JKAU: Sci., vol. 6, pp. 17-28 (1414 A.H. / 1994 A.D.)

Electrophoretic Analysis of Protein and Selected Enzymes of Acacia Seeds from Saudi Arabia

ALI A. AL-HELAL
Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT. The water-soluble, diluted salt-soluble and ethanol-soluble proteins of seed extracts from eight Saudi Arabia acacia species were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Qualitative differences were evident in the electrophoretic patterns of all protein fractions.

Also, the anodic isoenzyme patterns of amylase, glutamate oxaloacetate aminotransferase (GOT), non-specific esterase (EST) and phosphorylase in seed extracts were separated by polyacrylamide gel electrophoresis (PAGE). The studied enzymes were found to be highly polymorphic. It is clear from the results that the isoenzyme patterns can be used together with protein banding patterns as markers to estimate both genetic variation at biochemical level and taxonomical relationship among the species.

Introduction

The tropical and subtropical genus acacia (Mimosaceae) occurs on all continents except Europe and Antarctica. The number of species recorded in this genus ranges from 600 to 900[1]. Only 12 species have been reported in the different phytogeographic regions of the Kingdom of Saudi Arabia[2].

Acacia seeds of Saudi Arabia have been studied by a limited number of investigators, and only in regard to seed germination[3], protein of seedlings[4] and seed morphological differences between species[5]. There appears to be a lack of information concerning seed analysis and genetic variations at the biochemical level. However, acacia seeds of Australia have been analyzed by several investigators[6-9]. In a few cases, seed analysis has been employed in taxonomical studies[7,9].

Protein and isoenzyme analysis by gel electrophoresis has been used in higher plants to study various problems in breeding[10], genetics[11], taxonomy[12,13], physiology[14] ... etc. This work was initiated to estimate genetic variation within acacia species of Saudi Arabia by analyzing protein banding patterns and selected isoenzymes of seeds. Such analysis would provide a great deal of useful information, from both taxonomic and biochemical standpoints.

Material and Methods

The acacia seeds were supplied kindly by Dr. Al-Farraj, M.M., Department of Botany and Microbiology, College of Science, KSU (see reference[5] for seed collection).

Protein Extraction

The H_2O-soluble protein (albumin) was extracted by homogenizing 30 mg seed meal in 500 µl distilled water in a polyethylene tube, overnight at 4°C. The crude extract was centrifuged at 4000 rpm for 6 min and the supernatant was used as the albumin fraction. The pellet was washed with H_2O and extracted with 500 µl, 0.2 M tris/HCl buffer, pH 6.8, as described above, and the supernatant was used as the globulin fraction. The pellet was washed with the same buffer and extracted with 60% (w/v) ethanol as above, and the supernatant was used as prolamine fraction. Before electrophoresis, sodium dodecyl sulphate (SDS) and sucrose were added to the extract in total concentrations of 2% and 20% (w/v) respectively.

Enzyme Extraction

The enzymes were extracted with 0.2 M tris/HCl buffer, pH 6.8 as described before[11].

Electrophoresis and Staining

Electrophoresis and total protein staining were carried out as in reference[11]. Enzymes were detected on the gels as described in reference[15].

Results

H_2O-Soluble Protein

The results presented in Fig. 1 show the zymogram protein patterns of albumin fraction, extracted from the seeds, and analyzed by SDS-PAGE. It is clear from the figure that the species Tortilis had no clearly detectable protein bands, while the extracts from the other species were resolved into major and minor distinct electrophoretic bands. At least a total of 30 different major bands and several minor bands were resolved in all seed extracts. There were qualitative and quantitative differences between the species studied. The seed extracts from five species, namely, Laeta, Seyal, Nilotica, Farnesiana and Catechu appeared to have two common slow moving, heavely stained bands, although there were differences in their staining intensity and the relative mobility (Rf) between the different species.

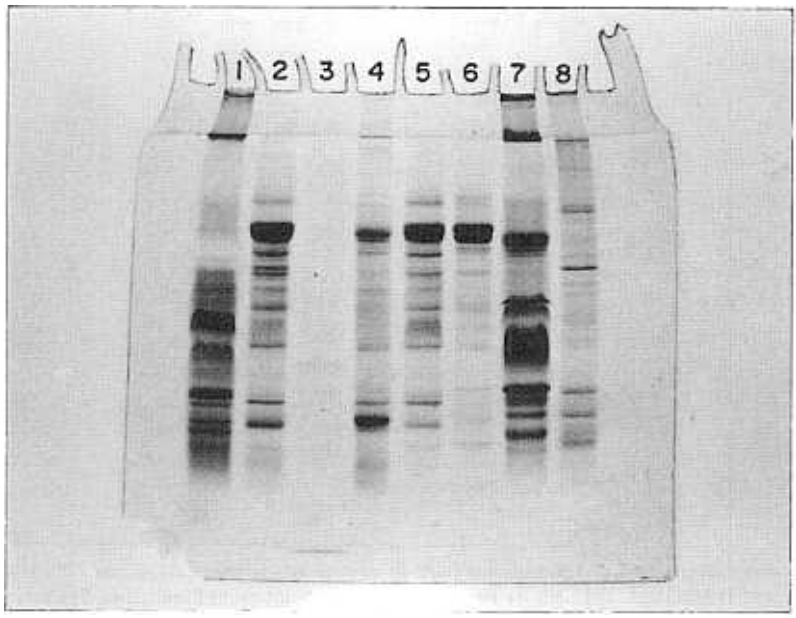


FIG. 1. Zymogram patterns of H_2O-soluble protein of acacia seed extracts on 17% SDS-PAGE. Track: 1) Mellifera, 2) Laeta, 3) Tortilis, 4) Seyal, 5) Nilotica, 6) Farnesiana, 7) Catechu, and 8) Prosopis. Sample loaded was 30 μl.

Tris Buffer-Soluble Protein

The protein zymogram patterns of globulin fraction are shown in Fig. 2. It is clear from Fig. 1 and Fig. 2 that there were several bands common to both albumin and globulin fractions, but more prominent in the albumin fraction. It is evident from the results that protein banding patterns of globulin fraction were species-specific.

Ethanol-Soluble Protein

The results presented in Fig. 3 show that seed extracts from the species Tortilis and Seyal had no bands in the prolamine fraction, while the seed extracts from the other species displayed several distinct minor bands, except the seed extracts from the species Nilotica and Farnesiana, where each extract contained 3 heavily stained bands.

Mixed Fractions of Proteins

The protein electrophoretic patterns of the mixed fractions appeared to be additive of the banding patterns of the separate fractions (Fig. 4). However, in the seed extract from the species Mallifera, two slow moving bands appeared only in the mixed fraction and they might represent subunits of aggregate proteins. Also, the

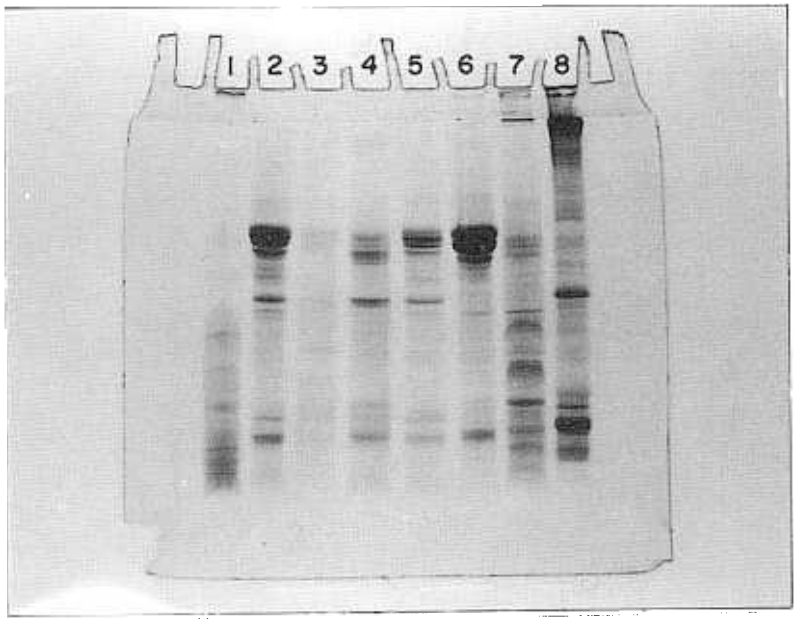


FIG. 2. Zymogram patterns of Tris-buffer-soluble protein of acacia seed extracts on 17% SDS-PAGE. Track: 1) Mellifera, 2) Laeta, 3) Tortilis, 4) Seyal, 5) Nilotica, 6) Farnesiana, 7) Catechu, and 8) Prosopis. Sample loaded was 50 μl.

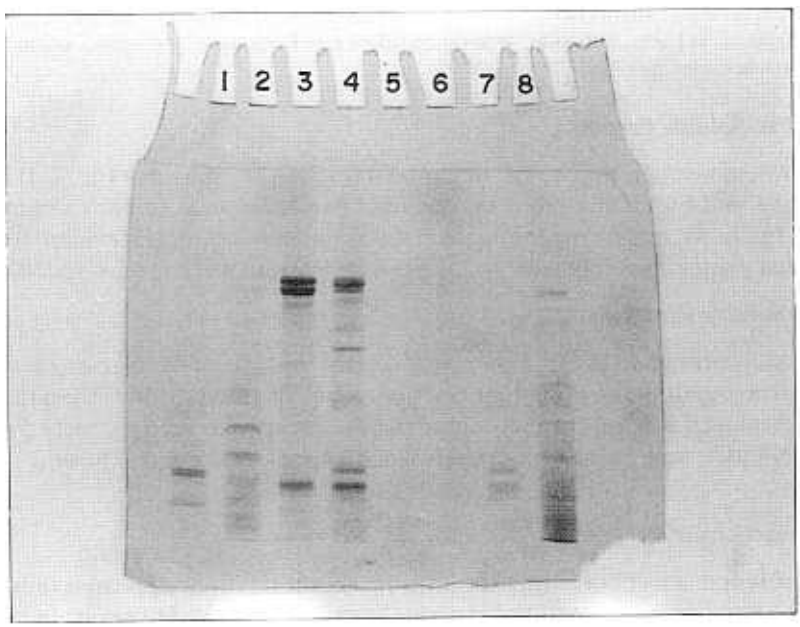


FIG. 3. Zymogram patterns of ethanol-soluble protein of acacia seed extracts on 17% SDS-PAGE. Track: 1) Prosopis, 2) Catechu, 3) Farnesiana, 4) Nilotica, 5) Seyal, 6) Tortilis, 7) Laeta, and 8) Mellifera. Sample loaded was 50μl.

electrophoretic protein patterns of mixed fractions from the species Prosopis had two slow migrating bands, and probably one fast moving band, that were not detected in the electrophoretic patterns of the separate fractions.

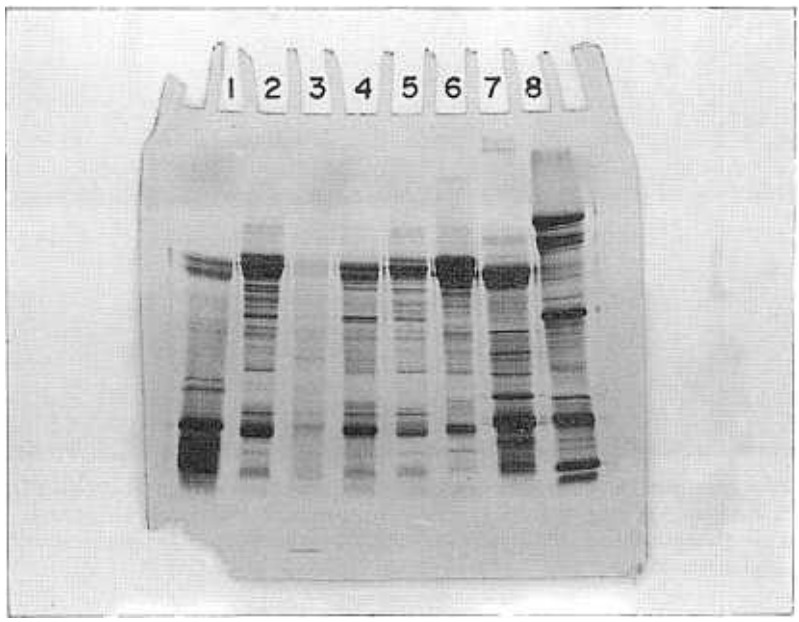


FIG. 4. Zymogram patterns of mixed protein fractions of acacia seed extracts on 17% SDS-PAGE. Track: 1) Mellifera, 2) Laeta, 3) Tortilis, 4) Seyal, 5) Nilotica, 6) Farnesiana, 7) Catechu, and 8) Prosopis. Sample loaded was 30 μl.

Amylase Patterns

The results in Fig. 5 show the electrophoretic patterns of amylase isoenzymes in gel incubated in a phosphate-free buffer. It is evident from the results that seed extracts from the species Seyal, Nilotica and Farnesiana had no apparent band, for amylase activities. The seed extract from the species Tortilis had a diffusible enzyme activity which was not separated to clear distinct electrophoretic bands. The seed extracts from the species Mellifera had two slow moving components and two fast moving components. The seed extract from the species Farnesiana displayed two very fast moving bands and the seed extracts from the species Catechu and Prosopis each had one very active component and several minor ones.

The results in Fig. 6 show the electrophoretic patterns of amylase and phosphorylase in gels incubated in a phosphate-containing buffer. It is evident from the figure that the zymogram patterns in gels incubated in phosphate-containing buffer contained bands that were not present in gel incubated in phosphate-free buffer and these bands represent phosphorylase activities.

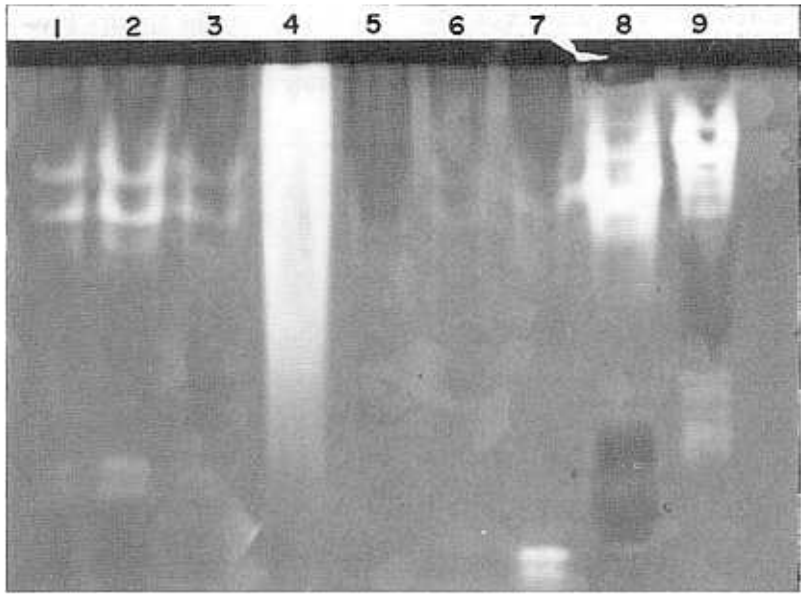
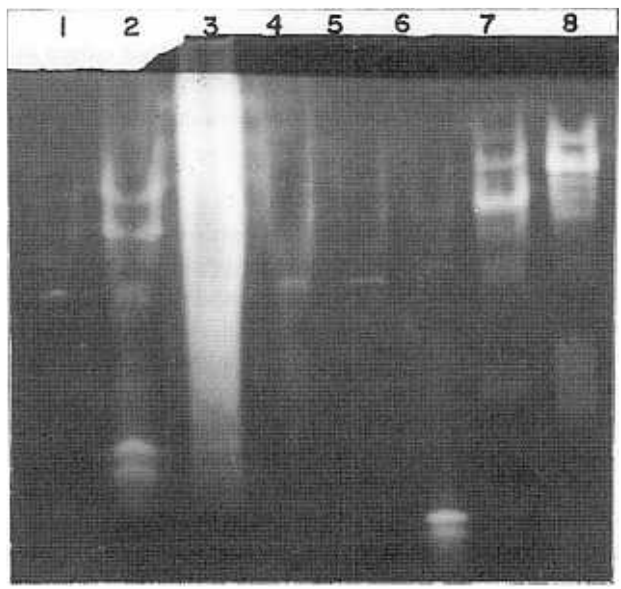


FIG. 5. Zymogram patterns of amylase isoenzymes of acacia seed extracts on 7.5% PAGE. Track: 1) and 2) Mellifera, 3) Laeta, 4) Tortilis, 5) Seyal, 6) Nilotica, 7) Farnesiana, 8) Catechu, and 9) Prosopis. Sample loaded was 30 µl in Track 1 and 50 µl in tracks 2 to 9. Gel was incubated in a starch solution free of phosphate.



Zymogram patterns of amylase and phosphorylase isoenzymes of acacia seeds extracts on 7.5% PAGE. Track: 1) Laeta, 2) Mellifera, 3) Tortilis, 4) Seyal, 5) Nilotica, 6) Farnesiana, 7) Catechu. and 8) Prosopis. Sample loaded was 50 µl. Gel was incubated in a starch solution containing phosphate.

Phosphorylase Patterns

A total of 7 phosphorylase isoenzymes were observed in all seed extracts and the number of bands present in each extract range from 2 to 3, except the species Tortilis which had phosphorylase activity. The species Laeta, Seyal, Nilotica and Catechu were identical in phosphorylase patterns (Fig. 7).

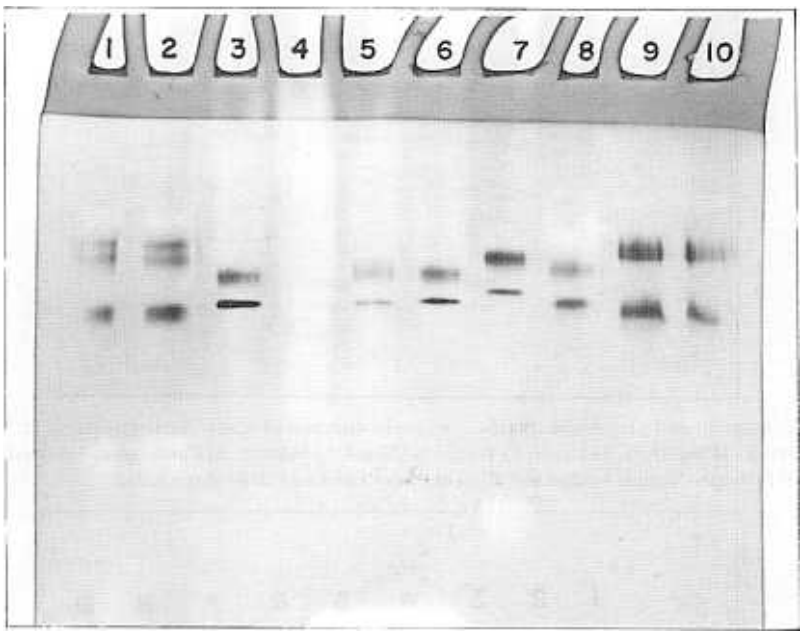


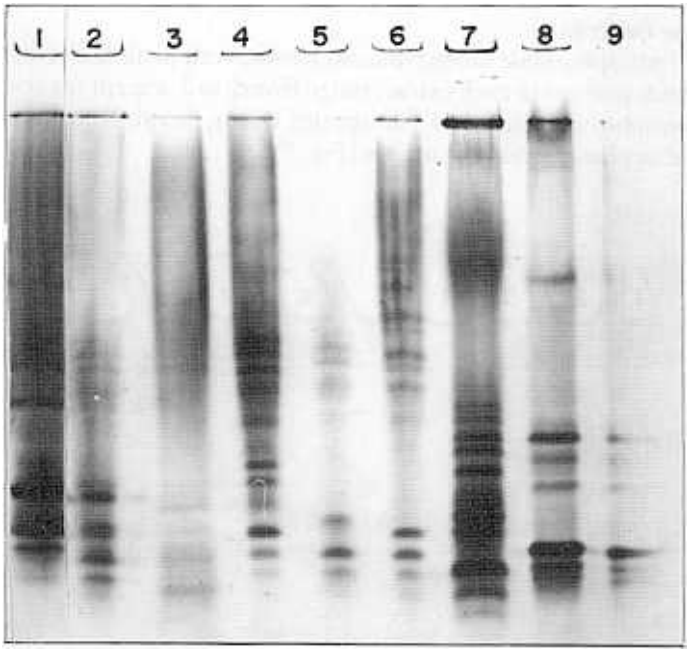
FIG. 7. Zymogram patterns of phosphorylase isoenzymes of acacia seed extract on 7.5% PAGE. Track: 1) and 2) Mellifera, 3) Laeta, 4) Tortilis, 5) Seyal, 6) Nilotica, 7) Farnesiana, 8) Catechu, 9) and 10) Prosopis. Sample loaded was 30 µl in Tracks 1 and 10 and 50 µl in Tracks 2 to 9.

EST Patterns

As shown in Fig. 8 that each seed extract displayed several distinct major and minor EST bands and all seed extracts studied differ in EST banding patterns :

GOT Patterns

The results in Fig. 9 and Fig. 10 showed that seed extracts from some species had low GOT activities and a large sample volume was used to resolve GOT components, while the extracts from other species were rich in enzyme activities and only small sample volume was required for bands resolution and using large sample volume resulted in a poor resolution. Each seed extract resolved into major and minor bands.



8. Zymogram patterns of non-specific esterase isoenzymes of acacia seed extracts on 7.5% PAGE. Track: 1) Mellifera, 2) Laeta, 3) Tortilis, 4) Seyal, 5) Nilotica, 6) Farnesiana, 7) Catechu, 8) and 9) Prosopis. Sample loaded was 50 μl in tracks 1 to 8 and 20 μl in track 9.

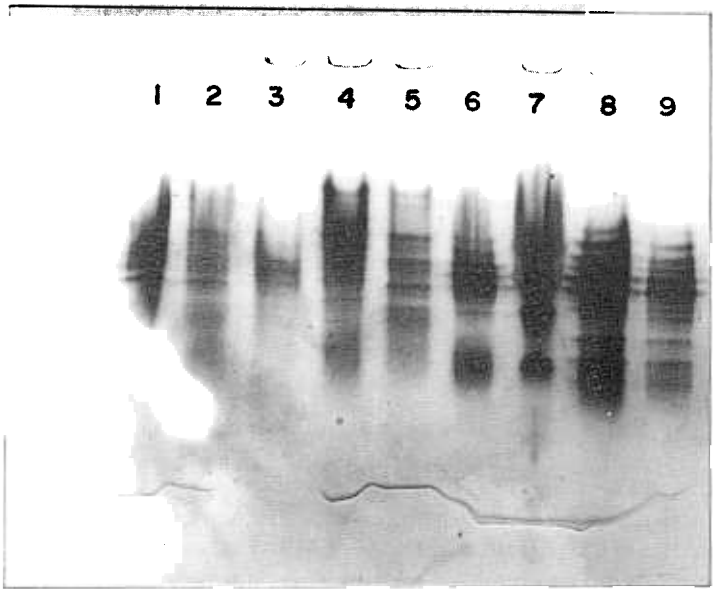


FIG. 9. Zymogram patterns of GOT isoenzymes of acacia seed extracts on 7.5% PAGE. Track: 1) and 2) Mellifera, 3) Laeta, 4) Tortilis, 5) Seyal, 6) Nilotica, 7) Farnesiana, 8) Catechu, 9) and 10) Prosopis. Sample loaded was 30 μl in tracks 1 and 10 and 50 μl in tracks 2 to 9.

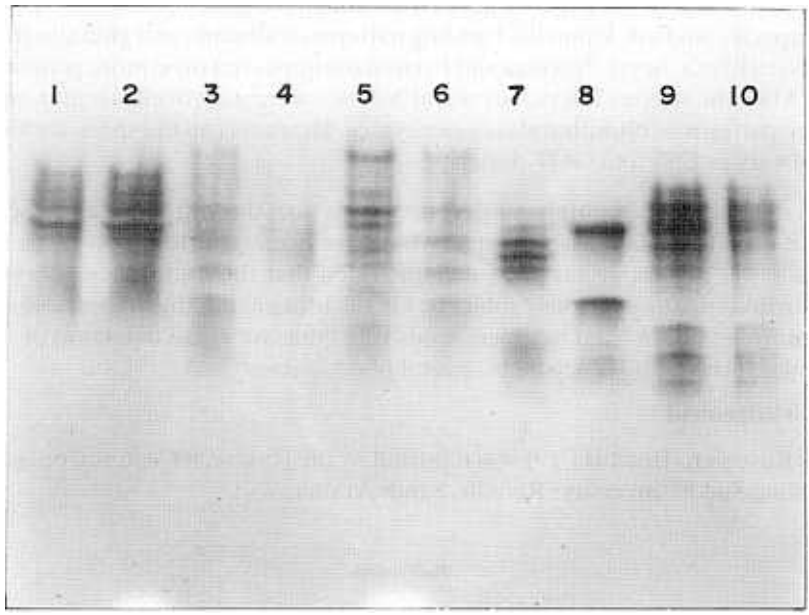


FIG. 10. Zymogram patterns of GOT isoenzymes of acacia seed extracts on 7.5% PAGE. Track: 1) Mellifera, 2) Laeta, 3) Tortilis, 4) Seyal, 5) Nilotica, 6) Farnesiana, 7) Catechu, 8) and 9) Prosopis. Sample loaded was 80 μl in tracks 1 to 8 and 60 μl in track 9.

Discussion

The use of chemical analysis of seeds, leaves and pollen grains for taxonomical studies for the genus acacia in the Kingdom of Saudi Arabia has been completely ignored despite the fact that such analysis would provide very useful information from both taxonomical and biochemical standpoints. The use of chemical analysis in taxonomy has been demonstrated for Australian acacia, using amino acids of seed globulin[7], free amino acids of seeds[6] and flavonoids of heartwoods and barks[8] as markers. However, using protein and isoenzyme patterns appears to be more valuable as markers for genetic analysis over the other biochemical markers, since proteins are nearly direct gene products and not products of biosynthetic reactions such as those leading to the production of compounds.

It is clear from the results that seed extracts from the species Tortilis had low protein content and low activities of enzymes, except amylase. This might be due to in part to the poor protein extraction due to the difficulty in obtaining fine seed powder.

The results at the present survey, describe the initial effort to use protein and isoenzyme patterns for genetic and taxonomical studies, protein, amylase, phosphorylase, GOT and EST patterns demonstrated a powerful tool in estimating the

degree of genetic variation between acacia species. The observations obtained from the present study, also verify, within certain limits, the genetic relationship between acacia species studied. Upon the banding patterns of albumin and globulin fractions, the species Laeta, Seyal, Nilotica and Farnesiana appeared to be more genetically related. Also, the species Laeta, Seyal and Nilotica were genetically similar, upon the banding patterns of phosphorylase isoenzymes. However, all the species which were studied vary in EST and GOT patterns.

The detection of multiple molecular forms for the enzymes amylase, phosphorylase EST and GOT is similar to what have been reported for a range of plant species[11, 15-19], where it has been demonstrated that the useful these enzymes are useful in investigating various problems. On the other hand, the presence of only one monomorphic GDH band in acacia seeds is in contrast to the detection of multiple molecular forms of this enzyme in several plant species[20, 21].

Acknowledgement

This Research (Bot/1411/19) was reported by the Research Center, College of Science, King Saud University, Riyadh, Saudi Arabia.

References

[1] **Hopper, S.T.** and **Maslin, B.R.**, Phytogeography of Acacia in Western Australia. *Aust. J. Bot.* **26:** 63-78 (1978).

[2] **Migahid, A.M.**, *Flora of Saudi Arabia*, Second edition, Riyadh Univ. Publication (1978).

[3] **Mahmoud, A.**, Germination of three desert acacias in relation to their survival in arid environment. *Proc. First Conf. Biol. Aspects of Saudi Arabia*, 74-94 (1977).

[4] **Al-Helal, A.A.**, Degradation of storage protein of *Acacia laeta* L. seeds during germination. *Qyton* **50**(1/2): 103-107 (1989).

[5] **Hassan, H.M.** and **Al-Farraj, M.M.**, Distribution and seed description of the genus *Acacia* (mimosoideae-leguminosae) in Saudi Arabia. *Arab Gulf J. Scient. Res.* **7**(2): 125-144 (1989).

[6] **Evans, C.S.**, **Qureshi, M.Y.** and **Bell, E.A.**, Free amino acids in the seeds of acacia species. *Phytochem.* **16:** 565-570 (1977).

[7] **Pettigrew, C.J.** and **Watson, L.**, Taxonomic patterns in amino-acids of acacia seed globulins. *Phytochem.* **14:** 2623-2625 (1975).

[8] **Tindale, M.D.** and **Roux, D.G.**, An extended phytochemical survey of Australian species of Acacia: chemotaxonomic and phytogenetic aspects. *Phytochem.* **13:** 829-839 (1974).

[9] **Murray, D.R.**, **Ashcroft, W.J.**, **Seppelt, R.D.** and **Lennox, F.G.**, Comparative biochemical and morphological studies of *Acacia sophorae* (Labill) R.B. and *A. longifolia* (Andrews) willd. *Aust. J. Bot.* **26:** 755-771 (1978).

[10] **Brown, A.H.D.**, **Matheson, A.C.** and **Eldrige, K.G.**, Estimation of the mating system of *Eucalyptus obliqua* L. Hérit. by using allozyme polymorphisms. *Aust. J. Bot.* **23:** 931-949 (1975).

[11] **Al-Helal, A.A.**, Amylase isoenzymes and protein of date palm (*Phoenix dactylifera* L.) Fruit. *Bot. Bull. Acad. Sinica* **29:** 239-244 (1988).

[12] **Natarella, N.J.** and **Sink, K.C.**, Electrophoretic analysis of proteins and peroxidases of selected petunia species and cultivars. *Bot. Gaz.* **136**(1): 20-26 (1975).

[13] **Cherry, J.P.** and **Ory, R.L.**, Electrophoretic characterization of six selected enzymes of peanut cultivars. *Phytochem.* **12:** 283-289 (1973).

[14] **Tsai, C.Y.** and **Nelson, O.E.**, Phosphorydases I and II of maize endosperm. *Plant Physiol.* **43:** 103-112 (1968).

[15] **Brewbaker, J.L., Upadhya, M.D., Makinen, Y.** and **Macdonald, T.,** Isoenzyme polymorphism in flowering plants. III. Gel electrophoretic methods and applications. *Physiol. Plant* **21:** 930-940 (1968).

[16] **Torres, A.M., Soost, R.K.** and **Diedenhofen, U.,** Leaf isoenzymes as genetic markers in citrus. *Amer. J. Bot.* **65**(8): 869-881 (1978).

[17] **Gates, P.** and **Boulter, D.,** The use of seed isoenzymes as an aid to the breeding of field beans (*Vicia faba* L.). *New Phythol.* **83:** 783-791 (1979).

[18] **Gorman, M.B.** and **Kiang, V.T.,** Variety-specific electrophoretic variants of four soyabean enzymes. *Crop Science* **17:** 963-965.

[19] **Matheson, N.K.** and **Richardson, R.H.,** Starch phosphorylase enzymes in developing and germinating seeds. *Phytochem.* **15:** 887-892 (1976).

[20] **Thurman, D.A., Palin, C.** and **Laycock, M.V.,** Isoenzymatic nature of L-glutamic dehydrogenase of higher plants. *Nature* **217:** 193-194 (1965).

[21] **Yue, S.B.,** Isoenzymes of glutamate dehydrogenase in plants. *Plant Physiol.* **44:** 453-457 (1969).

التحليل بالتفريد الكهربائي للبروتين وبعض الإنزيمات المختارة لبذور أكاشيا في المملكة العربية السعودية

علي عبد المحسن الهلال

قسم النبات والأحياء الدقيقة ، كلية العلوم ، جامعة الملك سعود

الريـــــاض ، المملكة العربية السعودية

المستخلص . لقد تم التحليل بالتفريد الكهربائي (SDS-PAGE) للبروتينات الذائبة في الماء والذائبة في المحاليل الملحية المخففة والذائبة في الكحول الأثيلي المستخلصة من بذور ثمانية أجناس من الأكاشيا . ويتضح من النتائج أن هناك فروقات في الحزم البروتينية بين الأجناس المدروسة .

كذلك تم فصل أيزو إنزيمات كل من الأميلاز وجلوتامات أوكسالو أسيتات ترانز أمينيز (GOT) والإستيرازات والفوسفوريلاز بالتفريد الكهربائي (PAGE) . ويتضح من النتائج أن هناك فروقات كبيرة في الحزم الأنزيمية بين الأجناس المدروسة ، وتدل النتائج على أن كل من الحزم البروتينية والأيزو إنزيمية يمكن استخدامها في دراسات وراثية وتقسيمية في الأكاشيا .