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SHORT COMMUNICATION

ELECTROPHORETIC ANALYSIS OF PROTEIN CHANGES DURING THE DEVELOPMENT OF THE FRENCH BEAN FRUIT

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Abstract—The protein complement of bean (*Phaseolus vulgaris*) seeds and pods was shown to change greatly during fruit development. Synthesis of storage protein was initiated when the seed attained 10 mm length, the subsequent accumulation rapidly diluting out other proteins readily detectable after electrophoresis on acrylamide gels of extracts from younger seeds. In contrast, the number of bands obtained on electrophoresis of proteins from the pod decreased as a result of degradative changes as the fruit matured.

INTRODUCTION

THE HIGH concentration of protein in leguminous seeds became evident from the studies of Osborne,[1] and physical characteristics of legume proteins were detailed by Danielsson.[2] Protein separation by electrophoretic techniques has been used in phylogentic studies of the Leguminoseae[3,4] and changes in spectra during germination have been described.[5]

The rapid synthesis of protein in growing bean seeds[6] supports other evidence indicating their potential value for studies on plant protein biosynthesis.[7] This paper reports the changes in protein profile observed after electrophoretic separation of extracts prepared from seeds and pods during development.

RESULTS

Consistent results were obtained for electrophoretic separation on acrylamide gels of protein extracts from seeds of similar length. No useful correlation between the protein complement of individual seeds and plant age, time from anthesis, position of pod on the plant or pod dimension was observed. A similar lack of relationship between seed age and development was noted by Loewenberg.[8] However, mean values for observations of growth or total metabolite content on many seeds or pods do give valuable data in relation to age.

The changes in protein profile during development that are illustrated in Fig. 1 can be correlated with the observations of Carr and Skene,[9] whose description of seed growth

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included data for both seed age and seed length. These authors noted that up to a length of about 9–11 mm the embryo grew until it filled the space available within the embryo sac. The profile shown after electrophoretic separation of proteins separated from 5–10 mm seeds (Fig. 1) therefore represents proteins of the seed coat, immature cotyledons and

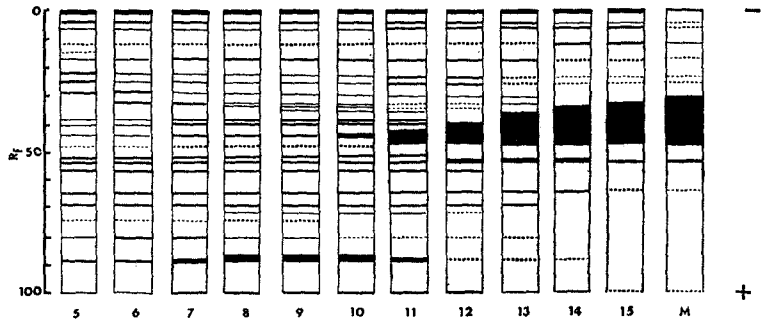

FIG. 1. ELECTROPHORETIC SEPARATION OF BEAN SEED PROTEINS DURING DEVELOPMENT.
The number below each gel diagram represents seed length (mm).

embryonic axis. A short lag followed this stage, and it seems likely that *m*RNA for globulins is synthesized in the newly forming cotyledon cells at this time; a rapid increase of storage protein is visible in gels of 10–13 mm seeds. Carr and Skene[9] commented: 'During the lag period a considerable revision of the pattern of metabolism of the seeds must take place, since when growth resumed changes associated with the onset of maturity began'.

Protein profiles for seeds larger than 13 mm did not change markedly (Fig. 1), although the total protein content per seed continues to increase during this phase of maturation.[6] The relatively large proportion of storage protein (R_f 38–42) tends to dilute out the other protein species so that it appears as a greatly overloaded band in the extract from 15 mm seeds. During the final stages of maturation much water is lost from the seed, and electrophoresis of protein extracts yields streaky patterns for the mature seed (Fig. 1, M).

The protein changes in mature pods appear to result from degradation during senescence rather than dilution effects from individual molecular species. While sharp bands were obtained from extracts prepared from pod sections corresponding to immature seeds

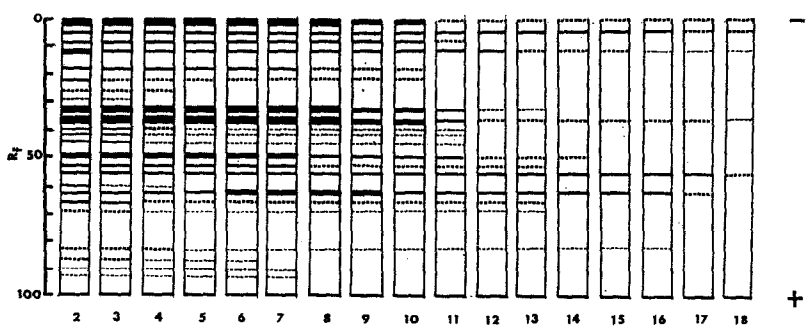

FIG. 2. ELECTROPHORETIC SEPARATION OF BEAN POD PROTEINS DURING DEVELOPMENT.
The number below each gel diagram refers to the seed length (mm) corresponding to the pod section extracted (see text).

2–10 mm long (Fig. 2), less distinct bands were noted for pods corresponding to 11 and 12 mm seeds. Proteins extracted from pods of maturing seeds (13 mm and longer) did not resolve well on electrophoresis, and even the rather clear bands at R_f 5, 11, 54 and 62 diminished as the pod dried out. Similar blurred patterns occur when protein extracts suffer degradation during preparation or storage. Seed and pod extracts from another commercially available bean variety ('Sprite') gave similar banding patterns to those described for 'Tendergreen'.

The data presented here indicate the rapid onset of storage protein synthesis in bean seeds commencing at the time the cotyledons fill the embryo sac. Under uniform culture conditions, this stage of development occurs 43 days after planting. Definition of the timing of the initiation of the synthesis of identifiable protein will facilitate further studies into the biochemistry of mechanisms for induction and accumulation of seed proteins.

EXPERIMENTAL

Plant culture. Bean seeds (*Phaseolus vulgaris* L. cv. Tendergreen) were obtained from Olds Seed Co., Madison, Wisconsin, U.S.A., and germinated in moist vermiculite for 7 days, The seedlings were transferred to plastic-lined fiberglass tanks containing a modified Hoagland's nutrient solution and cultured with continuous aeration under a programmed regime for light, heat and humidity in the University of Wisconsin Biotron.

Protein extraction from seeds and pods. Pods were slit open and the seeds and surrounding sections of pod sorted according to the length of the seeds. The material was then ground under N_2 with a VirTis '45' homogenizer in a protective buffer system[10] containing 5 mM dithiothreitol, 15% (w/v) insoluble polyvinylpyrrolidone and 0·1 M Hepes [1-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] brought to pH 7·4 with KOH. The extraction and dialysis solutions were maintained at 2% (w/v) NaCl to ensure solution of the globulin fraction. After filtration through acetate taffeta cloth and glass wool the solution was centrifuged, dialysed and then concentrated to approx. 10 mg protein/ml by placing the dialysis tubing in a trough of dry 'Aquacide II' (Calbiochem).

Electrophoresis. The extracts (0·5 mg protein/gel, measured by the Lowry[11] procedure) were separated electrophoretically[12] on 7% (w/v of monomer) acrylamide gels at pH 8·9 (Tris-glycine, 4°). Proteins were stained with aniline blue black, and R_fs assigned relative to the distance traveled by the bromophenol blue marker dye from the top of the separating gel.

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Key Word Index—Phaseolus vulgaris; Leguminosae; protein; developing seed.