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Original Research Article

Comparative Botanical and Genetic Diversity Study of Different Cultivars of *Sesamum indicum* L. Cultivated in Egypt

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ABSTRACT

Sesame (*Sesamum indicum* L.) is a plant belonging to the Family Pedaliaceae. It grows worldwide in India, Sudan and China. The high nutritive and curative effects of sesame favour its wide use in culinary and traditional medicines in many countries. No reports could be traced concerning the macromorphological and micromorphological characterization or genetic profiling of *S. indicum* L. cultivars (Shandawel-3, Giza-32 and Toshka) cultivated in Egypt. The establishment of proper identification and authentication of such valuable plant deemed necessary. Therefore, botanical and genetic diversity study of the three cultivars using RAPD markers were investigated. Detailed morphological study through examination of transverse sections in the lamina and midrib region, examination of surface preparations and powders of the three cultivars.Also, Genetic study was performed using twelve primers producing a total of ninety-nine RAPD fragments, of which twenty-five bands (25.25%) were polymorphic. The number of bands per primer ranged from two to fourteen, while the number of polymorphic bands ranged from zero to six and monomorphic bands ranged from two to twelve. Unique bands were observed with eleven primers. Unweighted pair group method using arithmetic average (UPGMA) clustering resulted in two major groups. Results showed that all cultivars have nearly similar macro and micromorphological characters but differ in the dimensions of certain elements with high level of genetic similarity. Thus, this study helped in providing a useful tool for identification of sesame for germplasm banks maintenance and effective parents selection in breeding programs.

Keywords: Genetic diversity, Macromorphology, Micromorphology, RAPD, *Sesamum indicum* L., UPGMA.

Introduction

Family Pedaliaceae is one of the most important families, comprising 16 genera and 50 species, distributed mainly in Africa, India, Sri Lanka, the Malayan islands and Australia.[1] Sesame is one of the members of the Family Pedaliaceae. It is extensively grown worldwide. The major producers of sesame are India, China and Sudan.[2] Although sesame is native to Africa, there is a belief that India was the actual origin.[3] Sesame is cultivated throughout the tropical and subtropical regions in India, Africa, southern United States, Mexico, Venezuela and China.[4] It is considered a good source of edible oil, proteins, vitamins, dietary fibers, omega-6 fatty acids and phenolics with potential pharmacological activities as anti-pyretic, anti-inflammatory, antioxidant, antimicrobial, antihypertensive and anticancer activities.[5] It has been widely cultivated in Egypt for its nutritive, curative effects and therefore, widely used in culinary and traditional medicines.[6] Nowadays, various methods are used for authentication of herbal medicines as morphological examination, chemical analysis and DNA molecular biology. The application of two or more methods for the authentication of herbal medicines may be used in critical cases. However, the most practical method for primary authentication is the ordinary light microscopy which is commonly used as it is a fast and low-cost method and uses small sample amount.[7] It is also, important to use genetic profiling in confirmation of plant identity. RAPD (randomly amplified polymorphic DNA) technique is an easy and fast method, making it ideal for genetic mapping, plant breeding programs and DNA finger printing.[8] This study aimed at the botanical study of the leaves of different cultivars of *S. indicum* L. cultivated in Egypt, as well as, estimation of the genetic polymorphism using RAPD-PCR-based DNA markers. Also, assessment of the genetic relationships among these cultivars, identification of the unique DNA markers and establishment of a typical fingerprint for each *S. indicum* L. cultivar.

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Material and Methods

Plant material

The leaves of the three cultivars of Sesame; Shandawel-3, Giza-32 and Toshka were collected in the period of June-September 2019 from Faculty of Agriculture, Cairo University, Egypt. Authentication of the plant was done by Professor Nagah Mohamed Sallam, Professor of Agronomy, Faculty of Agriculture, Cairo University. Voucher specimens [19.12.2018 (1-3)] for each cultivar was kept in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt. The leaves were used for both botanical study and genetic profiling.

Fresh samples of the leaves preserved in 70% ethanol containing 5% glycerin were examined. The leaves of each cultivar were separately dried, grinded to fine powder and stored in tightly closed dark

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containers for examination. The leaves of each cultivar were, separately, soaked in paraffin. A manual microtome was used to cut transverse sections of 10 to 15 µm thickness. Epidermises were obtained by scraping fresh plant samples. Examination of transverse sections, epidermises and powders of leaves was performed using light microscope (model XSP-13A), Shanghai, China.
The detailed botanical study included morphological study of leaves and micromorphological study through examination of the detailed transverse sections in the lamina and the midrib region, examination of surface preparations and powders of the three cultivars

Genetic profiling
Extraction, purification and estimation of DNA Concentration
The DNA of the three cultivars of *S. indicum* L. leaves were , separately, extracted and purified by DNeasy Plant Mini Kit (Qiagen Santa Clarita, CA).[9] For determination of DNA concentration, the DNA was diluted in distilled water in a ratio of 1:5. Samples of DNA were subjected to electrophoresis in 1% agarose gel against 10 µg of a DNA size marker (Lambda DNA digested with *Hind* III and Phi x 174 DNA digested with *Hae* III). This marker includes DNA fragments sizes ranging from 23130 bp to 310 bp and concentration ranging from 95 ng to 11 ng.

Randomly Amplified Polymorphic DNA (RAPD) Analysis
Twelve RAPD primers were used for polymorphism detection among the three cultivars. The synthesis of these primers was by Metabion Corp., Germany. The primers code and nucleotide sequences are presented in Table 1. The molecular size of the markers is in the range of 100-3000 bp. The reactions were performed in 25 µL volume composed of 1x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 0.2 µM of primer, 0.5 unit of *Taq* polymerase (Qiagen Ltd., Germany) and 50 ng of template DNA, in sterile distilled water.[9]
RAPD-thermocycling profile and detection of the PCR products was performed using Perkin Elmer thermal cycler 2400 programmed to fulfill 42 cycles for determination of PCR amplification of the DNA by applying the following temperature profile through different cycles: starting with an initial cycle for strand separation at 94°C for 5 min and then with 40 cycles comprised of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and an extension step at 72°C for 2 min. The final cycle was a polymerization cycle for 7 min at 72°C. PCR products were mixed with 2 µL gel loading dye and resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 mg/mL) in 1 x TBE buffer at 120 volt. A 1kb DNA ladder was used as molecular size standard. PCR products were visualized under UV light and documented using a ™XR+ Gel Documentation System (Bio-Rad).

A B C

Figure 1: Photographs of *S. indicum* L. herb cultivars
A: Shandawel-3, B: Giza-32, C: Toshka

Table 1: The codes and nucleotide sequences of RAPD-PCR primers

Primer	Sequence
A-12	5'-TCGGCGATAG-3'
A-13	5'-CAGCACCCAC-3'
A-14	5'-TCTGTGCTGG-3'
A-15	5'-TTCCGAACCC-3'
A-19	5'-CAAACGTCGG-3'
B-1	5'-GTTTCGCTCC-3'
B-2	5'-TGATCCCTGG-3'
B-3	5'-CATCCCCCTG-3'
B-5	5'-TGCGCCCTTC-3'
B-6	5'-TGCTCTGCCC-3'
B-7	5'-GGTGACGCAG-3'
B-15	5'-GGAGGGTGTT-3'

Results and Discussion

Botanical study
Macromorphological study
Sesamum indicum L. (Figure 1) is an erect branched annual herb up to 150 cm in height and about 1.5 cm in diameter. Leaves are petiolate, simple or palmately compound. The macromorphological measurements of different cultivars of *S. indicum* L. are listed in Table 2. Sesame leaves (Figure 2) located on the same plant show variation in shape and size. Usually, lower leaves are characterized by being broad and sometimes lobed with serrate margins. Young leaves are entire, lanceolate. The surface of leaves is hairy, dull and darkish green in colour. Leaf arrangement is opposite decussate. Leaves lamina are with asymmetric base and acute to acuminate apex showing more prominence of the midrib and veins on the lower surfaces with pinnate to pinnate reticulate venation. The lateral veins leave the midrib at 45º. They terminate in an intramarginal vein running around the lamina at 1 to 2 mm distance from the edge. The petiole is cylindrical, hairy and light green in colour. This observed macromorphological characters of sesame leaves are in agreement with the reported data.[10]

Micromorphological study
A transverse section through the lamina and the midrib region of the old leaves (Figure 3) shows dorsiventral arrangement. The midrib is more prominent on the lower surface.
The epidermis: in the surface view (Figure 4), the upper and lower epidermises are almost the same in shape. But, the cells of the lower epidermis are smaller in size. In transverse section they are square to rectangular and covered with cuticle. In surface view they are polygonal isodiametric cells with wavy anticlinal walls showing anomocytic stomata (sunken) and covered with smooth cuticle. The stomata are elliptical in shape. The results agreed with reported[11] data that the epidermal cells are mostly polygonal, tetragonal or isodiametric with wavy anticlinal walls showing anomocytic stomata. Also, agreed with reported results[12] showing that the leaf epidermis in dicotyledons is parenchymatous, the mesophyll contains parenchyma and collenchyma cells to provide support to the leaf. Upon examination of both upper and lower surfaces of the three cultivars, the following was found:
Glandular hairs with unicellular head and multicellular uniseriate stalk (2-4 cells) present only in Shandawel-3 and absent in other cultivars. Non-glandular multicellular uniseriate hairs (2-5 cells) with warty cuticle are found in Giza-32 and Toshka and absent in Shandawel-3.

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Peltate hairs are present only on the lower surfaces of the three cultivars. Peltate hair consists of multicellular head (4 cells) and unicellular stalk containing mucilage (detected with ruthenium red). The types of hairs observed were in accordance with reported[13] data on presence of various types of trichomes in twelve accessions of *Sesamum indicum* leaves such as unicellular glandular peltate, long and short unbranched uniseriate, capitate glandular, multicellular, multiseriate capitate glandular, scale and branched trichomes. Also, the presence of different types of hair as non-glandular uniseriate trichomes, peltate hairs containing mucilage which is in good agreement to our results was reported.[11] The reported glandular trichomes with either uniseriate or unicellular stalk and a small bicellular head was not detected.[11] Trichomes are important diagnostic features in family Pedaliaceae.[14]

The mesophyll: is differentiated into discontinuous upper palisade and spongy tissue. The palisade usually consists of one row of columnar cells with slightly wavy walls, interrupted in the midrib region and replaced by 3-4 rows of collenchymatous cells. The spongy tissue is formed of 4-5 rows of thin walled chlorenchymatous cells which are circular in shape.

The midrib: the cortical tissue of midrib consists of 3-4 rows of collenchymatous cells beneath the upper epidermis followed by 2-3 rows abutting the lower epidermis. The mesophyll consists of 5-7 rows of parenchymatous cells showing narrow intercellular spaces. The endodermis is undifferentiated.

The vascular tissue: consists of a collateral vascular bundle. The phloem consists of small thin cellulosic walled parenchymatous cells. The cambium is undifferentiated. The xylem consists of lignified vessels, wood parenchyma, with biseriate or triseriate medullary rays. The pericycle is parenchymatous. These results agreed with the reported[15] results that the cross section of the leaf showed parenchymatous epidermal cells, heterogeneous mesophyll that consists of palisade parenchyma and spongy parenchyma with few rows of collenchyma, and collateral vascular bundles are noticed with thick wall xylem vessels. The numerical values of different cultivars of *S. indicum* L. leaves are presented in Table 3.

Powders of different cultivars of the leaves

The powders of the three cultivars are green in colour with characteristic odour and taste and are microscopically characterized by the presence of fragments of the upper and lower epidermises showing polygonal isodiametric cells with wavy anticlinal walls and covered with thin, smooth cuticle with anomocytic stomata. Fragments of the neural epidermis showing slightly axially elongated sometimes isodiametric epidermal cells with thick anticlinal walls with no stomata. Fragments of lignified vessels with spiral, annular and pitted thickenings. Glandular hairs with unicellular head and multicellular uniseriate stalk (2-4 cells). Multicellular uniseriate non-glandular hairs (2-5 cells) with warty cuticle. Peltate hairs are present consisting of multicellular head (4 cells) and unicellular stalk containing mucilage (detected with ruthenium red) as illustrated in Figure 5. The microscopical measurements of the leaves of *S. indicum* L. different cultivars (µm) are presented in Table 4.

From the botanical study, it can be concluded that the three cultivars have nearly similar macro and micromorphological characters but differ slightly in dimensions (Table 4). Toshka had the largest leaves dimensions followed by Shandawel-3 and finally Giza-32. The powdered leaves of the three cultivars revealed the presence of more or less similar elements microscopically, they all have glandular, non-glandular hairs and peltate hair.

Genetic diversity study

The banding patterns generated by RAPD marker were compared to determine the genetic relatedness of the three cultivars (Table 5 and Figure 6), giving a score of (1) for the presence and (0) for the absence for clear and distinct amplification products. Bands with same mobility were scored as identical. Unique RAPD markers were identified to facilitate the discrimination between the studied cultivars. Unique markers are defined as bands that are present or absent in one cultivar and specifically useful in identifying cultivars. The similarity matrix was used in the cluster analysis. The observed data was organized by cluster analysis into meaningful structures for the development of taxonomies. At first, when each cultivar represents its own cluster, the distances between these cultivars are determined by the selected distance measure (Dice coefficient). If several cultivars have been linked together, the distance between the two clusters is calculated as the average distance between all pairs of cultivars in two different clusters. According to Unweighted Pair Group Method using Arithmetic Average (UPGMA).[16]

From Table 5 the results showed a total 99 of bands with 74 monomorphic bands and 25 polymorphic bands with polymorphic percentage of 25.25% and 25 unique bands were observed. The highest polymorphic percentage was observed with A14 primer (50%) followed by B1 and B3 primers (42.85%). On the other hand, no polymorphic percentage was observed with A19 primer. While the lowest polymorphic percentage was observed for A13 primer (7.69%). The UPGMA-dendrogram of RAPD analysis shown in Figure 6 divided the three cultivars into two main groups. The first group included Shandwael-3 alone while, the second group included Giza-32 and Toshka together. The highest similarity was observed between Giza-32 and Toshka (92%) followed by the similarity between Toshka and Shandawel-3 (91%) and the lowest similarity was between Giza-32 and Shandawel-3 (90%) (Table 6). RAPD technique is considered an easy and simple method for genetic study.[17] Thus, RAPD technique is useful for the systematics of sesame for germplasm banks maintenance and for effective parents' selection in breeding programs. Several studies reported the macromorpholgical and micromorpholgical study of sesame seed, as well as flower, stem and root.[15,18,19] However, to the best of our knowledge, no study has been conducted on the macromorphological and micromorphological characterization of *S. indicum* L. cultivars (Shandawel-3, Giza-32 and Toshka) leaves cultivated in Egypt.

This is the first report of the detailed botanical study (macromorphological and micromorphological characterization) and genetic profiling of the leaves of *S. indicum* L. cultivars (Shandawel-3, Giza-32 and Toshka) cultivated in Egypt.

Table 2: Macromorphological measurements of different cultivars of *S. indicum* L. (in cm)

Organ		Shandawel-3	Giza-32	Toshka
Herb		145 up to 150 cm in height and about 1.5 cm in diameter		
Young leaves	L	5-10.5 -16	5.5- 15.7 -19	4- 15.5 -20
	W	1-2.2-3.5	1.8-2.5-3.6	1.5-3-4.5
Old leaves	L	18- 25 -32.5	22.2- 29 -35.6	21- 26 -33
	W	8.5-10-12.5	10-15-16.5	12-15.5-17

L: length; W: width; cm: centimeter.

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Table 3: Numerical values of different cultivars of *S. indicum* L. leaves

Numerical Values	Shandawel-3	Giza-32	Toshka
Palisade ratio in upper epidermis	12	8	10
Stomatal number in upper epidermis	13	7	13
Stomatal number in lower epidermis	13	21	13
Stomatal index in upper epidermis	15.66 %	9.09 %	15.29 %
Stomatal index in lower epidermis	9.7 %	20.38 %	17.8 %
Vein islet number	23	17	19
Veinlet termination number	11.3	7.5	8

Table 4: Microscopical measurements of different cultivars of *S. indicum* L. leaves (in µm)

Element		Dimensions		
		Shandawel-3	Giza-32	Toshka
Upper epidermis	L	80.5-112.7-175.98	75.4-150-171.3	80.3-141.6-166.67
	W	39.5-66.6-116.9	38.3-58.3-86.4	39.5-66.7-110.5
Lower epidermis	L	81.2-125.4-175.3	55.1-100-192.1	80.33-100.5-135.25
	W	23-33.3-52.7	15.2-33.5-65.7	19.5-33.3-45.89
Stomata	D	120.3-166.67-210.33	114.6-141.67-170.8	95.5-175.5-211.33
Xylem	D	10.4-22.22-30.5	15.5-17.64-31.41	23.5-24-32.7
Peltate hair	D	45.5-50-55.55	49.5-52.94-119.64	65.42-75-97.74
Glandular hair	L	164.3-291.6-335.3	155.4-264.7-390.3	163.4-280.3-365.5
Non-glandular hair	L	120.1-193.33-390.92	110.11-235.3-445.5	116.64-260.85-382.3

L: length; W: width; cm: centimeter; D: diameter; µm: micrometer

Table 5: RAPD analysis of different cultivars of *S. indicum* L. using twelve primers

Primer	Total no. of bands	No. of monomorphic Bands	No. of polymorphic bands	Unique Bands	Percentage of polymorphism
A12	5	4	1	1	20
A13	13	12	1	1	7.69
A14	4	2	2	2	50
A15	7	6	1	1	14.28
A19	2	2	-	-	-
B1	7	4	3	3	42.85
B2	10	7	3	3	30
B3	14	8	6	6	42.85
B5	7	6	1	1	14.28
B6	7	6	1	1	14.28
B7	14	9	5	5	35.71
B15	9	8	1	1	11.11
Total	99	74	25	25	25.25

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Table 6: Similarity Index of *S. indicum* L. three cultivars

	Giza-32	Toshka	Shandawel-3
Giza-32	1.0	-	-
Toshka	0.92	1.0	-
Shandawel-3	0.90	0.91	1.0

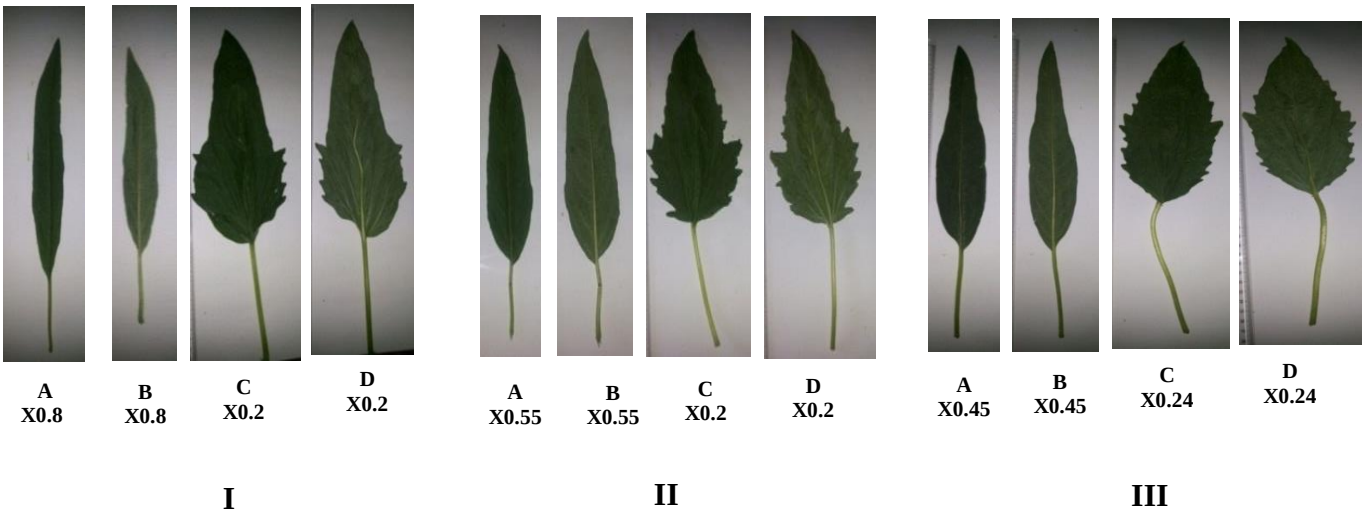

Figure 2: Photographs of different cultivars of *S. indicum* L. leaves
I- Shadawel-3 ,II- Giza-32 ,III- Toshka
A: Young leaf upper surface, B: Young leaf lower surface, C: Old leaf upper surface, D: Old leaf lower surface

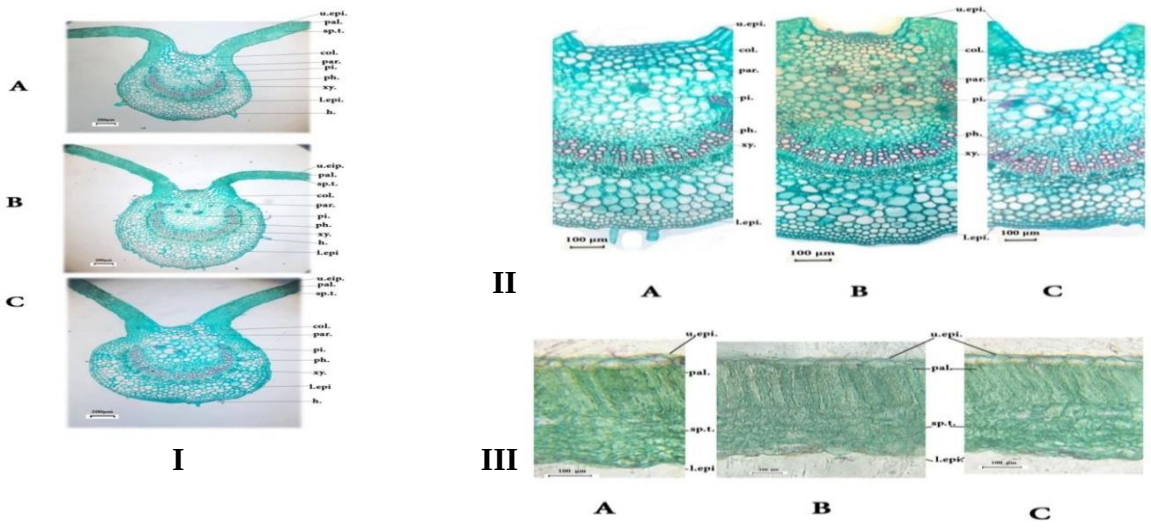

Figure 3: Microscopical study of different cultivars of *S. indicum* L. leaves
I-Transverse sections of *S. indicum* L. leaves (X 25)
II- Detailed sectors in the midrib (X 70)
III-Detailed sectors in the lamina (X80,60,80)
A. Shandawel-3, B.Giza-32, C.Toshka
col., collenchyma; h., hairs; l.ep., lower epidermis; pal., palisade ; par., parenchyma; ph., phloem; pi., pith; sp.t., sponge tissue; u.ep. upper epidermis; xy., xylem.

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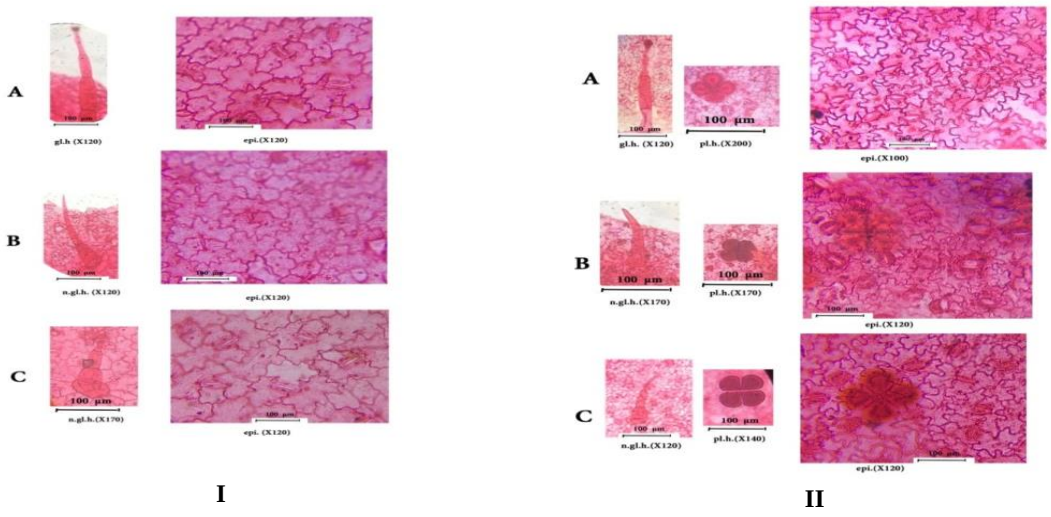

Figure 4: Surface preparation of *S. indicum* L. leaves
I-Upper surface , II-Lower surface
A.Shandawel-3, B.Giza-32, C. Toshka
epi.,epidermal cells; gl.h., glandular hair; n.gl.h., non glandular hair; pl.h, peltate hair

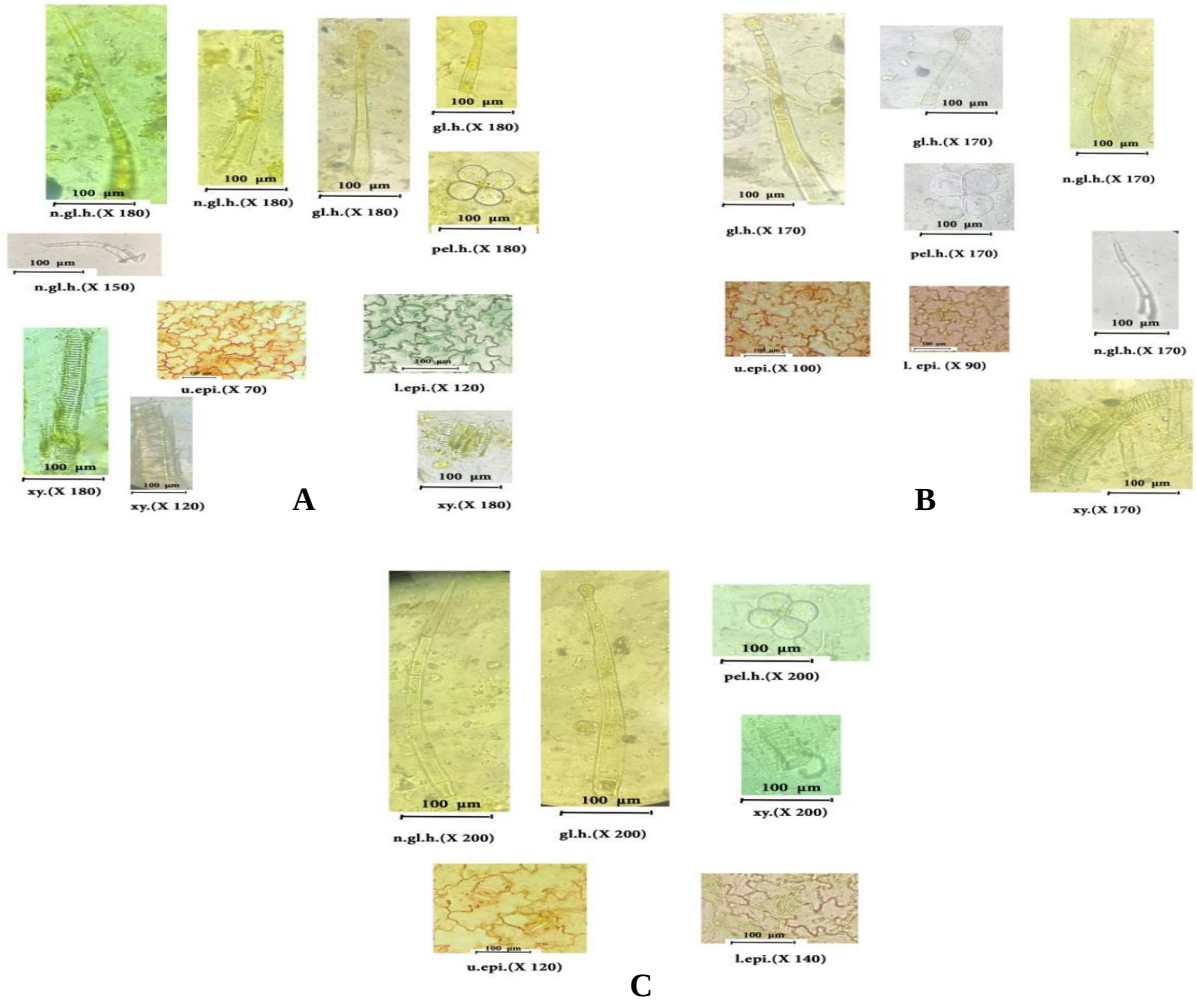

Figure 5: Powders of different cultivars of *S. indicum* L. leaves
A.Shandawel-3, B.Giza-32, C Toshka
gl.h., glandular hair; l.epi.,lower epidermal cells; n.gl.h., non glandular hair ; pl.h, peltate hair; u.epi.,upper epidermal cells; xy.,xylem.

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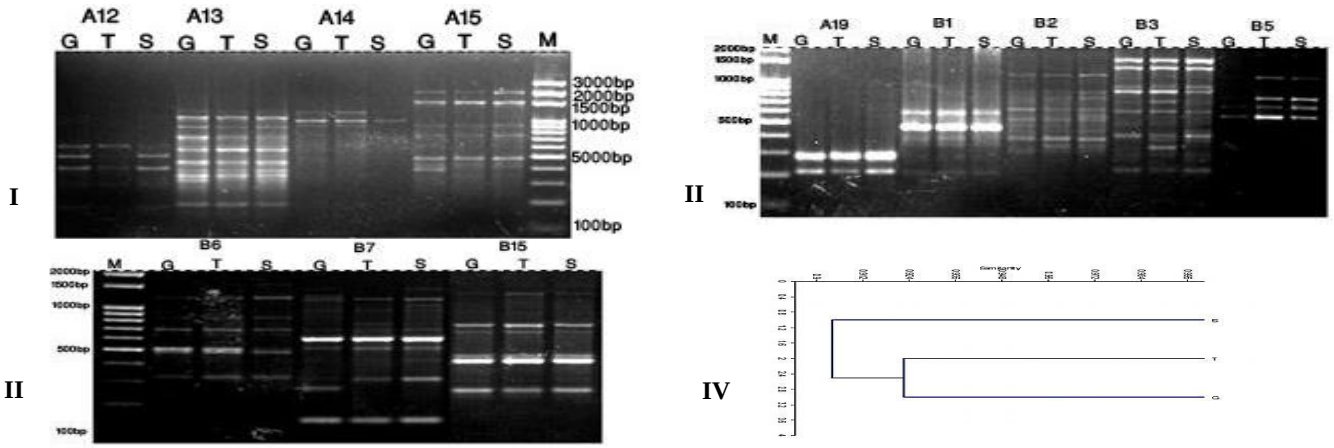

Figure 6: Photographs of DNA electrophoresis showing RAPD bands of *S .indicum* L. different cultivars with different primers
I:A12,A13,A14,A15 primers ; II**:**A19,B1,B2,B3,B5 primers ;III: B6,B7,B15 primers
M: marker, G: Giza-32,T: Toshka and S: Shandawel-3
IV: UPGMA-Dendrogram of RAPD analysis for *S. indicum* L. three cultivars

Conclusion

The three cultivars of *S. indicum* L. leaves showed some similarities in their morphological and anatomical levels, but there were differences at the molecular level. The RAPD technique was able to determine the polymorphism among them, which denotes the need for detailed study of the molecular differences among the studied cultivars. Thus, the combined detailed botanical study, as well as, DNA fingerprinting of *S. indicum* L. cultivars can be used for their authentication and selective discrimination of such valuable plant.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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