Vol. 49, No. 3
Feb. 5, 1980

ELECTROPHORETIC AND IMMUNOLOGICAL EVIDENCE ON PROTEIN DEGRADATION DURING SEED MATURATION IN *TEPHROSIA PURPUREA* (L.) PERS. (FABACEAE)

C. K. RAO

Department of Botany, Bangalore University, Bangalore 560 001

Abstract

Electrophoretic and immunological data are presented in support of the concept of degradation of certain protein components during seed maturation and dehydration in *Tephrosia purpurea*.

Introduction

MILLERD[1] suggested a possibility of certain amount of protein breakdown during seed maturation and dehydration in legumes. Electrophoretic and immunological evidence in support of this contention is presented here with reference to *Tephrosia purpurea* (L.) Pers.

Materials and Methods

Samples of dry (mature) and turgid (immature) seeds of *T. purpurea* were collected from Savanadurga (Karnataka State). Polyacrylamide gel electrophoresis was carried out using standard methods[2,3]. The gels stained with Coomassie blue were scanned on a Joyce Loebl chromoscan recording-integrating densitometer. The densitometer tracings were used to draw electrophoregrams. The standard gel pattern for *T. purpurea* was obtained by electrophoresing dry seed extracts of eight populations of the species from different parts of peninsular India[4]. Three replicates were studied per sample.

Antibodies were raised in a rabbit against defatted seed protein extract of *T. purpurea* from Waltair following standard methods[5,6]. A part of this extract (homologous antigen complex) was used as a standard for comparative purposes in subsequent studies. The double diffusion technique detailed by Ouchterlony[7,8] was employed loading the antiserum and the seed protein extracts (homologous and heterologous antigen complexes) in wells cut in 1·5% Difco bacto agar plates. Five replicates were maintained in various combinations of plating.

Results and Discussion

Electrophoretic Study

A comparison of gel patterns and the Rp values of the seed protein bands show that the dry seed extracts of Savanadurga populations have gel platterns identical with that standardised for *T. purpurea* (Table I). This pattern has five bands (A, B, D, E and F). Bands A, B and F were very dense and narrow (Fig. 1). The differences in the mobilities of the five bands fall within the normal range of population variation[4]. The immature seed extracts of the Savanadurga population showed bands A, B, D, E and F like the mature seeds but also have an additional diffuse band (C) (Table I; Fig. 1). While comparison of the electrophoregrams (Fig. 1) shows that the difference between mature and immature seeds lies in the bands C, D and E, the Rp values indicate that band C with a Rp value characteristic of its own (Table I), was not found in any other sample of the species studied[4]. Quantitative differences also were discernible in the bands of the mature and immature seeds as evidenced by the density and width of bands D and E. More particularly, band E was present in the immature sample in considerably lower quantities than in the dry seeds (Fig. 1).

Table I

Rp values of protein bands of T. purpurea *resolved by electrophoresis*

Band	Standardised for the species	Dry seed extracts*	Immature seed extracts*
(— ve end)			
A	0·12	0·13	0·12
B	0·22	0·21	0·19
C	..	..	0·32
D	0·53	0·52	0·55
E	0·81	0·78	0·76
F	0·91	0·90	0·89
(+ve end)			
Total number of bands	5	5	6

* From Savanadurga population.

Although identical gel patterns are indicative of identical protein components in closely related taxa, this is not always certain. Identity of proteins is more convincingly established by immunological

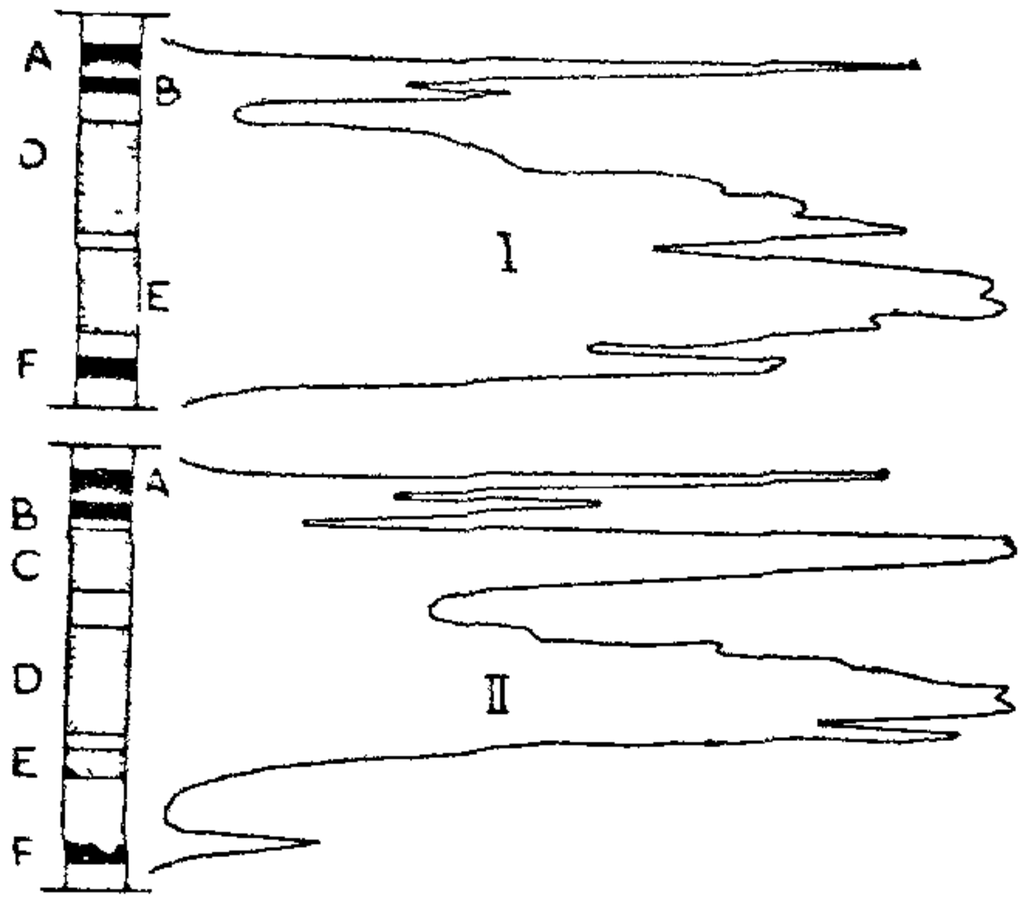

FIG. 1. Electrophoregrams and densitometer tracings of seed proteins of mature (I) and immature (II) seeds of *T. purpurea*.

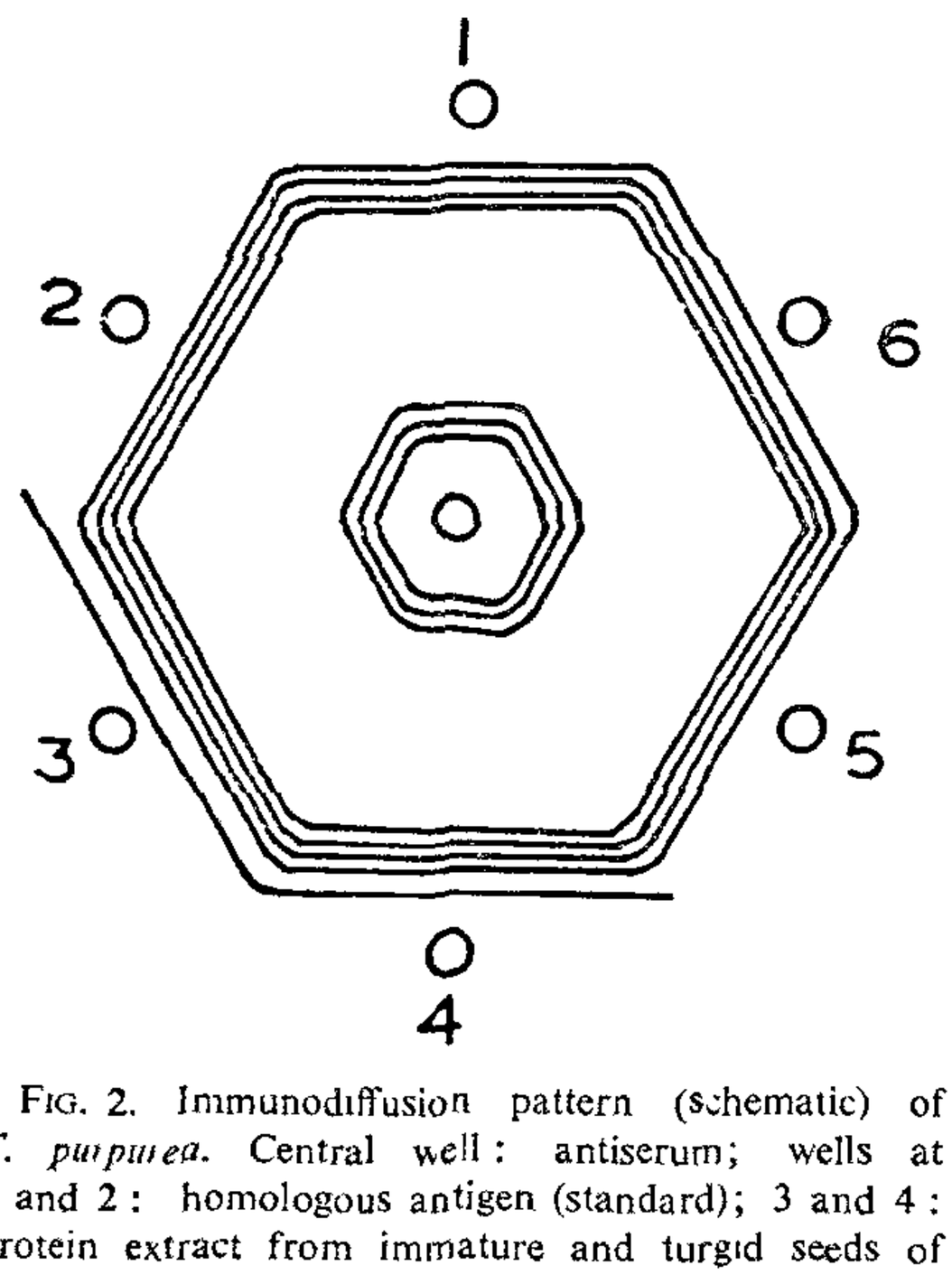

FIG. 2. Immunodiffusion pattern (schematic) of *T. purpurea*. Central well: antiserum; wells at 1 and 2: homologous antigen (standard); 3 and 4: protein extract from immature and turgid seeds of Savanadurga population. The seven concentric precipitin lines form the standard pattern for *T. purpurea*. The eighth line at wells 3 and 4 is the precipitin line formed by the protein component being degraded during seed maturation and dehydration; 5 and 6: Protein extracts from dry and mature seeds of Savanadurga population showing the same pattern as the standard.

methods. In addition, whether the band C represents a degrading protein in the immature seeds also cannot be conclusively proved by electrophoretic study even though its absence from the dry samples is an indirect indication of a degrading protein. It may even represent a dissociated component of an association complex of a protein. Legume seeds are known to contain proteins in association-dissociation complexes[1]. Immunological evidence was sought to elucidate these aspects.

Immunological Study

A protein extract (antigen complex) from seeds contains several antigenic components each forming an immunoprecipitin line on reacting with the antibody specific to itself and present in the antiserum. When antigen complexes of two taxa are plated in neighbouring wells the immuno-precipitin lines of all the antigen components identical between the taxa become confluent forming a smooth arc at the point of contact (Fig. 2).

The homologous antigen complex of *T. purpurea* formed seven immunoprecipitin lines, three lines proximal to the antiserum (central) well and four lines proximal to the antigen (peripheral) well (Fig. 2). Seed proteins of 18 populations of *T. purpurea* from different parts of peninsular India were studied to obtain a pattern of interpopulation variation in the immunodiffusion pattern of this species. No significant differences were found among the 18 populations indicating that the formation of seven immunoprecipitin lines is the standard pattern for *T. purpurea*. The dry seed samples from Savanadurga showed the standard pattern but the protein extracts from the immature and turgid seeds from this locality formed one or occasionally two sharply defined lines, closest to the antigen well, outside and in addition to the seven lines of the standard pattern (Fig. 2).

The antigenic potentialities of all protein components of an extract are not the same[7,8]. A protein component may be present in very small quantities but may be potentially more antigenic than the other components that may be present in larger amounts in the complex. The formation of precipitin lines is the function of the relative concentrations of the antigen and its antibody in addition to a few other factors like the density and the pore size of the gelified medium, temperature, etc.[7,8] The fact that additional precipitin lines are formed with the extracts of the immature seeds but not with those of the mature seeds shows that these protein components were present in the antigen complex used to raise the antibody but not in quantities sufficient for the formation of precipitin lines when tested against the antiserum with the dry seed extracts. Their presence in sufficiently large amounts in the immature seeds is indicated by

the formation of additional precipitin line(s) thus providing clear immunological evidence towards degradation of certain protein components during seed maturation and dehydration. Collation of electrophoretic and immunological data presented here indicate that presumably, band C in the gels represents the degrading protein and bands D and E the accumulating proteins.

Electrophoretic and immunological evidence presented here also emphasises the need for the precaution that all source material used in phytochemical studies should be in a comparable physiological state of development, no matter what class of chemical compounds are being investigated. On the other hand, in order to obtain all protein components in the seeds, samples of different ages need to be analysed.

ACKNOWLEDGEMENT

The author thanks Professor M. Nagaraj, Head of the Department, for facilities and encouragement, Dr. M. R. Suresh for help with methodology and the Director, University Botany Laboratory, Madras, for the use of the densitometer.

1. Millerd, A., *Ann. Rev. Plant Physiol.*, 1975, **26**, 53.
2. Ornstein, L., *Ann. N.Y. Acad. Sci.*, 1964, **121**, 321.
3. Davis, B. J., *Ibid.*, 1964, **121**, 404.
4. Rao, C. K., *Morphological, Cytological and Chemosystematic Studies on Some Indian Galegeae* (*Fabaceae*). Ph.D. Thesis, Andhra University, Waltair, 1978.
5. Ackryod, J. F., *Immunological Methods*, F. A. Davis, Philadelphia, 1964.
6. Kwapinski, J. B., *Methods for Serological Research*, John Wiley and Sons, New York, 1965.
7. Ouchterlony, O., In : *Immunological Methods*, Ed. J. F. Ackroyd, Blackwell Sci. Publ., Oxford, 1964.
8. —, In : *Handbook of Experimental Immunology*, Ed. D. M. Weir, Blackwell Sci. Publ., Oxford, 1967.

NATIONAL SYMPOSIUM ON MICROCHEMICAL TECHNIQUES

The National Symposium on Microchemical Techniques will be sponsored by the University Grants Commission, New Delhi, and the University of Mysore, Mysore, from 16th to 18th May 1980. The Symposium consists of plenary and invited lectures and will have contributed papers. Special lectures setting the theme of the Symposiun will be given by eminent scientists in India on the significance of analysis in research, technology and environment. Papers for presentation at the Symposium along with a copy of the abstract (100 words) should reach Prof. H. Sanek Gowda, Department of Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, on or before 31st March 1980.

DR. VIKRAM SARABHAI RESEARCH AWARDS

The following four distinguished scientists have been selected for the Dr. Vikram Sarabhai Research Awards, endowed by the Nadiad-based "Hari Om Ashram": Prof. A. B. Bhattacharya, Indian Institute of Technology, New Delhi; Dr. K. S. Krishnaswamy, Tata Institute of Fundamental Research, Bombay; Smt. K. Ghosh, Meteorological Office, New Delhi and Dr. S. C. Gupta, Vikram Sarabhai Space Centre, ISRO, Trivandrum. The awards, each carrying a medal and a cash prize of Rs. 8,000 will be presented on the birthday of late Dr. Vikram Sarabhai on August 12, 1980 at Physical Research Laboratory, Ahmedabad.

INDIAN INSTITUTE OF GEOLOGICAL EDUCATION, BANGALORE

A Seminar on "Invironmental Geology : Do Academic Programmes Meet Present Needs?" will be held during 9th to 11th August, 1980. Participants are requested to contact Prof. S. Sambe Gowda, Secretary, Indian Institute of Geological Education, Central College, Bangalore 560 001, for further particulars.