line has 10 plants. Seeds were planted in small pots in semi-shaded area. Young fresh leaves were collected randomly as one bulk for each genotype.

1. Preparation of the 70 % ethanol extracts.

Ethanolic extracts were obtained with 70% ethanolic solution as proceded by (Taha *et al*., 2015) with some modification. The supernatant mixture was evaporated under vacuum at 40 ^{o}C using a rotary evaporator (Laborota 4000-efficient, Heildolph, Germany).

All obtained residues were frozen dried using lyophilizer, obtained powders were kept in light-protected utensil at -18^{o}C until further use.

2. Determination of total polyphenols.

Total phenols concentration in all concentrates were estimated by a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer), as depicted by (Zheng and Wang, 2001) and the recommendation of (Society, 1990).

3. Estimation of total flavonoid content

Total flavonoid content was dictated by the technique of (Ordonez *et al*., 2006) with some alteration and calculated according to the following equation:

$$y = 0.02248x \quad R^2 = 0.992$$

Where: x is the absorbance and y is the concentration (µg QE). R^2=Correlation coefficient.

4. 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay.

The capacity of plant extracts to directly react with and quench free radicals was evaluated as described earlier by (Cheng *et al*., 2006). Percent DPPH radical scavenging activity was calculated as follows:

Percent radical scavenging activity= [(absorbance of control – absorbance of the test sample) / absorbance of control] × 100.

5. Reducing power activity Assay:

The reducing power was measured as portrayed by (Kuda *et al*., 2005). The absorbance was estimated by photometer at 700 nm. The measures were done in triplicate and the outcomes were communicated as mean qualities ± standard deviations. Expanded absorbance esteems show a higher reducing power.

6. DNA extraction:

After three weeks of sowing, young leaves were collected to separate genomic DNA utilizing DNA Extraction kit (Bio Basic kit, Canada).

7. RAPD –PCR and SCoT-PCR analysis:

12 RAPD primers (RA 1 to RA 12) and 10 SCoT primers (SCoT 1 to SCoT 10) were used in this study, the primers and their sequences are listed in Table (1).

Table 1. Sequences of 12 RAPD and 10 SCoT primers.

Primer	Sequences	Primer	Sequences
RA 1 **	5' ACT TCG CCA C 3'	SCoT 1	5' CAA CAA TGG CTA CCA CCC- 3'
RA 2	5' GTC GCC GTC A 3'	SCoT 2**	5' ACC ATG GCT ACC ACC GGC-3'
RA 3 **	5' TGA GCG GAC A 3'	SCoT 3**	5' CAA TGG CTA CCA CTA CAG -3'
RA 4 **	5' AGG GGT CTT G 3'	SCoT 4	5' CCA TGG CTA CCA CCG CAG-3'
RA 5 **	5' AGG GAACGA G 3'	SCoT 5**	5' ACG ACA TGG CGA CCA CGC-3'
RA 6	5' CCA CAG CAG T 3'	SCoT 6**	5' ACG ACA TGG CGA CCC ACA-3'
RA 7 **	5' CAA ACG TCG G 3'	SCoT 7	5' ACC ATG GCT ACC ACG GAG-3'
RA 8	5' GTG AGG CGT C 3'	SCoT 8	5' ACA ATG CTA CCA CCA AGC-3'
RA 9	5' TGC GCC CTT C 3'	SCoT 9**	5' CAC CAT GGC TAC CAC CAG-3'
RA 10 **	5' GTG AGG CGT C 3'		
RA 11	5' GTA GAC CCG T 3'	SCoT 10	5' ACG ACA TGG CGA CCA TCG-3'
RA 12	5' GTG AGG CGT C 3'		

**** Primers that amplified DNA fragments with extracted DNA samples.**

Polymerase chain reaction was conducted using (thermocycler), the reaction for all primers was done in a 20 µl total volume as follows:

Master mix:	1 µl	**DNA template:**	1 µl
Primer:	1 µl	**Distilled water:**	Up to 20 µl

PCR Amplifications conditions for both RAPD-PCR and SCoT-PCR techniques are shown in Table (2).

Amplification products were electrophoresed on 1.5 % agarose gel, and then gels were stained with ethidium bromide.

Table 2. PCR amplification conditions for both RAPD-PCR and SCoT-PCR approach.

Process	Temp.	Duration	No. cycles
initial denaturation	94°C	4 min	30-35
Denaturation	94°C	1 min	
Annealing	40°C -55°C *	1 min	
Elongation	72°C	3 min	
Final elongation	72°C	5 min	

*** Temperature was different depending on primer composition.**

8. Gels visualization:

Gels were displayed under ultraviolet light using (Bio-rad) gel documentation system.

Statistical analysis:

Experiments for determination of total phenolic, total flavonoids, and antioxidant properties using DPPH and reducing power assay were conducted in triplicates. Analysis of variance and significance of difference among means were tested by one-way ANOVA and performed with the statistical program MS Excel (Microsoft Office 2010 Professional).

Variables of RAPD and SCoT amplification products were scored across the lanes. The presence of amplified DNA bands; were scored as "1" and absence as "0" . The obtained data were compared to calculate similarity index and dendrogram by SPSS 14.0 evaluation version for both RAPD and SCoT techniques.

RESULTS AND DISCUSSION

Total phenols and flavonoids content:

The diversity with total phenolic compounds, as well as with total flavonoids was tabulated in the Table (3).

Table 3. Phenolic and flavonoids (as quercetin equivalent) contents of different species of fresh pepper leaves *(Capsicum annuum,* L.*)*

	Line Name	Origin	Total polyphenol mg/g extract	Flavonoids mg /g extract
*Capsicum annuum,*L.	R-15	China	21.78 ± 0.23	0.26 ± 0.04
	P-70	China	21.25 ± 0.41	0.41 ± 0.02
	ZUN-LA	China	21.78 ± 0.33	0.43 ± 0.02
	Z-1	China	24.58 ± 0.54	0.29 ± 0.04
	B-6	China	22.25 ± 0.13	0.22 ± 0.05
	ILICA-256	Egypt	53.78 ± 0.52	0.37 ± 0.01
	WAXY	Egypt	36.18 ± 0.32	0.36 ± 0.02

It can be noticed that ethanolic extracts (70%) of the four lines Z-1, ILICA-256, B-6 and WAXY recorded highest values of total phenolic compounds (53.78, 36.18, 24.58 and 22.25 mg/g respectively) comparing with R-15, ZUN-LA and P-70 (21.78, 21.78 and 21.25 mg/g, respectively) which showed the lowest mean values. Data in the same Table showed that the four lines ZUN-LA, P-70, ILICA-256, and WAXY had highest contents of flavonoids (0.43, 0.41, 0.37 and 0.36 mg/g, respectively) comparing with the other three lines Z-1, R-15 and B-6 (0.29, 0.26 and 0.22 mg/g, respectively) these results were in order with Gurnani *et al*., (2016) they reported that the amount of the total phenolic compounds and total flavonoid compounds in pepper extracts ranged from 7.95 to 26.15 mg/g gallic acid equivalents (GAE mg/g) and from 4.64 to 12.84 mg/g quercetin equivalents (RU mg/g) of the dry weight of residues, respectively. Phenolic compounds are secondary metabolites that can act as antioxidants due to their ability to donate hydrogen, quench singlet oxygen and act as metal chelators as stated by Michalak, (2006) who studied plant tolerance against heavy metal stress. Flavonoids consist of a large group of polyphenolic compounds, have benzo--pyrone structure, and provide benefits in multiple ways to plant tolerance as stated by Kumar *et al*., (2010) who studied antioxidant activity, total phenolics and GC-Ms *vitex Segunda.*

Free radical scavenging activity (RSA) DPPH assay:

DPPH is a steady and free radical. It is normally utilized as a substrate to assess *in vitro* cell reinforcement movement of concentrates of organic products, vegeTables, and restorative plants contrasted and different methods. Antioxidants can scavenge the radical by hydrogen donation, which causes a reduction of DPPH absorbance at 515 nm. This technique is broadly used to assess Antioxidants activity inside a generally brief time when contrasted and different strategies. The outcome obviously demonstrated (Table 4). The five lines ILICA-256, P-70, B-6 WAXY, and Z-1 had the highest percentage (21.49, 21.49, 18.86, 17.54 and 16.67 %, respectively comparing with lines ZUN-LA and R-15 (13.77 and 12.72, respectively). The antioxidant activity of five color species from *Capsicum annuum,*L. Red, Green, Yellow, Purple, and White were studied by Tinrat, (2016) and they were as follow: 54.72±8.06, 58.72±4.52, 17.2±2.19, 8.77±0.73 and 0.40±0.01 %, respectively and they stated that the ethanolic extracts of green *Capsicum annuum*, L. showed the highest DPPH value.

Table 4. Scavenging activity assay of pepper leaves ethanol extracts (70%) against DPPH free radical

	Line Name	Origin	% inhibition after 30 min.
*Capsicum annuum,*L.	R-15	China	12.72 ± 0.71
	P-70	China	21.49 ±0.41
	ZUN-LA	China	13.77 ±0. 74
	Z-1	China	16.67 ±0.66
	B-6	China	18.86 ± 0.89
	ILICA-256	Egypt	21.49 ±0.35
	WAXY	Egypt	17.54 ±0.44

Ferric reducing antioxidant power (FRAP) assay is a widely used method that uses antioxidants as reductants in a redox-linked colorimetric reaction, wherein Fe^{3+} is reduced to Fe^{2+}. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction at low pH causes the formation of a colored ferrous-probe complex from a colorless ferric-probe complex. Antioxidants are molecules which act as reducing agents by donating electrons to free radicals to stabilize them and minimize the damage caused by free radicals to DNA, cells and organ systems.

Fe^{3+} - Probe + Reducing Antioxidant gave Fe^{2+} - Probe.

The reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm according to the state by Chang *et al*., (2002). In this assay, the yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power. Reducing capacity was presented in Table 5, where lines ILICA-256, B-6, WAXY, P-70, Z-1, and ZUN-LA recorded highest values 1.136, 1.072, 1.00, 0.99, 0.988 and 0.83 comparing with R-15 (0.75). These diversities of antioxidant activity and of ferric reducing power, generally correlated with those diversities of total phenolic compounds and content of flavonoids compared with TBHQ and quercetin (2.581 and 1.752), respectively (Table 5). Tinrat, (2016) studied the reducing power activity of five species of *Capsicum annuum,*L. Red, Green, Yellow, Purple and White (17.36±1.67, 9.34±0.51, 20.14 , 22.44 and 0.30 mg /100g FW), respectively.

RAPD-PCR Polymorphism analysis:

Out of the 12 RAPD primers, only six detected genomic DNA fragments (RA 1, RA 3, RA 4, RA 5, RA 7 and RA 10) as shown in (Fig.1). Totally, 42 bands were scored using six RAPD primers to analyze diversity among seven pepper lines. A number of bands/ primer ranged from five bands (RA 7 primer) to 11 in RA 5 primer with average of seven bands /primer, their molecular weights were ranged from 79.72 bp to 2656.75 bp.

Bands were distributed as 20 polymorphic bands ranged from six bands with RA 5 primer to one band with primers RA 10 with an average of 3.33, a total average of polymorphism was 47.61%. Only one unique band was obtained in R-15 line using RA 1 primer with a molecular weight of 2043.55 bp.

Table 5. Determination of Ferric Reducing Antioxidant Power (FRAP)

	Line Name	Origin	Reducing power
*Capsicum annuum,*L.	R-15	China	0.75 ± 0.02
	P-70	China	0.99 ±0.05
	ZUN-LA	China	0.83 ±0.06
	Z-1	China	0.988 ± 0.03
	B-6	China	1.072 ± 0.04
	ILICA-256	Egypt	1.136 ± 0.04
	WAXY	Egypt	1.00 ± 0.07

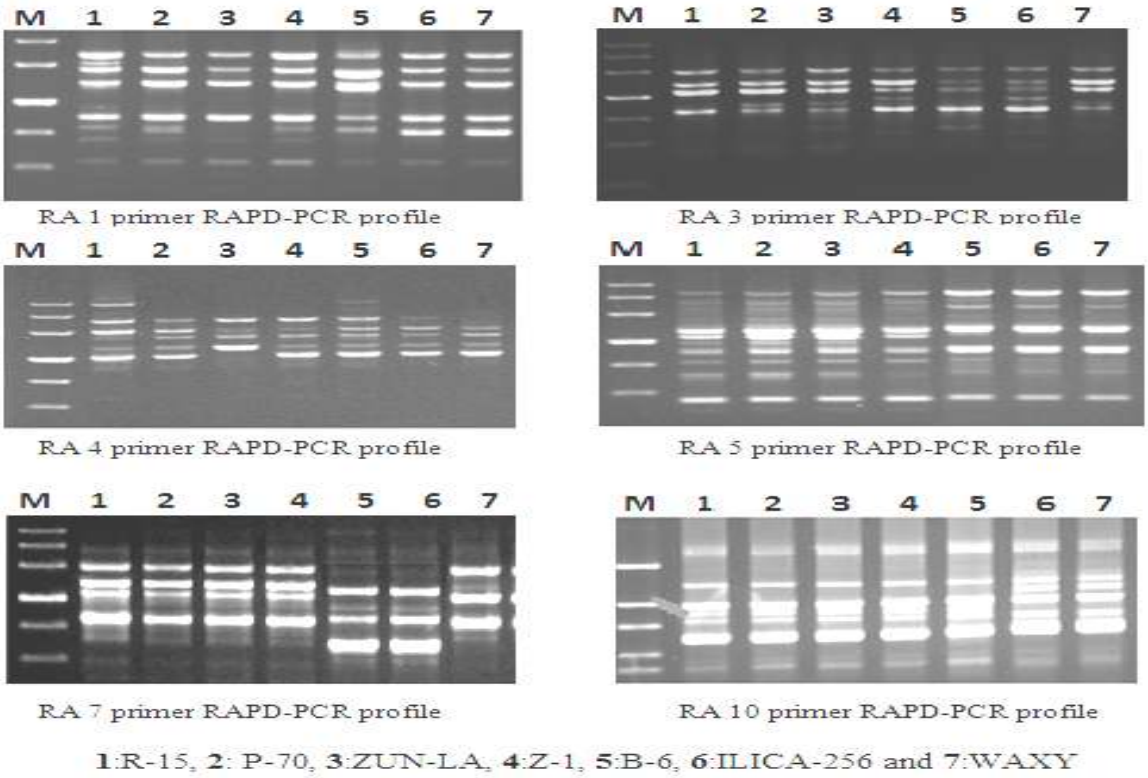

Fig. 1. RAPD-PCR profile using six RAPD primers with seven-pepper lines.

The highest polymorphic primer was RA 4 with 83.33%. While, the lowest polymorphism was obtained with primer RA 10 16.667% (Table 6).

RAPD – similarity index and dendrogram:

Comparative Similarity index demonstrates the diversity and relationship among the seven studied pepper lines using six RAPD primers (Table 7 and Fig. 2), genetic similarity between the seven studied pepper lines ranged from 0.952 between ILICA-256 and WAXY lines followed by 0.881 between B-6 and both lines ILICA-256 and WAXY with the same value of 0.881 was noticed between the two lines R-15 and P-70. While the lowest similarities were obtained between ZUN-LA and ILICA-256 with the same value of (0.643).

The greatest diversity was observed between the two lines ZUN-LA and ILICA-256 followed by the diversity between R-15 with both of the two lines ILICA-256 and WAXY.

These results proved that the seven studied lines showed relatively low diversity; keeping in view the previous results of total polymorphism ratio obtained by RAPD-PCR technique. This may owe to the same genetic origin that they share and those plants may be genetically closely related.

Dendrogram hierarchical cluster analysis was established on the basis of bands existence and disappearance. The clustering test from RAPD marker analysis assembled the seven pepper lines into three main clusters (Fig. 2). The first cluster A, include the three lines B-6, ILICA-256, and WAXY. While the second cluster B consists of the two lines R-15 and P-70. The third cluster C includes the two lines ZUN-LA and Z-1. These results indicated that these lines are genetically related and share the same genetic pool. In this study, RAPD markers scored comparatively minor genetic diversity among studied lines reflecting agreement with (Rana *et al*., 2014) who obtained 42% of polymorphism across pepper genotypes using RAPD primers.

Table 6. Gained bands using six RAPD primers in seven Pepper lines. Total, Monomorphic, polymorphic and unique bands.

Primer	Total no. bands	Monomorphic bands	Polymorphic bands		polymorphism
			Without unique	unique	
RA 1	8	5	2	1	37.50%
RA 3	6	4	2	0	33.33%
RA 4	6	1	5	0	83.33%
RA 5	11	5	6	0	54.55%
RA 7	5	2	3	0	60.00%
RA 10	6	5	1	0	16.667%
Total	42	22	19	1	47.61%

Table 7. Similarity index among the seven studied pepper lines based on RAPD –PCR analysis

Pepper lines	R-15	P-70	ZUN-LA	Z-1	B-6	ILICA-256	WAXY
R-15	1.000	0.881	0.738	0.786	0.786	0.667	0.667
P-70		1.000	0.857	0.810	0.810	0.738	0.786
ZUN-LA			1.000	0.810	0.714	0.643	0.690
Z-1				1.000	0.810	0.786	0.738
B-6					1.000	0.881	0.881
ILICA-256						1.000	0.952
WAXY							1.000

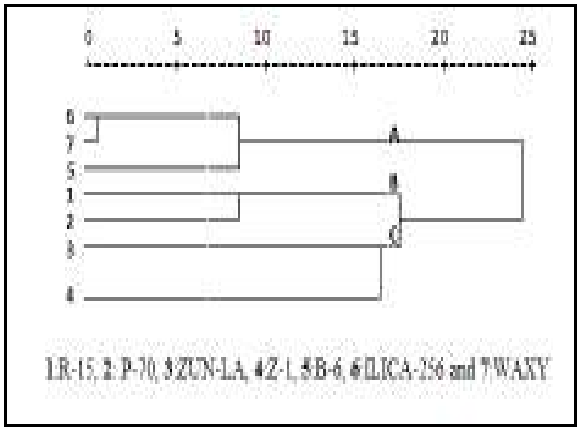

Fig. 2. Dendrogram presentation based on RAPD – PCR data among the seven Pepper lines

SCoT -PCR studies:

Out of the 10 SCoT primers, only five primers were able to detect genomic DNA fragments (Fig.3).

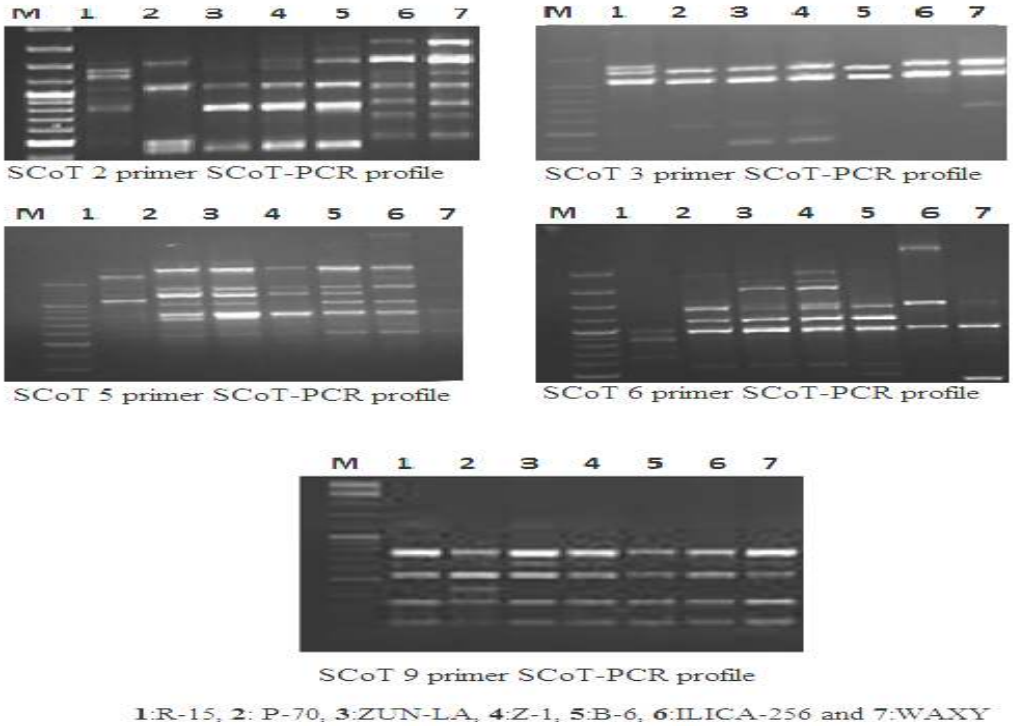

Fig. 3. SCoT-PCR profile using five SCoT primers with seven pepper lines.

Five SCoT primers (SCoT 2, SCoT 3, SCoT 5, SCoT 6 and SCoT 9) scored 38 bands in total. A number of bands ranged from six bands with (SCoT 3 and SCoT 9) to 11 bands with (SCoT 2 primer) with average of 7.6 bands /primer, with different molecular weights that ranged from 345.599 bp to 3851.873 bp.

Bands were distributed as 32 polymorphic and 6 monomorphic bands, with a high average of polymorphism (84.21%). Polymorphic bands ranged from 11 bands with (SCoT 2 primer) to 3 bands with primer (SCoT 9) with an average of 6.4. Appropriate numbers of unique bands were obtained with different SCoT primers distinguishing many specific markers for the studied pepper lines. The highest polymorphic primer was SCoT 2 and SCoT 5 with 100 % percentage. While the lowest polymorphism was obtained with primer SCoT 9 in percentage of 50 % (Table 8).

Table 8. Gained bands using five SCoT primers in seven Pepper lines. Total, Monomorphic, polymorphic and unique bands.

Primer	Total no. bands	Monomorphic bands	Polymorphic bands		polymorphism
			Without unique	unique	
SCoT 2	11	0	9	2	100.0%
SCoT 3	6	2	2	2	66.66%
SCoT 5	8	0	6	2	100.0%
SCoT 6	7	1	4	2	85.71%
SCoT 9	6	3	1	2	50.00%
total	38	6	22	10	84.21%

Results of Fig.4 and Table 9 showed the similarity matrix and dendrogram for the analysis of genetic diversity among the seven studied pepper lines. It is clear that line R-15 showed the lowest similarity with the other six studied lines 0.439 with P-70 line followed by 0.561 with lines ZUN-LA and Z-1.

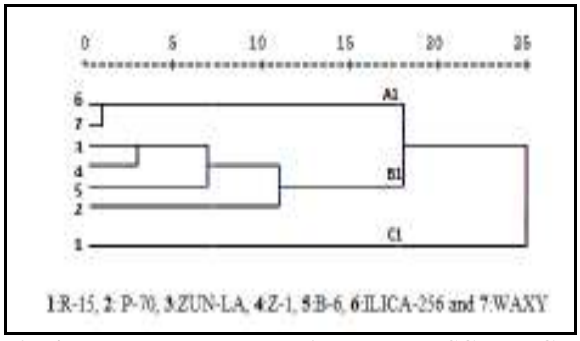

Fig. 4. Dendrogram presentation based on SCoT –PCR data among the seven pepper lines

Table 9. Similarity index among the seven studied pepper lines based on SCoT –PCR analysis

Pepper lines	R-15	P-70	ZUN-LA	Z-1	B-6	ILICA-256	WAXY
R-15	1.000	0.439	0.561	0.561	0.659	0.610	0.634
P-70		1.000	0.732	0.732	0.780	0.634	0.561
ZUN-LA			1.000	0.854	0.805	0.659	0.634
Z-1				1.000	0.805	0.659	0.585
B-6					1.000	0.854	0.732
ILICA-256						1.000	0.878
WAXY							1.000

This result suggests that R-15 line is genetically diverse with other six lines. The highest similarity was recorded between ILICA-256 and WAXY lines (0.878) followed by the similarity between B-6 with ILICA-256 (0.854), suggesting the same genetic origin that they share and those plants may be genetically closely related.

Keeping in view the previous results of total polymorphism ratio obtained by SCoT-PCR technique; it is obvious that SCoT –PCR is able to detect a high level of diversity and polymorphism. Dendrogram analysis was done on the basis of bands existence or disappearance. The clustering test from SCoT marker assembled the seven pepper lines into three main clusters (Fig. 4). The first cluster A1 contained two lines (ILICA-256 and WAXY) that indicate low genetic diversity. While, the second cluster B1 consists of four lines (ZUN-LA, Z-1, B-6, and P-70). The third cluster C includes one line (R-15). These results indicate that R-15 line is the most genetically diverse.

RAPD- SCoT specific marker analysis:

Regrettably, lines B-6 and ILICA-256 couldn't be distinguished with any specific marker of the studied primer group. The R-15 line was the most distinctive line that is characterized by the presence of five unique positive bands, 2034.55 bp with the primer RA 1, 1318.10 bp with primer SCoT 2, 3333.31 bp with primer SCoT 5, 3229.19 related to primer SCoT 3 and 904.81 bp with the primer (SCoT 6). It is also distinguished by two absent negative unique bands 1216.02 and 738.61 with the primer SCoT 5. Line P-70 was expressed by the presence of three positive unique bands weighted 447.29 pb, 1077.30 pb and 435.34 pb with primers SCoT 2, SCoT 5, and SCoT 9 respectively. P-70 was also characterized by the absence of one band weighted 252.92 pb with primer SCoT 9. While the three lines ZUN-LA, Z-1, and WAXY which were distinguished by one positive unique band for each that weighted 639.21 pb with SCOT 9 primer, 2262.82 pb with SCoT 6 primer and 826.03 pb with SCoT 9 primer SCoT 3 respectively (Table 10).

Table 10. Molecular weights of specific unique SCoT - RAPD positive and negative markers scored using five start codon targeted primers and five RAPD primers with seven-pepper lines.

	R-15	P-70	ZUN-LA	Z-1	WAXY
(RA 1)	2034.55				
SCoT 2	1318.10	447.29			
SCoT 3	3229.19				826.03
SCoT 5	3333.31 1216.02* 738.61*	1077.30			
SCoT 6	904.81			2262.82	
SCoT 9		252.92* 435.34	639.21		

(*)Negative specific bands

Pooled RAPD- SCoT Similarity index and dendrogram:

For gathered RAPD- SCoT investigation, the highest similarity (0.916) was observed between ILICA-256 and WAXY lines reflecting high genetic relationship, followed by (0.867) between B-6 and ILICA-256. While, the lowest similarity (0.639); was observed between R-15 and WAXY, followed by 0.651 between R-15 and both of ZUN-LA, ILICA-256 lines. It is worthy noted that the highest diversity appeared between R-15 and all studied lines, indicating different genetic resources (Table 11).

Dendrogram analysis showed that seven studied pepper lines were split into three main clusters; A2 that contain lines B-6, ILICA-256, and WAXY lines; indicating that these lines may be generated from the same parents. While, second cluster B2 includes also three lines ZUN-LA, Z-1, and P-70. While, the line R-15 is represented alone in a distinct cluster; reflecting high genetic diversity with the other lines (Fig. 5).

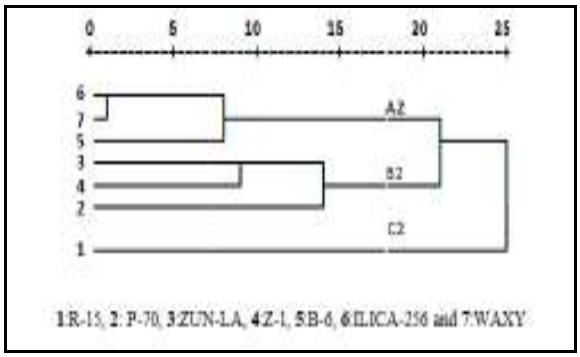

Fig. 5. Pooled dendrogram presentation based on RAPD – SCoT PCR data among the seven pepper lines.

Table 11. Pooled similarity index among the seven studied pepper lines based on RAPD – SCoT PCR analysis

Pepper lines	R-15	P-70	ZUN-LA	Z-1	B-6	ILICA-256	WAXY
R-15	1.000	0.663	0.651	0.675	0.723	0.639	0.651
P-70		1.000	0.795	0.771	0.795	0.687	0.675
ZUN-LA			1.000	0.831	0.759	0.651	0.663
Z-1				1.000	0.807	0.723	0.663
B-6					1.000	0.867	0.807
ILICA-256					.	1.000	0.916
WAXY							1.000

SCoT marker is the most efficient one of molecular markers (ie. RAPD, ISSR, and SCoT) that used to study genetic diversity, polymorphism, and marker index., these techniques could be used together for identification and fingerprinting of the plant genome. Similar investigations were recorded by (Arif *et al*., 2009). SCoT and RAPD markers are resembled, that they are random markers, but SCoT marker is preferable than RAPD for its higher reproducibility.

SCoT primers are longer (18-mer) than RAPD primers (10-mer) so it needs higher annealing temperature. Some studies reported that SCoT is better than RAPD and ISSR especially in evaluating genetic relationships. SCoT marker is a gene-targeted marker system that introduces highly specific and dependable data (Sankhla *et al*., 2015).

In this study, RAPD primers were able to express the adequate level of diversity among studied pepper germplasm and gave the too constringent number of unique bands to fingerprint all studied lines comparing with SCoT that scored a high level of diversity (84.21%).

In addition, abundant unique bands were obtained that presented powerful fingerprinting system for most studied samples in this result agree with (Aliki *et al*. 2015).

CONCLUSION

According to the results obtained, the studied pepper lines had levels of phenolic constituents that contribute to high antioxidant activity and may be considered as a good source of natural antioxidants. The high levels of total phenolic components were found in ILICA-256 and WAXY while, the high level of total flavonoids content was fundamentally highest in ZUN-LA which correlated with the highest antioxidant capacity.

This study was able to demonstrate and differentiate *Capsicum annuum,*L. genotypes using different molecular markers. Polymorphism obtained by SCoT markers was so numerous and could be used for molecular genetics studies of the pepper accessions, presenting high-valued reference for the diversity of germplasm, improvement of the present breeding programs, and conservation of the genetic resources of pepper species.

REFERENCES

Al-Qurainy, F., Khan, S., Nadeem, M. and Tarroum, M. (2015). SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm lines. Pakistan Journal of Botany, 47(6):637-643.

Aliki, X., Ioannis, G., Apostolos, K., Irini, O., Parthenopi, R. and Theodoros, M. (2015). Comparative analysis of genetic diversity in Greek Genebank collection of summer squash (*Cucurbita pepo*) landraces using start codon targeted (SCoT) polymorphism and ISSR markers. Australian Journal of Crop Science, 9(1):14-21

Amirmoradi, B., Talebi, R. and Karami, E. (2012). Comparison of genetic variation and differentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. Plant Systematics and Evolution, 298(9):1679-1688.

Arif, M., Zaidi, N., Singh, Y., Haq, Q. and Singh, U. (2009). A comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (*Dalbergia sissoo*). Plant Mmolecular Biology reporter,27(4):488-495.

Ashrafi S, Mugniéry D, van Heese EYJ, van Aelst AC, Helder J, Karssen G (2012) Description of Meloidoderita salina sp. n. (*Nematoda, Sphaeronematidae)* from a micro-tidal salt marsh at Mont-Saint-Michel Bay in France. ZooKeys, 249(1): 1–26.

Buso, G., Reis, A., Amaral, Z. and Ferreira, M. (2016). Novel and highly informative Capsicum SSR markers and their cross-species transferability. Genetics and molecular resaerches,15(3): 82-89 .

Chandra, D. and Choudhary, P. (2014). Diversity analysis of different accessions of Aloe barbadensis Mill.(*syn. Aloe vera L.*) collected from Rajasthan using RAPD marker system. The Bioscan,9(2):7-10.

Chang, L., Yen, W., Huang, S.C. and Duh, P. (2002). Antioxidant activity of sesame coat. Food chemistry, 78(3):347-354.

Cheng, Z., Moore, J. and Yu, L. (2006). High-throughput relative DPPH radical scavenging capacity assay. Journal of Agricultural and Food Chemistry, 54(20):7429-7436.

Eid, M. (2019). RAPD Fingerprinting and Genetic Relationships of Some Wheat Genotypes. International Journal of Genetics and Genomics, 7:(1): 1-11.

Frozza, C., Garcia, C., Gambato, G., Souza, M., Salvador, M. and Moura, S. (2013). Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. Food and Chemical Toxicology, 52(2):137-142.

Gurnani, N., Gupta, M., Mehta, D. and Mehta, B. (2016). Chemical composition, total phenolic and flavonoid contents, and in vitro antimicrobial and antioxidant activities of crude extracts from red chilli seeds (*Capsicum frutescens L.*). Journal of Taibah University for Science,10(6):462-470.

Hossain, S., Habiba, U., Bhuyan, SI., Haque, M., Begum, S. and Hossain D. (2014). DNA fingerprinting and genetic diversity analysis of chilli germplasm using microsatellite markers. Biotechnology,13(4):174-180.

Howard, L., Talcott, S., Brenes, C. and Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper lines *(Capsicum species)* as influenced by maturity. Journal of Agricultural and Food Chemistry, 48(5):1713-1720.

Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y. (2005). Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. Journal of Food Composition and Analysis, 18(7):625-633.

Kumar, P., Kumaravel, S. and Lalitha, C., (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. African Journal of Biochemistry Research,4(7):191-195.

Kwon, Y., Kim, K., Kim, D., Eun, M. and Sohn, J. (2002). Marker-assisted introgression of quantitative trait loci associated with plant regeneration ability in anther culture of Rice (*Oryza sativa L.*). Molecules and cells,14(1):24-28.

Luo, C., He, X., Chen, H., Ou, S., Gao, M. and Brown, J. (2011). Genetic diversity of mango lines estimated using SCoT and ISSR markers. Biochemical Systematics and Ecology, 39(4):676-684.

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies–. The American journal of clinical nutrition,81(1):230-242.

Mateos, R., Jiménez, A. and Román, P. (2013). Antioxidant systems from pepper (*Capsicum annuum,L.*): Involvement in the response to temperature changes in ripe fruits. International Journal of Molecular Science, 14(5): 9556–9580.

Máric, S., Laríc, S., Artincic, J., Pejíc, I. and Kozumplink, V. (2004). Genetic diversity of hexaploid wheat lines estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breeding, 123(4):366-369.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies,15(4): 210-224.

Ordonez, A., Gomez, J. and Vattuone, M. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food chemistry, 97(3):452-8.

Pawar, S.S., Bharude, N.V., Sonone, S.S., Deshmukh, R.S., Raut, A.K. and Umarkar, A.R. (2011). Chillies as food, spice and medicine: a perspective. International Journal of Pharmacy and Biological Sciences, 1(3): 311-318

Parr, A.J. and Bolwell, G.P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture, 80(7):985-1012.

Rana, M., Sharma, R., Sharma, P., Bhardwaj, S.V. and Sharma, M. (2014). Estimation of genetic diversity in Capsicum annuum,L. germplasm using PCR-based molecular markers. National Academy Science Letters, 37(3):295-301.

Randhir, R., Lin, Y. and Shetty, K. (2004). Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pacific journal of Clinical Nutrition,13(3): 15-28

Rathore, N.S., Rai, M.K., Phulwaria, M., Rathore, N. and Shekhawat, N. (2014). Genetic stability in micropropagated Cleome gynandra revealed by SCoT analysis. Acta physiologiae plantarum,36(2):555-559.

Renganathan, P., Ruíz, C., Hernández, G., Duraisamy, P. and Rueda, O. (2017). Evaluation of genetic diversity in germplasm of paprika (*Capsicum spp.*) using random amplifi ed polymorphic DNA (RAPD) markers. J Plant Sci Phytopathol, 1(9): 80-86.

Salam, (2015). Biochemical studies on peppers as speicific foodstuffs. Cu theses.

Sankhla, A., Malik, C. and Parashar, M. (2015). A Review on Start Codon Targeted (SCoT) Marker. The Journal of Plant Science Research, 31(2):153-160.

Sayed, L. (2016). Molecular characterization and genetic diversity of some egyptian citrus lines using RAPD and ISSRs markers. Egyptian journal of genetics and cytology, 44(2): 387-403.

Society AOC. Official methods and recommended practices of the American Oil Chemists' Society: AOCS-Pr.; 1990.

Taha, F., Elsaadany, S.S., Abo-Eyta, A.M. and Wahdan, K. (2015). Proximate Compositions, Phytochemical Constituents And Antimicrobial Activities Of Peels Extracts Of Bitter Orange And Banana. Zagazig J. Agric. Res., 42(6):1489-1497.

Thul, R., Coombes, S., Roderick, H. L., and Bootman, M. D. (2012). Subcellular calcium dynamics in a wholecell model of an atrial myocyte. Proc. Natl. Acad. Sci. U.S.A., 109(6): 2150–2155.

Tinrat, S. (2016). Antioxidant activities and total phenolic content of multi-colored fruits and vegeTables in Thailand. Asia-Pacific Journal of Science and Technology, 21(1):1-11.

Tiwari, G., Singh, R., Singh, N., Choudhury, D.R., Paliwal, R. and Kumar, A. (2016). Study of arbitrarily amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers for genetic diversity and population structure in Kalmegh [*Andrographis paniculata (Burm. f.) Nees*]. Industrial Crops and Products, 86(1):1-11.

Tsaballa, A., Ganopoulos, I., Timplalexi, A., Aliki, X., Bosmali, I. and Irini, N. (2015). Molecular characterization of Greek pepper (*Capsicum annuum,L*) landraces with neutral (ISSR) and gene-based (SCoT and EST-SSR) molecular markers. Biochemical Systematics and Ecology,59:256-263.

Tsonev, S., Todorova, V., Groseva, S., Popova, T. and Todorovska, E. (2017). Evaluation of diversity in Bulgarian pepper lines by agronomical traits and ISSR markers. Genetika,49(2):647-662.

Vera-Ramirez, L., Sanchez-Rovira, P., Ramirez-Tortosa, M., Ramirez-Tortosa, C., Granados-Principal, S. and Lorente, J. (2011). Free radicals in breast carcinogenesis, breast cancer progression and cancer stem cells. Biological bases to develop oxidative-based therapies. Critical Reviews in Oncology/Hematology,80(3):347-368.

Vivodík, M., Gálová, Z, Balážová, Ž. and Petrovičová, L. (2016). Start codon targeted (scot) polymorphism reveals genetic diversity in european old maize *(zea mays l.*) Genotypes. Potravinarstvo Slovak Journal of Food Sciences, 10(1):563-569.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids research.,18(22):6531-6535.

Wojtunik, K., Oniszczuk, A., Oniszczuk, T. and Waksmundzka, M. (2016). The influence of common free radicals and antioxidants on development of Alzheimer's Disease. Biomedicine & PharmaCotherapy,78:39-49.

Zheng, W. and Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. Journal of agricultural and food chemistry, 49(11):5165-5170.

دراسه مقارنه كيميائية و جزيئية على أنواع مختلفة من الفلفل (*Capsicum annuum*, L.)

هبة سيد احمد طه[1] ، طه فتحي طه[2] و محمد حامد عريشة[3]

[1] قسم الوراثـــة، كلية الزراعـــة ، جامعـة الزقازيق ، مصـــر

[2] قسم الكيمياء الحيوية ، كلية الزراعة، جامعة الزقازيق ، مصر

[3] قسم البساتيـــن ، كليـــة الزراعة ، جامعة الزقازيق ، مصـر

الفلفل (*Capsicum annuum*, L.) يعد محصول تجاري أساسي نشأ في المناطق الإستوائية والمعتدلة حول العالم. تم دراسة الفينولات الكلية ، ومحتوى الفلافونويد ونشاط مضادات الأكسدة بالإضافة إلى تنوعها الوراثي الجزيئي باستخدام نظامين مختلفين للبصمات الوراثية SCoT Primers و RAPD في أنواع مختلفه جينيا من الفلفل من منطقتين مختلفتين هما الصين ومصر، وقد أظهرت النتائج أن المستخلص الإيثانولي (70٪) من الأنواع الوراثية ILICA-256 و WAXY قد أحتوي على مستوى عالٍ من الفينولات الكليه (53.78 و 36.18 ملجم / غرام) بينما المستخلص الإيثانولي (70٪) من التراكيب الوراثية ZUN-LA و P-70 و ILICA-256 و WAXY كان محتوي الفلافونيدات فيها على النحو التالي ، 0.43 ، 0.41 ، 0.37 و 0.36 ملغم / غرام ، وأظهرت النتائج أن أعلى قيمة تم تسجيلها مع النوع الجينيZUN-LA . أظهر نتائج دراسه النشاط المضاد للأكسده أن مستخلصات P-70 و ILICA-256 و B-6 و WAXY و Z-1 لها نشاط أعلى مقابل مركب DPPH (21,49و 21,49 و و 17,54 و 16,67٪) على التوالي وكانوا أعلي من التراكيب الوراثية ZUN-LA ,R-15 (12,72) ,(13,77) ، ونتائج أختزال الحديديك FRAP في التراكيب الوراثية ILICA-256 ، B-6 ، WAXY ، P-70 ، Z-1 و ZUN-LA كانت 1.136 ، 1.072 ، 1.00 ، 0.99 ، 0.988و 0.83) ، على التوالي أعلي من R-15 (0.75) مقارنة مع TBHQ و كيرسيتين. فى هذه الدراسة تم استخدام ستة بادئات من نوع (RAPD) و خمسة بادئات من النوع SCoT . اظهر تحليل RAPD-PCR ان الاصناف المدروسه تتباينت وراثيا بنسبة 47.61% بينما سجلت بادئات الـ SCoT تباين وراثى بنسبة 84.21% . الاصناف B-6 وILICA-256 لم يتم تمييزها بأى معلم متخصص بينما السلالهR-15 كانت اكثر السلالات تميزا ، حيث انها تميزت بوجود خمسة حزم فريدة موجبة. P-70 تم التعبير عنها بوجود ثلاثة حزم فريدة موجبة وغياب حزمة واحدة فريدة. ZUN-LA و Z-1 وWAXY تم تمييزهم بوجود حزمة موجبة واحدة فريدة لكل منهم. من بيانات RAPD-SCoT المجمعة؛ الأصناف الأكثر ارتباطا وراثيا كانت (ILICA-256 و WAXY) بينما كان الصنف R-15 هوالأكثر إختلافا.