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Multiple Enzyme Forms of Tomato Seeds and Seedlings

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Proteins were extracted from dormant seeds, germinating seeds, and 12-day-old seedlings of Chico III and Homestead-24 tomato cultivars. Disc gel electrophoresis of the crude extracts showed that the number of enzyme forms differed with developmental stage of each cultivar. Generally the number of enzyme forms was greater in seedlings than in dormant seeds; exceptions were the numbers of enzyme forms of catalase and malic dehydrogenase, which decreased. In Homestead-24, the number of general protein forms was greater in seedlings than in dormant seeds. In Chico III, the number of protein forms was the same in dormant seeds and seedlings. The number of protein forms was lowest at the germinating seeds stage of both cultivars. Differences between the enzyme activities of the two cultivars were detected.

Seeds contain two types of proteins: metabolic proteins, both enzymatic and structural, which are concerned with cellular activities, and a second type, the storage or reserve proteins. Many of the former exist in multiple molecular forms known as isoenzymes. The latter have no enzymatic activity (Varner, 1965; Atschul et al., 1966). Both types of proteins function after promotion of seed germination. Reserve proteins are hydrolyzed by enzymatic proteins, and the degradation products are a source of nitrogen and carbon for the developing seedling (Oota et al., 1953).

Disc gel electrophoresis has been the most convenient method for the resolution of protein mixtures. Isoenzymes present in seed tissue (Cherry and Ory, 1973; Macko et al., 1967) and in other plant tissues (Kadam et al., 1973; Hall et al., 1969) have also been resolved into distinct patterns by this technique.

Numerous studies of enzyme activities in tomato fruit have been reported (Lee and MacMillan, 1968; Nakagawa et al., 1970; Hobson, 1967, 1974) and most concerned the enzymatic changes associated with ripening. Only few investigations have dealt with the identification of enzyme forms present in tomato seeds and seedlings and the changes those enzyme forms undergo during seed germination. A fundamental knowledge of the enzyme forms present in seeds and seedling would allow a better understanding of the biochemical steps that accompany the development of the plant. Any differences in enzyme distribution among varieties might enable the screening of plant crops for factors such as disease resistance and agronomic performance.

We undertook to identify and compare the enzyme forms of dormant seeds, germinating seeds, and seedlings of a firm (Chico III) and a soft (Homestead-24) variety of tomato, and to determine whether firmness of fruit could be related to the electrophoretic patterns of enzymes from plants at an early stage of development. We investigated the enzyme forms of acid and alkaline phosphatase, esterase, catalase, peroxidase, and malic dehydrogenase (MDH). Of these, only the isoenzymes of catalase, peroxidase, and MDH have well-understood genetics in studies on the physiological role(s) of specific enzymes (Scandalios, 1974).

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Table I. Soluble Protein and Enzyme Forms in Chico III and Homestead-24 Seeds and Seedlings[a]

	Chico III			Homestead-24		
	Dormant seed	Germinating seed	Seedling	Dormant seed	Germinating seed	Seedling
Soluble protein	24	15	24	20	16	28
Acid phosphatase	4	6	8	4	6	8
Malic dehydrogenase	6	6	5	6	6	5
Esterases	6	6	11	6	6	11
Peroxidase	2	3	12	2	3	12
Catalase	3	2	2	2	2	2
Alkaline phosphatase	0	2	5	0	2	3

[a] Figures represent the number of protein bands and enzyme forms detected.

EXPERIMENTAL SECTION

Extraction of Protein. *Dormant Seeds.* Chico III seeds were obtained from the Texas Agriculture Experiment Station, Weslaco, Texas, and Homestead-24 seeds were from a commercial source. The seeds were ground in a Wiley Mill equipped with a 20-mesh screen for sieving. One gram of dried ground seed was extracted for 1 h at 4 °C with 0.1 M Tris–HCl buffer (pH 8.0) that was 0.5 M sucrose, 0.006 M ascorbic acid, and 0.006 M cysteine–hydrochloride (Macko et al., 1967).

Germinating Seeds. One gram of seed was germinated on moist filter paper at room temperature. The 4-day-old germinating seeds were ground with a cold mortar and pestle and extracted by the same method as for dormant seeds.

Seedlings. The seeds were planted in vermiculite and exposed to direct sunlight and normal day and night temperatures. On the twelfth day the seedlings were removed from the vermiculite, washed, and dried. One gram of fresh tissue (12–14 whole seedlings) was ground and extracted the same way as germinating seeds. All extracts from seeds and seedlings were centrifuged at 12 000*g* for 30 min. An aliquot from the supernates was analyzed for protein and the remainder was dialyzed overnight at 4 °C against distilled water.

Protein Estimation. The protein content on an aliquot of the undialyzed extracts was determined according to Lowery et al. (1951).

Freeze-Drying. The dialyzed extracts were frozen with dry ice, freeze-dried under vacuum in a Stokes Food Freeze Drier, and stored at 0–4 °C.

Polyacrylamide Disc Gel Electrophoresis. Polyacrylamide gels (14%) were prepared according to Davis (1964). Protein extracts (200–250 μg) in 4 μL of 0.1 M Tris–HCl buffer (pH 8.0) were layered on the gel, and electrophoresis was carried out for 2 h at 3 mA–tube. To retard enzymatic reactions, electrophoretic separations were conducted at 5 °C. Immediately after electrophoresis, the gels were removed from the tubes and incubated in enzyme-specific substrate mixtures. This procedure yielded reproducible results from three extracts at each stage of development.

Enzyme Assays. The method of Desborough and Peloquin (1971) was used to detect acid phosphatase. Alkaline phosphatase was detected according to the method of Rudolph and Stahmann (1966). Nonspecific esterases and peroxidases were assayed by the methods of Brewbaker et al. (1968). Catalase activity was demonstrated by a negative staining technique (Macko et al., 1967). The malic dehydrogenase staining solution was that proposed by Thomas and Neucere (1973). General proteins were stained with a 1% solution of coomassie blue.

Densitometer Scans. The stained gels were scanned in a Gelman Digiscreen-M Scanner equipped with a standard, green gelatin, 525 nm filter and a stainless steel 0.2 × 2 mm slit. A Digiscreen recorder attached to the scanner recorded the absorption curve.

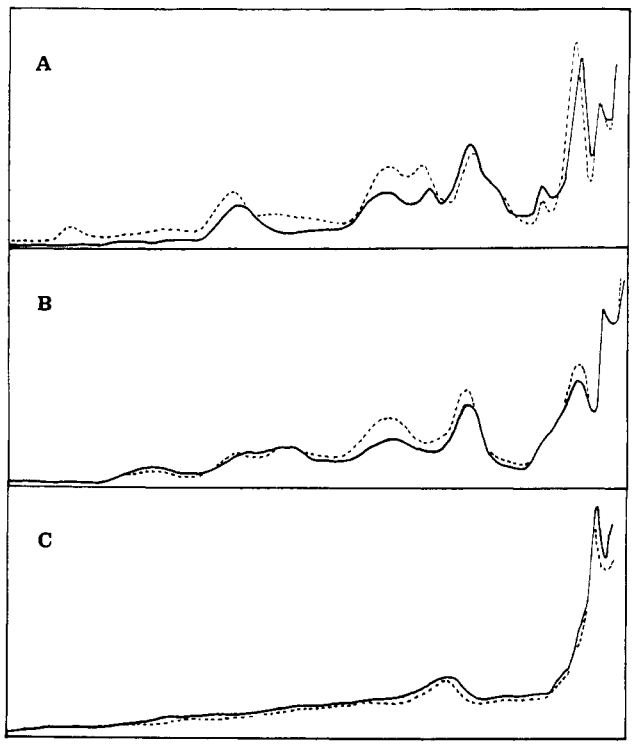

Figure 1. Densitometer trace of soluble proteins in dormant seeds (A), germinating seeds (B), and 12-day-old-seedlings (C) of Chico III (—) and Homestead-24 (- - - -).

RESULTS AND DISCUSSION

Dormant Seeds. Densitometer scans (zymograms) of the gels stained for soluble proteins from Chico III and Homestead-24 extracts are shown in Figure 1A. Each peak on the zymogram represents a protein band on the gel. Some faint bands on the gels that were visible to the eye were not recorded as peaks by the densitometer. Twenty-four general protein bands were detected visually on the stained gels for Chico III and 20 bands for Homestead-24 extracts (Table I). Diagrammatic representation of the electrophoretic separation of the enzymes assayed are shown in Figure 2A. The patterns were the same for both cultivars neither of which contained alkaline phosphatase in amounts detectable by the method used. The number of forms of each class of enzymes is tabulated in Table I. Most of the acid phosphatase, MDH, and esterase activity was concentrated in the fastest moving bands. The greatest peroxidase activity was shown by the slowest moving enzyme forms.

Germinating Seeds. An electrophoretic zymogram of Chico III and Homestead-24 protein extracts is shown in Figure 1B. The number of soluble protein forms was lower

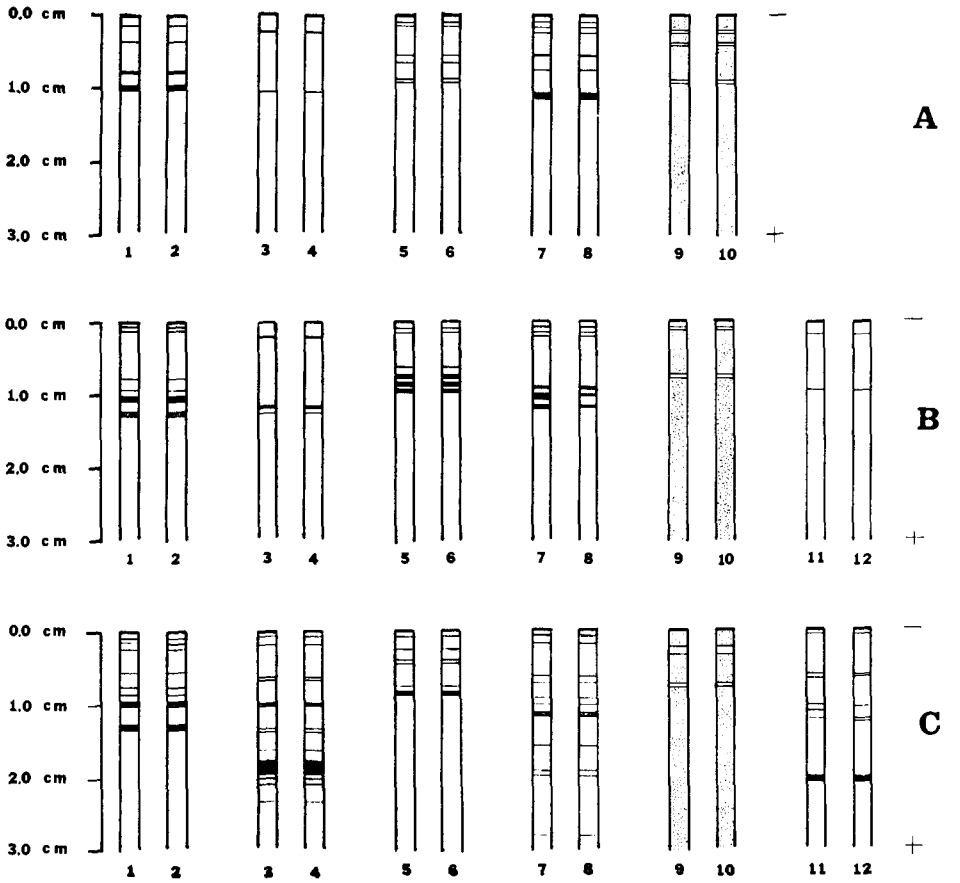

Figure 2. Diagrammatic representation of the disc gel electrophoretic separation of Chico III (1, 3, 5, 7, 9, 11) and Homestead-24 (2, 4, 6, 8, 10, 12) enzyme forms in dormant seeds (A), germinating seeds (B), and 12-day-old seedlings (C). Stained for acid phosphatase (1, 2), peroxidase (3, 4), malic dehydrogenase (5, 6), esterase (7, 8), catalase (9, 10), and alkaline phosphatase (11, 12).

in germinating seeds than in dormant seeds. This observation was anticipated since many storage proteins are hydrolyzed during seed germination. Visual and densiometric examination of the stained gels showed that each of the enzyme forms in Chico III extracts had a common form in Homestead-24 extracts (Figure 2B). The number of enzyme forms for each class of enzyme is tabulated in Table I. The two fastest esterase bands in Chico III extracts were always darker and wider than those of Homestead-24, indicating a higher activity.

Seedlings. Zymograms of the separated soluble proteins from 12-day-old Chico III and Homestead-24 seedlings were identical (Figure 1C). However, visual inspection of the stained gels showed four more protein bands in Homestead-24 than in Chico III. A comparative examination of the stained gels (Figure 2C) showed that the enzyme forms in Chico III and Homestead-24 were identical in number and mobilities. Alkaline phosphatase was the only enzyme exhibiting differences in activity between cultivars.

Both cultivars showed more enzyme forms of acid phosphatase, esterase, peroxidase, and alkaline phosphatase in seedlings than in dormant seeds (Table I). Similarly, more isoenzymes of acid phosphatase were observed in seedlings than in seeds of cucumber, pea, sunflower, pumpkin, and mung bean (Presley and Fowden, 1965). The number of enzyme forms of acid phosphatase, peroxidase, and alkaline phosphatase was greater in germinating seeds than in dormant seeds of Chico III and Homestead-24. Macko et al. (1967) reported that germinating seeds of wheat contained more peroxidase isoenzymes than the dormant seeds. Catalase and MDH showed decreases in the number of enzyme forms in seedlings relative to dormant seeds. A comparative examination of the stained gels revealed that the enzyme forms with highest activity tended to have greater mobility toward the anode.

A major problem in the interpretation of electrophoretic patterns is that, because of possible overlapping or identical electrophoretic mobilities any single band may not represent a single component. Thus in our work, for example, the sum of the bands made visible by the enzyme specific assays was usually greater than the total number of bands made visible by the stain for general proteins.

CONCLUSION

Disc gel electrophoresis of protein extracts from two tomato cultivars in dormant seeds, germinating seeds, and 12-day-old seedlings showed differences in the number of enzyme forms present. Differences in the number of soluble protein forms were also detected. A comparative examination of six enzyme systems in seeds and seedlings of Chico III, a firm fruit cultivar, and Homestead-24, a soft fruit cultivar showed no major differences between the two cultivars. Differences between the two cultivars were detected regarding the enzyme activities of esterases and alkaline phosphatases; however, evaluation of the differences was beyond the scope of the study since the metabolic significance of these enzyme forms has not been elucidated.

To establish the uniqueness of cultivars, such as Chico III and Homestead-24, by the multiple enzyme form technique requires that they differ in physiological character and in seedling or seed enzymes. This, however, may not always be possible. For example, Chico III and Homestead-24 plants produce fruit with very different

textural properties, yet they could not be distinguished on the basis of enzyme systems that were identified in extracts from seeds and seedlings. However, many other enzyme systems remain to be examined and evaluated. Thus, multiple enzyme expression in early stages of tomato plant development may still enable characterization of the plants for fruit firmness.

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Soluble Proteins and Enzymes as Indicators of Change in Peanuts Infected with *Aspergillus flavus*

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Buffered extracts of peanut (*Arachis hypogaea* Linn) seeds infected with four aflatoxigenic and five nonaflatoxigenic strains of *Aspergillus flavus* Link ex. Fries were examined electrophoretically for soluble proteins and selected enzymes. Quantitatively, soluble proteins in extracts of seeds infected 4 days were significantly lower than those in control seeds; however, the number of low molecular weight proteins in infected seeds increased. Enzyme patterns of extracts from seeds infected with aflatoxigenic *A. flavus* did not differ distinctively from patterns of seeds infected with nonaflatoxigenic strains. Esterase, leucine aminopeptidase, gluconate and alcohol dehydrogenase, and alkaline and acid phosphatase patterns in extracts could be distinguished between infected and control seeds.

Studies have shown that standard gel electrophoretic patterns of proteins extracted from viable peanut (*Arachis hypogaea* Linn) seeds are distinctly modified as a result of infection with species of *Aspergillus* and other fungi (Cherry et al., 1974, 1975, 1976; Cherry and Beuchat, 1976). Biochemical transformations include deletion of some proteins (including enzymes), intensification of others, and/or production of new components as evidenced by quantitative and qualitative changes in bands appearing in electrophoretic gels. These changes in band patterns indicate generally that biochemical mechanisms operative in saprophyte–seed interrelationships function efficiently and systematically in favor of the fungus. Studies suggested that leucine aminopeptidases, esterases, peroxidases, oxidases, and catalases, among other enzymes, were partially responsible for alterations in peanut proteins and for new bands appearing in gels. Many peanut enzymes remained active during invasion with Aspergilli. In some instances, intensification of activities was correlated with enzymes extracted from fungal mycelium collected from the infected seed surface. Quantitative and qualitative variations in gel patterns of certain enzymes also distinguished fungal mycelium grown on peanut seeds from that grown in a synthetic medium (Cherry et al., 1974).

Homologous protein fractions among various taxa can be useful in determining genetic relationships between organisms (Dessauer and Fox, 1964). Chang et al. (1962) demonstrated that protein complements of four *Neurospora* spp. were distinctive and recognizable. Shechter et al. (1966) reported that a number of homologous proteins present in *Microsporum* and *Trichophyton* were dissimilar to the protein complement of *Epidermophyton floccosum*, thus lending credence to the taxonomic placement of these species.

Protein homology does not always appear among taxonomically similar species of fungi, however. Kulik and Brooks (1970) examined three strains each of five members of the *Aspergillus flavus* group (*A. flavus*, *A. leporis*, *A. oryzae*, and *A. parasiticus*, and *A. tamarii*), *A. fumigatis*, and *A. ochraceus* using polyacrylamide gel electrophoresis. They concluded that although the seven taxa exhibited from 29 to 48 protein fractions, only two fractions were common to all taxa. Results did not support the contention that *A. flavus* and *A. parasiticus* may be more closely related to each other than to other members of the *A. flavus* group.

In a recent study, Schmidt et al. (1977) compared enzymes of aflatoxigenic and nonaflatoxigenic strains of *A. flavus* and *A. parasiticus* by polyacrylamide gel-slab

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