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# Cytogenetical Studies on Achene Colour Polymorphism of *Picris asplenoides* L. and *Urospermum picroides* L. (Asteraceae) in Egypt

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**Abstract:** Achene morphs of *Picris asplenoides* L. and *Urospermum picroides* L. were investigated in order to gain insight into its genetic variation based on the evidence obtained from karyotype analysis, electrophoretic pattern of achene proteins as well as nucleic acid analysis. In *Picris asplenoides* L., three achene morphs were observed from every inflorescence as follows: violet, brown and white, these morphs differ in their color. In the inflorescence of *Urospermum picroides* L., three achene morphs were differ also in their color were observed as follows: white, brown and black. All achene morphs of *Picris asplenoides* and *Urospermum picroides* were diploid, with ten chromosomes observed in somatic cells. Karyotype studies showed that the achene morphs of *Picris asplenoides* and *Urospermum picroides* have different karyotype formulae. However, the chromosome type nearly submetacentric (-) and nearly metacentric were common in all karyotype formulae of all different achene morphs of *Picris asplenoides* and *Urospermum picroides*. Not only the dissimilarity was found in the morphology of chromosomes but also in the Mean Chromosome Length (MCL) and Diploid Chromosome Length (DCL). Types and proportions of abnormalities for different achene morphs of *Picris asplenoides* and *Urospermum picroides* observed at mitotic division were analysed. The electrophoretic analysis of *Picris asplenoides* revealed the presence of fourteen bands of molecular weight ranging from 145.00 to 20.00 kD. The band with molecular weight 20.00 kD was restricted to brown achene from and can be used as molecular marker to distinguish brown achene form from violet achene form. The electrophoretic analysis of *Urospermum picroides* reveals the presence of nine bands of molecular weight ranging from 95.00 to 22.00 kD. The band with molecular weight 22.25 kD was restricted to white achene from and can be used as molecular marker to distinguish white achene form other achene forms. The nuclear DNA content for *Picris asplenoides* were 0.0295 and 0.0183 μg $g^{-1}$ fresh weight for violet and brown achene, respectively, while RNA content were 25.347 and 35.069 μg $g^{-1}$ fresh weight for violet and brown achene, respectively. The nuclear DNA content for *Urospermum picroides* were 0.093, 0.115 and 0.145 μg $g^{-1}$ fresh weight for brown, black and white achene, respectively while RNA content were 10.417, 17.361 and 21.528 μg $g^{-1}$ fresh weight for black, white and brown achene, respectively.

**Key words:** *Picris asplenoides*, *Urospermum picroides*, achene color polymorphism, karyotypes, protein profile, nucleic acids

## INTRODUCTION

Seed and fruit heteromorphism may be defined as the production by individuals of propagules differing in their behavior or other attributes including color, shape, size and presence or absence of dispersing capacities (Imbert *et al*., 1996). Much evidence shows that differences in seed morphs may result in different dispersal, dormancy and germination patterns (Schmitt *et al*., 1985; Venable and Levin 1985; Telenius and Torstensson, 1989) as well as divergent growth and fitness of the resulting plants (Walter, 1984; Schnee and Walter, 1986).

The existence of heteromorphic fruits has been well described in a number of families including Asteraceae, Chenopodiaceae, Brassicaceae and Poaceae (Tanowitz *et al*., 1987). The production of heteromorphic seeds has been shown for many plant species inhabiting unpredictable environments, such as frequently disturbed habitats (Harper, 1977) arid and semi arid environments (Venable and Lawlor, 1980; Ellner and Shmida, 1984).

Achene polymorphism in Asteraceae most likely spreads germination out in space and time and thereby increases the number of safe sites an individual parent can exploit in disseminating offsprings (Mc-Evoy, 1984). The production of different fruit morphs by an individual

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has other advantages including the reduction of the competition and mating between siblings and protection against seed predation (Ellner and Shmida, 1984; Schoen and Lioyd, 1984; Venable, 1985; Tanowitz *et al*., 1987). Seed heteromorphism has been also assumed to increase adaptation in highly variable environments because different seed types have been shown to function differentially in dispersal (e.g., *Gymnarrhena micrantha*, Koller and Roth, 1964; *Picris echioides*, Sorensen, 1978 and *Crepis sancta*, Imbert *et al*., 1996), within or among year time of germination (*Senescio jacoboea*, MC-Evoy, 1984; *Heterotheca latifolia*, Venable and Levin, 1985 and *Hemizonia increscens*, Tanowitz *et al*., 1987).

Heteromorphic fruits (achenes) have been reported in Asteraceae (Zohary, 1950; Täckholm, 1974; Boulos, 1995). The family Asteraceae is one of the largest families of angiosperms (1535 genera and 23000 species; Bremer, 1994) and is considered by most taxonomists the highest in the scale of evolution.

However, no study to our knowledge examined the genetic variability which correlates with fruit color polymorphism in *Picris asplenoides* and *Urospermum picroides*. This investigation is carried out in order to gain insight into its genetic variation based on evidence obtained from karyotype analysis, electrophoretic pattern of achene proteins as well as nucleic acid analysis.

## MATERIALS AND METHODS

**Achene collection:** Viable achenes of the studied taxa were collected from 50 mature individuals. Viable achenes of *Picris asplenoides* were collected from sand dunes region of Idko, El-Behera Governorate, but viable achenes of *Urospermum picroides* from Dakahlia Governorate.

Viable achenes of *Picris asplenoides* were separated into three morphs according to their color to violet, brown and white (Fig. 1A-C). Achenes of *Urospermum picroides* were divided into three forms also according to their color to white, brown and black (Fig. 6). All morphs of the studied taxa were observed in every inflorescence.

The studied taxa were identified by Dr. Ibrahim A. Mashaly Professor of Plant Ecology and Flora, Department of Botany, Faculty of Science, Mansoura University, Egypt.

**Cytological analysis:** All achenes of the studied taxa were germinated except white achene form of *Picris asplenoides*, several trials were carried out to enhance germination but this achene form failed to germinate. The highest percentage of germination was obtained in temperature ranging from 17-20°C.

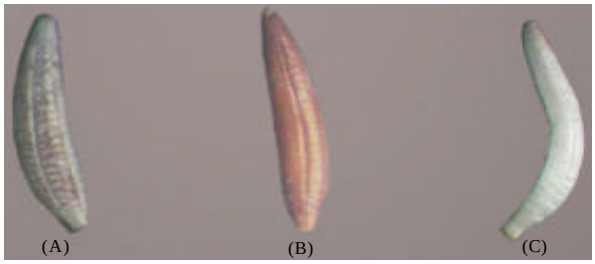


Fig. 1: Photograph of different achene morphs of *Picris asplenoids*, (A) violet achene form, (B) brown achene form and (C) white achene form (X = 10)

Actively growing root tips were fixed in solution of 1:3 glacial acetic acid and absolute ethanol (v/v) and stored in refrigerator. Examination of roots was done in permanent root tip squash preparations by using 2% aceto orcein after acid treatment (Chattopadhyay and Sharma, 1988). The nomenclature system for chromosome type was determined as described by Abraham and Prasad (1983). Karyotypes analysis was carried out using Micro measure computer program (Reeves, 2001). The mean measurements of three cells for each achene were used to construct the karyotype.

**Protein analysis:** According to Bradford (1976) total protein extract of achenes were analyzed to determine the total protein of the achenes. For electrophoresis analysis, the method for discontinuous SDS-PAGE techniques was based on that of Laemmli (1970). The analysis percentage of the bands were carried out using BIO-RAD Video denistometer.

The gel was photographed and analyzed using BIO-RAD Video documentation system, Model Gel Doc, 2000. The percentages of polymorphic bands were determined according the following equation:

$$\text{Polymorphic bands (\%)} = \frac{(\Sigma\text{bands for each sample} - \Sigma\text{common bands for all samples})}{\Sigma\text{bands for all samples}} \times 100$$

**Nucleic acid analysis:** For nucleic acids extraction, the method based on that of Shibko *et al.* (1967). DNA was estimated by diphenylamine colour reaction described by Burton (1968). RNA was determined following the method by Dische (1962).

## RESULTS AND DISCUSSION

### Karyotype analysis

***Picris asplenoides* L.:** Chromosomal measurements for two achene morphs of *Picris asplenoides* Table 1. The

Table 1: Somatic chromosome measurements of *Picris asplenoides*

| | Chromosome length (Um) | | | | | |
|---|---|---|---|---|---|---|
| Achene morphs | Long arm (L) | Short arm (S) | Total chr. length | Arm ratio (L/S) | Cent index | Chromosome type |
| Violet | 2.351 | 1.051 | 3.402 | 2.236 | 0.309 | nsm(-) |
| | 2.213 | 0.858 | 3.071 | 2.578 | 0.279 | nsm(-) |
| | 1.978 | 0.985 | 2.963 | 2.009 | 0.332 | nsm(-) |
| | 1.811 | 1.095 | 2.906 | 1.654 | 0.377 | nsm(-) |
| | 1.500 | 1.056 | 2.556 | 1.421 | 0.413 | nm |
| Brown | 3.201 | 1.046 | 4.247 | 3.061 | 0.246 | nsm(+) |
| | 2.973 | 1.579 | 4.552 | 1.883 | 0.347 | nsm(-) |
| | 2.649 | 1.050 | 3.699 | 2.523 | 0.284 | nsm(-) |
| | 2.010 | 1.034 | 3.044 | 1.944 | 0.34 | nsm(-) |
| | 2.329 | 1.578 | 3.907 | 1.477 | 0.404 | nm |

Table 2: Karyotype parameters of somatic chromosomes of different achene morphs of *Picris asplenoides*

| No. | Achene morphs | DCL | MCL | TCV | S (%) | TF (%) | A1 | A2 | Syi index | Rec index | Karyotype formula |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Violet | 29.457 | 2.946 | 4.753 | 0.35 | 32.57 | 0.486 | 0.099 | 0.329 | 0.866 | 8 nsm(-) + 2 nm |
| 2 | Brown | 38.424 | 3.842 | 4.552 | 0.25 | 31.90 | 0.498 | 0.140 | 0.318 | 0.844 | 2 nsm(+) + 6 nsm(-) + 2 nm |

DCL: Diploid complement length; A1: Intrachromosomal asymmetry index; MCL: Mean chromosome length; A2: Interchromosomal asymmetry index; TCV: Total chromosome volume; Syi: The symmetric indices; S (%): Symmetry percent; Rec: Resemblance between chromosomes

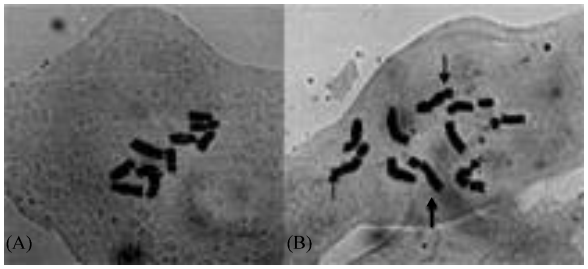


Fig. 2: Somatic cells of *Picris asplenoides* with choromosome number (2n = 10), (A) violet achene form and (B) brown achene form, arrows refer to secondary constriction

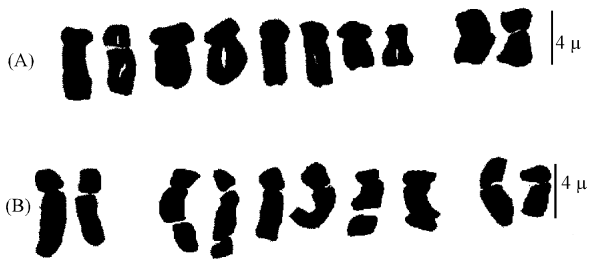


Fig. 3: Karyogram of different achene morphd of *Picris asplenoides*, (A) violet achene, (B) brown achene, bar = 4 µ, arrows refer to the secondary constriction

taxon under study is diploid, ten chromosomes are observed in somatic cells of different achene morphs (Fig. 2A-B) which coincides with earlier cytological report on *picris asplenoides* by Brullo *et al.* (1977) and Morton (1977).

Karyogram of each achene morphs is shown in (Fig. 3A-B). The chromosome number, arm ratio, centromeric index, Diploid Complement Length (DCL), Mean Chromosome Length (MCL), Total Chromosome Volume (TCV), symmetry percent (S%), total form percentage (TF%), intrachromosomal asymmetry index ($A_1$), interchromosomal asymmetry index ($A_2$), symmetric indices (Syi), resemblance between chromosomes (Rec), as well as karyotype formulae of all achenes are shown in Table 2.

From the point of karyotype formula view, the chromosome type nearly submetacentric (-) and nearly metacentric are representing in all karyotype formula of two achene forms of *Picris asplenoides*. Nearly, submetacentric (+) is represented only in brown achene form.

There are substantial differences in karyotype formula among all achene forms of *Picris asplenoides*. It is evident from the karyotype studies that none of the achene forms have an identical chromosome set. Not only the dissimilarity was found in the morphology of chromosomes but also in the MCL and DCL. Brown achene form recorded the highest values of DCL and MCL (38.424 and 3.842 µ, respectively). However, violet achene form was recorded the smallest DCL and MCL (29.457 and 2.946 µ, respectively).

The evolution of karyotype is estimated by the indices of symmetry. These values range theoretically from 0 to 100 for Rec and Syi indices (Greilhuber and Septa, 1976); from 0 to 50 for TF (%) (Huziwara, 1962) as well as symmetry (S%). A karyotype with high values of these indices is considered low evolved. According to these parameters, violet achene form recorded the highest values of Rec index, Syi index, TF% and S% (0.866, 0.329, 32.57 and 0.35, respectively) so, this achene form may be considered as less evolved one. Brown achene form recorded the lowest values of Rec index, Syi index, TF%

Table 3: Mitotic abnormalities percentage for different achene morphs of root tips of *Picris asplenoides*, total number of cells examined equal 10,000

| | | Abnormal cells (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Interphase | | Prophase | Metaphase | | | | | | |
| Achene morphs | Normal cells (%) | Micro nucleus | Multi nucleated cells | Micro nucleus | Disturbed | Polyploidy | Non congression | Fragmentation | Chromosome ring | Stickiness | Micro nucleus |
| Violet | 96.78 | 0.00 | 0.00 | 0.00 | 0.991 | 0.389 | 0.318 | 0.142 | 0.071 | 0.00 | 0.00 |
| Brown | 97.28 | 0.986 | 0.179 | 0.0299 | 0.00 | 0.00 | 0.209 | 0.00 | 0.00 | 0.359 | 0.06 |

| | | Abnormal cells (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Anaphase | | | | Telophase | | | |
| Achene morphs | Normal cells (%) | Bridge | Late separation | Laggard | Bridge and laggard | Late separation | Bridge | Laggard | Total abnormalities |
| Violet | 96.78 | 0.495 | 0.283 | 0.142 | 0.18 | 0.142 | 0.035 | 0.035 | 3.22 |
| Brown | 97.28 | 0.209 | 0.09 | 0.06 | 0.00 | 0.299 | 0.149 | 0.209 | 2.72 |

Table 4: Somatic chromosome measurements of *Urospermum picroides*

| | Chromosome length (Um) | | | | | |
|---|---|---|---|---|---|---|
| Achene morphs. | Long arm (L) | Short arm (S) | Total chr. length | Arm ratio (L/S) | Cent index | Chromosome type |
| White | 1.501 | 0.854 | 2.355 | 1.757 | 0.363 | nsm(-) |
| | 1.320 | 0.763 | 2.083 | 1.730 | 0.366 | nsm(-) |
| | 1.082 | 0.580 | 1.662 | 1.866 | 0.349 | nsm(-) |
| | 1.530 | 1.031 | 2.560 | 1.484 | 0.403 | nm |
| | 1.277 | 0.777 | 2.054 | 1.643 | 0.378 | nm |
| Brown | 1.549 | 0.786 | 2.336 | 1.971 | 0.337 | nsm(+) |
| | 1.378 | 0.819 | 2.197 | 1.682 | 0.373 | nsm(-) |
| | 1.140 | 0.532 | 1.672 | 2.145 | 0.318 | nsm(-) |
| | 1.118 | 0.502 | 1.621 | 2.225 | 0.310 | nsm(-) |
| | 1.150 | 0.850 | 2.000 | 1.353 | 0.425 | nm |
| Black | 2.675 | 0.757 | 3.462 | 3.536 | 0.220 | nsm(+) |
| | 2.085 | 1.209 | 3.294 | 1.724 | 0.367 | nsm(-) |
| | 2.030 | 1.196 | 3.227 | 1.697 | 0.371 | nsm(-) |
| | 1.473 | 0.760 | 2.384 | 1.938 | 0.340 | nsm(-) |
| | 1.329 | 0.918 | 2.246 | 1.447 | 0.409 | nm |

and S%, so this achene form may be considered more evolved one (0.844, 0.318, 31.9 and 0.25, respectively).

According to Zarco (1986), the intrachromosomal and interchromosomal asymmetry indices ($A_1$ and $A_2$, respectively) defined clear differences among achene morphs. In general, high $A_1$ and $A_2$ values were scored in species with higher degrees of variation in chromosome length (Kamel, 1999). Since, brown achene form have the highest values of $A_1$ and $A_2$ (0.498 and 0.14 μ, respectively), they may be considered as the most advanced achene form. Violet achene form recorded the lowest values of $A_1$ and $A_2$ (0.486 and 0.099 μ, respectively), so, that this achene form may be considered less evolved one.

Some mitotic abnormalities have been recorded (Table 3, Fig. 4, 5) where, the total abnormalities percentage was ranging from 2.72 in brown achene form to 3.22 in violet achene form Table 3.

***Urospermum picroides*:** Chromosomal measurements for different achene morphs of *Urospermum picroides* (Table 4). The taxon under study is diploid, ten chromosomes are observed in somatic cells of different achene morphs (Fig. 6A-C) which coincides with earlier cytological report by Loeve and Kjellqvist (1974), Nazorva (1975) and Kuzmanov and Jurukova (1977).

There are substantial differences in karyotype structure among the different achene morphs of *Urospermum picroides*. Not only the dissimilarity was found in the morphology of chromosomes but also in the Mean Chromosome Length (MCL). The highest value (2.878 μ) was recorded in black achene form, while the lowest value (1.951 μ) was recorded in brown achene form. Also, the highest Diploid Chromosome Length (DCL) was found in black achene form (28.784 μ) and the lowest in brown achene form (19.511 μ) (Table 5). The study on *Urospermum picroides* confirmed the remarkable degree of chromosome variability among achene forms studied. It is evident from the present karyotype analysis that none of the achene forms of *Urospermum picroides* have identical chromosome sets but two chromosome types (nearly submetacentric (-) and nearly metacentric) were common in all karyotype formulae (Fig. 9A-C).

According to S%, TF%, Rec index and Syi index parameters, white achene form recorded the highest

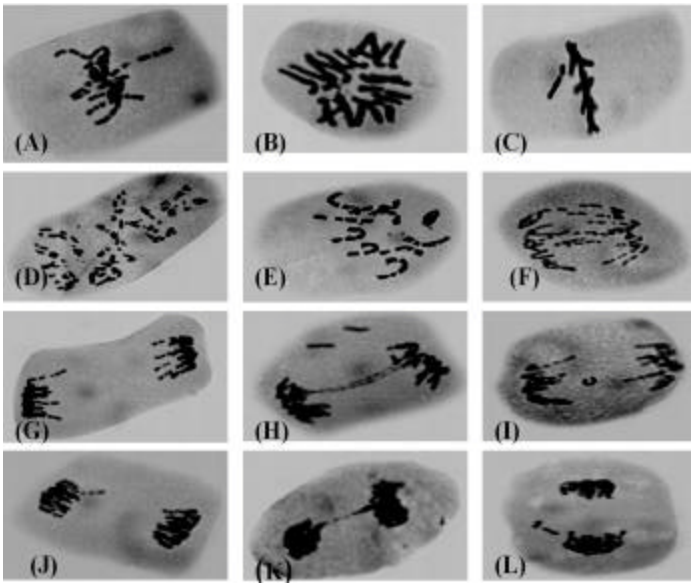


Fig. 4: Chromosomal abnormalities of *Picris asplenoides* (Violet achene form). (A) Disturbed metaphase, (B) polyploidy, (C) non congression at metaphase, (D) fragmentation, (E) chomosome ring at metaphase, (F) bridge at naphase, (G) late separation at anaphase, (H) bridge and laggard at anaphase, (I) laggard at anaphase, (J) late separation attelophase, (K) bridge at telophase and (L) laggard at telophase ($\chi = 1000$)

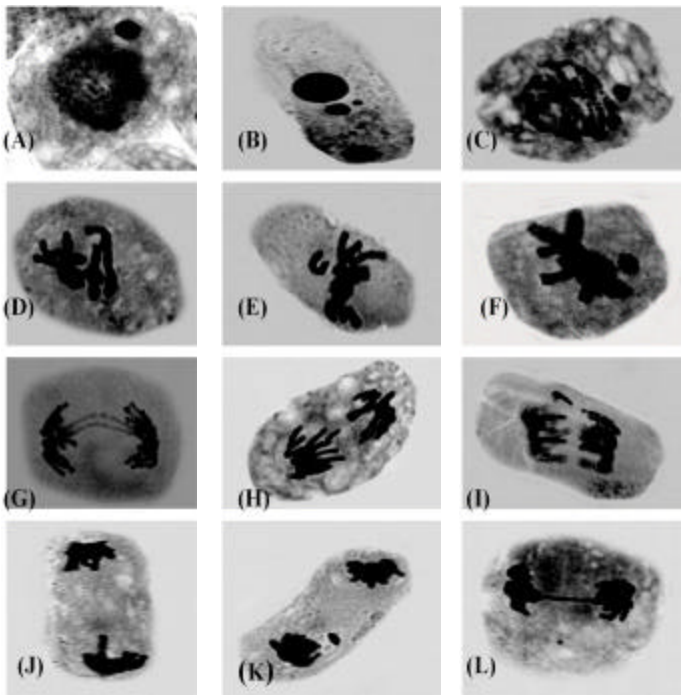


Fig. 5: Chromosomal abnormalities of *Picris asplenoides* (Brown form). (A) Mironucleus in interphase, (B) multinucleated cells, (C) micronucleus in prophase, (D) stickiness at metaphase, (E) non congression at metaphase, (F) micronucleus at metaphase, (G) bridge at anaphase, (H) late separation at anaphase (I) laggard at anaphase, (J) late separation attelophase, (K) laggard at telophase and (L) bridge at telophase ($\chi = 1000$)

Table 5: Karyotype parameters of somatic chromosomes of different achene morphs of *Urospermum picroides*

| Achene morphs | DCL | MCL | TCV | S (%) | TF (%) | A1 | A2 | Syi index | Rec index | Karyotype formula |
|---|---|---|---|---|---|---|---|---|---|---|
| White | 21.134 | 2.113 | 28.552 | 0.351 | 36.591 | 0.44 | 0.152 | 0.363 | 0.826 | 6 nsm(-) + 4 nm |
| Brown | 19.511 | 1.951 | 12.094 | 0.323 | 35.502 | 0.445 | 0.154 | 0.353 | 0.835 | 8 nsm(-) + 2 nm |
| Black | 28.784 | 2.878 | 16.112 | 0.282 | 33.705 | 0.44 | 0.194 | 0.347 | 0.831 | 2 nsm(+)+ 6 nsm(-)+2 nm |

DCL: Diploid complement length; A1: Intrachromosomal asymmetry index; MCL: Mean chromosome length; A2: Interchromosomal asymmetry index; TCV: Total chromosome volume; Syi: The symmetric indices; S%: Symmetry percent; Rec: Resemblance between chromosomes; TF%: Total form percentage

Table 6: Mitotic abnormalities percentage for different achene morphs of root tips of *Urospermum picroides*, total nnmber of cells examined equal 10,000

| | | | Abnormal cells (%) | | |
|---|---|---|---|---|---|
| No. | Achene morphs | Normal cells (%) | Polyploidy | Bridge at anaphase | Total abnormalities |
| 1 | White | 98.07 | 1.737 | 0.193 | 1.93 |
| 2 | Brown | 97.62 | 2.38 | 0.00 | 2.38 |
| 3 | Black | 98.44 | 1.56 | 0.00 | 1.56 |

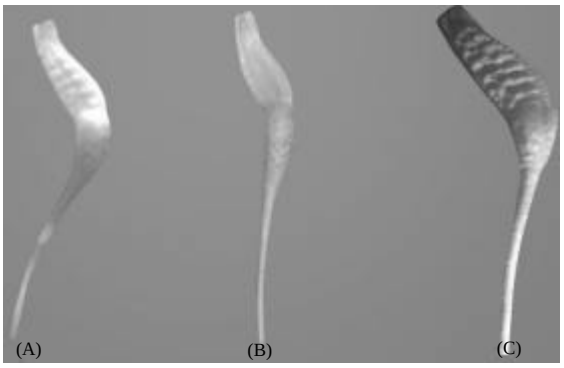


Fig. 6: Photograph of different achene morphs of *Urospermum picroides*, (A) white achene form, (B) brown achene form and (C) black achene form (X = 10)

values of S%, TF% and Syi index (0.351, 36.591 and 0.363, respectively) so, this achene form may be considered as less evolved one. Brown achene form had the highest value of $A_1$ (Intrachromosomal asymmetry) (0.445), whereas black achene form had highest value of $A_2$ (interchromosomal asymmetry) (0.194). Thus these achene forms may be considered more advanced than white achene form.

Some mitotic abnormalities have been recorded (Table 6, Fig. 8A, B) where, the total abnormalities percentage was ranging from 1.56 in black achene form to 2.38 in brown achene form. Polyploidy was observed in all achene forms, but bridge at anaphase was recorded in white achene form only.

**Achene proteins**

***Picris asplenoides*:** The total achene protein percentage was ranging from 1.10 in brown achene form to 2.28 in violet achene form (Fig. 7A-C).

The electrophoretic analysis was revealed the presence of fourteen bands of molecular weight ranging from 145.00 to 20.00 kD. Thirteen bands are common in

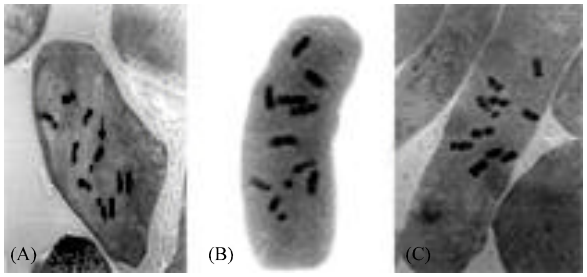


Fig. 7: Somatic cells of *Urospermum picroides* with chromosome number (2n = 10), (A) black achene form, (B) brown achene form and (C) white achene form, arrows refer to secondary constriction

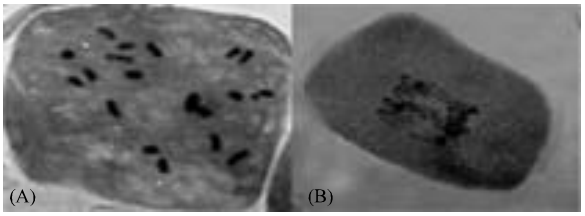


Fig. 8: Chromosomal abnormalities of *Urospermum picroides*, (A) polyploidy and (B) bridge at anaphase

two achene forms, but the band with molecular weight 22.00 kD was restricted to brown achene form, so that this band can be used as molecular marker to distinguish brown achene form from violet achene form.

Nevo *et al.* (1998) indicated that micro climatic conditions generate both protein and DNA patterns of polymorphism that parallel macroscale environmental pattern. The protein profile of achene forms is shown in Table 7 and Fig. 10.

The percentages of polymorphic bands of the studied taxon are shown in Table 7. The percentage of polymorphic bands being the lowest (0) in brown form and the highest (0.07) in violet form.

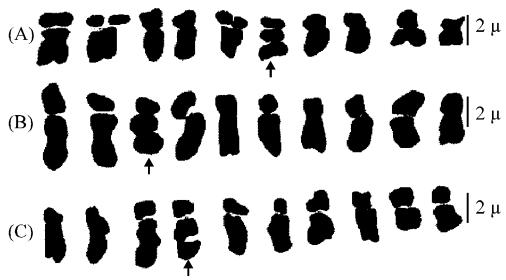


Fig. 9: Karyogram of different achene morhs of *Urospermum picroides*, (A) white achene, (B) brown achene and (C) black achene form, bar = 2 µ, arrows refer to secondary constriction

Table 7: Achene proteins attributes, DNA and RNA of different morphs of *Picris asplenoides*

| Morphs | Violet | Brown |
|---|---|---|
| **Achene protein analysis** | | |
| 145.00 | 1 | 1 |
| 102.00 | 1 | 1 |
| 85.00 | 1 | 1 |
| 74.50 | 1 | 1 |
| 67.00 | 1 | 1 |
| 62.00 | 1 | 1 |
| 57.00 | 1 | 1 |
| 46.50 | 1 | 1 |
| 45.00 | 1 | 1 |
| 42.00 | 1 | 1 |
| 31.00 | 1 | 1 |
| 27.5o | 1 | 1 |
| 26.5o | 1 | 1 |
| 20.00 | 0 | 1 |
| Total bands | 13 | 14 |
| Polymorphic bands (%) | 0.07 | 0.00 |
| Total achene protein (%) | 2.28 | 1.10 |
| DNA (µg $g^{-1}$) | 0.0295 | 0.0183 |
| RNA (µg $g^{-1}$) | 25.347 | 35.069 |

Table 8: Achene proteins attributes, DNA and RNA of different morphs of *Urospermum picroides*

| Morphs | White | Brown | Black |
|---|---|---|---|
| **Achene protein analysis** | | | |
| 95.00 | 0 | 1 | 1 |
| 58.00 | 1 | 1 | 1 |
| 51.50 | 1 | 1 | 1 |
| 42.25 | 0 | 1 | 1 |
| 40.00 | 1 | 1 | 1 |
| 36.50 | 1 | 1 | 1 |
| 22.75 | 1 | 1 | 1 |
| 22.25 | 1 | 0 | 0 |
| 22.00 | 0 | 1 | 1 |
| Total bands | 6 | 8 | 8 |
| Polymorphic bands (%) | 0.11 | 0.33 | 0.33 |
| Total achene protein (%) | 2.65 | 2.87 | 3.89 |
| DNA (µg $g^{-1}$) | 0.145 | 0.093 | 0.115 |
| RNA (µg $g^{-1}$) | 17.361 | 21.528 | 10.417 |

***Urospermum picroides*:** The total achene protein percentage was ranging from 2.65 in white achene form to 3.89 in black achene form (Table 8). The protein profile of Achene forms is shown in Fig. 11.

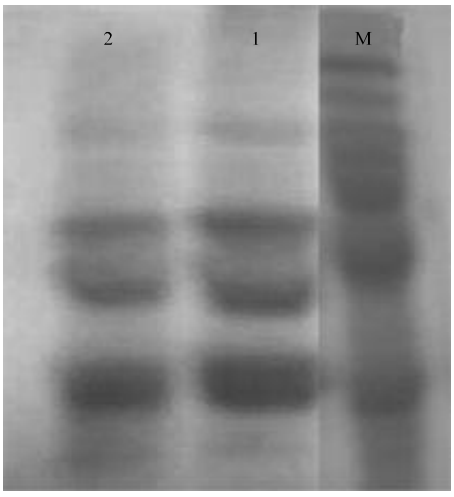


Fig. 10: Polyacrylamide gel showing achene protein bands of different morphs of *Picris asplenoides*. (M) Marker, (1) brown form and (2) violet form

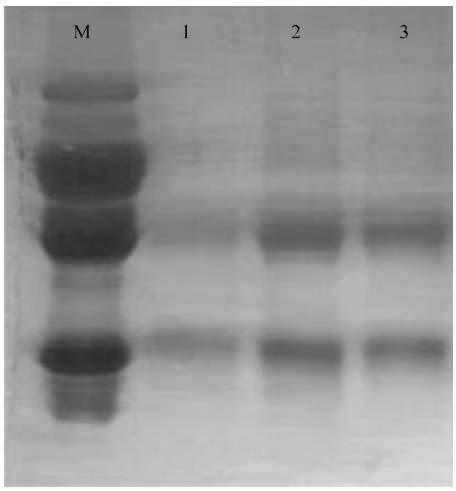


Fig. 11: Polyacrylamide gel showing achene protein bands of different morphs of *Urospermum picroides*, (M) Marker, (1) white form, (2) brown form and (3) black form

The electrophoretic analysis revealed the presence of nine bands of molecular weight ranging from 95.00 to 22.00 kD. Five bands are common in all achene forms with molecular weight (58.00, 51.50, 40.00, 36.50 and 22.75 kD). The bands with molecular weight 95.00, 42.25 and 22.00 kD were present in brown and black achene forms. The band with molecular weight 22.25 kD is restricted to white achene form sothat; this band can be used as molecular marker to distinguish white achene form other forms.

The percentages of polymorphic bands of the studied taxon are given in Table 8. The percentage of polymorphic bands being the lowest (0.11) in white form and the highest (0.33) in brown and black forms.

**DNA and RNA content**

***Picris asplenoides*:** DNA localized in cell nuclei codes most of the genetic information of an orgamism (Dolezel *et al*., 1998). From Table 7 it is clear that, the nuclear DNA contents ranges from 0.0183 μg $g^{-1}$ in brown achene form to 0.0295 μg $g^{-1}$ in violet achene form. The RNA content ranges from 25.347 μg $g^{-1}$ in violet achene from to 35.069 μg $g^{-1}$ in brown achene form.

***Urospermum picroides*:** From Table 8 it was clear that, the nuclear DNA contents ranged from 0.093 to 0.145 μg $g^{-1}$. The maximum value of 0.145 μg $g^{-1}$ was recorded in white achene form and the minimum value of 0.093 μg $g^{-1}$ was recorded in brown achene form. The RNA content ranges from 10.417 to 21.528 μg $g^{-1}$. The highest content value was found in brown achene form 21.528 μg $g^{-1}$, the lowest value 10.528 μg $g^{-1}$ was found in black achene form.

## REFERENCES


Abraham, Z. and P.N. Prasad, 1983. A system of chromosome classification and nomenclature. Cytologia, 48: 95-101.

Boulos, L., 1995. Flora of Egypt, Checklist. 1st Edn., Al Hadara Publishing, Cairo, Egypt, ISBN: 977-5429-08-0.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Bremer, K., 1994. Asteraceae: Cladistics and Classification. Timber Press, Portland, Oregon, ISBN-10: 0881922757.

Brullo, S. G. Majorana, P. Pavone and M.C. Terrasi, 1977. Numeri cromosomici per la flora Italiana. Inform. Bot. Ital., 9: 40-55.

Burton, K., 1968. A study of the condition and mechanism of the diphenylamine reaction for the colourimetric estimation of DNA. Biochem. J., 62: 315-323.

Chattopadhyay, D. and A.K. Sharma, 1988. A new technique for orcein banding with acid treatment. Stain Technol., 63: 283-287.

Dische, Z., 1962. Color Reactions of Pentoses. In: Methods in Carbohydrates Chemistry, Whistler, R.L. and M.L. Wolfrom, (Eds.), Academic Press, New York, pp: 484-488.

Dolezel, J., J. Greihuber, S. Lucretti, A. Meister, M.A. Lysak, L. Nardi and R. Obermayer, 1998. Plant genome size estimation by flow cytometry: Interlaboratory comparison. Ann. Botany, 82: 17-26.

Ellner, S. and A. Shmida, 1984. Seed dimorphism in relation to habitat in genus *Picris* (Compositae) in Mediterranean and Arid regions. Isr. J. Bot., 33: 25-39.

Greilhuber, J. and F. Septa, 1976. C-banded karyotypes in the *Scilla hohenackeri* group. *S. persica* and *Puschkinia* (Liliaceae). Plant Syst. Evol., 126: 149-188.

Harper, J.L., 1997. The Population Biology of Plants. Academic Press, London.

Huziwara, Y., 1962. Karyotypic analysis in some genera of compositae, VIII. Further studies on the chromosomes of *Aster*. Amer. J. Bot., 49: 116-119.

Imbert, E., J. Escarre and T.J. Lepar, 1996. Achene dimorphism and among-population variations in some biological traits in *Crepis sancta* (Asteraceae). Int. J. Plant Sci., 157: 309-315.

Kamel, E.A., 1999. Karyological studies on some taxa of the Asteraceae in Egypt. Comp. News I., 33: 1-18.

Koller, D. and N. Roth, 1964. Studies of the ecological and physiological significance of amphicarpy in *Gymnarrhena micrantha* (Compositae). Am. J. Bot., 51: 26-35.

Kuzmanov, B. and P. Jurukova, 1977. In IOPB chromosome number reports LV III. Taxon, 26: 557-565.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage $T_4$. Nature, 227: 680-685.

Loeve, A. and E. Kjellqvist, 1974. Cytotaxonomy of Spanish plants. IV. Dicotyledonous: Caesalpiniaceous- Asteraceae- Lagascalia, 4: 153-211.

Mc-Evoy, P.B., 1984. Dormancy and dispersal in dimorphic achenes of Tansy ragwort, *Senecio jacobaea*. Oecologia, 61: 160-168.

Morton, J.K., 1977. A cytological study of the compositae (Excluding *Hieracium* and *Taraxacum* ) of the British isles. Watsonia, 11: 211-223.

Nazorva, E.A., 1975. Chromosome numbers of some species of armenian flora. Biol. Zurn Arm., 1: 95-97.

Nevo, E., B. Baum, A. Beiles and D.N. Johnson, 1998. Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the fertile crescent. Genet. Resour. Crop Evol., 45: 151-159.

Reeves, A., 2001. Micro measure: A new computer program for the collection and analysis of cytogenetic data. Genome, 4: 439-443.

Schmitt, J., D. Ehrhardt and D. Swartz, 1985. Differential dispersal of self fertilized and outcrossed progeny in jewelweed (*Impatiens capensis*). Am. Nat., 126: 570-575.

Schnee, B.K. and D.M. Walter, 1986. Reproductive behaviour of *Amphicarpaea bracteata* (Leguminosae), an amphicarpic annual. Am. J. Bot., 73: 376-387.

Schoen, D.J. and D.G. Lioyd, 1984. The selection of cleistogamy and heteromorphic diasporas. Biol. J. Linn. Soc., 23: 303-322.

Shibko, S., P. Koivistoven, C.A. Tratnyek, A.R. Newhall and L. Friedman, 1967. Method for sequential quantitative separation and determination of protein, RNA, DNA, lipid and glycogen from a single liver homogenate or from subcellular fraction. Annal. Biochem., 19: 415-528.

Sorensen, A.E., 1978. Somatic polymorphism and seed dispersal. Nature, 276: 174-176.

Täckholm, V., 1974. Student's Flora of Egypt. Cairo University Press, Cairo, Egypt.

Tanowitz, B.D., P.F. Salopek and B.E. Mahall, 1987. Differential germination of ray and disk achenes in *Hemizonia increscens* (Asteraceae). Am. J. Bot., 74: 303-312.

Telenius, A. and P. Torstensson, 1989. The seed dimorphism of *Spergularia marian* in relation to dispersal by wind and water. Oecologia, 80: 206-210.

Venable, D.L. and L. Lawlor, 1980. Delayed germination and dispersal in desert annuals: Escape in space and time. Oecologia, 46: 272-282.

Venable, D.L., 1985. The evolutionary ecology of seed heteromorphism. Am. Nat., 126: 577-595.

Venable, D.L. and D.A. Levin, 1985. Ecology of achene dimorphism in *Heterotheca latifolia*: I. achene structure, germination and dispersal. J. Ecology, 73: 133-145.

Walter, D.M., 1984. Differences in fitness between seedlings derived from cleistogamous and chastogamous flowers in *Impatiens capensis*. Evolution, 38: 427-440.

Zarco, C.R., 1986. A new method for estimating karyotype asymmetry. Taxon, 35: 526-530.

Zohary, M., 1950. Evolutionary trends in the fruiting heads of compositae. Evolution, 4: 1403-1409.