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Histological Studies of some Brassicaceous Samples and the Genetic Variability Analysis using SCOT Markers

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ABSTRACT



Brassicaceae family (Cruciferae) known as mustard family, it is one of the largest families of flowering plants which have 372 genera and 4060 species all around the world. This study aims to investigate comparatively the anatomical structure of some plant samples of this family. Anatomical sections were taken on both stems and leaves, and 42 different qualitative characters were recorded in a data matrix. The results were numerically analysis to know the similarity between these samples by draw a dendrogram. Then the anatomical results were used to determine the genetics relationships between studied cultivated genera and the wild genera in order to includ in the breeding and improvement programs. Scot methods was chosen as it is fast, effective and gives accurate results. Start codon targeted (SCoT) polymorphism are one of the common markers were used to assess genetic diversity in some genera in Brassicaceae family. Seventin genera of the Brassicaceae have been used with ten SCoT primers. Totally, 174 bands were scored out of which 84.5% showed polymorphism, where 149 polymorphic bands and 25 monomorphic bands were observed. The results indicated that the existence of a significant degree of genetic similarity ranged from 0.62 to 0.88, where the highest mean value was 0.88 between genera 12 (Morettia canscens) and 13 (Moricandia siniaca) and the lowest mean value was 0.62 between genera 2 (Brassica napus) and 15 (Sinapis alba).

Keywords: Genera in Brassicaceae – Histology - Genetic variability – SCoT markers – Dendrogram.

INTRODUCTION

Brassicaceae, was usually called Cruciferae or mustard family, comprise many economically important species that are grown worldwide. They have been traditionally consumed in the human diet as fresh and preserved vegetables, vegetable oils and condiments from ancient times to the present time. They represent a monophyletic group distributed all over the world. In the world, there are about 372 genera and 4060 species of Brassicaceae family (Al-Shehbaz et al., 2006).

By researching the genus Brassica, it was found that it has a very long evolutionary process. The genus firstly beganed in domestication as vegetables, followed by as edible oilseed crops (Prakash et al., 2009 and Kaur et al., 2014). It has three diploid species, B. rapa (2n=20, AA genome), B. nigra (2n=16, BB genome) and B. oleracea (2n=18, CC genome), and three amphidiploid species, B. juncea (2n=36, AABB genome), B. carinata (2n=34, BBCC genome) and B. napus (2n=38, AACC genome) (Jiang et al., 2015). Then explained the relationship between these species a long time ago (Nagaharu, U., 1935). Each of them was different, such as turnip root vegetables, cabbage Chinese for green leaves, cauliflower and broccolini for flowering (Baker et al., 2017). Consequently, it is possible to develop new varieties and the possibility of adapting them to give the higher yield by increasing the genes within them. As wild mustard can contained in the breeding programs and general wild species must be analyzed at the molecular

level to find out the degree of genetic affinity between them ter working on the techniques of molecular parameters. The ISSR technique is one of the fast, distinct, and accurate molecular techniques, as it has short iterations that recognize parts of DNA and bind to it along the genome, so many alleles can be observed (Safari et al., 2013). A technique has been developed to explore the genetic diversity and molecular differences of different plants such as ginger (Baruah et al., 2019), cassava (Afonso et al., 2019), wheat (El-Sherbeny et al., 2020), flax (Ahmed et al., 2019) and asparagus (Chen et al., 2020), in addition, this technique was used to Brassica by (Kalia et al., 2017; Koch et al., 2017 and Mohammadin et al., 2017).

Cross Mark

The aim of this study is to determine the genetic relationship between the different cultivated and wild genera of the Brassicaceae family in order to be included in the breeding and improvement programs. The ScoT method was chosen because of fast, effective and gives accurate results.

MATERIALS AND METHODS

The plant samples were collected from five different locations: Al-Qalubia (Q), Suez-Cairo Road (SR), Cairo (C), North Coast Road (NC) and Saint Catherine (SC). These samples contain (17) taxa all belonging to Brassicaceae.

The studied samples of the Brassicaceae were selected for this study. All obtained samples were shown in Table 1.

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No.	Plants	Sources	No.	Plants	Sources
1-	Alyssum maritimum (L.)Lam.	Q	10-	Malcolmia africana (L) R.Br.	SC
2-	Brassica napus L.	Q	11-	Matthiola incana R.Br.	Q
3-	Cakile maritima. Scop	NR	12-	Morettia canescens Boiss.	SČ
4-	Diplotaxis harra (Forssk.) Boiss.	SR	13-	Moricandia sinaica Boiss.	SC
5-	Eruca sativa Mill.	Q	14-	Raphanus sativus L.	NR
<u>5</u> -	Farsetia aegyptia Turra	SR	15-	Sinapis alba L.	NR
7-	Iberis amara. L.	Q	16-	Sisymbrium irio L.	SR
3-	Isatis microcarpa Boiss.	SČ	17-	Zilla spinosa (L.) Prant.	SR
9-	Lepidium sativum L.	Q			

Table 1. List of Brassciaceae studied samples and their sources.

Materials:

1- Sections of stems and leaves:

According to the method suggested by Sass (1958) .Samples of stems and leaves were prepared, then by using rotary microtome, sections were cut at the thickness of 15 microns and mounted on slides with the aid of egg-albumin as an adhesive. Sections on the slides were stained with Safranin and Light green, and Canada balsam used as mounting medium. Sections in such cases are microscopically examined for the different microphotographs which can be explored for the different tissues and component.

2- DNA extraction and	purification CTAP
CTAB buffer	Microfuge tubes

Mortar and Pestle	Liquid Nitrogen
Absolute Ethanol (ice cold)	70 % Ethanol (ice cold)
65° C water bath	Water (sterile)
Chloroform: Iso Amyl Alcohol (24:	1) Agarose
6x Loading Buffer	1x TBE solution
Agarose gel electrophoresis system	Ethidium Bromide solution
CTAB extraction buffer: 1L	
100 mM Tris = 12.1g	1.4 M NaCl = 85g

100 mM Tris = 12.1g 20 mM EDTA =7.44g

2% (w/v) CTAB (Hexadecyl triammonium bromide) =20g immediately before use 0.3% β-mercaptoethanol were added **TE buffer:**

10 mM Tris-HCl	1mM EDTA
10X TBE buffer:	
750 mM Tris = 108g	900 mM Boric acid =55g
2 mM Na ₂ -EDTA =7.44g	In one latter dH ₂ o
10/ 1	

1% Agarose gel:

1 g Agarose dissolved in 100 ml TBE DNA Extraction **Procedure:**

DNA extraction according to Porebski *et al.* (1997) with some modifications: A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al.* (1997) was adopted as follows:

 Prepare CTAB buffer use within 2-3 days, store capped: Add polyvinyl pyrrolidone mol. weight 40,000 (PVP-40) and β-mercaptoethanol and stir to dissolve before starting extractions:

CTAB PVP-40 β-merc

- 1.5 ml 0.06g 7µl
- 2- Grind tissue sample in a mortar with liquid N. Add 1.5 ml of CTAB buffer and grind samples with pestle.
- **3-** Transfer solution to a 2 ml tube. Add 2µl of RNase A and mix by inverting. Incubate samples at 65°C for 30min. Centrifuge for 5 minutes at maximum speed (10000 rpm), after centrifuge transfer the supernatant to a new 2 ml tube.
- 4- Add 500µl of 24:1 Chloroform-Isoamyl Alcohol and mix well to form an emulsion by shaking tubes with hands.
- 5- Centrifuge for 5 minutes at maximum speed (10000 rpm).a. Following centrifugation, you should have three layers: top: aqueous phase, middle: debris and proteins,

bottom: chloroform. Proceed to the next step quickly so the phases do not remix. Pipette off the aqueous phase (top) taking care not to suck up any of the middle or chloroform phases. Place the aqueous phase into a new labeled 1.5 ml tube. Estimate the volume of the aqueous phase. This should be approximately 750µl. Add one volumes of cold isopropanol.

- **6-** Let sit in freezer for 45 min to an hour. Centrifuge for 5 min at maximum speed. Orient tubes in an equal fashion to facilitate subsequent removal of supernatant without disturbing resultant DNA pellet.
- 7- Pipette off the liquid, being careful not to lose the pellet with your DNA. The DNA pellet at this stage is very loose and difficult to see. Add 700 μ l of cold 70% Ethanol and invert once to mix. Centrifuge for 1min at maximum speed.
- 8- Dry the pellet in a vacuum centrifuge or on a hot plate at 55° C. Resuspend samples with 100 µl of water (dH₂O). Allow to resuspend for 1hr at 55° C before using.

Estimation for the DNA concentration:

- **a- Agarose Gel:** Run 2 μ l of the parents DNA samples on 1% agarose gel in comparison to 10 μ l of a DNA size marker (100bp DNA ladder). To estimate DNA concentration, compare the degree of fluorescence of the DNA sample with the different bands in DNA size marker.
- **b-** NanaDrop: the concentration of genomic DNA will be estimated by NanoDrop and if necessary it will be diluted with distilled water to adjust for PCR amplification.

Analysis Start Codon Targeted (SCoT)

SCoT-PCR Reactions

Ten SCoT primers were used in the detection of polymorphism as shown in Table (2). The amplification reaction was carried out in 25 μ l reaction volume containing 12.5 μ l Master Mix (), 2.5 μ l primer (10pcmol), 3 μ l template DNA (10ng) and 7 μ l dH₂O.

OR	
5xbuffer	5ul
Mgcl ₂ (25mM)	2ul
dNTPs (10mM)	0.5ul
Table 2 Duine one CCel	T name and Cognones

Table 2. Primers	SCOT name and Sequence
Primer Name	Sequence
SCoT-01	5'-ACGACATGGCGACCACGC-3'
SCoT-02	5'-ACCATGGCTACCACCGGC-3'
SCoT-03	5'-ACGACATGGCGACCCACA-3'
SCoT-04	5'-ACCATGGCTACCACCGCA-3'
SCoT-05	5'-CAATGGCTACCACTAGCG-3'
SCoT-06	5'-CAATGGCTACCACTACAG -3'
SCoT-12	5'-CAACAATGGCTACCACCG -3'
SCoT-16	5'-CCATGGCTACCACCGGCA-3'
SCoT-21	5'-CCATGGCTACCACCGGCC-3'
SCoT-35	5'-AACCATGGCTACCACCAC-3'
Primer (10pmol)	(SCoT) 2.5

DNA (10ng/ul) 3ul

To a DNA Delement

Taq DNA Polymerase (5u/ul)0.2uldH2O up to 25ul0.2ul

The rmocyling Profile PCR:

PCR amplification was performed in a Perkin-Elmer/GeneAmp[®] PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 50°C for 1 min, and an elongation step at 72°C for 1 min. The primer extension segment was extended to 7min at 72°C in the final cycle.

Detection of the PCR Products:

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

RESULTS AND DISSCUTION

1- Anatomical characters:

The current results include 41 qualitative characters recorded for the studied 17 brassicaceous taxa according to the anatomical features of both stems and leaves . The results put in a comparative manner in the data-matrix (Table3).

The recorded 41 qualitative characters obtained from the investigation of both stems and leaves from 17 Brassicaceae taxa.

Stem:

- 1 outline shape : angled + / rounded -
- 2 epidermal surface: smooth + / rough -
- 3-epidermal hypodermis present + / absent -
- 4 epidermal secretory cells present + / absent -
- 5 epidermal amorphous inclusions present + / absent –
- 6-cortex: parenchyma cells only +/ not so -
- 7 cortex collenchyma cells present +/absent -
- 8 cortex sclerenchyma cells " +/ " +/ " -
- " 9 - cortex secretory cells
- 10- cortex prismatic oxalate crystals present + / absent -
- 11- cortex amorphous inclusions present + / absent -

- 12-Pericycle: sclernchyma cells present + / absent -
- 13- Pericycle secretory cells present + / absent -
- 14- Phloem: secretort cells present + / absent -
- 15- Phloem oxalate crystals present + / absent -
- 16-Xylem vesels: tyloses present + / absent -
- 17- Xylem vesels oxalate crystals present + / absent -
- 18- Pith zone : solid + / hollow -

19-Pith zone parenchyma +/ only parenchyma&sclernchyma-

- 20- Pith zone secretory cells present + / absent -
- 21- Pith zone prismatic crystals present + / absent -

Leaf or leaflet:

- 22- Upper epidermis: smooth + / rough
- 23- Upper epidermis large cell present+/ absent -
- 24- Upper epidermis secretory cells present + / absent -
- 25- Upper epidermis amorphous inclusions present + / absent-
- 26-Mesophyll: dorsiventral + / isobilateral -
- 27-Palisade tissue: one layer + / more than one layer -
- 28-Palisade tissue extended over the midrib zone +/ not so -
- 29- Spongy tissue: secretory elements present +/ absent -
- 30 Spongy tissue : amorphous inclusions present + / absent -
- 31- Midrib zone : upper epidermis concave + / convex -
- 32 -Midrib zone collenchyma cells present + / absent -
- 33 -Midrib zone with one vascular bundle + / with more than one
- 34 -Midrib zone bundle cresent shape + / rounded -
- 35- Midrib zone bundle sheath of fibers present + / absent -
- 36- Midrib zone druses oxalate crystals present + / absent -
- 37- Lower epidermis: smooth + / rough -
- 38- Lower epidermis : large cell present+/ absent_-
- 39- Lower epidermis :secretory cells present + / absent -
- 40- Lower epidermis : amorphous inclusions present + / absent-
- 41- Mesophyll consists of palisade and spongy +/ consists of palisade or spongy -

Table 3. Data matrix of 41 qualitative characters recorded comparatively to 17 brassicaceous taxa depending on the anatomy of stems and leaves.

Sp/ch	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
1	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+
2	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	-	+
3	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-	+	-	+	+	-	-	+	-	-	+	+
4	-	+	-	+	-	+	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-
5	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+
6	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+	-	+	+	-	-	+	-	-	+
7	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-	+	+	-	-	+
8	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-
9	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	-	-	+
10	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
11	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-
12	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	-	+	+	-	+	+	-	+
13	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-
14	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+
15	-	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	+
16	-	+	+	-	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+
17	-	+	+	-	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-	-	+	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+

A) Transverse sections of stems:

The stem outline shape is mostly rounded as in Allysum maritimum (Fig.1) but in two taxa the stem takes triangle shape as in Morettia canescens (Fig. 2). Epidermal surface is mostly smooth as in Eruca sativa. while it is rough in few taxa as in Cakile maritimum. The presence of the hypodermal layer is recorded in some samples as in Zilla spinosa (Fig.4). Secretory cells recorded only in Diplotaxis hara, while amorphous inclusions are observed only in two taxa as in Zilla spinose (Fig.7). The cortical cells are usually parenchyma as in Brassica napus, while some taxa have few

collenchyma cells besides the parenchyma as in Sinapis alba (Fig.5), or few sclerenchyma cells besides the parenchyma cells as in Zilla spinose (Fig. 8). Secretory cells

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and the druses oxalate crystals are observed only in the cortex of *Diplotaxis harra*, while the amorphous inclusions recorded in the cortex of three taxa such as *Sisymbrium irio* (Fig.7). In the pericyclic layer sclerenchyma cells are noticed in three taxa such as *sinapis alba* (Fig.6), also secretory cells are recorded only in the pericyclic layer of *Morettia canescens*. In phloem tissue secretory cells observed only in *Morettia canescens* and the druses crystals showed only in Raphanus sativus. Tyloses in xylem vessels observed only in *Moricandia sinaica* and druses crystals recorded in xylem parenchyma of *Zilla spinosa*. The pith zone is usually solid . except in *Brassica napus* (Fig. 3) which has hollow pith zone. In some taxa the pith zone consists of paranchyma alternated with sclerenchyma cells such as *Zilla spinose* (Fig.9) while in the most samples the pith zone consists of parenchyma cells only such as *Alyssum maritimum* (Fig. 1). Secretory cells in pith zone noticed only in *Morettia canescens* and druses crystals recorded in the pith zone also in one taxa (*Cakile maritima*).



Fig.1 Allysum maritimum with rounded ,parenchymal cells only and solid pith zone ,Fig 2 Morittia cances with 1 trianglde stem and solid pith zone and Fig,3 Brassica napus with rounded stem and hollow pith zone. all x 10

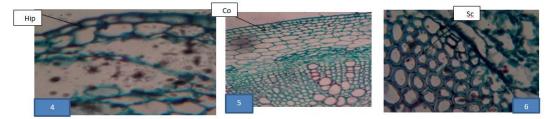
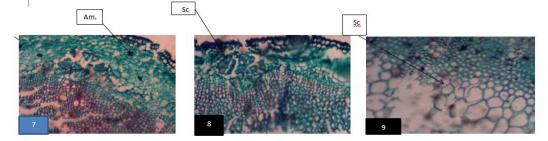


Fig. 4. the hypodermal layer in the stem of *Zilla spinosa*, and Figs. (5 and 6 cortical collenchyma and schlernchyma cells in the pericyclic layer in the stem of *Sinapis alba*. (All x10). Hip= the hypodermal layer. Sc= schlerenchyma cells and co= collenchyma cells.



Figs.7-9. T.S. in the stem of Zilla spinose: 7, amorphous inclusions and 8, schlerenchyma cells respectively at x 10 and Fig 9 schlerenchyma in the pith zone at x 40. Am= amorphous inclusions and Sc= schlerenchyma cells.

B) Leaf vertical sections:

The investigation of the leaves vertical sections of the studied seventeen samples showed some variations (table 4). The upper epidermal layer is usually with smooth surface as in *Diplotaxis harra* (Fig. 11) except in *Farsetia aegyptia* and Cakile *maritime* (Fig. 10) which have rough surfaces. Large cells are noticed in most examined taxa as in *Farsetia aegyptia* (Fig. 10). Secretory cells are recorded only in two taxa which are *Diplotaxis harra* and *Matthiola incana*, also the amorphous inclusions bodies are observed only in *Morettia cances*. The mesophyll tissue is mostly dorsiventral such as Cakile *maritime*, while it is isobilateral in some samples such as *Diplotaxis harra* (Fig. 11). The palisade tissue under the upper epidermis is usually in more than one layer as in *Moricandia sinaica* (Fig.13) while it is rarely in

one layer such as *Zilla spinose*, and the palisade layers extended over the midrib zone in some taxa as in *Diplotaxis harra* (Fig. 12). Secretory elements are recorded in the spongy tissue three studied taxa such as *Eruca sativa*, also amorphous inclusions are observed in this tissue in *Zilla spinosa* alon. Upper epidermis is concave above the midrib zone of some samples as in *Cakile maritimume* (Fig. 14) or it is convex above the others such as *Alyssum maritimum* (Fig. 13). Collenchyma cells is absent in midrib zone in most examined taxa such as *Alysum maritimum* (Fig. 13) but it is present in the other taxa such as *Sinapis alba* (Fig. 15). The midrib zone is mostly has one vascular bundle as in *Sinapis alba* (Fig.15) except some samples which have more than one bundle such as *sisymbrium irio* (Fig.16). The vascular bundle in the midrib is mostly rounded in shape as in

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sisymbrium irio (Fig.16) except five taxa only have bundle with cresent shape such as *Moricandia sinaica*. The main bundle in the midrib surrounded with sheath of fiberes only in two taxa *Farsetia aegyptiaca* and *Morettia cances*. Druses oxalate crystals are recoded only in two studied samples *Diplotaxis harra* and *Morettia cances* (Fig. 17). The lower epidermis is mostly with smooth surface as in *Diplotaxis harra* (Fig. 11) except five taxa which have rough epidermal surface such as *Farsetia aegyptiaca* (Fig.10). Large cells observed in the lower epidemis in most samples such as *Farsetia aegyptia* (Fig10.). Secretory cells recorded in most samples as in *Malcolmia africana*, while amorphous inclusions observed in few taxa as in *Moricandia sinaica*. The mesophyll tissue consists of palisade cells and spongy cells in most taxa as in *Brassica napus*, except four taxa the mesophyll consists of only palisade cells only or spongy cells as in *Diplotaxis harra* (Fig.11), observed that upper and lower epidermal cells were smooth and found the large cell in 13 species as *sisymbrium irio*. Mesophyll, it contains two types cell were observed according to the shape and position to each cell. The first type was Palisade tissue was observed at in the upper epidermis only (dorsiventral) a number of the species under study, such as *Alyssum maritimum* and *Brassica napus*, While it was found on both epidermis (isobilateral) as *Diplotaxis harra*, palisade tissue consists of one layer in three species *Isatis microcarpa*, *Morettia canescens* and *zilla spinosa*, while more than layer in fourteen speceis as *Alyssum maritimum*. Also extended over the midrib region in - *Iberis amara*, and *Eruca sativa*.

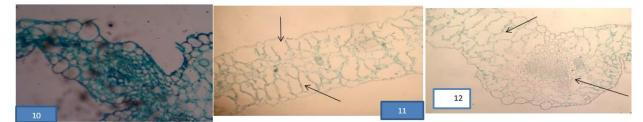
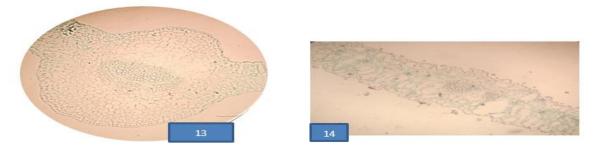


Fig. 10. V.S.of leaf as the following large cells on both upper and lower epidermis of *Farsetia aegyptia*. at x 5,Fig 11 palisade tissue found on mesophyll isobilateral, mesophyll consists of only palisade ,upper and lower smooth of *Diplotaxis harra* at x 5,12 palisade layers extended over the midrib zone - *Diplotaxis harra*



Figures: 13. Cross-section of leaf as the following upper epidermis, covex13- Alyssum maritimum concave : 14- Cakile maritimum at x 5



Figures. (15-17) Cross-section of leaf as the following collenchyma cells in midrib zone and one bundle: 15- *sinapis alpa* and more than bundles in midrib zone16- *sisymbrium irio* 17- *Diplotaxis harra* druses crystals All at x 10

Analysis variations bsed on (SCoT) analysis (SCoT) analysis:

In this study ten primers were chosen to find out the genetic relationship among 17 genera of Brassicaceae produced a total of 174 bands in the profiles Table 15 and Figure 19. Totally, 141 polymorphic, 25 monomorphic, and 8 unique bands were acquired. The primers SCoT-01, SCoT-05, SCoT-06, SCoT-12 and SCoT-21 have generated unique bands. In Table 5 there was a unique band with the primer SCoT-01 at the molecular weight 720bp. As well as, in Table 9 at the molecular weight 170 and 200 bp there was two

unique bands with the primer SCoT-05. In addition, in Table 10 there was a unique band with the primer SCoT-06 at the molecular weight 240bp. Likewise, at the molecular weight 240bp there was a unique band with the primer SCoT-12 in Table 11. Also in Tables 13, 14 there was a unique band with the primer SCoT-21 at the molecular weight 1550bp. However, in the remaining Tables 6, 7, 8, and 12. There were no any unique bands and always recommend using primers that can be obtained, including unique bands, these results were in agreement with those obtained by El-Hefnawy (2020).

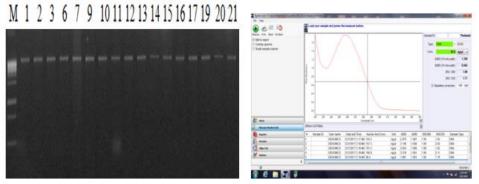


Figure18.	Concentration DNA	using Agarose Gel

Table4. C	Concentration DNA	using Nan	o Drop
Sample	Concentration	Sample	Concentration
1	103.5	13	111.5
2	157.3	14	116.3
3	101.2	15	115.2
6	98.4	16	130.9
7	125.5	17	90.3
9	107.5	19	98.7
10	89.5	20	85.3
11	88.1	21	70.1
12	107.5		

The highest number of bands was (24) obtained from SCoT-12 but the lowest total number of bands was (11) resulted by SCoT-35, these results were in agreement with Abdelmigid et al. (2012) who showed that the polymorphism rate was 87% and the number of polymorphic bands per primer was 13.4 in Brassica napus. The results showed that there are high genetic differences as a result of the polymorphism of genera Brassicaceae. The highest percentage of polymorphism was 95% obtained from (SCoT-05) while, the lowest percentage of polymorphism was 73% obtained from (SCoT-35). Also, in (SCoT-04 and SCoT-06) the percentage was 81% and the total average of polymorphism of 17 genera Brassicaceae was 84.5%. The total mean of band frequency was 0.53 ranged from 0.5 to 0.6, the lowest values of mean band frequency were produced of primers (SCoT-01, SCoT-03, SCoT-05, SCoT-06, SCoT-12, SCoT-16 and SCoT-36) while, the highest values of mean band frequency were produced of primers (SCoT-02, SCoT-04 and SCoT-21) these results agreed with Das et al. (2014) who indicated that the use of ten primers amplified a total number of 353 bands

under 93 loci through 5 mung bean genotypes with an average of 9.3 loci/primer appearing in general polymorphism of 52.7%. However, Mahjoob *et al.* (2016) also showed that higher *MI* value (6.4) than our study using 13 ISSR markers for 35 Brassica genera. The difference between *MI* values with respect to previous studies could be because of different accessions and primers used (Akçali-Giachino, 2020).

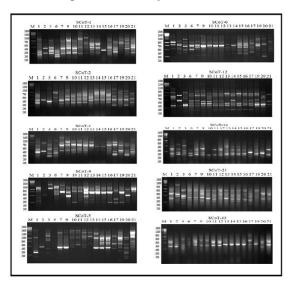


Fig. 19. DNA polymorphism using SCoT with ten primers in 17 genera of Brassicaceae.

Table 5. Primer SCoT-01	, molecular weight (bp) and	presence or absence	bands for	17 genera	of Brassicaceae.

ScoT-1									Ger	nera of	Brass	icacea	e					
MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Frequency
1800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.1
1500	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0.1
1300	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0.2
1050	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
910	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	0.4
880	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0.4
740	1	1	1	0	0	1	0	1	0	1	1	0	0	1	1	1	1	0.6
720	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0.1
700	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0.1
610	1	0	0	0	1	1	0	0	1	1	1	0	0	0	1	1	1	0.5
530	0	1	1	0	0	1	0	0	0	1	0	0	0	0	1	1	1	0.4
460	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0.9
420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
380	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	1	0.4
340	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0.8
300	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	0	0	0.6
280	1	0	0	0	0	1	1	1	0	1	0	0	0	0	1	1	0	0.4
250	0	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	0.8
220	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0.2
200	1	0	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0.4
160	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0.2

ScoT-2		Genera of Brassicaceae																
MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Frequency
1600	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.1
1250	0	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0.4
970	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	1	1	0.6
800	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9
680	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
600	1	0	0	1	1	1	0	1	1	1	1	0	0	1	0	1	0	0.6
550	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0.5
500	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	0.6
460	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
380	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0.2
330	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0.9
280	0	0	1	0	1	1	0	1	0	1	0	0	0	0	0	0	1	0.4
240	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0.3
220	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.1

Table 6 . Primer SCoT-02, molecular weight (bp) and presence or absence bands for 17 genera of Brassicaceae.

Table 7. Priı	ner SC	oT-()3, n	nolec	ular	weig	ght (b	p) and	d pres	sence	or abs	sence	bands	s for 1	7 g	enera	of Bra	assicaceae.
ScoT-3										lenera								
MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Frequency
2000	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.1
1700	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0.1
1350	1	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0.4
1150	1	0	0	0	1	1	0	1	1	1	0	1	1	1	1	0	0	0.6
960	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0.4
760	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9
650	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
560	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0.8
470	0	0	0	1	1	0	0	1	1	0	0	1	0	0	0	1	0	0.4
430	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0.3
370	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1	0	0.8
320	1	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0.3
310	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0.2
280	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0	1	1	0.5
250	1	0	1	1	0	0	0	0	1	0	0	0	0	1	1	1	1	0.5
200	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	0.4
180	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1

Table 8. Primer SCoT-04, molecular weight (bp) and presence or absence bands for 17 genera of Brassicaceae.

ScoT-4	Genera of Brassicaceae																	
MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	- 16	17	Frequency
1900	0	0	1	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0.4
1600	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0.1
1500	1	1	1	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0.5
1300	1	1	1	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0.6
1100	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9
970	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9
880	1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0.4
850	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
780	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
630	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0.1
530	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
460	1	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0.5
420	1	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1	0	0.7
330	1	0	0	1	0	1	1	1	1	1	0	0	1	1	1	0	1	0.6
290	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0.2
240	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	0.5

Table 9. Primer SCoT-05, molecular weight (bp) and presence or absence bands for 17 genera of Brassicaceae.

ScoT-5									G	enera								
MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Frequency
2000	1	0	1	0	0	1	1	1	1	0	0	0	0	0	1	0	1	0.5
1800	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0.2
1700	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	1	0.4
1500	1	0	1	0	1	1	1	1	1	1	0	0	0	0	1	1	1	0.6
1300	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	0.6
1200	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
1150	1	0	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	0.8
1050	0	0	1	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0.6
930	0	0	1	0	1	0	0	1	1	0	0	1	1	1	1	1	1	0.6
870	1	0	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	0.8
760	1	0	0	0	1	1	1	1	1	0	1	1	1	0	1	0	1	0.6
650	0	0	0	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0.6
580	1	0	1	0	1	0	1	1	1	1	0	0	0	0	1	1	1	0.6
510	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	0	1	0.6
450	0	1	0	0	1	1	1	0	1	0	1	1	1	1	1	1	1	0.7
410	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0.3
340	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0.3
300	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0.4
220	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.1
200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.1
170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.1
160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.1

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Tabla	10. Prim	on SCo'	т 06	mala	aulan u	oicht ((bp) o	nd nno	co n oo (n oba	nao ho	nda 1	7	0.000 0	fDra	caiooo	
ScoT-6			1-00,	mole	cular w	eigint	(up) a	Gen	era of I	Brassic	aceae	mus 1	/ gen		n Dra	ssicaci	
MW	1	2			5 6		8	9	10	11	12	13	14	15	16	17	Frequency
1850 1650	0 1	0 0			$ \begin{array}{ccc} 0 & 1 \\ 0 & 0 \end{array} $	0 0	$\begin{array}{c} 0\\ 0\end{array}$	0 0	$\begin{array}{c} 0\\ 0\end{array}$	0 0	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$1 \\ 0$	$\begin{array}{c} 0\\ 0\end{array}$	0 1	0.2 0.1
1450	1	0				0	0	0	0	0	1	1	0	1	1	0	0.4
1250	1	0	0	0	1 0		0	0	0	0	1	1	1	1	0	0	0.4
1150	0	1		1	1 0		0	0	0	0	0	0	1	0	1	1	0.4
1050 960	1	0 1		1 0	$ \begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array} $	1 0	1 0	$1 \\ 0$	$\begin{array}{c} 0\\ 0\end{array}$	0 0	1 1	1 1	1 1	$\begin{array}{c} 0 \\ 1 \end{array}$	$\begin{array}{c} 0 \\ 1 \end{array}$	$1 \\ 0$	0.7 0.4
840	1	1		1	1 1	1	1	1	1	1	1	1	1	1	1	1	1.0
800	1	1		1	1 1	1	1	1	1	1	1	1	1	1	1	1	1.0
780 730	1	1 0		1 1	$ \begin{array}{ccc} 1 & 1 \\ 1 & 1 \end{array} $	1	1 0	$1 \\ 0$	$1 \\ 0$	1 1	1 0	1	1 1	1	1 1	$1 \\ 0$	1.0 0.6
630	0	0		0	1 1 1	1	1	1	1	1	1	1	1	1	0	1	0.0
570	1	1			0 0		0	0	0	0	0	0	1	0	0	0	0.2
530	1	1		1	1 1	1	1	1	1	1	1	1	1	1	1	1	1.0
470 420	0 0	0		1 1	$ \begin{array}{ccc} 0 & 0 \\ 1 & 0 \end{array} $		0 0	0 0	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	1	1	$\begin{array}{c} 0\\ 0\end{array}$	1 0	$1 \\ 0$	$\begin{array}{c} 0\\ 1\end{array}$	0.4 0.4
390	Ő	ŏ		0			1	1	ŏ	Ő	0	0	1	ŏ	Ő	0	0.3
350	0	0			0 0		0	0	1	1	1	1	0	1	0	0	0.4
310 280	0 0	1 0		0 1	$ \begin{array}{ccc} 0 & 0 \\ 1 & 1 \end{array} $	$\begin{array}{c} 0\\ 0\end{array}$	0	0 0	$\begin{array}{c} 0\\ 0\end{array}$	0 0	$\begin{array}{c} 0\\ 0\end{array}$	1 0	$\begin{array}{c} 0\\ 0\end{array}$	1 0	1 1	$\begin{array}{c} 0\\ 0\end{array}$	0.2 0.2
240	1	0					0	0	0	0	0	0	0	0	0	0	0.2
	11. Prim	er SCo'	-	-	cular w	veight ((bp) a	nd pre	sence of	or abse	ence ba	nds f	or 17	-	ra of 1	Brassi	
ScoT-1	Table 11. Primer SCoT-12, molecular weight (bp) and presence or absence bands for 17 genera of Brassicaceae.ScoT-12Genera of BrassicaceaeWW 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 FrequencyFrequencyVIW 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 Frequency																
<u>MW</u> 1600	$\frac{1}{1}$ $\frac{2}{0}$	$\frac{3}{0}$	<u>4</u> 0		<u>6 7</u> 0 0	<u>8</u> 1	<u>9</u> 1	<u>10</u> 0	$\frac{11}{0}$	$\frac{12}{0}$	$\frac{13}{0}$	$\frac{14}{0}$	$\frac{15}{0}$	<u>16</u> 0	$\frac{17}{0}$		Frequency 0.2
1450	1 0	1	0	0	0 0	1	1	0	Õ	0	0	0	0	0	0		0.2
1200 1100	$\begin{array}{ccc} 0 & 0 \\ 1 & 0 \end{array}$	$\begin{array}{c} 0\\ 1\end{array}$	$\begin{array}{c} 1\\ 0\end{array}$		$ \begin{array}{ccc} 1 & 0 \\ 1 & 1 \end{array} $	1 1	1	$\begin{array}{c} 0\\ 1\end{array}$	$1 \\ 0$	1 1	1	1	1		0		0.6 0.8
960	1 1	1	1	1	1 1	1	1	1	1	1	1	1	1	1	1		1.0
930 840	$\begin{array}{ccc} 0 & 0 \\ 0 & 1 \end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	1 1	1	$ 1 1 \\ 1 1 $	1	1	1 0	1	$1 \\ 0$	1	1	1	$1 \\ 0$	1		0.8 0.7
760	1 0	1	1	0	1 0	Ō	1	1	Ō	1	1	Ĩ	1	1	Ō		0.6
700	1 0	0	0		0 1	0	0	0	0	0	0	0	0	1	0		0.2
630 600	$ \begin{array}{ccc} 0 & 0 \\ 1 & 0 \end{array} $	1 1	$\begin{array}{c} 0\\ 0\end{array}$		$ \begin{array}{ccc} 0 & 1 \\ 0 & 1 \end{array} $	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	0	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	0	0	0		0.1 0.2
570	0 0	0	0		0 0	0	0	1	0	1	1	0	1	1	0		0.3
500 460	$ \begin{array}{ccc} 0 & 1 \\ 0 & 0 \end{array} $	$\begin{array}{c} 0\\ 0\end{array}$	1		$ \begin{array}{ccc} 1 & 0 \\ 1 & 1 \end{array} $	1 1	1			$\begin{array}{c} 0\\ 0\end{array}$	1	1	1	0	0		0.6 0.5
450	0 0	0	1	0	0 0	0	0	0	ĩ	Õ	Ō	0	Ō	1	1		0.2
400 380	$ \begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array} $		$\begin{array}{c} 1\\ 0\end{array}$		$ \begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array} $	1 1	1					$\begin{array}{c} 1\\ 0\end{array}$		$1 \\ 0$	0		0.9 0.1
350	0 0	1	ŏ	0	0 0	0	0	ŏ	ŏ	0 0	ŏ	ŏ	1	1	ŏ		0.2
340 320	$ 1 1 1 \\ 1 1 $	1 1	1		$ \begin{array}{ccc} 1 & 1 \\ 1 & 1 \end{array} $	1	1	1	1	1	1	1	1	1	1		$1.0 \\ 1.0$
280	$\begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array}$	1	0				$\stackrel{1}{0}$	0	0	$\stackrel{1}{0}$	0	0	1	1	0		0.2
270	1 1	0	1		$ \begin{array}{ccc} 0 & 0 \\ 0 & 0 \end{array} $	0	0	0	0	0	0	0	0	0	1		0.2
240	$\begin{array}{cc} 0 & 0 \\ 2100 & 0 \end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	1 1		$\begin{array}{ccc} 0 & 0 \\ 0 & 0 \end{array}$	$\begin{array}{c} 0\\ 1\end{array}$	$\begin{array}{c} 0\\ 1\end{array}$	0 1	$\begin{array}{c} 0\\ 1\end{array}$	$\begin{array}{c} 0\\ 1\end{array}$	$\begin{array}{c} 0\\ 1\end{array}$	$\begin{array}{c} 0 \\ 1 \end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	0	$\begin{array}{c} 0\\ 0\end{array}$		0.1 0.5
	12. Prim	er SCo'	T-16.	mole	cular w	veight ((bp) a	nd pre	sence o	or abse	ence ba	unds f	or 17	gener	ra of]	Brassi	caceae.
ScoT-1								Ĝen	era of B	rassica	ceae			0			
MW 1350	<u> </u>	$\frac{2}{0}$		5 0	<u>6</u> 0	7 0	8 0	1	10 1 0 () () 0	0) (0	0	17 0	Frequency 0.1
1050	0	0 1	l 0	0	0	0	0	0	1 () () 0	0			0	0	0.1 0.2
790 610	$\begin{array}{c} 0\\ 0\end{array}$	$ \begin{array}{ccc} 0 & 0 \\ 1 & 1 \end{array} $		$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	0 0	0 0	0 0	1 (1			1			00	$\begin{array}{c} 0\\ 0\end{array}$	0.2 0.2
550	0	0 () 0	0	0	0	0	0	1 () () 1	0) (Ő	0	0	0.1
460 400	1 1	$ \begin{array}{c} 0 & 0 \\ 1 & 0 \end{array} $		1	$\begin{array}{c} 0\\ 1\end{array}$	0 1	0	0 1	$ \begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array} $			0			00	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0.4 \\ 0.4 \end{array}$
320	1	0 1	1	1	1	0	ī	1	1 1	1	1	0)	1	0	1	0.8
250 220	1	1 (1 1 1		1	0 1	0 0	0	0 1	0 1			1	. () 1	1 1	0	$\begin{array}{c} 0.4 \\ 0.8 \end{array}$
200	1	1 1	ı 1 I 1	1	1	1	1	1	1 1		1	1		1	1	1	1.0
180	1	1 1		1	1	1	1	1	1 1		1	1		1	1	1	1.0
160 130	1 0	$\begin{array}{c} 0 & 0 \\ 0 & 0 \end{array}$		$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 1\\ 0\end{array}$		$ \begin{array}{c} 1\\ 0 \end{array} $	$ \begin{array}{c} 1\\ 0 \end{array} $	$ \begin{array}{ccc} 1 & 1 \\ 1 & 1 \end{array} $			1			$ \begin{array}{c} 1\\ 0 \end{array} $	$ \begin{array}{c} 1\\ 0 \end{array} $	$\begin{array}{c} 0.8\\ 0.4\end{array}$
	13. Prim	er SCo'	T-21.	mole	cular w	eight ((bp) a	nd pre	sence (or abse	ence ba	nds f	or 17	gener	ra of 1	Brassi	caceae.
ScoT-2								Ger	era of I	Brassic	aceae			0			
MW 1550	$\frac{1}{0}$	$\frac{2}{1}$ $\frac{3}{0}$		<u>5</u> 6 0 0		8 0	9 0	<u>10</u> 0	$\frac{11}{0}$	12 0	<u>13</u> 0		1 <u>4</u> 0	15 0	16 0	<u>17</u> 0	Frequency 0.1
1150	1	0 0	1	1 0	0 (0	Õ	0	0	0	0		0	0	1	Õ	0.2
990 940 800	$\begin{array}{c} 0\\ 1\end{array}$	$ \begin{array}{ccc} 1 & 0 \\ 1 & 1 \end{array} $	$\begin{array}{c} 0\\ 1\end{array}$	$ \begin{array}{ccc} 0 & 0 \\ 1 & 0 \end{array} $		$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0 \\ 1 \end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$	$\begin{array}{c} 0\\ 0\end{array}$		$\begin{array}{c} 0\\ 0 \end{array}$	$\begin{array}{c} 0\\ 0 \end{array}$	$\begin{array}{c} 0\\ 0\end{array}$		0.1 0.4
800	1	1 1	1	1 1	1	1	1	1	ľ	1	Ĩ		1	1	ĭ 1	1	1.0 1.0
600 520	1		$\stackrel{1}{0}$	$ \begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array} $) 1	1	$\stackrel{1}{0}$	1	1	1			$ \stackrel{1}{0} $	$ \stackrel{1}{0} $	0	$\overset{1}{0}$	0.4
440 370	0		1	1 0 1 1		1	1	1	1	1	1 0		1 0	1 0	0 1	0 1	0.4 0.7 0.8
330 300	1	1 Ī	1	1 1	1	1	1	1	1	1	1		1	1	1	1	1.0
300 260	0 0	$\begin{array}{ccc} 1 & 1 \\ 0 & 0 \end{array}$	1 1	$ \begin{array}{ccc} 1 & 1 \\ 1 & 1 \end{array} $	1	1 1	$\begin{array}{c} 1\\ 0 \end{array}$	1 1	1 1	1	1		1	$\begin{array}{c} 0 \\ 0 \end{array}$	$1 \\ 0$	$\begin{array}{c} 1\\ 0\end{array}$	0.9 0.6
180	0	$\begin{array}{ccc} 0 & 0 \\ 0 & 0 \end{array}$	1	<u>1</u> 1		$ \stackrel{1}{0} $	0	$\overset{1}{0}$	$ \stackrel{1}{0} $	$\overset{1}{0}$			$ \stackrel{1}{0} $	0	0	0	0.8

ScoT-35				ć						G	enera	of Bra	ssicac	eae				
MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Frequency
1150	0	0	0	0	0	1	1	0	0	0	1	1	0	1	0	0	0	0.3
820	0	1	1	0	1	1	1	0	0	0	1	1	0	0	0	0	1	0.5
730	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0.1
650	0	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0.5
570	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	0.4
490	1	1	0	1	1	0	0	1	1	0	0	0	0	0	1	1	0	0.5
430	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
390	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
370	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
300	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0.3
260	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.2

Table 14. Primer SCoT-35, molecular weight (bp) and presence or absence bands for 17 genera of Brassicaceae.

Table 15. Ten SCoT primers in the present investigation, monomorphic bands (MB), polymorphic bands (PB), total bands (TB), unique bands, percentage of polymorphic bands (PB%) and mean of band frequency.

	bands (1B), unique bands, per centage of polymorphic bands (FB %) and mean of band frequency.													
Primers	MB	PB (with.unique)	PB (without.unique)	ТВ	Unique bands	PB%	Mean of band frequenc							
SCoT-01	2	19	18	21	1	90	0.5							
SCoT-02	3	12	12	15	0	80	0.6							
SCoT-03	1	16	16	17	0	94	0.5							
SCoT-04	3	13	13	16	0	81	0.6							
SCoT-05	1	21	19	22	2	95	0.5							
SCoT-06	4	17	16	21	1	81	0.5							
SCoT-12	3	21	20	24	1	88	0.5							
SCoT-16	2	12	12	14	0	86	0.5							
SCoT-21	3	10	8	13	2	77	0.6							
SCoT-35	3	8	7	11	1	73	0.5							
Total	25	149	141	174	8	845	5.3							
Average	2.5	14.9	14.1	17.4	0.6	84.5	0.53							

Similarity matrices based on SCoT markers:

With respect to the dendrogram generated by Jaccard similarity coefficient (Figure 20 and Figure 21), relationships among the 17 of genera of Brassicaceae used in this research were evaluated by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters established using SCoT markers were found in Fig. 20 and Table 15.

There are many researches examining the relationships in the Brassicaceae family by using microsatellite markers (El-Esawi *et al.*, 2016; Singh *et al.*, 2018; Thakur *et al.*, 2017). In addition, there are many studies in which specifically ISSR markers used to determine the genetic relationship in the Brassicaceae family (Khalil & El-Zayat, 2019; Safari *et al.*, 2013; Shen *et al.*, 2016; Wang *et al.*, 2017).

Cluster analysis using SCoT data grouped the 17 genera of Brassicaceae into one main group with Jaccard's similarity coefficient ranging from 0.0 to 1.0. The similarity displayed among all genera ranged from 0.62 to 0.88. The highest value (0.88) was recorded between 12 (*Morettia canscens*) and 13 (*Moricandia siniaca*) but the smallest value (0.62) was recorded between 2 (*Brassica napus*) and 15 (*Sinapis alba*).

The results from this study showed a medium level of similarity between (3, 17), (4, 6), (4, 13), (4, 14), (6, 10), (8, 14), (8, 17), (9, 10) and (12, 14).

The main group contained two subgroups, the first included four genera of Brassicaceae, and the second group contained two subgroups too, the first of them consisted of two genera of Brassicaceae and the second contained two subgroups too, the first of them consisted of four genera and the second group included six genera of Brassicaceae. These results showed that cluster analysis of SCoT markers used genetically to differentiate between genera of Brassicaceae, so a large number of primers were recommended to be used. These results were in agreement with (Singh *et al.*, 2011 and kaur *et al.*, 2016).

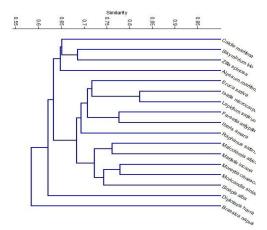


Figure: 20. Dendrogram established with UPGMA-based cluster analysis of 17 genera Brassicaea family.

containe	ontained two subgroups too, the first of them consisted of																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100																
2	59	100															
3	64	60	100														
4	60	60	55	100													
5	64	65	62	69	100												
6	67	59	62	67	72	100											
7	67	59	66	62	66	78	100										
8	67	56	64	63	70	70	75	100									
9	69	57	62	63	73	70	71	82	100								
10	61	56	63	58	59	68	67	70	68	100							
11	60	62	59	65	68	71	75	73	71	76	100						
12	57	<u>52</u>	58	60	64	67	66	67	66	70	76	100					
13	60	56	60	65	64	70	68	70	73	70	74	78	100				
14	63	60	60	66	70	69	70	66	72	65	71	63	71	100			
15	63	56	62	57	63	69	66	70	74	71	73	71	78	69	100		
16	68	58	63	62	63	65	64	61	63	62	64	60	64	64	73	100	
17	62	56	67	59	64	66	66	70	64	58	64	57	61	61	65	68	100

Figure 21. Similarity matrices based on Jaccard similarity coefficients of SCoT markers of 17 genera of Brassicaceae.

According to the results obtained from the anatomical and genetical studies the recorded characters were transformed to qualitative characters and analyzed by Multi Variate Statistical Package (MVSP)This program represents the similarity or dissimilarity between the studied taxa in a form of dendrogram (Fig. 20).

As shown in the dendrogram the studied plant samples classified into 4 clusters. The first cluster include 4 species (taxa 1-3-16-17) the maximum similarity (0.68) is between Sisymbrium sp. and Zilla sp. while the minimum similarity (0.65) is between the previous two species and both Cakile sp. and Alyssum sp. The second cluster include 11 taxa (5-15) this cluster split into 3 groups the first group A contain 5 species (no.5-9), the maximum similarity (0.82) is between Isatis sp. and Lipidium sp. while the minimum similarity (0.72) is between both Farsetia sp. and Iberis sp. and Eruca sp. the second group B include only one species Raphanus sp. which has 0.70 similarity with the other taxa in the same group. The third group include 5 species (taxa 11-15) and the maximum similarity (0.73) is between Morettia sp. and Moricandia sp. while the minimum similarity (0.75) is between Sinapis sp. and the other taxa in the same group. The third cluster include only one taxa (Diplotaxis harra.) which has (0.63) similarity with the species of the second cluster. The fourth cluster include also one taxa (Brassica napus.) which has (0.52) similarity with the previous taxa.

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الدراسات النسيجية لبعض عينات الكرنب الصغير وتحليل التباين الوراشي باستخدام علامات SCOT شاهيناز الشحات أحمد على¹، سحرفتحى محمد الحفناوى¹، محمد قدرى محمد حمزة ²وممدوح محمد زايد² اقسم العلوم البيولوجية والبيئية-كلية الإقتصاد المنزلى جامعة الأزهر ²قسم النبات الزراعى- كلية الزراعة-جامعة الأزهر

الفصيلة الصليبية (Cruciferae) المعروفة باسم علنة الخردل ، وهي واحدة من أكبر فصلتل النباتات الزهرية التي تضم 372 جنما و 4060 نوعًا في جميع أنحاء العالم . تهف هذه الدراسة إلى مقارنة التركيب التشريحي لبعض العينات النباتية لهذه الفصيلة تم أخذ المقاطع التشريحية على كل من السيقان والأوراق ، وتم تسجيل 41 صفة كمية مختلفة في مصفوفة البيانات (Data matrix) . تم تحليل النتائج عدديا لمعرفة التشابه بين هذه الحينات عن طريق رسم معامل التشابه الجينى بين الانواع (dendrogram) . ثم استخدمت النتائج التشريحية التحديد العلاقات الوراثية بين الأجناس المزروعة المدروسة والأجناس البرية من أجل تضمينيا في برامج التربية والتصبين. تم اختيل طرق (Scor) . ثم استخدمت النتائج نتائج دقيقة . تعد تعدد الأشكل المستهدف من كودون البداية (Scor) واحدة من العلامات الشائعة التي تم استخدامها لتقبير التوع الجيني في بعض الأجناس في الفصيلة الصليبية. تم استخدام سبعة عشر نوعًا من النباتات الصليبية مع عشرة بادائت Scor (Scor) واحدة من العلامات الشائعة التي تم استخدامها لتقبير التوع الجيني في بعض الأجناس في الفصيلة الصليبية. تم استخدام سبعة عشر نوعًا من النباتات الصليبية مع عشرة بادائت Scor (Scor) واحدة من العلامات الشائعة التي تم استخدامها لتقبير التوع الجيني في يعض الأجناس في الفصيلة الصليبية. تم استخدام سبعة عشر نوعًا من النباتات الصليبية مع عشرة بادئات Scor (Scor) واحدة من العلامات الشائل واحدة ، منها 5.45 أظهرت حزم متعددة الأشكل ، حيث لوحظ 149 حزم استخدام سبعة عشر نوعًا من النباتات الصليبية مع عشرة بلديات عن من عالي من المنات والحبني في يعض الأحداس 149 مع الأشكال و 25 حزمة أحديد الشكل. أشارت النتائج إلى أن وجود درجة معنوية من التشابه الجبني تراوحت بين 6.00 و 8.00 ، حيث كانت أعلى قيمة متوسطة 8.00 بين الأمكال و 20 حزمة المالي المال و 20 حزم متعداة المالي النبات الن الن التي الذي التربي الشكال و 20 مؤلف في الماليات النتائي الماليم و 3.00 متصدة الشكل، أشارت النتائي الماليمان الأخلس 10.00 و 10 منالي النتائي الفي متوسطة للقيمة كان 9.00 بين الأجناس 20 (Morettia canscers) و 20 مقام متوسطة القيمة كان 9.00 بين الأجناس 20 والمت بين الأجلس 20 و 3.00 متوسطة 10.00 و 10 ملائي م