

Phylogenetic Relationship and Similarity Indices of Some *Acacia* Species Using Seed Protein Analysis

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ABSTRACT

Seed protein patterns were investigated in seven *Acacia* species by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Total number of polymorphic bands represented was forty two. Phylogenetic analysis based on the protein pattern showed that *A. nilotica nilotica* and *A. nilotica tomentosa* were very close to each other. Great similarity in their electrophoretic patterns supports their classification as subspecies of *A. nilotica*. There is also great similarity between *A. nilotica* and the cultivated species, *A. farnesiana*. The data show that *A. laeta*, *A. seyal*, *A. etbaica*, *A. tortilis raddiana* and *A. pachyceras* were separated singly one by one due to their characteristic protein pattern which is unique to each of them.

Key Words: *Acacia* species; Similarity; Protein pattern; SDS-PAGE; Conservation

INTRODUCTION

There are 129 *Acacia* species in Africa. They are intermediate in plant succession and colonize degraded land. They restore soil fertility and can be maintained indefinitely in agricultural systems. Despite their benefits, they are disliked for their thorns and invasiveness (Barnes 2001). *Acacia* are allelopathic, and their toxic aqueous leachates are used to detect differences in the patterns of expression of cytoplasmic root proteins in crop plants, indicative of biochemical alterations at the cellular level. Mujoo *et al.* (2001) suggested that the triterpenoid saponins from *A. victoriae*, an Australian desert tree, have potential as novel anti-cancer agents.

Previous studies on the seeds suggested that *Acacia* species are a possible source of protein for human use (Bukhari, 2002). Acacias obtain their nitrogen from ground water rather than from the atmosphere, but can produce more crude protein per hectare than many grain crops: the protein content of *A. mellifera* (41.6%) was close to that of soybean (42.8%) (Prakash *et al.*, 2001). They are also useful as fodder for livestock (Ramirez *et al.*, 2000; El-Seed *et al.*, 2002). Bedouin use *Acacia* leaves and pods for fuel and fodder, and also for health remedies (Ashkenazi, 1995), but there are problems of identification. It is possible for example that the various subspecies of *A. tortilis* are more or less interchangeable with regard to their products but not for their ecological requirements; similarly, problems in interpreting data also occur with *A. nilotica* if the relevant subspecies is not mentioned.

Phylogenetic relationships among taxa are particularly valuable for conservation management of threatened taxa in an evolutionarily diverse flora (Byrne *et al.*, 2001). The phylogeny of some Egyptian *Acacia* species and subspecies was investigated in this study.

MATERIALS AND METHODS

Seeds of the following *Acacia* species were kindly provided by the Faculty of Science, South Valley University, Aswan, Egypt: *pachyceras* O. Schwartz (= *gerrardii* Benth. subsp. *negevensis*), *tortilis* (Forssk.) Hayne (subsp. *raddiana* (Savi) Brenan), *etbaica* Schweinf., *seyal* Del., *laeta* R.Br., *farnesiana* Domin and *nilotica* (L.) Del. (subsp. *nilotica* and *tomentosa* (Benth.) Brenan)] (Fig. 1).

Samples were prepared as follows. Half a gram of seeds of each species were placed in liquid nitrogen and then ground to a fine powder and 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 & made up to 100 mL with distilled water). The mixture was left for 5 h at 4°C, centrifuged for 20 min at 5000 rpm and then aliquots of the supernatants analyzed by slab gel electrophoreses.

Electrophoresis (Laemmli, 1970) used 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 *Acacia* polypeptides.

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 finger print bands have been scored, the data are ready for interpretation. Quantitative evaluation of the protein pattern by eye may be sufficient to give a quick answer to the investigation problem. The gel was also scanned using a LKB Recording Laser Densitometer equipped with LKB Recording

Integrator. Pairwise similarities of the species used a 'similarity index', S (Nei & Li, 1979), calculated from band-sharing according to the formula:

$$S = 2 N_{ab} / (N_a + N_b)$$

Where N_a and N_b represent the total number of bands present in lanes a and b, respectively, and N_{ab} is the number

Fig. 1. The legumes of different *Acacia* species showing their shapes



A. farnesiana



A. pachyceras



A. laeta



A. seyal



A. etabica



A. nilotica tomentosa

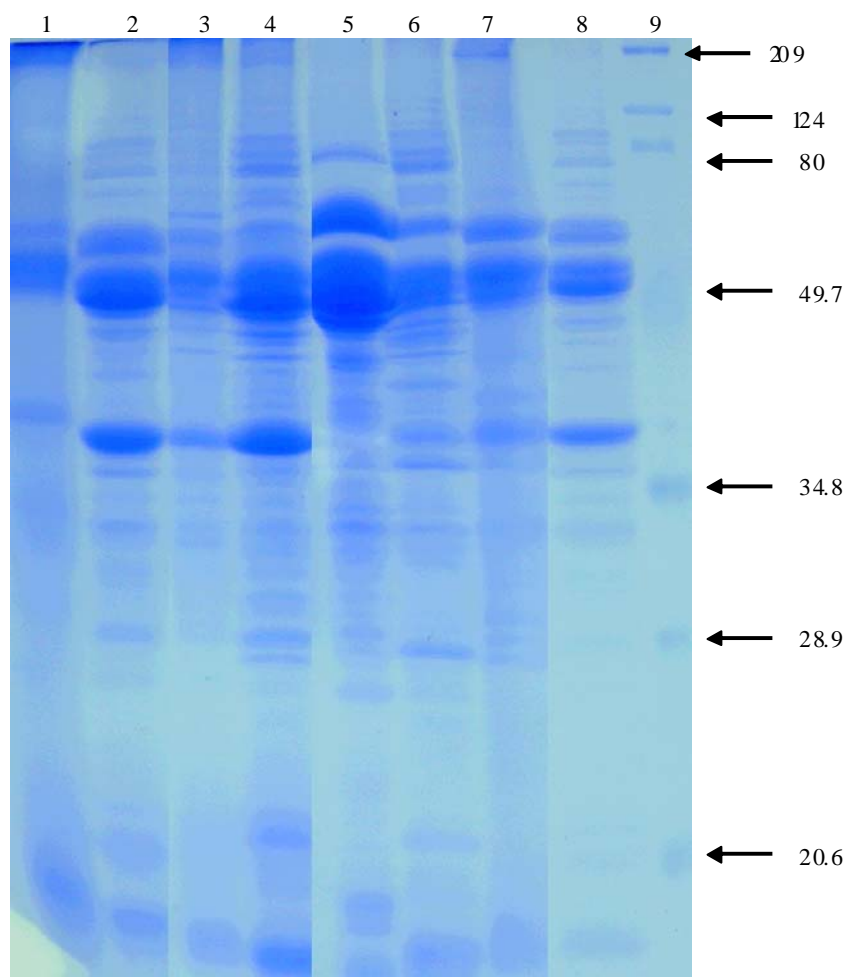


A. nilotica nilotica



A. tortilis raddiana

Fig. 2. Electrophoretic patterns of the seeds of *Acacia* species: 1: *A. pachyceras*, 2: *A. tortilis raddiana*, 3: *A. nilotica tomentosa*, 4: *A. etabica*, 5: *A. seyal*, 6: *A. farnesiana*, 7: *A. nilotica Nilotica*, 8: *A. laeta*. Number 9 is a wide range molecular weight standard (from top to bottom, 209, 124, 80, 49.7, 34, 28.9 & 20.6 KDa)

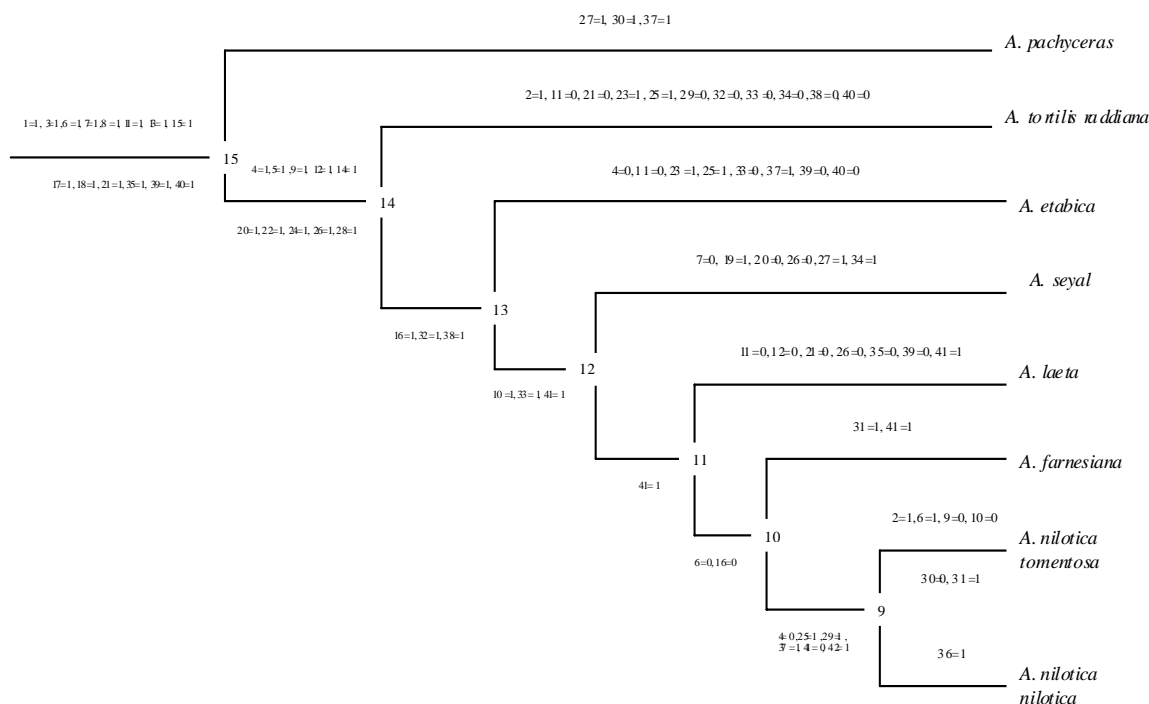


All the *Acacia* species studied here except *A. nilotica* and *A. farnesiana* are endanger of extinction. *A. pachyceras* suppose to be the oldest species since it has the fewest bands, lacking the bands of molecular weights 96, 51, 39, 37 and 32 KDa found in all other species (Table I). If priority-setting processes based on the present study were to be applied to these species, *A. pachyceras* would have a greater value for genetic conservation management in Egypt. There is widespread concern over the mortality of *A. tortilis* and *A. pachyceras* in the Negev desert (Danin, 2000). Increased risk of extinction may be due to habitat destruction, and fragmentation of populations (Shrestha *et al.*, 2002), and also because of environmental, demographic and genetic stochasticity (Fisher & Matthies, 1998). This idea was supported by Ward and Rohner (1997), who concluded that the total mortality of *Acacia* trees varies widely and may reach as high as 61% in some populations, reputedly an effect of water limitation imposed by anthropogenic misuse.

The phylogenetic analysis through protein marker

gives us a general idea about these seven acacian species which can help with the limited data already available about the relatedness between them. Also, this paper presents and sheds light on the *Acacia* proteins (*nilotica* & *farnesiana*) which grow on the Nile banks of Delta region and occasionally in desert with reasonable water resources. The other ones (*pachyceras*, *tortilis raddiana*, *etabica*, *seyal* & *laeta*), which grow in much arid environment of Egyptian desert with shortage of water, both categories showed variations in their protein patterns. Therefore, very much attention should be given to arid species since they are under severing threats ecologically. Furthermore, DNA markers should be used to show the genetic structures and variations within the populations of each species, to measure the degree of risk facing these populations. The long term goal is to help conserving these valuable plants which showed some signs as an important resource for desert ecosystem and as a refuge for many wild animals in arid environment.

Fig. 3. Cladogram of the seven species of *Acacia* showing the relationships between them based on seed protein patterns. Each number at the node or branching represents a hypothetical ancestor and the number on the lines correspond to the characters tabulated in table 1(data matrix). (length = 72, ci = 64)



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