Egyptian Journal of Biology, 2003, Vol. 5, pp 27-34
© Printed in Egypt. Modern Press. **Egyptian British Biological Society (EBB Soc)**

Variation of seed protein of *Alkanna orientalis* subpopulations in relation to geographical isolation in St Katherine Protectorate, Sinai, Egypt

Somia S El-Akkad[1*] and Mona M Ali[2]

1. Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt
2. Biotechnology Research Centre, Suez Canal University, Ismailia, Egypt

ABSTRACT

Alkanna orientalis was surveyed from four wadi systems (El-Arbaein, El-Tofaha, El-Dir and Abu Seila), and two open flat sites (beginning of wadi El-Sheikh and El-Rasis) in the St. Katherine Protectorate to look at the variation within and among individuals in these sites using total seed storage proteins. A total of 55 clearly distinguishable polymorphic bands were observed from 78 individual plants collected from these six sites. Some bands were found to be common to all subpopulations, but each subpopulation was characterized by its own band(s). Shannon's diversity index was calculated for each subpopulation showing that subpopulations contain different levels of diversity with an overall average of $H_s = 2.97$. The level of variation was very low within populations as compared with the very high difference detected among them. The six subpopulations were found to contain different levels of protein diversity (as measured by Shannon's index of diversity): wadi El-Tofaha, Abu Seila, El-Arbaein and El-Dir contained the lowest diversity, while El-Rasis and El-Sheikh subpopulations contained the greatest diversity. Geographically, the first four sites are closed wadi systems (narrow wadi beds bounded by high mountains) and hence probably having very restricted gene flow with each other, while the last two sites (El-Rasis and El-Sheikh) are open flat areas expected to be subjected to occasional gene flow via seed transport from the other four wadis.

KEYWORDS: Boraginaceae, protein pattern, diversity index, dendrogram, arid environment

INTRODUCATION

Alkanna orientalis (Boraginaceae) is a viscous, glandular-hairy, yellowish green, short-lived perennial that grows to 1 m in diameter in sandy and rocky wadies: it only occurs in the St Katherine Protectorate within Egyptian ecosystems. The world distribution of *A. orientalis* stretches from Algeria to southern Greece, Turkey, Syria, Palestine and Sinai (Boulos 2000), but only at high elevations: it occurs at about 1500 m and above in the Sinai mountains. Subpopulations are fairly large, from around 50 plants in wadi El-Tofaha to several hundreds in the other subpopulations (Gilbert *et al*. 1996). *A. orientalis* has been studied as one of the medically important plants grown in Sinai: pyrrolizidine alkaloids were detected using GLC and GLC- MS (Hammouda *et al*. 1992; Roeder *et al*. 1992; El-Shazly *et al*. 1998).

Previous studies by Willmer *et al.* (1994) and Gilbert *et al.* (1996) have provided a detailed background of morphological variation between subpopulations of *A. orientalis* at St. Katherine. Wolff *et al.* (1997) studied the plants using RAPDs (Randomly Amplified Polymorphic DNA); the data showed that there were genetic differences among all subpopulations, with wadi El-Dir being the most genetically different from the other subpopulations of El-Arbaein, El-Tofaha and El-Rasis. Their results suggest that most gene dispersal is between wadies El-Tofaha and El-Arbaein subpopulations and the plain (El-Rasis) subpopulation.

The work of Willmer *et al*. (1994) and Gilbert *et al.* (1996) explored the possibility that morphological variation in the *Alkanna* population is determined by the activity of the main plant pollinator (*Anthophora pauperata*), which can potentially transfer pollen grains between subpopulations. However, the data of Wolff *et al.* (1997) showed that floods may

* Address for Correspondence

play the main role in gene flow between subpopulations by moving the seeds between sites. This work aims to add more information about the genetic diversity of *A. orientalis* subpopulations in the area by adding more sites within the protectorate and using different genetic markers (protein). This is recommended when studying genetic diversity of populations and species, and especially when exploring relationships among populations (Nieto-Lopez *et al.* 2003; Karihaloo *et al.* 2002).

MATERIALS AND METHODS

Plants were collected in 2003 from *Alkanna orientalis* growing in St. Katherine from four wadis (El-Arbaein, El-Dir, El-Tofaha and Abu Seila) and two open plains (an open flat area of wadi El-Sheikh near the town of St Katherine, and El-Rasis). All study sites occur within 10 km of the town of St Katherine (see Figure 1). The area of study is described in detail in Wolff *et al.* (1997), but extra sites are included here: Abu Seila, a narrow small wadi behind the plain of El-Raha opposite the mouth of wadi El-Dir; and wadi El-Sheikh, which starts from the mouth of El-Dir and El-Raha plain and continues towards the west to the Gulf of Suez - this wadi drains the water from all the studied wadis, hence its importance in this study.

Dry seeds were taken from randomly collected plants during late April and early May (the end of the season, before seed dispersal). The number of plants analyzed were 18 from wadi El-Arbaein, 14 from wadi El-Dir, 18 from El-Tofaha, 9 from Abu Seila, 8 from El-Rasis and 10 from Wadi El-Sheikh.

Half gram of *Alkanna* seeds was ground to a fine powder and then mixed with 1.5 ml extraction buffer (10 g sucrose, 5 ml 2-mercaptoethanol, 2 g SDS and 2.422 g Trizma base, pH adjusted to 8.5 and made up to 100ml with distilled water), vortexed and left overnight at 4°C. It was then centrifuged at 5000 rpm for 20 min. and the supernatent transferred to a fresh tube. Aliquots of the supernatant were analyzed by slab gel electrophoresis (Laemmli 1970) using 12% polyacrylamide gels. A wide range of standard proteins of known molecular weights (20.6, 28.9, 34, 49.7, 80, 124, 209 KDa) were run on a corresponding gel and used for characterization and determination of molecular mass of *Alkanna* polypeptides. The protein of each individual plant was extracted separately and applied to the electrophoresis unit in a separate lane; the experiment was repeated twice for all individuals in all localities, and a consistent protein pattern (molecular weight and concentration) was found for all individual plants.

Following electrophoresis the gel was stained with a solution containing 0.002% Commassie Blue-R, and then de-stained with a mixture of glacial acetic acid, methanol and water. Once the position and matches of fingerprint bands had been scored, the data were ready for scanning using a LKB Recording Laser Densitometer equipped with LKB Recording Integrator.

The data matrix was built based on the presence/absence of bands, and used to construct a neighbour-joining (NJ) tree of individuals using Euclidean distances computed between all pairs of individuals. Shannon's index of diversity was calculated from the frequencies of the protein bands within each subpopulation and also overall subpopulations (King & Schaal 1989), to obtain estimates of the within-subpopulations genetic diversity (H_s) and total diversity in the population (H_r). The equation used was: $H = -\sum p_i \log_2 p_i$, where p_i is the frequency of a band. A one-way ANOVA was used to test for differences among sites using SPSS version 11.0. A dendogram of the relationships among individuals was constructed using the UPGMA method on the matrix of Euclidean distances.

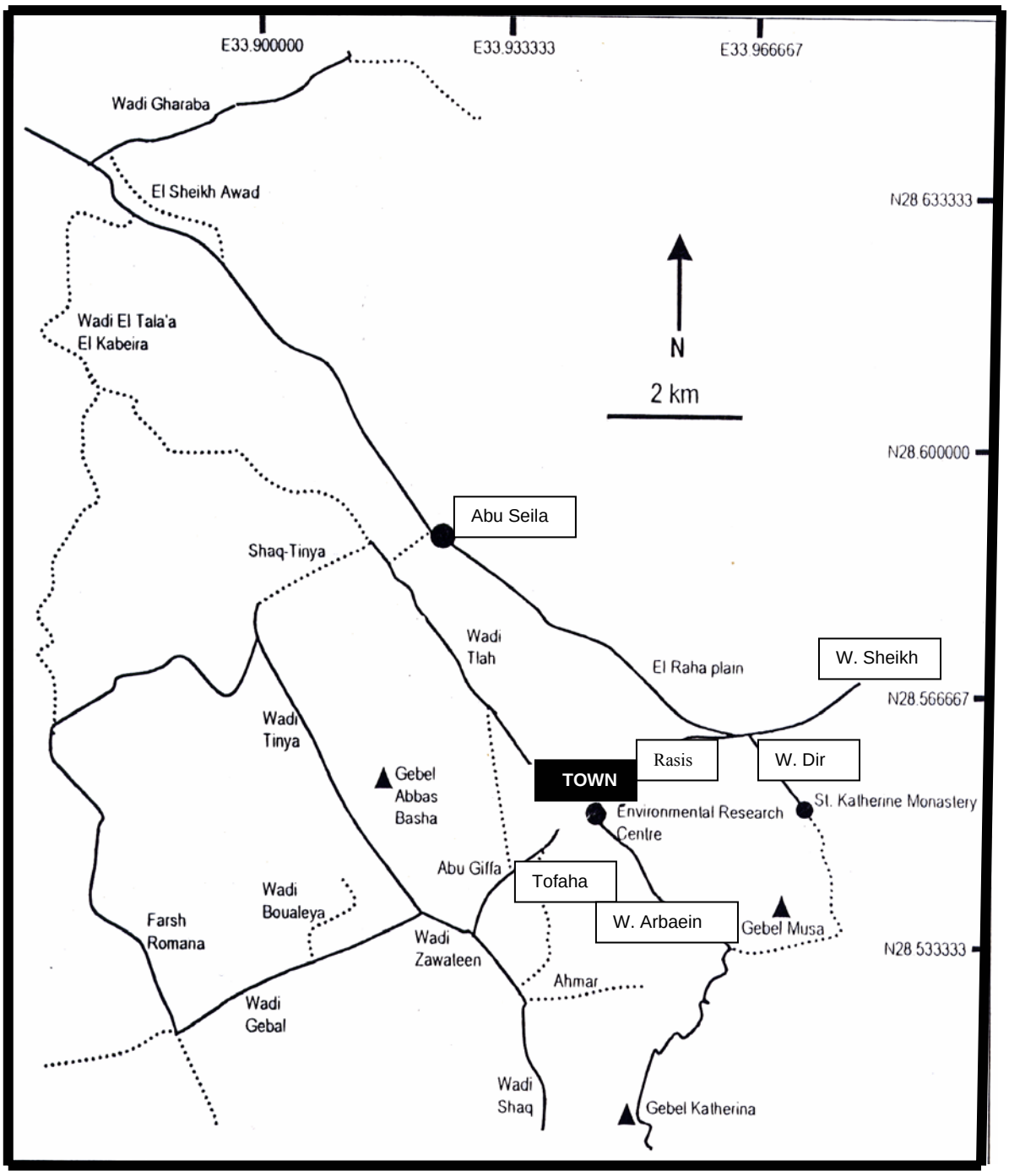

Figure 1: Map of the St Katherine town showing the study sites (names within square boxes) where *Alkanna orientalis* individuals were sampled.

RESULTS

Using SDS-PAGE, the seed storage protein from 78 individuals of *Alkanna orientalis* gave a total of 55 separate polymorphic bands. These bands were distributed along the gel with molecular weights ranging from 137 to 6 KDa. A considerable amount of genetic variation within and between subpopulations was evident in both the number of bands and their molecular weights. The number of bands obtained from plants of each location ranged from 19-39 bands depending on the site of collection. Individuals from wadi El-Sheikh

were found to have the highest number of bands (39 bands), whereas wadi El-Arbaein subpopulation possessed lowest number of bands (19 bands).

The data matrix based on protein pattern (Figure 2) shows that there are some bands in common among all subpopulations, with molecular weights of 15, 28 and 49 KDa. It was observed also that each subpopulation was characterized from the rest by the presence or absence of one or more bands. Wadi El-Tofaha has unique bands (at 5, 30, 44, 67 and 77 KDa) which characterized its individuals from all others. El-Tofaha lacks two bands (22 and 45 KD) which are found in all other subpopulations. Wadi El-Sheikh was characterized and separated from the other subpopulations by the presence of bands with molecular weights 48,100, 118 and 163 KDa. Abu Seila, El-Dir and El-Arbaein were characterized also by bands of molecular weights 68, 10 and 91 KDa respectively. El-Rasis had no characteristic bands.

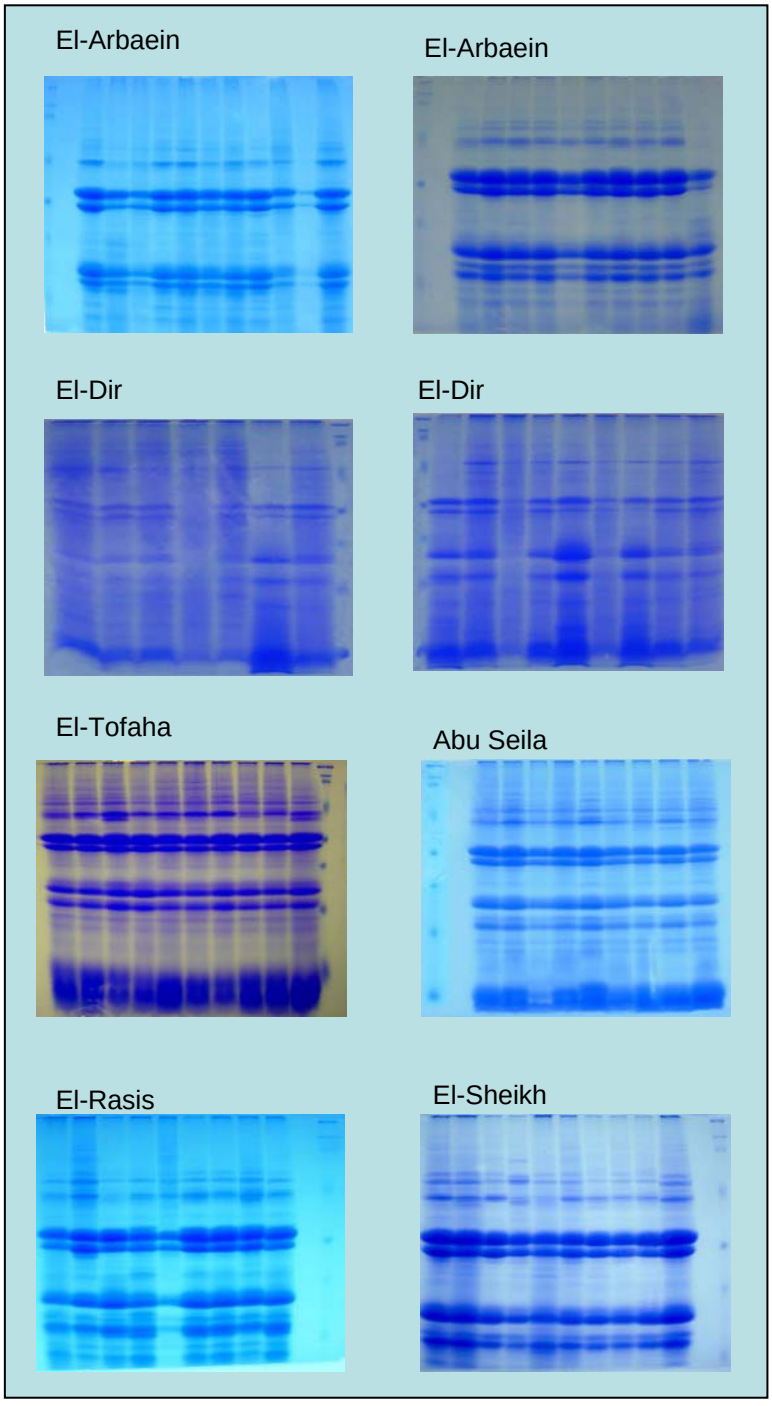

Figure 2: Protein pattern of subpopulations of *Alkanna orientalis* collected from different wadi systems in the St Katherine Protectorate. Each lane presents an individual plant. There are standard markers either on the right or left side of each gel, with molecular weights (from top to bottom) of 209,124, 80, 49.7, 34, 28.9, 20.6 KDa.

Shannon's diversity index was calculated overall for each subpopulation in turn (H_s for El-Arbaein = 1.33, El-Dir = 2.83, El-Tofaha = 0.391, Abu Seila = 1.2, El-Rasis = 8.6, El-Sheikh = 3.45), showing that subpopulations contain different levels of diversity with an overall average of H_s = 2.97. Shannon's diversity index for the total population was estimated to be H_t = 25.07, and the proportion of total diversity distributed within populations (H_s/ H_t = 0.12) was found to be smaller than that present among subpopulations [(H_t- H_s)/ H_t = 0.88].

Diversities for individual plants were used to find out if there are any differences among the subpopulations of *Alkanna orientalis* in the six sites, using a one-way Anova. This showed highly significant differences among sites ($F_{5,72}$ = 25.3, P<0.0001), as shown in Figure 3. Wadi El-Sheikh is different from other wadis with a higher individual diversity, whilst wadi El-Arbaein has a very low individual diversity.

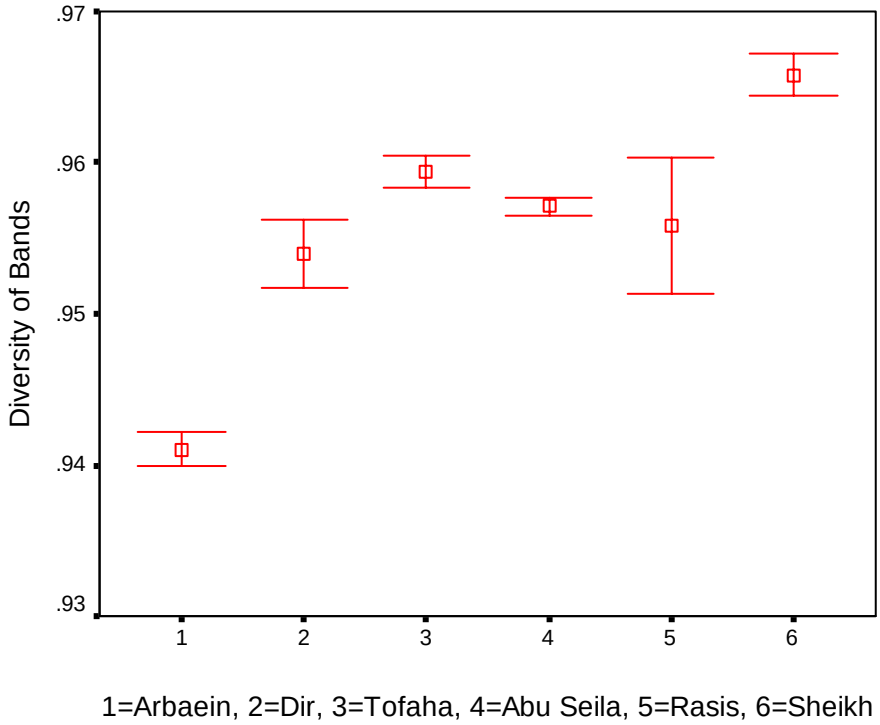

Figure 3: The difference in the mean individual diversity of bands based on the protein pattern data among the six sites, showing the highly significant differences among *Alkanna orientalis* subpopulations in different sites

To depict the relationships among individuals based on their protein profile, a neighbour-joining (NJ) tree was constructed from the matrix of Euclidean distances among individuals. It is evident from the tree (Figure 4) that in general individuals from a given subpopulation tend to cluster together, and are therefore more genetically similar than individuals from different subpopulations.

The cluster analysis of the protein-pattern characters indicate that the *Alkanna* population in St. Katherine consists of two major groups, one comprising wadi El-Tofaha and the other all remaining subpopulations. The second group was subdivided into two branches: the first branch has two clusters representing wadi El-Sheikh and Abu Seila; and the second branch has three clusters representing wadi El-Dir, El-Rasis and El-Arbaein. It is clear from these results that the wadi El-Tofaha subpopulation has the lowest genetic

diversity of all subpopulations under study since it has the smallest diversity index (0.391) and its individuals cluster together in a separate group.

DISCUSSION

The present study is a continuation of study began by Willmer *et al.* (1994) and Gilbert *et al.* (1996) who provided a detailed background of morphological variation between subpopulations of *A. orientalis* at St. Katherine. It was postulated that because of pollinator behaviour, gene flow by the pollinator would be highly restricted (Willmer *et al.* 1994) and this might bring about genetic divergence among subpopulations. Genetic evidence suggested that limited gene flow and drift were the main factors that have led to the evolution of these genetic differences (Wolff *et al.* 1997), and confirmed that the high mountain ridges between the wadis at St Katherine make movement by bees across the ridges unlikely. From the pattern of genetic relationship, seed transport by flash floods, which occur very regularly in Sinai at the end of winter, seemed to be a more likely cause of extensive gene flow in the population of *A. orientalis* at St Katherine. The protein analysis by SDS- PAGE of mature seeds from *A. orientalis* reported here also shows that that these subpopulations located in different wadis are genetically different from each other.

The analysis of the banding patterns suggest that most gene dispersal is between El-Sheikh and Abu Seila subpopulations. Gene dispersal is also between wadi El-Dir, El-Rasis, and El-Arbaein subpopulations. Wadi El-Tofaha subpopulation is the least genetically diverse (as recorded by Shannon index) and the most genetically different of the six subpopulations under study (as shown in the cluster analysis), probably reflecting the fact that plants sampled from wadi El-Tofaha were the most distant from all other plants sampled, and that wadi El-Tofaha is the most isolated from the other subpopulations.

The six subpopulations were found to contain different levels of protein diversity (as measured by Shannon's index of diversity): the wadi El-Tofaha, Abu Seila, El-Arbaein and El-Dir contained the smallest diversity, while El-Rasis and El-Sheikh subpopulations contained the greatest diversity. Geographically, the first four sites are narrow wadi beds bounded by high mountains, whilst the other two sites are open flat areas and are expected to be subjected to occasional gene flow via seed transport from the wadi subpopulations.

From the results of both the present and the previous study (Wolff *et al.* 1997) on *Alkanna* populations in St Katherine, the level of inter-population variation differs according to the degree of polymorphism of the corresponding markers. Both proteins and RAPDs proved to be polymorphic markers, but the former were more variable and more able to distinguish among the different subpopulations. Wolff *et al.* (1997) found that four subpopulations ("Plain" i.e. El-Rasis, El-Arbaein, El-Dir and El-Tofaha) contained similar levels of diversity and that the within-subpopulation diversity was greater than among subpopulations. The results of the present study suggest that subpopulations contain different levels of genetic diversity, and that the within-subpopulation diversity (0.12) is smaller than among subpopulations (0.88). This indicates that the protein method was more able to distinguish among these subpopulations, an idea confirmed by Nieto-Lopez *et al*. (2003) working on the genetic diversity in wild Spanish populations of *Thinopyrum junceum* and *Thinopyrum junceiforme* using endosperm proteins and PCR-based markers.

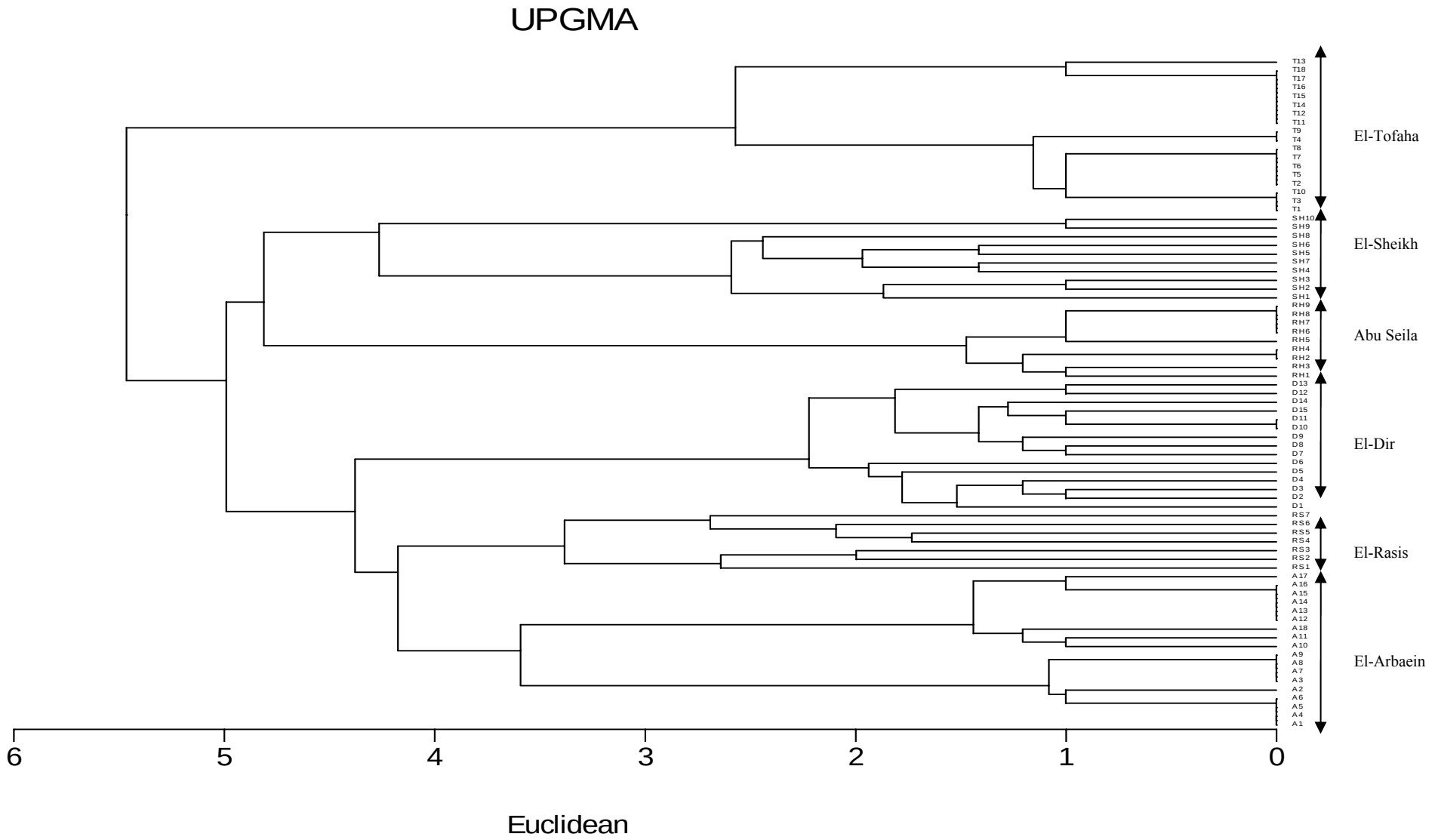

Figure 4: Dendogram showing the similarity indices of the populations of *Alkanna orientalis* in different wadi systems in St Katherine Protectorate.

REFERENCES

Boulos L (2000). Flora of Egypt. Al-Hadara publishing, Cairo, Egypt, 188pp.

El-Shazly A, El-Domiaty M, Witte L & Wink M (1998) Pyrrolizidine alkaloids in members of the Boraginaceae from Sinai (Egypt). *Biochemical Systematics & Ecology.* 26 (6): 619- 636.

Gardiner SE, Forde MB & Slack CR (1986) Grass cultivar identification by sodium dodecylsulphate polyacrylamide gel electrophoresis. *New Zealand Journal of Agricultural Research* 29: 193-206.

Gilbert F, Willmer P, Semida F, Ghazoul J, Ghazoul J & Zalat S (1996) Spatial variation in selection in a plant pollinator system in the wadis of Sinai, Egypt. *Oecologia*. 108: 479- 487.

Hammouda FM, Ismail SI, Hassan NM, Tawfiq WA & Kamel A (1992) Pyrrolizidine alkaloids from *Alkanna orientalis* (L.) Boiss. *Qatar University Science Journal* 12: 80-82.

Karihaloo JL, Kaur M & Singh S (2002) Seed protein diversity in *Solanum melongena* L and its wild and weedy relatives. *Genetic Resources & Crop Evolution.* 49(6): 533- 539.

King LM & Schaal BA (1989) Ribosomal DNA variation and distribution in *Rudbeckia missouriensis*. *Evolution* 43: 1117- 1119.

Laemmli, UK (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 277: 680- 685.

Nieto–Lopez RM, Soler C & Garcia P (2003) Genetic diversity in wild Spanish populations of *Thinopyrum junceum* and *Thinopyrum junceiforme* using endosperm proteins and PCR–based markers. *Hereditas* 139(1): 18- 27.

Willmer P, Gilbert F, Ghazoul J, Zalat S & Semida F (1994) A novel form of territoriality: daily paternal investment in an anthophorid bee. *Animal Behaviour*. 48: 535- 549.

Wolff K, El-Akkad S & Abbot J (1997) Population substructure in *Alkanna orientalis* (Boraginaceae) in the Sinai Desert, in relation to its pollinator behaviour. *Molecular Ecology.* 6: 365- 372.

الملخص العربى

الإختلافات فى بروتينات بذور عشائر نبات اللوبيد (*Alkanna orientalis*) وعلاقته بالعزل الجغرافى فى محمية سانت كاثرين - جنوب سيناء - مصر

سمية صلاح الدين العقاد[1] - منى محمود على[2]

1- قسم علم النبات - كلية العلوم - جامعة عين شمس - العباسية - القاهرة
2- معهد التقنية الحيوية - جامعة قناة السويس - الإسماعيلية

تم أثناء هذه الدراسة حصر لنبات اللوبيد (*Alkanna orientalis*) فى أربعة أودية ضيقة بمحمية سانت كاثرين وهم: وادى الأربعين - وادى التفاحة - وادى الدير - وادى أبو سيلة بالإضافة إلى اثنين من المناطق المنبسطة والواسعة فى المحمية وهما وادى الشيخ ومنطقة الرصيص.

تم جمع بذور بعض الأفراد من تلك الوديان وتحليل تركيبها البروتينى بإستخدام جهاز التفريد الكهربائى لدراسة الأختلافات بين الأفراد وبعضها البعض داخل الوادى الواحد وكذلك بين الأفراد فى الوديان المختلفة.

تم تمييزعدد 55 من البروتينات المتباينة فى بذور ثمانية وسبعون من أفراد تم جمعهم بصورة عشوائية من مناطق الدراسة. بعض البروتينات التى تم حصرها كانت ممثلة فى جميع الافراد فى تحت العشائر المختلفة والبعض الأخر كان مميزاً لكل تحت عشيرة.

باستخدام النتائج السابقة تم تعيين معدل شانون للتنوع (Shannon diversity index) سواء بين الأفراد داخل نفس المنطقة أو بين الأفراد فى المناطق المختلفة ولقد أثبتت النتائج أن التحت عشائر تحتوى على مستويات مختلفة من التباين بين افرادها، وأن مستوى التباين كان منخفضا داخل كل تحت عشيرة اذا ما قورن بالتباين الموجود بين كل تحت عشيرة وأخرى.

تقترح النتائج أن المناطق فى محمية سانت كاثرين تعمل بصورة منعزلة عن الأماكن القريبة منها مما يوحى بوجود عزل بينها وخصوصاً فى مناطق الوديان حيث ثبت أن وادى التفاحة والأربعين وأبو سيلة والدير تحتوى على تنوع بروتينى منخفض مقارنة بأفراد وادى الشيخ والرصيص والتى يزداد التنوع البروتينى لأفرادها.

من الناحية الجغرافية نجد أن مناطق الوديان الأربعة: الأربعين - التفاحة - الدير - أبو سيلة هى وديان ضيقة توجد فى أماكن مرتفعة ويحدها جبال شاهقة الأرتفاع وبناء على ذلك فهى تحتوى على أنسياب جينى محدود فيما بينها. وعلى النقيض فى منطقتى وادى الشيخ والرصيص وهى مناطق واسعة، مفتوحة بالإضافة إلى إنخفاضها مقارنة بالوديان الأخرى مما يجعلها عرضة لانتقال بذور أفراد الوديان المختلفة اليها من خلال حركة المياه أثناء الأمطار أو السيول وهذا يؤدى بدوره إلى حدوث انسياب جينى على فترات.