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Some Genetic and Molecular Studies on *Calotropis procera*

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CALOTROPIS PROCERA, as one of medicinal plant in risk of extinction in Egypt, due to human activities, was selected to identify some of its cytological, histological and biochemical characters. The studied samples were collected from the garden of Al-Azhar University, Cairo, Egypt. Identification of DNA and protein fingerprints for such plant was carried out. Various methods for seed preservation and storage were tested and evaluated for seed viability after exposure to cryopreservation. Ten primers were used to amplify its genomic DNA, A9 primer was able to produce unique specific bands. Seed storage protein was fractionated into subunits with MWs ranged from 11.11 to 94.5 Kda. The obtained results showed the stability of *C. procera* proteins after prolonged periods of storage (~ 4 years).

Keywords: *Calotropis procera*, RAPD-PCR, SEM, Protein bands, Metal content and Cryopreservation.

Calotropis procera (Ait.) R. Br., (Asclepiadaceae) is a common perennial small tree of the milkweed family known locally as *Usher* or *Oshaar* and in English as Calotrope or Sodom apple. Its botanical name is derived from *Kalos* (beautiful) and *tropis* (ship's keel) with *procera* (tall), from the shape of the fruit of the closely related *Calotropis gigantea* of India. It is widely distributed in the Egyptian deserts (Täckholm, 1974).

The plant is not used as forage because of its milky poisonous sap. It can grow under harsh conditions and it is also resistant to fire, most insects, animals and birds. It excretes certain toxins into the soil which inhibit the growth of seedlings of other species.

Usher has large fleshy leaves (4-12 cm), broadly ovate, obovate or elliptic pale green and covered with a mealy bloom, the apex rounded, apiculate, the base auriculate. Their woolly under sides, serve to insulate the stomata from desiccation by desert winds and harbor insect eggs (Larson, 1983). Flowers pedicellate; sepals 4-6 X 3-4 mm, ovate, acute, hairy outside; corolla 2-2.5 cm broad, lobes ovate, spreading, purple at the upper, white at the base, pinkish outside; follicle 8-14 X 6-9 mm subglobose to obliquely ovoid. Its fruit is a spongy apple-green globe measuring up to 15cm, called a *Sodom apple* (a post-Biblical name). It is filled with seeds attached to silky hairs which help their dispersal by wind. The seeds are 6-8 X 5-6 mm, ovate, coma 2.5-4mm. When cut, the corky white bark excretes a copious caustic and irritant milky sap (latex),

from which several poisons have been isolated and identified. It is produced in specialized cells or tube- like structures known as laticifers and is a by-product of chemical processes in the photosynthetic conversion of sunlight to energy and organic molecules by Wang and Huffman (1981).

Traditionally, the Bedouin used the wood and bark of this plant to produce fine charcoal in making gunpowder, stem fibers for fishing lines and nets and the floss for stuffing pillows. The plant as a whole is purgative and anthelmintic (kills intestinal worms). The root bark is used to treat dysentery, as a diaphoretic (causing perspiration) and as an expectorant. The flowers are bitter and considered to be digestive, stomachic, tonic and to cure inflammation, the latex is supposed to be used as an abortifacient by Atal and Sethi (1962).

Kalita and Saikia (2004) evaluated chemical constituents and energy content of some latex bearing plants as potential renewable sources of energy and chemicals. Plant parts (leaf, stem, bark) and also whole plants were analyzed for elemental composition, oil polyphenol, hydrocarbons, crude protein, α-cellulose, lignin and ash. The study indicated that the tested plant species (*Plumeria alba, Calotropis procera, Euphorbia nerrifolia and Nerium indicum*) might be suitable as alternative source of hydrocarbons and other phytochemicals. The study showed also that the highest gross heat value was exhibited by *C. procera* (6145 cal/g).

Iwalewa *et al.* (2005) evaluated the aqueous extract of *C. procera* for its spasmolytic effect using *in vitro* trachea smooth muscle chain of Guinea-pig. Their results indicated that the extract (50, 100, 200 micrg/ml) showed a dose-dependent relaxant activity.

Identification of a novel cardenolide (2-oxovoruscharin) from *C. procera* and the hemisynthesis of novel derivatives were reported by Van Quaquebeke *et al.* (2005). Their report indicated that of 27 compounds that they hemi-synthesized, one displayed *in vitro* antitumor activity on a panel of 57 human cancer cell lines similar to taxol and higher than SN-38 (the active metabolite) of irinotecan, two of the most potent drugs used in hospitals to combat cancer.

Zhao *et al.* (2005) investigated the effect of modified vitrification techniques on cryopreservation using a new ice blocking agent, Supercool X1000 and the studied material was shoot tips of potato cultivars "Superior" and "Atlantic". Their results showed that treatments with 0.1 and 1 Supercool X1000 significantly improved survival by 55% in Superior and 71.3% in Atlantic, respectively. Their study showed also that after cryopreservation, verified shoot tips resumed growth within a week in the investigated medium.

Gwo *et al.* (2005) reported that cryopreservation of algae could prevent genetic drift and minimize labor costs compared to the current method of maintenance and subculturing. Their study aimed to assess the viability of the microalgae (*Nannochloropsis oculata*) directly after thawing and measure the

algal concentration after 2-30 days of growth. The results of such study showed that, direct freezing of algae in liquid nitrogen resulted in a severe loss of viability but the tested modified cryopreservation protocol, using five cryoprotectants, proved to be more appropriate for the preservation of *N. oculata.*

The scope of this study was to identify some cytological, histological and biochemical characters of *C. procera* plant. Identification of DNA and protein fingerprints for such plant was carried out. Various methods for seed preservation and storage were tested also to evaluate the seed viability after exposure to such strategies for cryopreservation.

Material and Methods

Plant materials

The studied seeds, leaves and stems were collected from *Calotropis procera* grown in the garden of Al-Azhar University, Cairo, Egypt. The voucher specimen was deposited in the herbarium of Botany Department, Faculty of Science (Girls).

1- Cytological and histological studies of C. procera plant

For cytological study, root tips were collected at 0.5-1 cm from 5- days old seedlings and treated in ice-cold water, fixed, hydrolyzed with 1N HCl, stained in 1% acetoorcein and squash preparations were made for traditional and image investigations. Image investigations included chromosome length, nuclear dimensions, nuclear area, cell dimention and cell area. Untreated group of roots was prepared for mitotic index (MI), percentage of mitotic stages and percentage of chromosomal aberrations.

For histological study, samples of root, stem and leaf were fixed in FAA solution (formalin: acetic acid: ethyl alcohol 70% 5: 5: 90 v/v) and paraffin wax method was used to investigate various tissues of embedded samples after serial sectioning by microtome (Johansen, 1940) and observed by Olympus CH 40 microscope (15x3.5x40x).

Scanning Electron microscope (SEM) was used according to Schneider and Cerarlquiest (1998) to investigate ultrastructure of young and large leaves surfaces (morphological fingerprints) and seeds at various stages of maturity to show the silky hairs attachment. For SEM investigations, the samples were fixed in FAA at room temperature for 24 hr and dehydrated in a graded alcohol series. The specimens were then dried to the critical point with liquid CO_2, sputted with gold and viewed with JEOL JSM, 5500 LV. at an acceleration voltage of 20 KV.

2- RAPD analysis

Randomly amplified polymorphic DNA (RAPD) technique was used in this study to identify *C. procera* genome. RAPD assay was done at the Agriculture Genetic Engineering Research Institute (AGERI), Giza, Egypt. DNA was

extracted from the young leaves of this plant. DNA amplification reactions were performed as described by Williams *et al.* (1990). Ten arbitrary 10-base primers from Operon Technologies, Inc., USA (Table 1) were used. The PCR reaction mixture consisted of 1x PCR buffer, 1.5mM $MgCl_2$, 0.02 mM each of dATP, dTTP, dCTP and dGTP, 1 unit Taq DNA polymerase (Perkin-Elmer), 1 ng/µl DNA template and 0.2 mM primer in sterile pure water for a total volume of 25 µl. The reaction mixtures were overlaid with one drop of mineral oil in order to avoid evaporation. The amplification was performed in a DNA thermal cycler (Perkin Elmer Cetus 480), programmed as following: 1 cycle of 94°C for 5 min. (initial strand separation), followed by 40 cycles for 1 min. at 94°C (denaturation), 1 min. at 36°C (annealing), 2 min at 72°C (elongation), 1 cycle of 7 min. at 72°C (final extension) and 4°C (infinitive). After amplification, PCR products of the studied genome as well as –ve control, were separated by electrophoresis in 1% agarose gel, stained with ethidium bromide and photographed under UV light using black and white Polaroid films. Band fragments were scored as present (1) or absent (0). Dendrogram was calculated by using unweighted pair group method with arithmetic averaging (UPGMA).

TABLE 1. Oligonucleotide primers used in the present study

Primer	Nucleotide sequence 5`-3`
A3	AGT CAG CCAC
A7	GAA ACG GGTG
A8	GTG ACG TAGG
A9	GGG TAA CGCC
A20	GTT GCG ATCC
B8	GTC CAC ACGG
B12	CCT TGA CGCA
B14	TCC GCT CTGG
B15	GGA GGG TGTT
B17	AGG GAA CGAG

3-Banding pattern of extracted seed protein of C. procera plants (protein fingerprints)

Total protein extracted from the seeds of *C. procera* plants was analyzed according to Laemmli (1970). Such seeds were of 4 seasons from the same plants: 2000, 2001, 2002 and 2003.

Seed samples were first washed, surface-sterilized, defatted three times then dried at 4 °C for 30 min (Stegemann *et al.*, 1987).

Protein was extracted with Tris/Boric buffer pH 8.9 (Tris 125 mM and Boric acid 19 mM) including 0.01% sodium azide. The extraction was done by grinding 0.5 gram defatted powder sample, 2 volume Aluminum Oxide (Alcoa) and 10 ml extraction buffer in a mortar, followed by shaking for 60 min. at 4 °C and finally by centrifugation at 10.000 rpm. The supernatant was collected and kept at 4 °C for subsequent assays. The extracted protein was measured by using UV Spectrophotometry at 280 nm. Reagents, samples preparation, gel

preparation staining and destaining were performed as described by Abd El-Kawy (2005)

4- Assessment of C. procera elements (Biochemical investigation)

Analysis of *C. procera* leaf elements was carried out in the Medical Mycology Lab., the Regional Center for Mycology and Biotechnology, Al-Azhar University. The following elements were analyzed from leaf sample; Na, Mg, Si, P, S, Cl, Ca, Cu, and Zn using the x-ray detection of the scanning Electron Microscope (JEOL JSM, 5500 LV).

5- Viability of C. procera seeds after Cryopreservation methods

Seeds of the investigated plant were evaluated for ability to germinate after exposure to various methods of preservation and storage strategies.

Seeds were surface sterilized in commercial bleach (pril) for 3 min, and 10% Clorox several times, washed in sterile distilled water several times. Such seeds were grouped and exposed to the following preservation methods: deep freezing treatments (-5°C& -20°C); liquid nitrogen (LN) treatment (immersion) and low temperature storage 5°C. Another group of surface-sterilized seeds, as control, were germinated at room temperature (27± 2°C) in aseptic water-agar medium (8 g/l), on Vermiculite and on wetted cotton. After incubation in dark for one week, germination was observed for all the above seed groups.

Results

1- Cytological and histological studies of the wild C. procera plant

Cytological study included karyotype identification, mitotic behaviour, description of interphase cells.

For karyotype identification, number of chromosome was count from well-spread metaphase cells from different roots and chromosome length was measured. Figure 1 clearly shows that somatic cell of *C. procera*, collected from Al-Azhar University, contains 22 chromosomes. Data obtained from image analysis system confirmed the above information and showed that the mean length of chromosome equals 0.18 μm, the minimum and maximum lengths of chromosomes were 0.08 and 0.40 μm, respectively. In case of interphasic cells, data obtained from image analysis system indicated that mean nuclear diameter ranged from 2.4 to 9.3 μm^2 and mean cell diameter ranged from 10.2 to 19.0 μm^2 with standard and average deviation of 0.07>0.05. While cell dimensions were 6.02 X 2.95 μm, nuclear dimensions were 4.01 X 2.17 μm.

Mitotic response illustrations revealed that the rate of cell division, mitotic index, (under lab conditions) was 34.94 and the percentage of total abnormalities reached the value of 31.85. Analysis of mitotic index data showed an accumulation of cells in prophase (86.13 %) and the marked percentage of chromosomal aberrations was restricted in prophase stage. Microscopic

observations revealed also that stickiness of chromosomal materials was the notable chromosomal aberrations observed. Such aberrations may be due to several environmental responses.

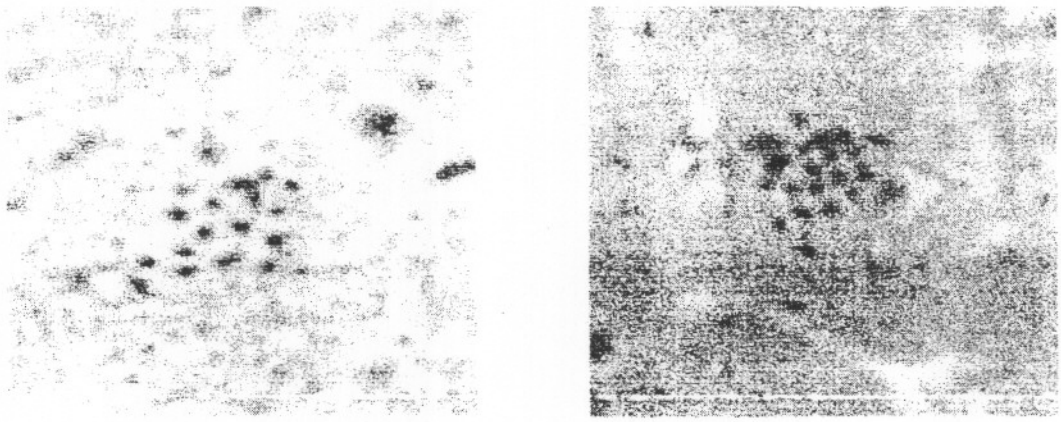


Fig. 1. Scattered chromosome at metaphase stage in *C. procera* 2n = 22 (X1000).

For histological identification, a transverse section (T.S.) in the root of *Calotropis procera* (Fig. 2), showed irregular to nearly rounded section in outline. It showed an irregular brownish cork cells surrounding a wide cortex, having on the inner side a diffuse ring of separated small groups of phloem, then a well distinct cambium layer, followed by a proportionally very wide region of secondary xylem having the diarch primary xylem in the center but mostly eccentrically situated. Medullary rays are numerous, and well visible. Pith was totally absent.

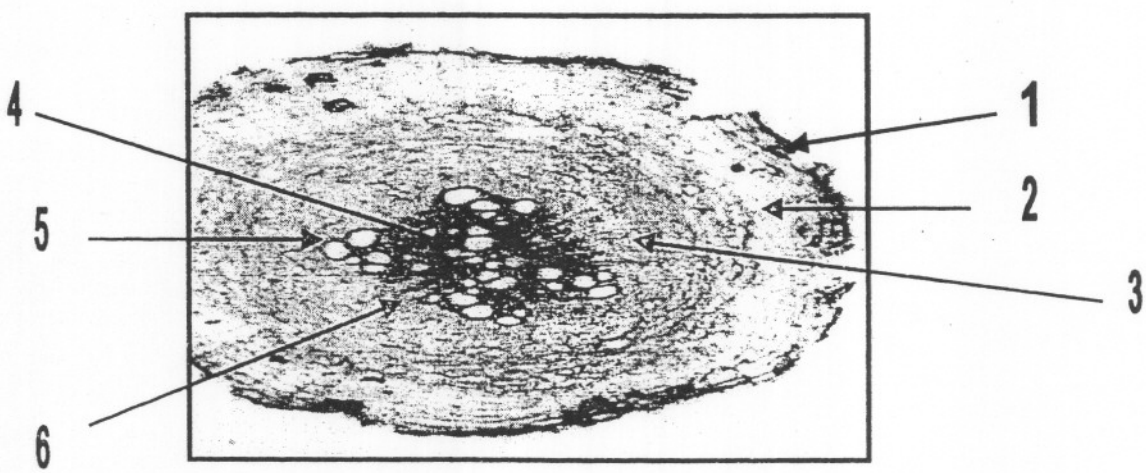


Fig. 2 . Transverse section of root of *C. procera* plant (X = 50).
1= Cork 2= Cortex 3= Phloem 4= 1^{ry} Xylem 5= 2^{ry} Xylem 6= Secondary medullary ray fiber

A transverse section in the stem (Figs. 3, 4 & 5) was more or less quadrangular to rounded in shape in its outline, showing an epidermis, followed by a somewhat wide cortex, limited on the inner side by a starch-sheath, then a pericycle with groups of non-lignified fibers, followed by a continuous ring of vascular tissues, surrounding a wide parenchymatous pith, in which are scattered numerous small strands of perimedullary phloem, as well as, numerous laticiferous tubes are found in the cortex, phloem and pith.

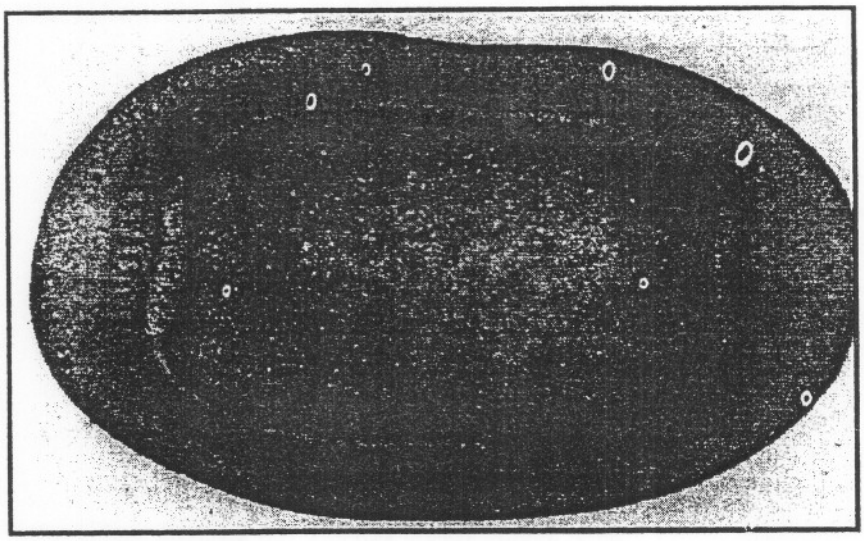


Fig. 3. T.S. of stem of *C. procera* (node 3) (X 50).

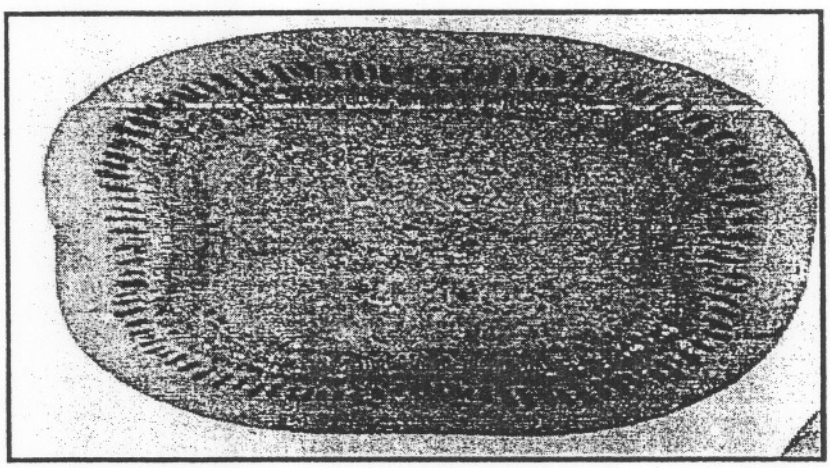


Fig. 4. T.S. of stem of *C. procera* plant (node 6) (X 50).

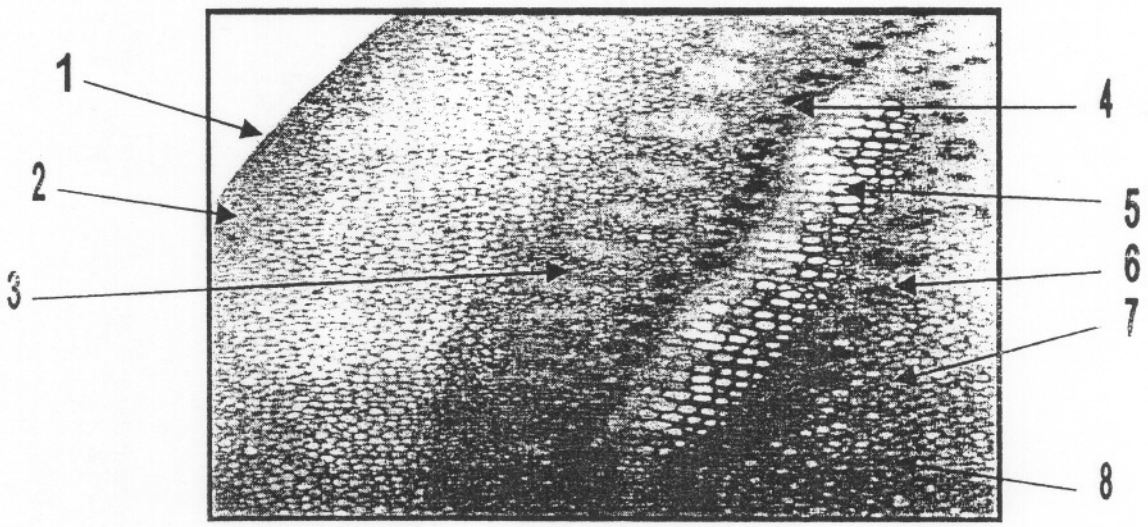


Fig. 5. Bicollateral vascular bundle of *C. procera* stem (X100) .
1- Epidermis 2- Collenchyma 3- Pericycle 4- Phloem 5- Xylem 6- Phloem 7- Latex tube 8- Pith

The tissues of the leaf of *Calotropis procera* as seen in atransverse section

Figures. 6, 7 & 8 comprised the upper and lower hairy epidermises, enclosing in between the isobilateral mesophyll, transversed by the vascular strands of the midrib and veins, with an incomplete starch sheath. Laticiferous tubes, as well as, supernumerary groups of peri-medullary phloem were observed clearly in Fig. 8.

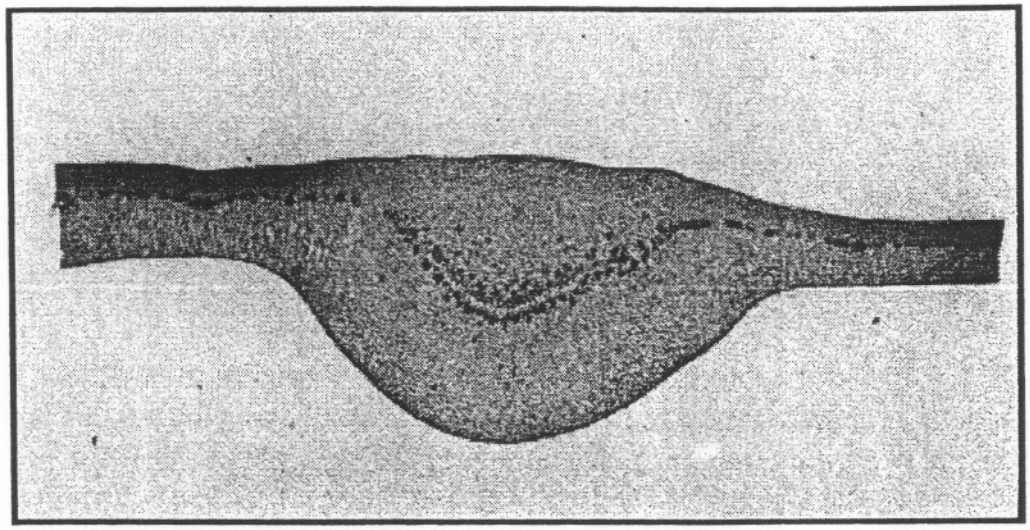


Fig. 6 . T.S. of the first leaf of *C. procera* plant (X100) .

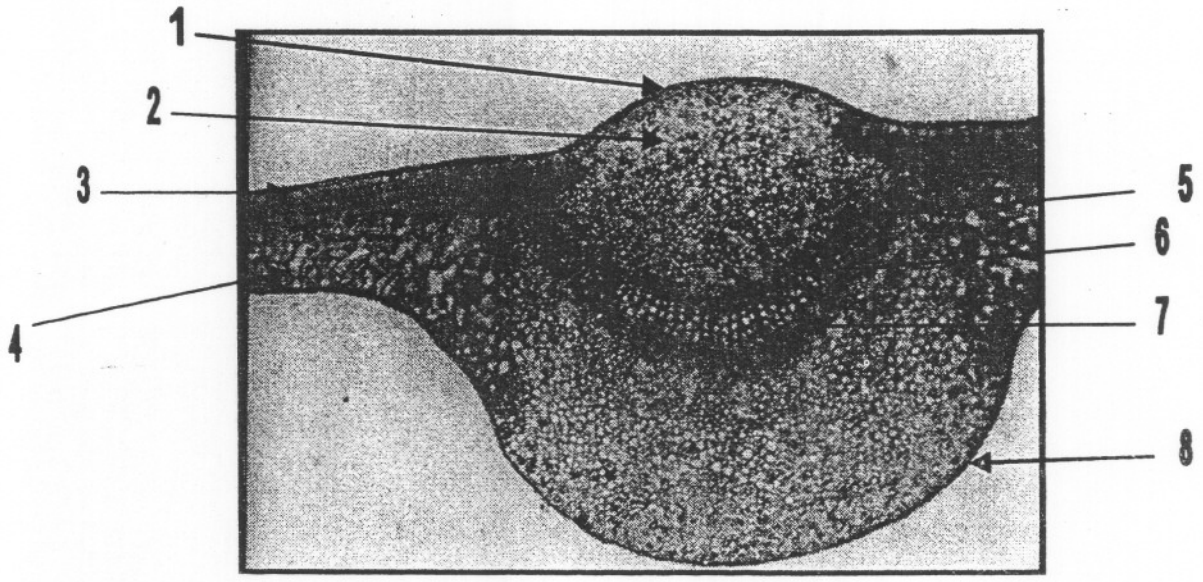


Fig. 7.T.S. of the 4th leaf of *C. procera* plant (X100).

1- Upper epidermis 2- Collenchyma 3- Upper palisade tissue 4- Loose lower palisade tissue

5- Perimedullary phloem 6- Xylem 7- Pericycle 8- Lower epidermis

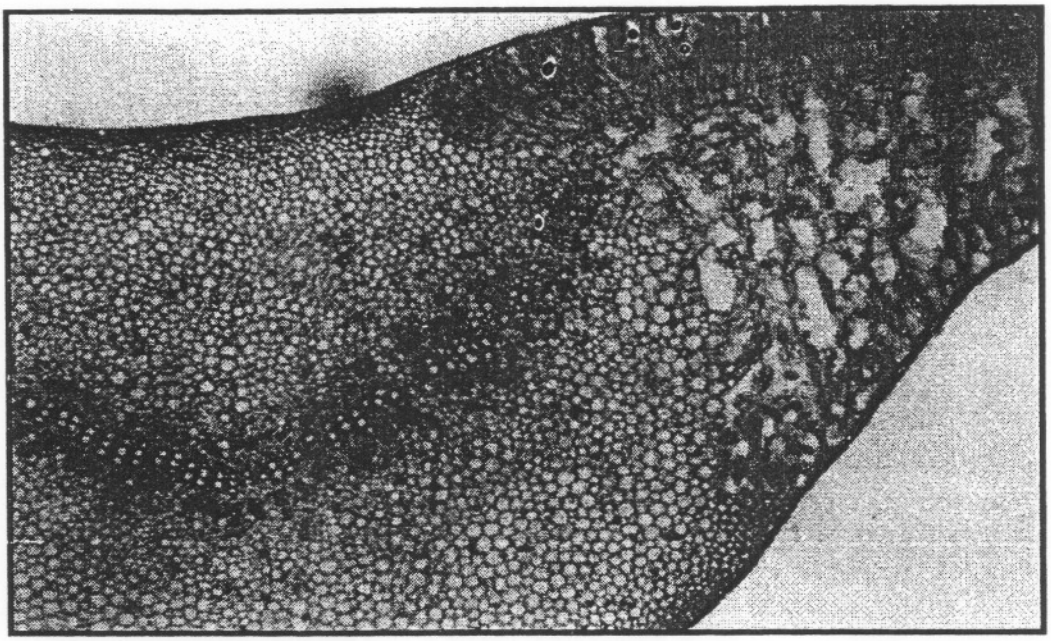


Fig. 8 . *C. procera* 4th leaf showing vascular bundle and mesophyle tissue (X400) .

Scanning electron microscope (SEM), as a morphological fingerprint, was used to investigate ultrastructure surface (upper & lower) of *C. procera* young and old leaves as well as seed surface. Figs. 9 & 10 show the narrow openings (stomata) found in the epidermis of upper and lower surface of the leaves. The stomata are surrounded by two guard cells (kidney-shaped), their wall is unevenly thickened. The wall surrounding the pore is thickened and inelastic due to the presence of secondary layer of cellulose and the remaining part of the wall remains thin and elastic. The radial wall has micellae. These Figs also showed the subsidiary cells (accessory cells). It is clear that the stomata are found on both surfaces of the leaf and the number of stomata varies in the upper and lower surfaces.

Metcalfe and Chalk (1950) stated that the stomata of *C. procera* are anomocytic or anisocytic or paracytic or diacytic. While Stace (1965) reported that the stomata are actinocytic or cyclocytic type. In the present study the stomata were found to be anomocytic.

In case of trichomes, scanning electron microscopic illustration of *C. procera* leaf surfaces are shown in Figs. 11 a&b and 12 a&b. According to Uphof (1962) these trichomes are non-glandular, unicellular, unbranched and uniseriate. Figure 13 illustrates the seed shape of *C. procera* plant. It is clear that the seeds are irregularly flattened, thick membranous with pale brown margin and on the narrow end bear a tuft of very long silky plume, which is 8-10 times longer than the seed (Fig. 14) . The seeds are up to 0.8 cm in length and up to 0.5 cm in width. SEM was used also to investigate the attachments of silky hairs with the studied large seed (Fig. 15 a, b & c) and in small seed (Fig. 16 a & b).

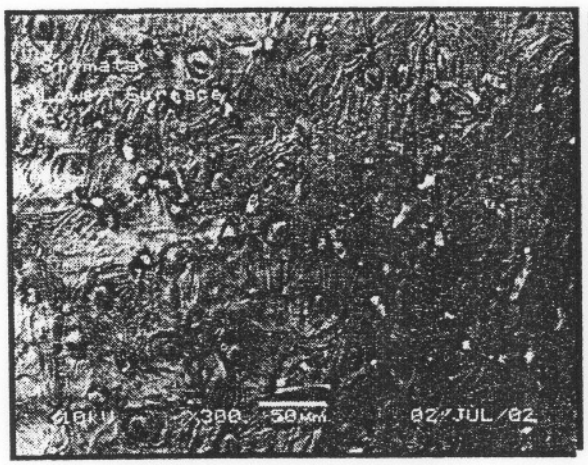


Fig.9.SEM photograph showing stomata in the upper surface of *C. procera* leaf X300.

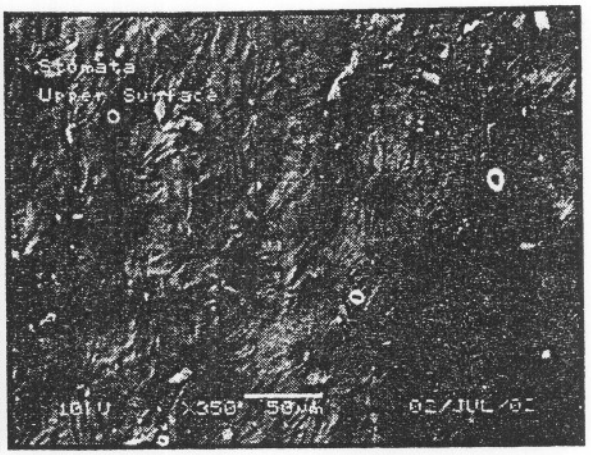
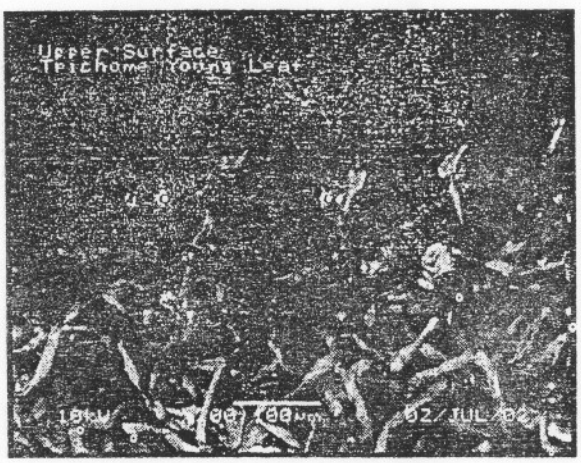


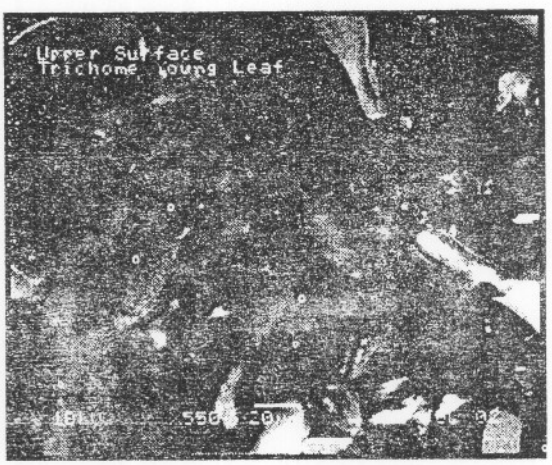
Fig.10. SEM photograph showing stomata of leaf lower surface in *C. procera* X350.

Metcalfe and Chalk (1950) stated that the stomata of *C. procera* are anomocytic or anisocytic or paracytic or diacytic. While Stace (1965) reported that the stomata are actinocytic or cyclocytic type. In the present study the stomata were found to be anomocytic.

In case of trichomes, scanning electron microscopic illustration of *C. procera* leaf surfaces are shown in Figs. (11 a&b and 12 a&b). According to Uphof (1962) these trichomes are non-glandular, unicellular, unbranched and uniseriate. Figure 13 illustrates the seed shape of *C. procera* plant. It is clear that the seeds are irregularly flattened, thick membranous with pale brown margin and on the narrow end bear a tuft of very long silky plume, which is 8-10 times longer than the seed (Fig. 14). The seeds are up to 0.8 cm in length and up to 0.5 cm in width. SEM was used also to investigate the attachments of silky hairs with the studied large seed (Fig. 15 a, b and c) and in small seed (Fig. 16 a and b).

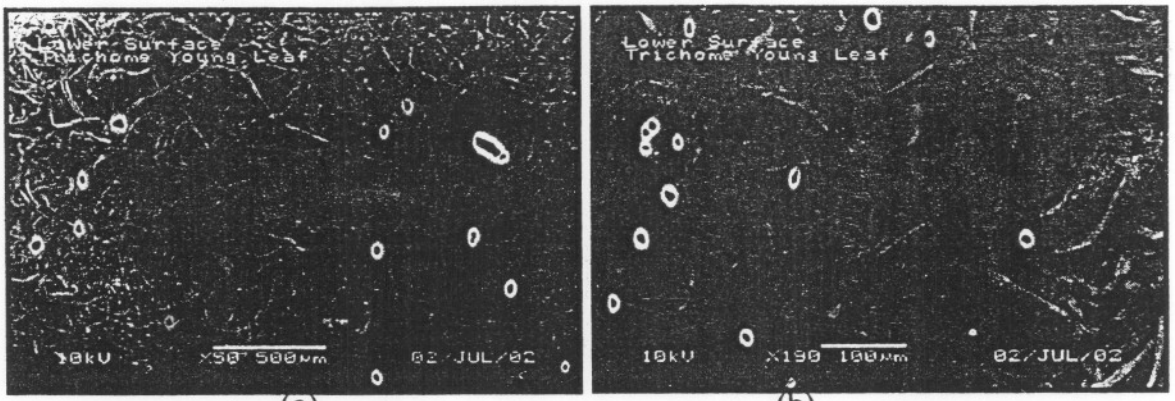


(a)



(b)

Fig. 11. SEM photographs showing trichome in upper leaf surface of the studied sample a) X 200 b) X 550.



(a) (b)

Fig. 12. SEM photographs showing trichome in lower leaf surface of the investigated sample a) X 50 b) X 190.

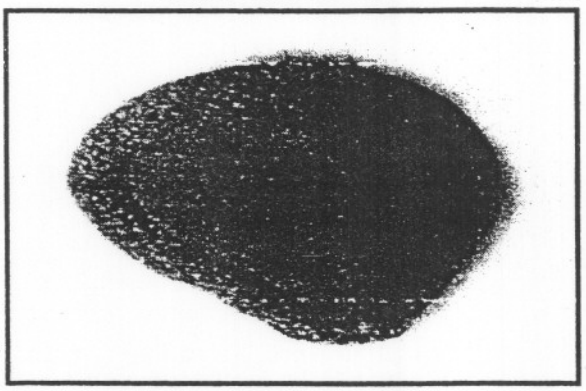
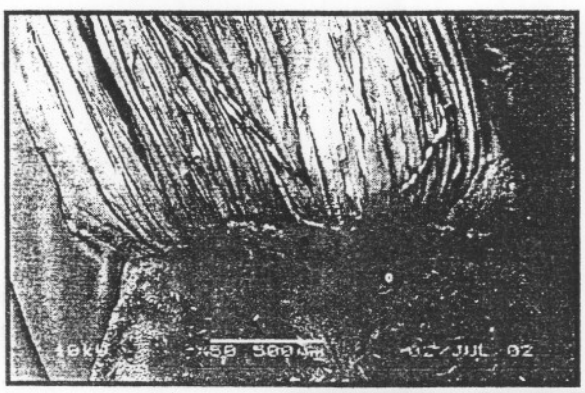
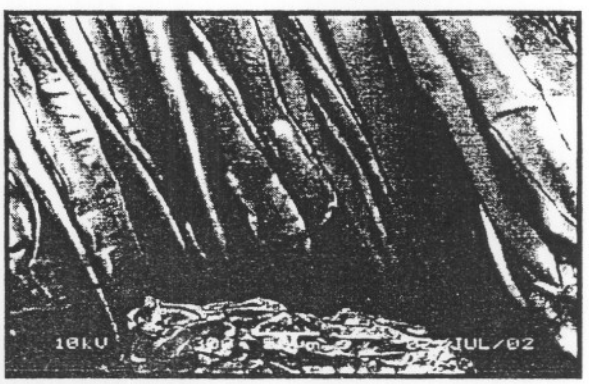


Fig. 13: Shape of mature seed of *C. procera* (X100).

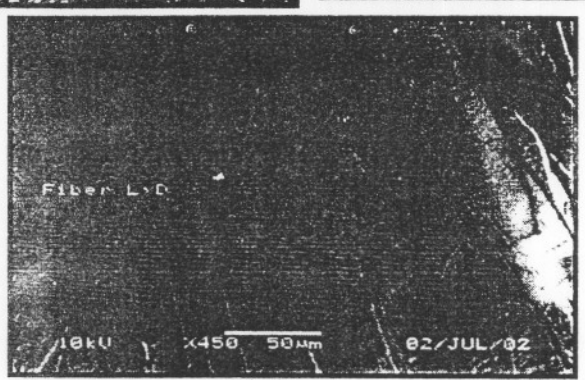
Fig. 14 The attachments of the silky hairs (X400).



(a)

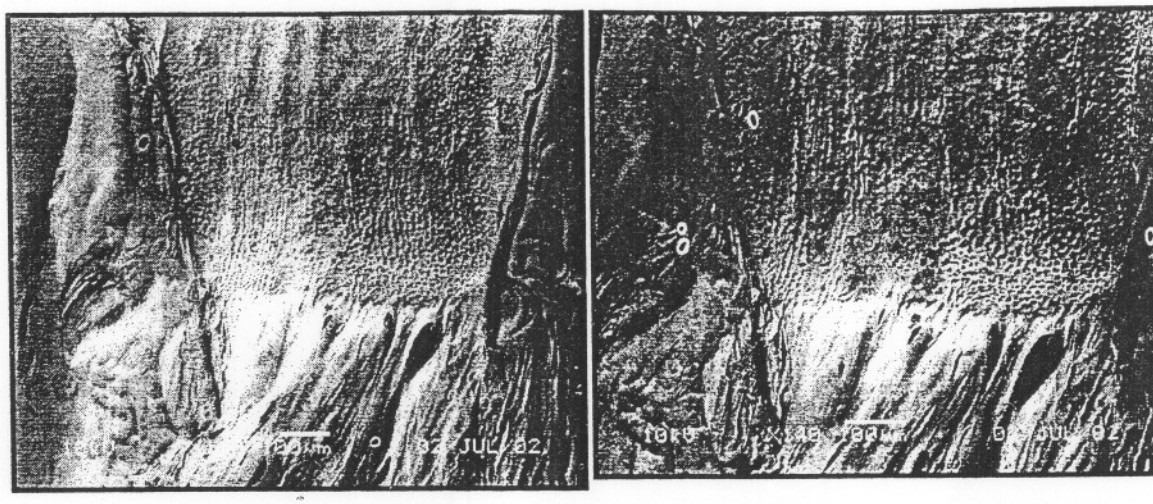


(b)



(c)

Fig. 15. SEM photographs showing attachment of silky hairs of large seed of *C. procera*. a) X 50 b) X 300 c) X 450



(a) (b)

Fig. 16. SEM photographs showing attachment of silky hairs in micropyle in small seed of *C. procera*, a) X 110 b) X140.

2- Identification of C. procera genome (DNA fingerprints)

As far as is known, this is the first molecular investigation for *C. procera*. Therefore, the present PCR-RAPD profiles will help in the future to detect such plant as fingerprints. Ten primers were used to amplify genomic DNA from *C. procera* plant leaves (Fig. 17). The ten primers produced a total of 21 reproducible bands or fragments. The PCR analysis of investigated plant with the different primers resulted in the amplification of 6-12 DNA fragments, ranging in sizes from ~0.5 Kb to ~ 2 Kb. Primer A9 revealed 4 bands only with molecular weights (MWs), ranging between 1Kb and ~ 1.7 Kb. (lane 4), 3 bands of them with MWs 1 Kb to ~ 1.3 Kb and the later one is more than 1.6 Kb in size. Primers A7 and B17 (Lanes 2 & 10) produced the largest number of products. Of the ten random primers tested for detecting genomic DNA of *C. procera*, primers A20, B8 and B15 (lanes 5, 6 & 9) showed variations in the in the RAPD products. The UPGMA dendrogram obtained from distance matrix clearly indicated that primers (A7 & B17) are the two primers that share the largest amount of RAPD fragments. The most distinct fragments were distributed as follows: one corresponding to primer B17; a specific DNA fragment with markable intensity about 1.3 Kb in size (lane 6) was characterized by primer B8. Using primer A20 (lane 5) a Ca ~ 200 bp band characterized the *C. procera* genome. Such fragments yielded by the previous primers must be taken in consideration for identifying *C. procera* genome.

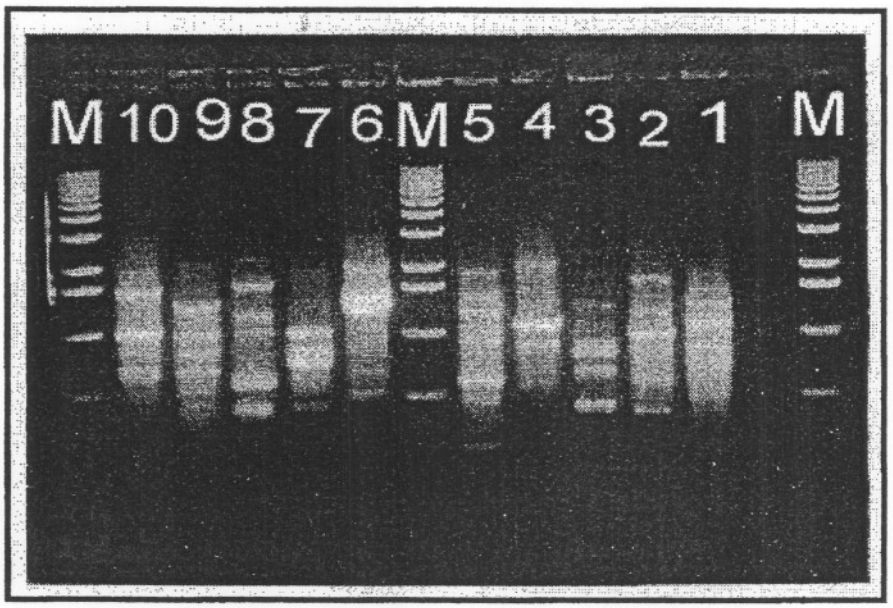


Fig. 17. DNA fingerprints of RAPD products obtained by PCR amplification of DNA isolated from *C. procera* leaves.

3- Banding patterns of extracted seed storage protein of C. procera plants (protein fingerprints)

Characterization of *C. procera* seed storage protein was estimated by SDS polyacrylamide gel electrophoresis technique and the seed samples for this experiment were collected from season 2000, 2001, 2002 and 2003. Electrophoretic profiles of the seed proteins of the studied samples are shown in Fig. 18 and the molecular weights (MWs) of the protein fractions (protein bands) and the relative percentage of such subunits are listed in Table 2. Analysis of gel lanes was documented using transilluminator, and image analysis software. As shown in Table 2, *C. procera* seed protein was fractionated into subunits with MWs generally, ranging from 11.11 to 94.5Kda. The maximum number of bands (9) was recorded for seed samples of seasons 2000 and 2002. Table 2 shows that electrophoretic banding patterns for *C. procera* storage protein extracted from the studied samples were similar but not identical, most recorded bands were similar in their MWs but differed in their relative % (intensity). For example, protein subunits of MWs 84.8 Kda were estimated in seed sample of season 2000 in relative % of 4.25 while its content reached 17.66 in season 2002. Also, protein fractions of MW 11.2 Kda ranged from 18.73 (2002) to 30.07 (2003). Table 2 shows also that seed protein of *C. procera* sample of 2002 was characterized by the presence of band with MW 94.5 Kda and relative % about 13.57, specific band with MW 79.0 and relative % of value 4.43 was identified from sample collected from season 2000.

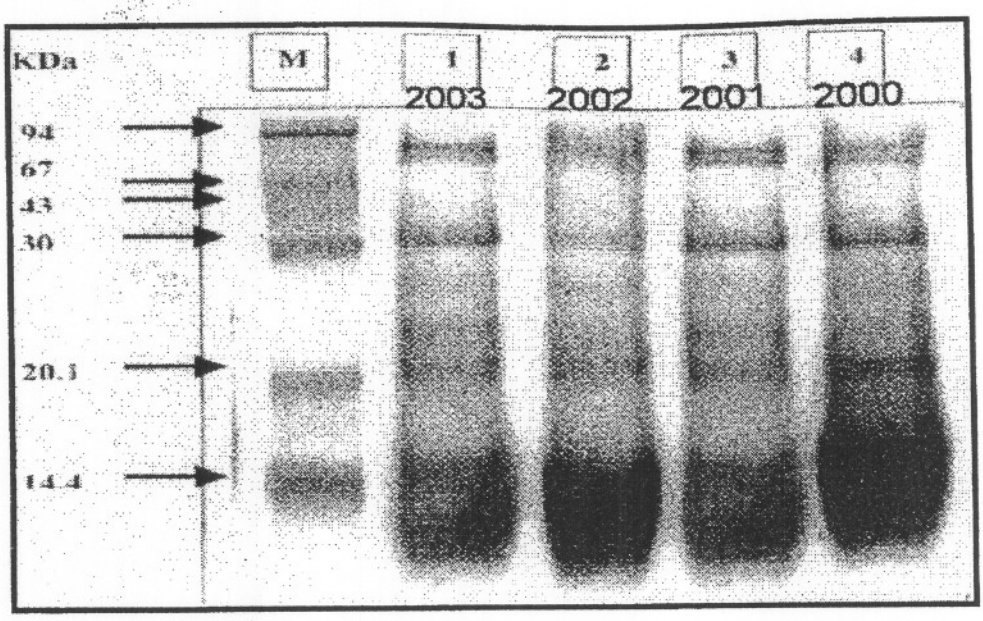


Fig. 18. SDS-PAGE (12%) separation of total proteins of the investigated seed samples.

TABLE 2. Number of bands, relative percentage and molecular weight of the total soluble protein bands after separation on SDS-PAGE (12%) of the investigated seeds of the seasons 2000-2003 of *C. procera*

Band number	Molecular weight (Kda)	Relative %			
		Season 2000 (lane 4)	Season 2001 (lane 3)	Season 2002 (lane 2)	Season 200 (lane 1)
1	94.5	----	----	13.57	----
2	84.8	4.25	14.55	17.66	15.11
3	79.0	4.43	----	----	----
4	45.7	4.68	4.12	4.69	3.65
5	31.9	11.88	11.58	8.82	11.17
6	25.3	9.59	9.41	6.98	8.74
7	22.8	7.62	8.42	3.96	7.97
8	20.8	16.05	9.01	9.59	8.74
9	15.0	16.06	14.85	16.05	14.55
10	11.2	25.43	28.6	18.73	30.07

4- A assessment of C. procera elements (Biochemical investigation)

Leaf sample of *C. procera* was used to assay its elements quantitatively using scanning electron microscope and X-ray detection. The studied sample analysis (Fig. 19) showed variation in the metal contents. Calcium is an abundant element in *C. procera* leaves collected from the garden of Al-Azhar University, zinc and copper are found in more than one form (four), such data can be considered as fingerprints for the studied plant.

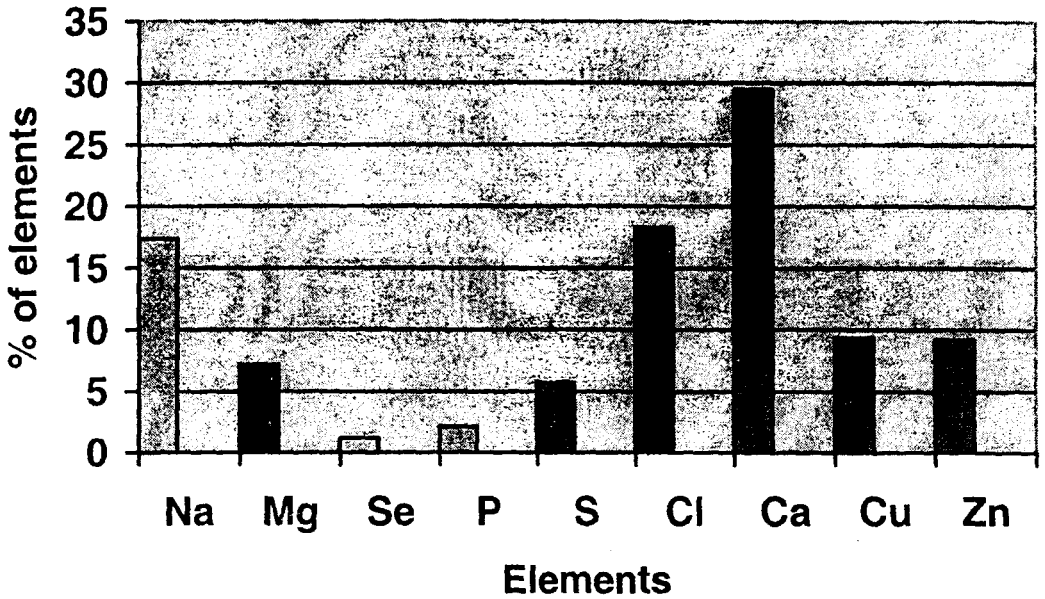


Fig. 19. Histogram showing the percentage of the different metals in the leaf sample of *C. procera*.

5-Viability of C. procera seeds after cryopreservation methods

Success in cryostorage was evaluated in this study on the basis of germination tests after low temperature deep freezing (5, -5 and -20 °C) and liquid nitrogen (LN) immersion treatments. Germination tests showed that viability of seeds exposed to LN and different temperatures were approximately equal to the control (90%) seed viability. Such observation indicated that none of the tested treatments adversely affected seed viability.

Discussion

Attention is focused to produce biocrude from number of latex-producing plants; one of the most important plants in this context is *Calotropis procera*. Latex can be used to make commercially desirable products required by the petrochemical industry. Additionally, latex from *C. procera* is widely used in folk medicine as a rich source of biological active compounds capable of promoting benefits such as control of dermal fungal infections, antimicrobial activities and pain relief among other useful properties. Alencar *et al.* (2004) studied the anti-inflammatory effect of *C. procera* latex. They suggested that the tested sample act over a wide spectrum as a novel anti-inflammatory agent. Soares *et al.* (2005) concluded that the protein fraction derived from the whole latex possesses antinociceptive activity.

On the other hand, numerous researches were biased towards the toxic effects of *C. procera* plant and its injuries effects in animal diet. In spite of this, Abbas *et al.* (1992) noticed animals during the drought season, browsing of *C. procera*.

They stated that if the leaves were mixed with a larger amount of dry desert grass, animals did even better and gained weight. They reported also that sometimes, camel- men force their animals to browse in fields of *C. procera* for days to cure outbreaks of night-blindness resulted from Vitamin A deficiency caused by long day season.

Histological investigations of *C. procera* plant collected from the garden of Al-Azhar university indicated that the obtained anatomical investigation is similar to those obtained from previous studies (Rizkallah, 1967) on such plant collected from sandy soils of Egypt, particularly in Upper Egypt. In case of the cytological study, MI of somatic cells, which is a positive reflection of the capacity of a cell population to grow, exhibited a marked value under lab conditions with noticeable percent of chromosomal aberrations. The image analysis showed interesting information on the chromosomes and interphasic cells of the studied plant.

Identification of DNA and protein fingerprints are one of the important information obtained in the present study. To our knowledge, DNA and protein fingerprints for *C. procera* have not been reported to date.

DNA fingerprints in this study were illustrated by RAPD-PCR (Williams *et al.*, 1990). Arbitrarily primed PCR (AP-PCR) is based on the primise that, because of its complexity, eukaryotic nuclear DNA may contain paired random segments that are complementary to single decanucleotides and furthermore, these segments have the correct orientation and are located close enough to each other for PCR amplification. The DNA fragments can be separated by gel electrophoresis and the DNA variation is detected by pattern of DNA bands. RAPD markers provide a fast and easy approach for taxonomical classification and making fingerprints for many plant species especially those in risk of extinction such as *C. procera*. Chen *et al.* (2005) evaluated the genetic diversity of *Coelonema draboides* species between individuals and among populations using RAPD markers. They came to the conclusion that population in different habitats should be studied and protected; so as to retain as much genetic diversity as possible.

In the present study the tested primers were able to anneal and produce PCR products indicating their ability to amplify random sequences from total *C. procera* DNA extracted from bulked-leaves. The bands obtained were in a molecular mass range of 0.2-2 Kb.

The DNA fingerprint pattern generated by the different primers comprised from 5-8 bands, primers A7 & B17 produced the highest number of fingerprints and consequently, gave us the higher background and pictures about *C. procera* DNA. Amplified products obtained by A9, for example, were characterized by specific bands and were also considered as fingerprints, such data will serve as a base to establish genetic structure for the studied plant.

The present study also showed the protein fingerprints for the investigated plant indicating the presence of protein fractions of MW ranged, generally, from 11.2 to 84.8.

On the other hand, investigating the variability in seed storage proteins in *C. procera* on the basis of SDS-PAGE showed, generally, that there was no remarkable change in the seed storage proteins in the four samples studied (2000-2003). The stability of proteins and enzymes is usually the factor that limits their usefulness, the most interesting results obtained from this study was the stability of *C. procera* proteins and consequently several enzymes after prolonged periods of storage (~ 4 years) and reflect the ability of this plant to adapt the harsh environmental conditions.

Leaf sample of *C. procera* was used to assay its elements quantitatively using scanning electron microscope and x-ray detection. Sample analysis showed differences in metal contents. Calcium was abundant element in *C. procera* leaves collected from the garden of Al-Azhar University, zinc and copper were found in more than one form (four) and can be considered as a biochemical fingerprints for the studied plant.

Of all cryopreservation techniques, vitrification is one of the most popular (Lambrides *et al.*, 2000 and Matsumoto *et al.*, 2001). The basic principle is complete vitrification of the cytoplasm during freezing (in liquid nitrogen by direct immersion); as a result ice-crystals are rarely formed (Towill, 1995). This effect is further reduced by rapid thawing after cryopreservation as this is used to avoid recrystallisation and cell death of explants (Sakai *et al.*, 1990). Vitrification has major advantages: its application is easy, reduced the need for expensive equipment, may avoid damaging consequences of ice crystal formation, and larger explants can be used (Towill, 1995 and Verleysen *et al.*, 2005). Success in cryostorage was evaluated in this study on the basis of germination tests after low temperature deep freezing (5, -5 and -20 °C) and LN immersion treatments. Germination tests showed that viability of seeds exposed to LN and different temperatures were approximately equal to the control seed viability. Such observation indicated that none of the tested treatments adversely affected seed viability.

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دراسة وراثية وجزيئية على نبات العشار

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تهدف هذه الدراسة الى اجراء بعض الدراسات لأحد النباتات البرية المعمرة وهو نبات العشار المصرى لما له من استخدامات طبية كثيرة ويعتبر أيضا من محاصيل الألياف ومصدر مهم لاستخراج الطاقة.

تناول البحث الدراسات الآتية:

أ- الدراسات السيتولوجية :- أمكن التعرف على عدد الكروموسومات (٢٢) وقياس معدل الانقسام وقياس معدل الشذوذ الكروموسومى وبعض القياسات للخلايا فى مرحلة الطور البينى باستخدام طريقة تحليل الصور البيولوجية بالبرمجيات والطرق التقليدية.

ب- الدراسات الهستولوجية : أمكن عمل ق.ع من خلال بلوكات الشمع للجذر والساق والاوراق وأمكن عمل دراسات بالميكروسكوب الماسح للتعرف على الذوائد الشعرية والثغور وسطح البذرة وموضع خروج الألياف من البذرة.

ج- دراسات على المستوى الجزيئى :

- أمكن عزل د ن ا الجينومى وعمل بصمة وراثية من خلال ال -(PCR RAPD) وأظهرت النتائج أن هناك بادئان لهم نتائج هامة وكذلك بادئ آخر (A9) الذى يعتبر المفتاح الجينومى لهذا النبات.

- أمكن التعرف على حزم البروتين المخزن فى البذرة من خلال طريقة التفريد الكهربى والتعرف على حزم البروتين المصاحبة لتغير عوامل البيئة من خلال أربع مواسم مختلفة لتفسير ظاهرة الوراثة الموجهة حيث لوحظ أن فى بذور سنة ٢٠٠٢ أحد الحزم ذات وزن جزيئى مميز.

د- دراسات خاصة بتحليل العناصر الصغرى : وذلك من خلال القياس المباشر بواسطة أشعة X حيث كان عنصر الكالسيوم هو أكبر العناصر فى الأوراق وهذا متوافق علميا. وكذلك وجود العناصر الصغرى والنادرة مثل الزنك والنحاس والسلينيوم وهذه العناصر الصغرى تميز هذا النبات فى تحمله للظروف المعاكسة حيث أمكن اعتباره من النباتات المنظفة للبيئة حيث أن المعلوم أن الزنك يرتبط مع بروتين معين ليكون عامل نسخ لجين ما يسمح بنسخ هذه الجينات بمعدلات سريعة مكونا ما يسمى ميتالوثيونين وهذا أساسى وضرورى لتحمل النباتات للجفاف وله تطبيقات هامة فى مجال الهندسة الوراثية وهندسة البروتين.

ه- دراسات على حيوية البذور بعد حفظها بطرق مختلفة : وقد أظهرت النتائج حيوية البذور بعد معاملات الحفظ المستخدمة فى هذه الدراسة لحماية هذا النبات المهدد بالانقراض من نتيجة التوسع العمرانى والصناعى.