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DISTRIBUTION OF *HYOSCYAMUS* SPECIES IN EGYPT AND THEIR GENETIC RELATIONSHIPS

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The loss of biodiversity has become an issue of great global concern and the identification of species status had been recognized as an urgent task. Egypt is among the hot arid regions of the world, where only little attention has been given to its threatened plant species. The genus *Hyoscyamus* (Solanaceae) is among the high medicinal potentiality genera in Egypt. Eight *Hyoscyamus* species were reported in the Egyptian flora. Assessment of the *Hyoscyamus* species was carried out during this work, the results revealed that: *Hyoscyamus albus* L., *H. desertorum* (Aschers. ex Boiss.) Täckh. are endangered species; *H. pusillus* L. is highly endangered; *H. boveanus* (Dunal) Aschers. & Schweinf. Is an endemic vulnerable species; *H. aureus* L. may be now extinct in Egypt; *H. niger* L. and *H. reticulatus* L. have never been recorded from Egypt in wild localities and *H. muticus* L. is the common species in the genus. Now, it is reasonable to conclude that only five *Hyoscyamus* native species may be present in Egypt. Genetic relationships between four of these species were constructed based on three isozyme systems: Esterase (EST, Glutamate Oxalo-acetate Transaminase (GOT) and Peroxidase (PRX). The enzyme polymorphism among the studied species was used to construct a dendrogram using the statistica multivariate analysis program. The obtained data display the possible origin of *H. boveanus* from *H. muticus* in the Sinai mountainous region. The genetic relationships between *Hyoscyamus* species reflect the urgent need for their conservation as a potential gene pool.

Keywords: species assessment, Egyptian flora, *Hyoscyamus*, endangered, endemic, isozyme.

The arid lands of the Middle East are not very productive in terms of plant biomass per unit area and need to be utilized with care to maintain a balance between productivity and harvesting, collection, cutting and grazing (Zaman, 1997). The loss of biodiversity has become an issue of great global concern (Heywood, 1995). Species used in the production of pharmaceuticals are among the highly valuable species. Their wild relatives are economically important because: (1) these species may contain the same active constituents; (2) some of these plants may be used for production of the active constituent precursor and (3) favor the environment for creation of new economic hybrids.

The genus *Hyoscyamus* is among the medically important genera in the Egyptian flora and is represented by six or eight species as cited by different authors. Täckholm (1974) and Boulos (1995 and 2002) reported that the genus is represented by seven species: *Hyoscyamus albus, H. aureus, H. boveanus, H. desertorum, H. muticus, H. pusillus* and *H. reticulatus*. Hepper (1998) in his taxonomic revision of the family Solanaceae, described eight species in Egypt and added *H. niger* to the seven species mentioned by Boulos (1995). However, Hepper (cited by Boulos, 2002) in his last treatment of the Solanaceae referred to only seven species excluding *H. niger* which he added earlier. The problem here is the difference in the number of *Hyoscyamus* species cited by different authors; no one of these treatments refers to the species status. Assessment of *Hyoscyamus* species is reported in this work, based on field surveys, literature data and herbarium specimens.

Isozymes have been used in genetics for defining systematic phylogentic relationships and to assess the genetic divergence between taxa (Tanksley, 1983; Bonnin *et al.*, 1996; Yang *et al.*, 1996). Isozyme polymorphism among the economic species provides a base for species propagation and conservation, especially the vulnerable, endangered and wild crop-related species. In closely related species, parallel homologous variation is reflected in the polymorphism of the same allozymes (Yaaska, 1988).

The present study aims at: (1) assessment of the genus *Hyoscyamus* status and its distribution in Egypt; (2) determination of the genetic relationships among the species still extant in Egypt; and (3) tracing the possible origin of the endemic species.

MATERIALS AND METHODS

Plant Material

Seeds of *Hyoscyamus* species under investigation were collected from the field during May 2001, 2002 and 2003. *H. albus* was collected from Wadi El-Amr, a branch of Wadi El-Arish, Sinai; *H. boveanus* from Burg El-Arab, western Mediterranean strip; *H. desertorum* from Nekhel, North

Sinai; while *H. muticus* was from the Cairo-Alexandria desert road. The author failed to collect *Hyoscyamus pusillus* during this work due to its scarcity in Egypt. Dry seeds were germinated in the greenhouse under the same conditions. The first four foliage leaves were selected for isozyme study.

Species Distribution

The distribution figures presented in this work are based on the compiled data from the specimens deposited in different Egyptian Herbaria (Cairo University, Agricultural Research Center, National Research Center) and Kew herbarium (after Hepper, 1998), and records the field trips during May of 2001-2003. Literature data were also used, following Tãckholm (1974); Danin *et al.* (1985); Boulos (1995, 1997 and 2002); El-Gazzar *et al.* (1995); Boulos and Barakat (1998); El-Kady *et al.* (1998); Hepper (1998); Al-Gohary (1999); Abd El-Ghani (2000) and Hegazy *et al.* (2001).

Isozymes

Esterase (EST), glutamate oxalo-acetate transaminase (GOT), and peroxidase (PRX) were assayed using 100 mg freshly harvested leaves of the studied species according to the method of Stegemann *et al.* (1983). A polyacrylamide standard gel was prepared by dissolving 8.55 g acrylamide and 0.45 g bisacrylamide in 150 ml Trisborate buffer 0.125 M Tris, pH 8.9. After filtration, 145 ml of this monomer solution were used to prepare the gel by adding 50 mg sodium sulphate, 0.1 ml TEMED, and 2.8 ml ammonium persulphate. PAGE electrophoresis was performed in a Biometra apparatus using Slab gels (11 x 12 cm).

After sample application, electrophoresis was carried out at 20 mA and 120 Volt for 20 minutes and then at 40 mA and 200 Volt for 4 hours. Esterases were detected by incubating the gel for one hour at room temperature, in 200 ml phosphate buffer (0.15 M, pH 7.2) containing α-naphthyl acetate (40 mg) and fast blue RP salt (100 mg). Peroxidases were incubated for 5 minutes in a mixture of 15 ml benzidine (10 %), 85 ml ethanol (95 %), 100 mM K-acetate pH 4.67 and 0.2 ml H_20_2 (30 %). Glutamate oxaloacetate transaminases were detected by incubating the gel for one hour at room temperature in 25 ml substrate solution (200 ml H_20, 146.1 mg of α-ketoglutaric acid, 532.4 mg L-aspartic acid, 2 mg PVP-40, 20 mg EDTA and 5.68 g Na_2HPO_4, pH 7.4), 25 ml water and 50 mg fast blue BB salt. After incubation, gels were fixed in 50 % glycerol for one hour and soaked in water for several hours. Zymograms were photographed. The resulted bands were analyzed using the Gel-Pro Analyzer V.3.0 computer program. Only anodic bands were analyzed. Bands were characterized by their rates of migration and, in the case of esterase, also by color (α-esterase dark brownish to blackish, β-esterase light to dark reddish, α/β intermediate (Lange and Schifino-Wittmann, 2000). The rate of migration is generally calculated for each band by dividing the distance migrated by the band by the distance migrated by the front line.

Statistical Analysis

Multivariate analysis for the isozyme zymogram data was carried out using hierarchical clustering analysis. Isozyme bands were scored as presence or absence on the zymograms. If the band was present in other genotypes it was designated 2, if not shared in another genotype it was designated 1, and 0 if absent. The genetic diversity was based on shared and unique polymorphic bands, in addition to the percentages of the developed bands measured using Gel-Pro Analyzer V. 3.0 computer program; both have been used to construct the phylogenetic dendrogram Statistica Programme for Windows Release 4.5, copyright by StatSoft, Inc. (1993).

RESULTS

Species Distribution

Hyoscyamus albus L. : The species distribution is given in figure (1). The author collected the species for the first time from Wadi El-Amr, Wadi El-Ansh attribute, Sinai in May 2003. The southern species limit is 30° 30' N while the western species limit is 2 9°30'E.

H. aureus L. : Danin *et al.* (1985) reported the species presence in mountain crevices in the area outlined in Sinai (Fig. 1). The only specimen deposited in Egypt was collected from Ataka mountain, eastern desert, in 1951 (Hepper, 1998). The western species limit was 32° 18' E while the southern limit was 29°15' N. The field excursions during this work and the recent literature data failed to trace this species.

H. boveanus (Dunal) Aschers. and Schweinf. : The species was recorded from Sinai to 1982 (Danin *et al.*, 1985). The last record for this species in the eastern desert was in 1878, in Wadi Abu-Marwa, Gulf of Suez. The species is an endemic; its recent records during this work (Fig. 1) display the changes in species distribution and give a general indication of the future northward expansion of the species. The southern species limit is 24° 6' N and the species is distributed between 29° 48' E to 34°30' E.

H. desertorum (Aschers. ex Boiss.) Täckh.: The species is mainly distributed in Sinai (Fig. 1). One specimen was collected from Saint Antonias Monastery, eastern desert in 1887; now the species is extinct from the area of this monastery. But about 250 km south of this site, Hegazy *et al.* (2001) collected the species from Shayeb El-Banat mountain group. Now the western species limit is 33° E and the southern limit is 27° 33` N.

H. muticus L. The southern species limit in Egypt is 22°48' N and the western species limit is 26° E (Fig. 1). New localities for the species were traced by El-Gazzar *et al.* (1995), in Ras Mohammed, Nabq and Abu Galum, S. Sinai. The author collected the species from the Gulf of Suez in May 2002. A flourishing community was found in Sallum plateau, western sector of the Mediterranean strip in April 2003 (Abd El-Ghani, personal communication, May 2003).

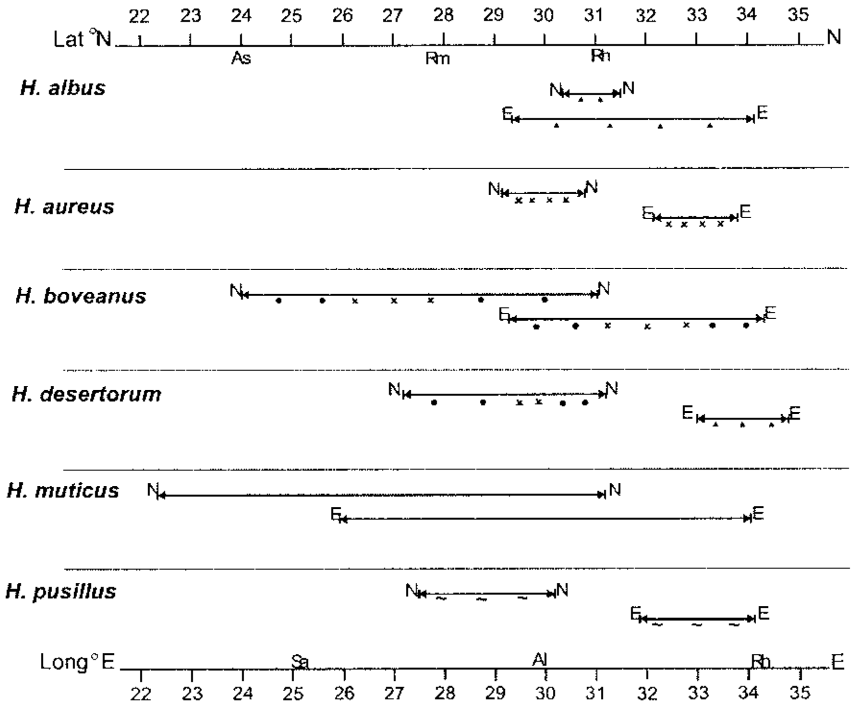

Fig (1). Diagrammatic representation of the geographical distribution of *Hyoscyamus* species in Egypt.

(⟷ Continuous presence, ~ =Rarely present, ▴ =Occasionally found, × =Extinct, • =Scattered patches)

(As= Aswan, Rm= Ras Mohammed, Rh= Rafah, Al= Alexandria, Sa= Sallum)

H. niger L. The species reported by Hepper (1998) as belonging to the Egyptian flora is based on cultivated specimens in an experimental garden. The species never recorded from Egypt in wild localities during this work.

H. pusillus L. The species was never recorded out of Sinai (Fig. 1). Recently the species was collected from El-Aqaba Gulf, S. Sinai (El-Kady *et al.,* 1998) and reported also among the species in Saint-Katherine Protectorate (Protectorate database). The western species limit is 32° 36' E and it extends to Central Asia; the southern species limit is 27° 33` N.

H. reticulatus L. The only sample deposited in Egypt was from Khan-Yunis, Palestine, Boulos (2002) gives it for Sinai.

Isozymes

Esterases (EST)

The developed zymogram showed a minor inter-specific variability among the studied *Hyoscyamus* species. These variabilities were mainly noticed in α and β-esterases bands. A total of eight esterase bands were detected in the four studied species; two bands in each species. However, α and β-esterases bands were detected in each species, differences in bands concentrations were notable. α-esterase was detected in higher concentrations in *Hyoscyamus albus* and *H. desertorum* than in *H. muticus* and *H. boveanus*. On the other hand, the latter two species contained higher concentrations of β -esterases bands.

Peroxidases (PRX) and Glutamate Oxalo-acetate-Transaminase *(GOT)*.

The developed zymograms in both enzymes showed a little polymorphism between the studied species. A total of eight bands were detected in each enzyme, two bands for each studied species. One of the two patterns of bands was detected in *Hyoscyarnus albus* and *H. desertorum*, and the other appeared in *H. muticus* and *H. boveanus*.

Concentrations of the developed bands of the three enzymes: EST, PRX and GOT are given in table (1). Bands concentration and loci polymorphism were used to construct the dendrogram (Figure 2). The dendrogram showed a close uniformity between *H. muticus* and *H. boveanus*.

TABLE (1). Results of Esterases, Peroxidases and Glutamate Oxalo-acetate-Transaminase isozymes for the studied *Hyoscyamus* species.

Enzyme	*Hyoscyamus albus*	*H. boveanus*	*H. desertorum*	*H. muticus*
	Percentages for the bands concentration			
α- esterases	67.4	23.3	63.2	33.6
β- esterases	23.7	70.8	32.2	54.5
Peroxidase				
Band 1	41.8	0.0	39.5	0.0
Band 2	32.5	0.0	29.8	0.0
Band 3	0.0	45.7	0.0	45.3
Band 4	0.0	45.3	0.0	50.2
Glutamate oxaloacetate transaminase				
Band 1	0.0	43.7	0.0	45.3
Band 2	0.0	47.3	0.0	50.0
Band 3	37.5	0.0	39.6	0.0
Band 4	56.4	0.0	29.9	0.0

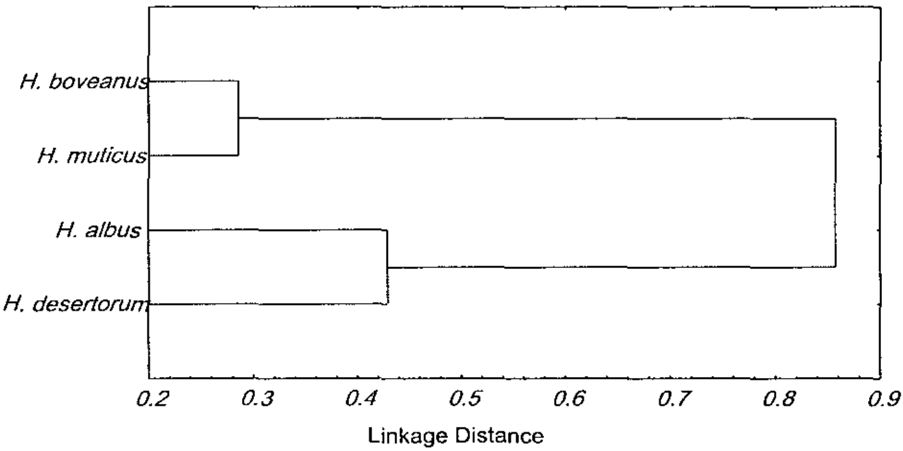

Fig. (2). Dendrogram of the studied *Hyoscyamus* species.

DISCUSSION

Species Status

Hyoscyamus albus, H. desertorum and *H. pusillus* were recorded in annual form, however, *H. albus* can grow in both annual and perennial forms. The dominance of the annual form is shown in the accidental vegetation type. Kassas (1966) used the term accidental vegetation, where precipitation is so low and falls are so irregular that no permanent vegetation exists, vegetation is restricted to patches in wadis and runnels. Accidental vegetation consists of annual, potential annual or potential perennial species. These plant species can grow as long as water persists in soil (Abd El-Ghani, 2000).

Generally, *Hyoscyamus albus* and *H. desertorum* were traced in species-poor communities. This phenomenon is a general feature for the Egyptian desert because of water stress. Plants responded to water scarcity by a rapid decline of biomass production and decreasing population size (Springuel, 1997). Sinai is the main area for *H. desertorum* distribution; only one site was traced in the eastern desert after 110 years from the last species record (Hegazy *et al.*, 2001). On the other hand, *H. pusillus* was never recorded out of Sinai; recently, individuals were traced in South Sinai runnels (El-Kady *et al.*, 1998), after 42 years from the last record for the species. It is reasonable to treat *Hyoscyamus albus* and *H. desertorum* as endangered species because of their restricted distribution (Fig. 1), dominance of the annual form, sparse growth communities, drought stress, and low migration potentiality of both species. The scarcity of *H. pusillus* confirms its treatment as a very rare species.

H. aureus, the species collected from Sinai to 1982 (Danin *et al.,* 1985) has not been traced in Egypt during the last twenty years. The author's field survey indicates that the species is extinct in Egypt. Actually, drought is the major growth limiting factor in the Egyptian desert (Amer and Sheded, 1998). The species extinction from Egypt is not only of national loss, but also of global concern. The percentage of the species loss in an arid region, with low biodiversity, is much higher than that in more species rich regions (McNeely, 2003).

H. boveanus the potential changes in distribution and a general indication of the expected future changes in its distribution are clearly seen in figure (1). The northward species migration, and the species extinction from the eastern desert, may relate to the severe aridity of this area especially during recent decades. The area receives no rain for years and may receive 22-25 mm/year (Hegazy and Amer 2001). Moisture content and low soil fertility are the main factors leading to vegetation changes in the Egyptian desert (Sharaf El-Din and Shaltout 1985, Amer and Sheded, 1998; Abd El-Ghani 2000). With drought stress affecting the plant it is considered among the vulnerable species.

H. boveanus is an endemic species recorded in Sinai for the first time; its origin is believed to be in Sinai. It then migrated southwest to the eastern desert through the Red Sea wadis, then to the western desert after crossing the Nile. The species in Sinai occupied the area and adapted over millions of years. Species isolated for a long time have evolved into new ones which remain in restricted distribution and are unique endemic species (Ghabbour, 1997).

Hyoscyamus muticus is the most common species among these studied species (Fig. 1). It is the only species extending westward to Libya and southward to Sudan and eastward to India (Boulos, 2002). This extensive distribution reflects the highest adaptive characters of the species to environmental conditions, wide ecological range and its high migration potentiality. *H. muticus* grows in perennial flourishing communities in desert wadis and fine sandy plains. The fine sandy soil supports its perennial growth. The dry upper layer of the surface acts as a protective layer; moisture is stored in subsurface layers and the presence of the permanently wet sub-surface layer is a well known phenomenon in Egyptian deserts (Kassas and Batanouny 1984).

However, although *H. muticus* is a common species in Egypt, its growth and distribution are under pressure of collection for medicinal purposes and the opportunities for sandy plains and wadis.

Hyoscyamus niger and *H. reticulates,* the first was found once in 1936 in an experimental garden. Both *H. niger* and *H. reticulatus* have never been recorded in wild localities in Egypt, so do not belong to the Egyptian flora.

Isozymes

Although most of the *Hyoscyamus* species growing in Egypt showed high morphological diversity, very little is known about the extent of genetic variation within these species. Isozymes were addressed here to clarify the inter-specific genetic relationships. Isozymes merely represent different structural configurations of the same polypeptide chain of an enzyme (Weeden, 1983).

EST, PRX and GOT electrophoretic patterns showed variability in the anodal region among the four *Hyoscyamus* species studied. The species showed a fixed heterozygous electrophoretic phenotype consisting of multi-isozyme variants specified by more than one gene. The number of bands observed on a gel for each enzyme may be resulted from several factors: (1) number of coding genes, (2) their allelic state (homozygous or heterozygous), (3) quaternary structure of the protein products and (4) their sub-cellular compart-mentalization (Wendel and Weeden, 1989).

EST, PRX and GOT electrophoretic patterns showed the presence of two bands for each enzyme (Table 1), this indicating that the studied *Hyoscyamus* species are heterozygotes with monomeric enzymes. In the absence of post-translation modification, heterozygous plants will be in two bands for monomeric enzymes (Crawford, 1983).

The uniformity between the electrophoretic patterns obtained from EST, PRX and GOT between *Hyoscyamus albus* and *H. desertorum* is expected from the annual form of both species and their tendency to grow in desert small wadis and runnels. Similarly the uniformity between isozyme patterns in *H. boveanus* and *H. muticus* was expected from their perennial growth form and their habitats.

The constructed dendrogram (Fig. 2) showed a close affinity between *H. boveanus* and *H. muticus*. The dendrogram supports the author's postulation of the origin of *H. boveanus* from *H. muticus* in Sinai mountainous area. Hepper (1998) mentioned that dried *H. boveanus* specimens are not easily differentiated from *H. muticus* because of their close similarity.

CONCLUSION

At least *Hyoscyamus* species now grow in Egypt. *H. pusillus* is very rare. The genetic relationships between the studied *Hyoscyamus* species reflect the potential importance of these wild species as a gene pool for the medicinally important *H. muticus*. Egypt is the far western species limit for four of the studied species. *H. boveanus* may have originated from *H. muticus* in Sinai.

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دراسة توزيع جنس السكران في مصر والعلاقة الوراثية بين أنواعه

وفاء محروس عامر

المعشبة – قسم النبات – كلية العلوم – جامعة القاهرة – الجيزة ١٢٦١٣ – مصر

تنتمي مصر إلى المنطقة الحارة الجافة من العالم والتي يتضائل فيها الاهتمام بالأنواع المهددة بالإنقراض. وتحتوي الفلورة المصرية على العديد من الأنواع الإقتصادية منها جنس السكران (الفصيلة: الباذنجانية) ويشتهر هذا الجنس بالأهمية الطبية. وقد تم تسجيل ثمانية أنواع تنتمي لهذا الجنس في الفلورة المصرية. وقد قامت هذه الدراسة بإعادة تقييم لهذا الجنس من حيث عدد الأنواع وتوزيعها ودرجة إنتشارها والعلاقات الوراثية بين هذه الأنواع. وأسفرت هذه الدراسة عن أن الفلورة المصرية تحوي خمسة أنواع فقط من جنس السكران وتم تحديد توزيعها الجغرافي بخطوط الطول والعرض. وجاء تقييم تواجد الأنواع كالاتي: نوعان مهددان بالانقراض هما الهيسيامس ألبص وديزرتورم ونوع شديد الندرة هو الهيسيامس بوسلس ونوع واحد متوطن هو الهيسيامس بوفيانص. أما الهيسيامس أوريس فقد انقرض من مصر والهيسيامس ريكليناتس لم يسجل في مصر. أما الهيسيامس ميوتكس فهو النوع الأكثر شيوعا في مصر.

وقد تمت دراسة العلاقات الوراثية من خلال دراسة لثلاثة من الأنزيمات المتناظرة هي: الإستيراز والبيروكسيداز وجلوتاميت أوكسالوأسيتات وقد تبين أن الهيسيامس بوفيانص المتوطن في مصرنتج من الهيسيامس ميوتكس في جبال سيناء. وتثبت هذه العلاقات أهمية الحفاظ على الأصول الوراثية للأقارب البرية للهيسيامس ميوتكس ذو الأهمية الطبية حتى نضمن استمراريته في انتاج هُجن قادرة على التأقلم مع المستجدات المناخية.