Cytogetnecal and ecological studies of some wild congeneric species in the Solanaceae distributed in upper Egypt

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ABSTRACT. Cytogetnecal and ecological studies in some species of the Solanaceae were made in the Mediterranean-type ecosystem in Upper Egypt. The chromosome complement of Datura innoxia (2n=24) consisted of 24 median-centromeric chromosomes; that of D. stramonium (2n=24) consisted of 22 median- and two submedian-centromeric chromosomes; that of Hyoscyamus albus (2n=48) consisted of 62 median- and six submedian-centromeric chromosomes, that of H. muticus (2n=28) consisted of 21 median- and seven submedian-centromeric chromosomes; that of Withania somnifera (2n=36; new count) consisted of 27 median- and nine submedian-centromeric chromosomes; and that of Solanum nigrum (2n=72) consists of 68 median and four submedian-centromeric chromosomes. Thirty-three reproducible polymorphic bands were resulted after four RAPD-PCR primers; those bands were used for studying the genetics similarity among the species. Average similarity coefficient was ranged from 0.05 to 0.47. RAPD bands resulted after PCR: D. innoxia and D. stramonium showed five bands each; H. albus and H. muticus showed two and one bands respectively; W. somnifera seven bands and S. nigrum showed 13 bands, different patterns were produced among congeneric species. Ionic status of hot water extract of the species revealed that Hyoscyamus species was more accumulated Na⁺ and Ca²⁺, while Datura species accumulated more K⁺ and Mg²⁺ and W. somnifera and S nigrum were intermediate. These strategies of ionic accumulation were in relation to the different chromosome number of species, which might help for surviving the species in desert ecosystem.

KEYWORDS: Chromosomes, Ecosystem, Ionic status, RAPD, Solanaceae.

Cytogenetical and ecological investigations in some species of the Solanaceae are very important from taxonomical and ecological point of view, that can lead to more intensive partitioning of the dried habitats, evolution of gender dimorphism in desert plants and may play important role in consequences of genetic and genomic changes of natural population (Solties et al. 2003).

The Solanaceae in Egypt consists of eight genera and 33 species (Tackholm 1974; Boulos 1995). Datura, Hyoscamus, Solanum and Withania are taxonomically quite complex since they got so much confusion in morphological markers. They are medicinally important among the family members (Porter 1959; Jennifer and James 1997).

Since use of chromosomal data to solve systematic problems of higher plants were introduced early in the last century (e.g., Tahara 1915), its importance has been growing gradually and fast as the nature of chromosomes became understood, and the methodologies for the study of chromosomes have been developed (e.g., Tanaka 1959, 1960). Chromosome counts of the Egyptian plant species have been received attention of many authors (e.g., Amin 1972a, b; Badr 1995; Badr et al. 1997; Mohamed 1997; Abd El-Twab et al. 2008). Some cytotaxonomic studies in the Solanaceae have been reported (Philomina 1980; Badr et al. 1997; El-Nahas et al. 2000).

Molecular markers provide useful information insight into the classification which have been available for these several years and developed to overcome the problems associated with the morphological characters (Awashi et al. 2004; Ruangadtapha et al. 2007). The commonly used polymerase chain reaction (PCR)-based DNA marker system is Random Amplified Polymerase DNA (RAPD) (Williams et al. 1990; Staub et al. 1996). RAPD method depends on the amplification of DNA sequences by using single, short and random oligonucleotides. This method has advantages of being sensitive, quick to perform and applicable to many samples (Babaoğlu et al. 2004). Applied RAPD in many plants of different families insure the efficiency of RAPD in genomic identification. RAPD was applied to identify genomic relationships of wild congeneric species (Abd El-Twab and Zahrani 2010), cultivars (Ruangsuttapha et al. 2007), as well as to make molecular and cytogenetic analysis of some taxa in the Solanaceae (Shedai et al. 2007).

Plant responses to water scarcity are complex, involving many adaptive strategies. Under field conditions, these responses can be synergistically or antagonistically modified by superimposition of other stresses. Plant strategies to cope with drought normally involving a mixture of stress avoidance and tolerance strategies that varies with species genotype. This complexity is well illustrated in Mediterranean type ecosystem, where plants with predominant drought-avoidance strategies (e.g. deep rooted perennials or winter/spring annuals). These strategies mediated by gene expression to improve plant function under these stresses (Bohnert and Sheveleva 1998). Thus, under future scenarios of more arid climates due to global environmental changes, water limitation may be a critical constraint to plant especially in desert
This study aims to investigate 1) the karyomorphological constituents by using the aceto-carmine, 2) phylogenetic relationships of congeneric species in the Solanaceae distributed in Upper Egypt studied by RAPD markers and 3) the inorganic osmoticum accumulation among these species.

**Materials and Methods**

**Plant materials.** The species studied were *Datura innoxia* Mill., *D. stramonium* L., *Hyoscyamus albus* L., *H. muticus* L., *Withania somnifera* (L.) Dun. and *Solanum nigrum* L. The plants used were collected from nature in three sites namely: Site 1: at 28° 07′ with ASL 130 ft; Site 2: at 28° 27′ 46.70″ N and 30° 51′ 55.83″ E with ASL 138 ft; and Site 3: 28° 24′ 23.72″ N and 30° 34′ 30.39″ E with ASL 161 ft during the dry season. The voucher herbarium specimens were deposited in the Herbarium, Department of Botany and Microbiology, Faculty of Science. Minia University, Egypt.

**Chromosome preparation.** The method followed Kondo *et al.* (1992) and Abd El-Twab *et al.* (2008). Examination of slides was done by Zeiss microscope and photographs for good plates were taken with a digital camera “Ulead explorer”. Analysis of chromosome complements and accumulation of data was based on the microphotography.

**Plant genomic DNA extraction.** Plant genomic DNA extraction followed Abd El-Twab and Zahran (2008).

**Random Amplified Polymorphic DNA (RAPD) analysis.** The ready-to-go RAPD analysis beads and primers 1, 2, 3 and 4 were used. The procedure followed the manufacturer’s protocol (GE Healthcare Bio-Science AB). PCR amplification was done with four RAPD primers using 100 ng of genomic DNA. The thermal cycler (Thermo Hybaid) was operated as follows: 1 cycle at 95 °C for 5 min; 40 cycles at 95 °C, 36 °C and 72 °C for 40 sec, 1 min, and 2 min, respectively; and a final amplification at 72 °C for 10 min.

**Gel-electrophoretic analysis.** Gel electrophoretic was used to determine the presence/absence of the total genomic DNA and size of the DNA fragments after RAPD loaded using loading buffer in 2.0% Agarose Gel, which carry DNA from negative to positive side. DNA was stained in gel by ethidium bromide (0.5 μg/ml), which combined with DNA fragments and give violet light under UV light and photographed using a digital system. Ladder DNA (vivantis VC 100bp Plus DNA ladder, Product No.: NL0401). Fragments with size 500bp and 1000bp are higher in intensity in comparison to other bands to serve as orientation points. Usage: 0.1μl of the DNA ladder per 1mm of lane.

**Calculation of DNA concentration and purity.** The total genomic DNA concentration μg/ml and purity were calculated by the equations: 50×260OD×100/1000 and 260/280, respectively (Sambrook *et al.* 1989).

**Data analysis.** RAPD markers produce DNA amplification signals that can be converted into measurements of similarity or dissimilarity (DNA electrophoretic patterns contain scorable bands assigned to specific positions in an individual lane). Pairwise similarity of the genotypes or genetic phenotypes represented in the different lanes can be quantified using indexes or coefficients of similarity. These estimators define “genetic distances” that portray DNA divergence between organisms in phenetic and cladistic analysis (Huang *et al.* 2000). For each primer, the consistent amplified products were recorded. The polymorphic fragments (RAPD) were named by the primer code followed by the size of the amplified fragment in base pairs. The presence of a specific product was noted whatever the intensity of the band. Each marker was assumed to correspond to a locus with two alleles (presence or absence of the band).

**Calculation of the similarity matrix (Jaccard) and Cluster analysis.** PAST computer program was used for a hierarchical clustering analysis based on the unweighted pair group method with arithmetic mean to generate a dendrogram and to describe relationships among genotypes.

**Ionic analysis.** Three replicates of each plant species were oven-dried to use for analysis. In the hot water extract of plant samples cations and anions were measured: Sodium and potassium were measured by flame photometer method as Williams and Twine (1960). Calcium and magnesium were determined by versine titration methods as Schwarzenbach and Biedermann (1948). Phosphorus was measured colorimetrically as described by Woods and Mellon (1958). Chloride was determined according to Jackson (1962). Nitrate was assessed by method of phenoldisulphonic acid as Richards (1969).

**Results**

The chromosome numbers and karyotypes of six taxa belonging to the Solanaceae collected in different habitats were reported in Upper Egypt. The present chromosome number of *Datura innoxia* was 2n=24 (Figs.1 and 2A); its chromosome complement had mean length of 59.01 μm (Table 1) and consisted of 24 median-centromeric chromosomes. *Datura stramonium* L. had the chromosome number of 2n=24 (Figs. 1 and 2B); its chromosome complement had mean length of 74.13 μm (Table 1) and consisted of 22 median and two sub-median centromeric chromosomes. *Hyoscyamus albus* L. had the chromosome number of 2n=68 (Figs. 1 and 2C); its chromosome complement had mean length of 124.11 μm (Table 1) and
consisted of 62 median and six sub-median centromeric chromosomes. *Hyoscyamus muticus* had the chromosome number of 2n=28 (Figs. 1 and 2D); the chromosome complement had mean length of 72.21 μm (Table 1) and consisted of 21 median and seven sub-median chromosomes. *Withania somnifera* had the chromosome number of 2n=36 reported here for the first time (Figs. 1 and 2E); the chromosome complement had mean length of 116.84 μm (Table 1) and consisted of 27 median- and nine submedian-centromeric chromosomes. The present count chromosome number of *Solanum nigrum* had 2n=72 (Figs. 1 and 2F); the chromosome complement had mean length of 129.24 μm (Table 1) and consisted of 68 median and four sub-median chromosomes.

Table 1. Chromosome number, length, morphology, chromosome complement lengths and locality of the species studied in the Solanaceae distributed Upper in Egypt

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome no. 2n</th>
<th>Chromosome length μm</th>
<th>Total Chromosome complement Lengths μm</th>
<th>Chromosome morphology</th>
<th>habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shortest ± SD</td>
<td>Longest ± SD</td>
<td>shortest</td>
<td>longest</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>metacentric</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sub-metacentric</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>24</td>
<td>1.71 ± 0.05</td>
<td>3.32 ± 0.06</td>
<td>57.34</td>
<td>60.08</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>24</td>
<td>2.42 ± 0.07</td>
<td>3.62 ± 0.13</td>
<td>73.07</td>
<td>75.07</td>
</tr>
<tr>
<td><em>Hyoscyamus albus</em></td>
<td>68</td>
<td>1.24 ± 0.08</td>
<td>2.85 ± 0.05</td>
<td>119.35</td>
<td>127.18</td>
</tr>
<tr>
<td><em>Hyoscyamus muticus</em></td>
<td>28</td>
<td>1.87 ± 0.09</td>
<td>3.52 ± 0.03</td>
<td>71.22</td>
<td>73.23</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>36</td>
<td>1.93 ± 0.13</td>
<td>4.91 ± 0.09</td>
<td>115.31</td>
<td>118.38</td>
</tr>
<tr>
<td><em>Solanum nigrum</em></td>
<td>72</td>
<td>1.12 ± 0.05</td>
<td>2.87 ± 0.04</td>
<td>129.24</td>
<td>132.73</td>
</tr>
</tbody>
</table>

length of 130.98μm (Table 1) and consists of 68 median and four sub-median-centromeric chromosomes.

RAPD primers revealed bands polymorphism (Fig. 3). Each of the primers was tested on all samples studied, and were selected for genotype analysis because their patterns were reproducible and stable, while monomorphic loci were not recorded. Polymorphic bands were selected for identifying the genetic similarity for the group of species. Genetic distances were calculated for all the species studied and dendrogram were obtained with the PAST computer program.

Thirty three reproducible polymorphic bands were resulted after four RAPD-PCR primers; those bands were used for studying the genetics similarity among the species. The average similarity coefficient was ranged from 0.05 to 0.47 (Table 2). Datura innoxia and D. stramonium showed five (15.2%) bands each, Hyoscyamus albus and H. muticus showed two (6.1%) and one (3.0%) bands, respectively, Withania somnifera showed seven (21.1%) bands and Solanum nigrum showed 13 (39.4%) bands, different patterns were produced among congeneric species showing that the RAPD method is able to identify the genetic variations of the taxa and can rapidly and easily differentiate among them. Species relationships based on the data matrix of RAPD markers were analyzed by using the dendrogram drawn from the UPGMA cluster analysis (Fig. 4). Two main groups of the six species were clustered into the Clades 1 and 2. Clade 1-(3 and 4) consisted of; Clade 3 of H. muticus (one RAPD band) and Clade 4 of D. stramonium (five RAPD bands). Clade 2 is divided to 5 and 6-(7)-(9 and 10) and 8). Clade 5 consisted of H. albus (two RAPD bands); Clade 8 consisted of D. innoxia (five RAPD bands); Clade 9 consisted of W. somnifera (seven RAPD bands); and Clade 10 consisted of S. nigrum (13 RAPD bands).

Succulence among different species. The water status and succulence are the most important features for plant to ensure the biological activities especially under xeric conditions. So, the selected species maintain relatively high succulence ratio to facilities the metabolic process, to overcome the adverse environmental stress as drought stress. The plants tend to readjust its internal osmotic pressure quickly by accumulation of inorganic solutes and on long term by accumulation of organic solutes (Lew 1996). Hyoscyamus species were less affected by the environmental conditions than other studied species. Thus, its succulence was higher (8.83 and 5.58 %) for H. muticus and H. albus, respectively, than other species probably 2-3 folds higher. S. nigrum was more affected by drought prevailing condition so it has lower degree of succulence 2.94 %. Datura species as well as W. somnifera were in between and had succulence percentage 3.32 and 3.15 and 3.65 for D. innoxia, D. stramonium and W. somnifera, respectively. This means that among the studied species Hyoscyamus species tend to maintain the

<table>
<thead>
<tr>
<th>Jaccard Measure</th>
<th>D. innoxia</th>
<th>D. stramonium</th>
<th>H. albus</th>
<th>H. muticus</th>
<th>W. somnifera</th>
<th>S. nigrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Datura innoxia</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. stramonium</td>
<td>0.0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyoscyamus albus</td>
<td>0.37</td>
<td>0.1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. muticus</td>
<td>0.0</td>
<td>0.16</td>
<td>0.2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>0.4</td>
<td>0.16</td>
<td>0.3</td>
<td>0.12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>0.35</td>
<td>0.35</td>
<td>0.29</td>
<td>0.05</td>
<td>0.47</td>
<td>1</td>
</tr>
</tbody>
</table>
played a major role in osmotic adjustment so; they were Calcium and magnesium as inorganic compatible solutes in Solanum nigrum other species this may be to avoid sodium injury.

Enhance plant growth (Sbabala et al. 2003). For Datura innoxia leaves Mg++ content was 11.28 meq/l higher than other species. Calcium may have the lowest ratio of Na+/K+ (0.88 meq/l). This due to high accumulates of K+ (5.67 meq/l) in the plant. The succulence of Hyoscyamus species was positively correlated with both of total cations and anions.

Table 3. Ionic status of shoots of selected species (meq)

<table>
<thead>
<tr>
<th>Species</th>
<th>Succ.</th>
<th>Na+</th>
<th>K+</th>
<th>Ca++</th>
<th>Mg++</th>
<th>Cl−</th>
<th>NO3−</th>
<th>PO4−</th>
<th>total anions</th>
<th>total cations</th>
<th>Eff. salinity</th>
<th>Na+/K+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Datura innoxia Mill</td>
<td>3.31</td>
<td>5.07</td>
<td>5.67</td>
<td>7.60</td>
<td>11.26</td>
<td>4.00</td>
<td>0.43</td>
<td>0.38</td>
<td>4.81</td>
<td>29.60</td>
<td>8.91</td>
<td>0.89</td>
</tr>
<tr>
<td>Datura stramonium L.</td>
<td>3.14</td>
<td>5.16</td>
<td>4.86</td>
<td>6.50</td>
<td>9.28</td>
<td>1.10</td>
<td>0.41</td>
<td>0.43</td>
<td>1.93</td>
<td>25.81</td>
<td>8.20</td>
<td>1.06</td>
</tr>
<tr>
<td>Hyoscyamus albus L.</td>
<td>5.58</td>
<td>6.25</td>
<td>3.24</td>
<td>23.30</td>
<td>8.40</td>
<td>13.29</td>
<td>1.05</td>
<td>0.84</td>
<td>15.18</td>
<td>41.18</td>
<td>7.37</td>
<td>1.92</td>
</tr>
<tr>
<td>Hyoscyamus maticus L.</td>
<td>8.83</td>
<td>5.43</td>
<td>3.64</td>
<td>24.60</td>
<td>9.48</td>
<td>9.99</td>
<td>0.97</td>
<td>0.88</td>
<td>11.85</td>
<td>43.16</td>
<td>4.88</td>
<td>1.49</td>
</tr>
<tr>
<td>Withania somnifera (L.) Dun.</td>
<td>3.64</td>
<td>4.98</td>
<td>5.67</td>
<td>9.90</td>
<td>2.96</td>
<td>7.89</td>
<td>0.79</td>
<td>0.59</td>
<td>9.27</td>
<td>23.51</td>
<td>6.44</td>
<td>0.87</td>
</tr>
<tr>
<td>Solanum nigrum L.</td>
<td>2.93</td>
<td>4.35</td>
<td>2.02</td>
<td>13.90</td>
<td>3.65</td>
<td>9.69</td>
<td>0.69</td>
<td>0.44</td>
<td>10.82</td>
<td>23.93</td>
<td>8.14</td>
<td>2.14</td>
</tr>
</tbody>
</table>

Cations accumulation. The data tabulated in Table 3 showed that Hyoscyamus species more accumulated Na+ (6.25 and 5.43 meq/l) for H. albus and H. maticus, respectively than the other species studied reflecting the dependency of species on Na+ to overcome the high external conditions. While S. nigrum was less accumulated for Na+ 4.35 meq/l D. stramonium, D. innoxia as well as W. somnifera were moderately accumulated Na+ (5.16, 5.07 and 4.98 meq/l, respectively. The advantage of high Na+ content apparently to compete other cations as K+ and Mg++ (Okusnya and Ungar 1984) and Ca ++ (Lohaus et al. 2000). Under drought conditions both D. innoxia and W. somnifera tended to accumulate more K+ (5.67 meq/l) than other species this may be to avoid sodium injury. Solanum nigrum had the lowest accumulation of K+ ions 2.02 meq/l. Calcium and magnesium as inorganic compatible solutes played a major role in osmotic adjustment so; they were accumulated in high concentrations compared with Na+ and K+ (Table 3). Calcium was accumulated higher 24.6 meq/l than magnesium 9.48 meq/l in Hyoscyamus species more than two times. The high content of calcium in leaves of Hyoscyamus species was in concomitant with its succulence which reflects the role of Hyoscyamus leaves as water storage for this plant. In desert under arid conditions Hyoscyamus species was dependent on inorganic ions so, its content of Na+, Ca++ and Mg++ were mostly higher than other species. Calcium may significantly alleviate detrimental effects of Na+ and enhance plant growth (Sbabala et al. 2003). For D. innoxia Mg++ content was 11.28 meq/l higher than other species this means that D. innoxia had ability to accumulate more Mg++ compared with other studied species so, this cation played a main role for survived this plant under desert conditions.

The data of the present work showed that plants absorbed and accumulate higher contents of both inorganic solutes according to the type of stress. The high amount of Na+ in the external media gave a good indication that species can tolerate with stress by different mechanisms, so where sodium was lower in soil supporting growth of Hyoscyamus species was 2.2 meq/l and 2.3 meq/l (unpublished data). This means that, the species tend to accumulate more Na+ in plant tissues 6.25 meq/l for H. albus and 5.43 meq/l for H. maticus tissues. Datura species did not show that. S. nigrum and W. somnifera grew in soil with relatively high Na+ content but excluded Na+ (Table 3). The ability to limit Na+ transport as in case of W. somnifera and S. nigrum and to reduce Na+ accumulation in the plant tissues was in agreement with (Razmjoo et al. 2008). Whish is critical for maintenance of high growth rate and production and metabolic process in elongated cells.

Anions accumulation. Under xeric conditions, the high accumulation of Cl− generally was higher in Hyoscyamus species 13.29 and 9.99 meq/l for H. albus and H. maticus, respectively which was reflected its sensitivity to drought, and its dependence on inorganic ions to overcome the external stress. Most studied species showed high ability to accumulate more Cl− under the adverse conditions. The low Cl− content as anionic osmoticum (1.10 meq/l) in D. stramonium was attributed to its tolerance against prevailing ecological conditions.

For NO3− and PO4−3 there contents were lower in all selected species except for Hyoscyamus species. The small amount of PO4−3 may be reflecting the minor role of PO4−3 in surviving plants under drought conditions. The narrow differences between both anions NO3− and PO4−3 in all studied species reflecting its tolerance to environmental conditions. The accumulation of PO4−3 may reflect the need to synthesize organic solutes to avoid Na+ injury (Table 3).

Relation of Na+/K+ ratio and the effective salinity. The high ratio of Na+/K+ in S. nigrum 2.15 meq/l compared with other selected species not due to high content of sodium but due to the plant accumulate the lowest amount of K+ among other species this means that this species takes up large amounts of Na+ while still maintaining K+ uptake (Glenn and O’Leary 1984). On the other hand W. somnifera had the lowest ratio of Na+/K+ (0.88 meq/l). This due to high accumulate of K+ (5.67 meq/l) in the plant. The succulence of Hyoscyamus species was positively correlated with both of total cations and anions.
DISCUSSION

The present counts of chromosome number were convenient with previously reported counts in *Datura innoxia* of 2n=24 (Palomino et al. 1988), *D. stramonium* of 2n=24 (Bir et al. 1978; Hill 1995; Murin 1997; Badr et al. 1997); *Hyoscyamus albus* L. of 2n=68 (Andreev 1981; Srivastava and Lavania 1987; Aboucaya and Verlaque 1990; Diosdado et al. 1993; Badr et al. 1997). *Hyoscyamus muticus* of 2n=28 (Srivastava and Lavania 1987; Tyagi and Dubey 1989; Misra et al. 1991; Badr et al. 1997 and Mohamed 1997). Only Bahl and Tyagi (1992) reported that *H. muticus* had 2n=30. *Withania somnifera* of 2n=36 considered as a new count and different from that was reported as 2n=48 (Bir and Neelam 1980; Renard et al. 1983; Slavik et al. 1993; Badr, et al. 1997; Sundari et al 1999). The present count chromosome number of *Solanum nigrum* of 2n=72 was convenient with previously reported counts (Rao and Kumar 1980; Sidhu 1979; Symon 1981; Bir and Sidhu 1979; Probatova and Sokolovskaya 1981; Singh and Roy 1982; Bhiravamurti and Rethy 1983). *Solanum nigrum* was reported as to have various chromosome counts of 2n=24 and 48 (Kulkarni and Hegde 1983) and 2n=60 (Magulave 1984).

Based on the cluster analysis, which was carried out using eight morphological characters, the taxa were classified into two main groups included *Hyoscyamus albus* and *Hyoscyamus muticus* as ditantly related to the other three genera, *Withania somnifera* is more closely related to *Solanum nigrum* than *Datura stramonium* and *Datura innoxia*, which clustered together (data not shown).

The information of chromosomal data can be very useful in verifying the integrity of species and in those instances when species have different chromosome numbers, for providing a reliable character to separate species (Kondo et al. 1992). This is especially true when chromosomal difference is correlated to morphological variation (Tanaka 1969). Karyotype evolutions are one of the most important aspects of the whole evolutionary processes (Imai et al. 1986) and considered as an isolating mechanism in speciation and have their own evolutionary trends independent of genetic evolution (Imai et al. 2001). Therefore, karyotype evolution generally tends towards an increasing number and terminal centromeric chromosomes (acrocentric). The opposite tendency, the reduction of chromosome number and formation of median centromeric chromosomes (metacentric) are primitive (Imai et al. 2001). Accordingly, among the diploid species, the chromosome complement of *D. innoxia* is considered the most primitive karyotype (all chromosomes of the complement are median-centromeric chromosomes). The evolution of chromosome shape, size, composition, number and redundancy might result in a wide diversity of karyotypes. The taxa in this study showed variations in visible numbers of satellites in each chromosome complements at mitotic metaphase by Carmen staining, probably due to either too much condensation of satellite by 8-hydroxyquinoline and/or buried satellite in chromosome arm or too much physically stretched secondary constriction (Kondo et al. 1996). Therefore the exact sat-chromosome numbers could not be detected in the mitotic plates of the present taxa. The species in the family Solanaceae had a various basic chromosome number of x = 7, 9-12, 14 and 17 (Badr et al. 1997). Therefore, it was assumed that the Solanaceae might have originated from a taxon with basic chromosome number of X = 7 or 8, which give rise to other taxa by aneuploidy and/or polyploidy. By advancing evolution in different secondary lines, diversification of chromosome complement could be predicted to occur through additional cycles of polyploidy or reduction in chromosome number after polyploidy (El-Nahas et al. 2000). The present results showed that the species in Solanaceae have chromosome number of X = 12, 14 and 17.

The chromosomal modifications in different taxa were usually associated with structural changes as fragmentation, deletion and reciprocal translocation that lead to overall decrease in the chromosome length (Kondo et al. 1999; Abd El-Twab et al. 2004). In the present study, even the chromosome number of both *H. albus* (2n=68, X=17) and *H. muticus* (2n=28, X=7/14) was different as well as the basic chromosome number, but were clustered together after RAPD (0.2 similarity coefficients). The genomic DNA of *H. albus* was genetically more closely similar to the *D. innoxia*, *W. somnifera* and *S. nigrum* than *H. muticus* (Jaccard coefficient was 0.37, 0.3, 0.29 and 0.2, respectively). *D. innoxia* showed genetically distant to *D. stramonium* (Jaccard coefficient= 0.0), while it showed closely related to *W. somnifera* (coefficient=0.4). These data might indicate that genomes of *D. innoxia* and *D. stramonium* (both 2n=24) have different ancestors or one of them might had mutation of genome deletion after development.

Based on the present chromosome and RAPD study, it is now clear that the genome of *S. nigrum* is quite complexed. As it has several RAPD bands and various chromosome numbers and is considered as a widely distributed polyploid species with naturally occurring diploid (n=12), tetraploid (n=24) and hexaploid (n=36) forms.

The ecological study indicated that *Hyoscyamus* species were distributed in the wild habitats where the sodium was lower (2.2 meq/l), while Datura, *S. nigrum* and *W. somnifera* were salty lover, which grew in with relatively high sodium content habitats. It was observed (Table 1 and Fig. 4) that in spite of *Datura* had same chromosome for both species (2n=24), the chloride accumulation in their tissues was varied from 4 for *D. innoxia* to 1.1 meq/l for *D. stramonium*, this might be explained, convenient and support their position in two different clusters in the dendogram. *Hyoscyamus* species had two different chromosome numbers. *Hyoscyamus*
Na+ accumulation with mostly high values of effective ionic strategies. These strategies of ionic accumulation could help for surviving these species in desert ecosystems, and was able to identify genetic variations of the taxa to tolerate high salinity but in different pathways of genetic ability. Therefore, increasing the chromosome numbers or polyploidy level might support the genetic ability of these species in desert ecosystem, and was able to identify genetic variations of the taxa to differentiate rapidly and easily among them.

**Literature Cited**


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