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SOLUBLE PROTEIN AND ENZYME PATTERNS IN SHOOTS OF SLASH PINE UNDER DIFFERENT NUTRITIONAL REGIMES*

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Abstract—The potential for using gel electrophoresis techniques in nutrition studies is demonstrated. Total soluble proteins separated in acrylamide gels are influenced by substrate nutrient levels. Isozymes containing Cu and Fe (polyphenol oxidase, peroxidase, and ascorbic acid oxidase) were strongly influenced by excess P in the substrate. Total soluble proteins and isozymes (e.g. peroxidase), whose activities are influenced by cations (e.g. Mn), were also shown to be influenced by substrate nitrogen level. These assays may be more sensitive to mineral deficiencies or excesses than gross tissue analysis.

INTRODUCTION

CERTAIN cations are activators or components of the molecular structure of various enzymes. Thus, internal concentration of these nutrients should influence enzyme activity. This hypothesis might be developed into a method for defining deficient, optimum, or toxic levels of these enzyme-related cations.

Separation of soluble plant proteins by polyacrylamide gel techniques was described recently by Steward and Barber.[1] This method is potentially useful in studying the effects of different nutritional regimes on soluble proteins containing active enzymes. In this preliminary investigation, acrylamide gel electrophoresis techniques were used to determine whether various nutritional regimes would produce demonstrable differences in soluble protein and isozyme patterns of peroxidase, polyphenol oxidase, and ascorbic acid oxidase.

RESULTS

Patterns of total soluble protein in pine seedling shoots grown for 3 months in sand flats are shown diagrammatically (Fig. 1). Each of four flats was fertilized with either a complete or modified 1/10 strength Hoagland's nutrient solution. Extracted tissue from seedlings grown in the flat fertilized with 1/10 Hoagland's with supplemental P had three less bands than tissue from seedlings fertilized with complete 1/10 Hoagland's. Omitting Cu or Fe from the complete nutrient solution resulted in two and one less bands, respectively, than found in extracts of seedlings fertilized with complete 1/10 Hoagland's.

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[1] F. C. STEWARD and J. T. BARBER, *Ann. N.Y. Acad. Sci.* **121**, 525 (1964).

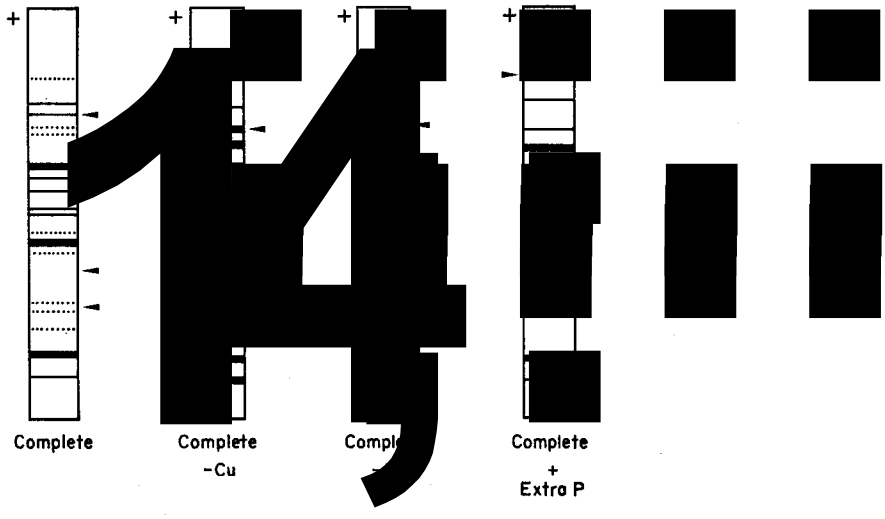

FIG. 1. ACRYLAMIDE GELS SHOWING TOTAL SOLUBLE PROTEIN BANDS FROM SHOOTS OF SEEDLINGS GROWN UNDER FOUR NUTRIENT REGIMES.

The isozyme patterns of peroxidase, an Fe containing enzyme, and polyphenol oxidase and ascorbic acid oxidase, both Cu containing enzymes, were separated electrophoretically from the same acetone powder as used for total soluble protein. The number of isozymes for each of these enzymes was reduced in extracts of seedlings fertilized with the nutrient solution supplemented with extra P (Fig. 2).

Nitrogen applications to seedlings growing on Leon fine sand also produced demonstrable changes in enzyme patterns in seedling foliage. Peroxidase isozymes (Fig. 3a) and Fe containing protein (Fig. 3b) patterns from extracts of foliage from 7-month-old seedlings are shown. Nitrogen at a rate of 100 ppm reduced the number of peroxidase isozymes from three to two when compared to unfertilized seedlings on Leon soil. The procedure used for separating Fe containing proteins yielded the same number of bands for fertilized and unfertilized seedlings, although the band patterns were different.

DISCUSSION

These data indicate that changes in enzyme patterns and total soluble protein do occur under different substrate nutritional regimes and that these changes can be demonstrated on acrylamide gels. This study did not attempt to determine the causes of the changes in enzyme activity or number of total soluble protein, but several hypotheses can be advanced. High levels of P in the growth media perhaps precipitated certain micronutrients such as Cu and Fe, thereby rendering them unavailable for uptake by the plant. Thus, insufficient quantities of these elements are present for incorporation into enzymes. Micronutrient deficiencies resulting from excess P fertilizer is a frequent consequence in agriculture.[2] Another possibility is that P uptake is increased by high substrate P, precipitating certain micronutrients within the plant tissue. The increasing use of P fertilizers in the southeastern U.S. pine forests prompted our interests in the consequences of high levels of P in the growth medium.

[2] S. J. LOCASCIO, P. H. EVERETT and J. G. A. FISKELL, *Proc. Am. Soc. Hort. Sci.* **92**, 583 (1968).

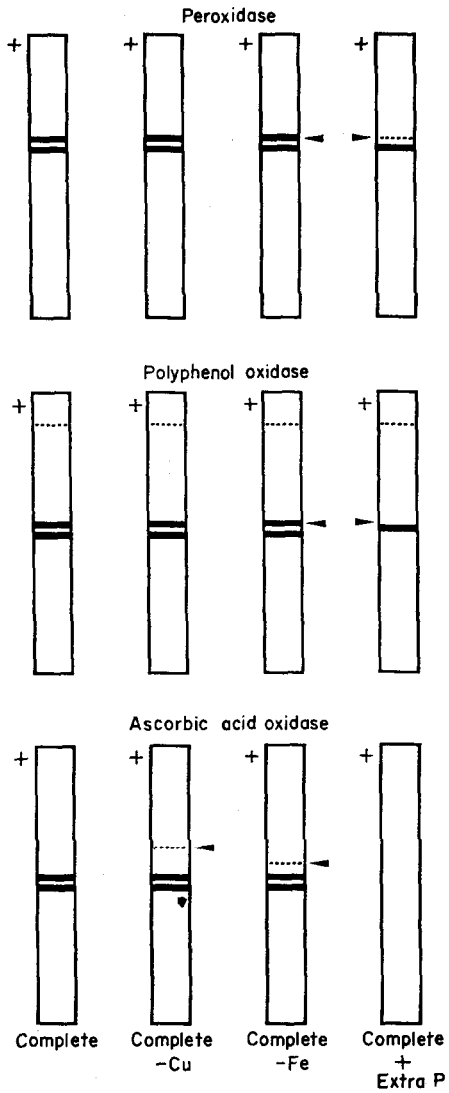

FIG. 2. ISOZYME PATTERNS OF PEROXIDASE, POLYPHENOL OXIDASE, AND ASCORBIC ACID OXIDASE AS INFLUENCED BY VARIOUS NUTRITIONAL REGIMES.

Omitting Cu or Fe from the nutrient solution had no effect on the number of isozymes of peroxidase and polyphenoloxidase. These young pine seedlings probably received sufficient Cu and Fe from the seed so that enzymes containing these micronutrients were unaffected. Activity differences in specific isozymes may have occurred but were not discernible by the visual assay procedures. Supplementing 1/10 Hoagland's with extra P resulted in no isozymes of ascorbic acid oxidase.

Nitrogen applied at 100 ppm to Leon fine sand reduced the number of peroxidase bands and altered the pattern of the Fe containing proteins. A possible explanation is that the application of N reduced the soil pH thereby increasing Mn availability for uptake.[3] Mn is

[3] G. W. BENGTSON, unpublished data (1968).

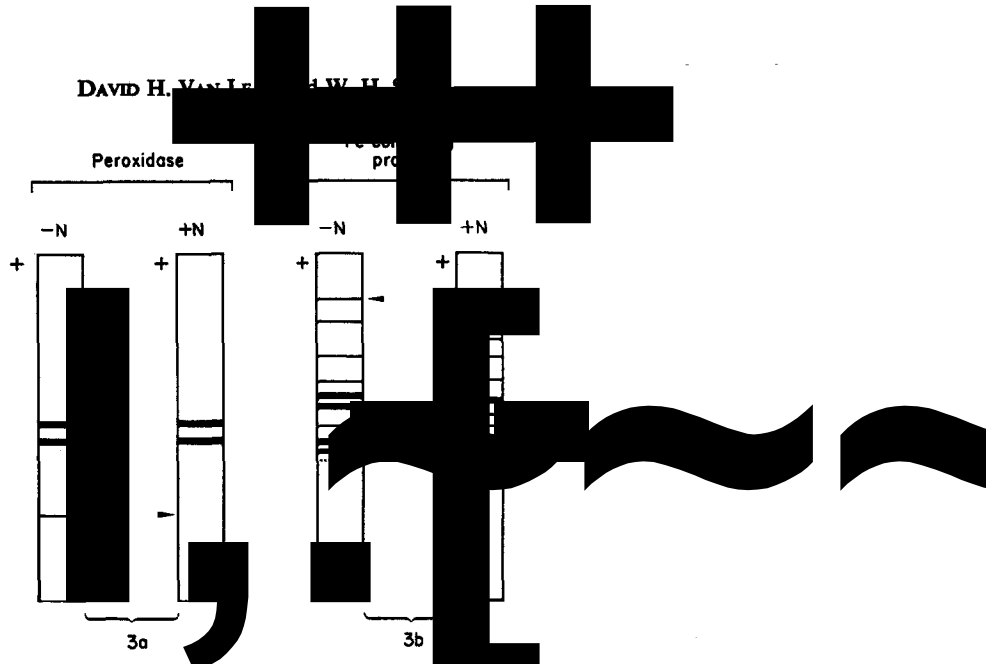

FIG. 3. ISOZYMES OF PEROXIDASE (3a) AND Fe CONTAINING PROTEI[redacted]EEDLING FOLIAGE AS INFLUENCED BY NITROGEN APPLICATION TO LEON FINE SAND.

antagonistic to Fe uptake and thus Fe may have been deficient. Thus, activity of peroxidase, an Fe containing enzyme, may have been reduced. Alternatively, peroxidase, an auxin oxidase, is reportedly sensitive to either low or high levels of Mn, *per se*.[4]

Tissue nutrient analysis, although valuable, involves many problems limiting its usefulness.[5] With further refinement, electrophoretic protein enzyme assays may be a useful technique to complement tissue analysis in seeking answers to diagnostic problems.

EXPERIMENTAL

Half-sib seedlings of slash pine (*Pinus elliottii* var. *elliottii* Engelm.) were grown in flats of acid-washed sand or in pots of Leon fine sand. Each of the four sand flats was fertilized with one of the following nutrient solutions: (1) 1/10 strength Hoagland, (2) 1/10 Hoagland minus Cu, (3) 1/10 Hoagland minus Fe, (4) 1/10 Hoagland with a double level of P. Nutrient solution was added to each flat five times over a 2-month period. Between nutrient supplements, flats were watered as needed. Pots of Leon fine sand were fertilized with 0 and 100 ppm N from urea added in solution to the soil surface after seedlings were about 2 months old.

Tissue analyzed for total soluble protein and enzyme activity included the apical centimeter of shoots from eighty seedlings grown in sand flats. Only foliage was sampled for seedlings grown in Leon soil. Polyacrylamide gel electrophoresis of tissue acetone-powder extracts was conducted essentially as described by Durzan[6] on duplicate mixed samples. In 100 ml of extracting solution were 15·0 g of urea, 0·5 g $K_2S_2O_5$, 1·0 g ascorbic acid, 0·1 g Cleland's reagent, 4 ml 10% Tween 20 and Tris to pH 8·5. When ascorbic acid oxidase activity was assayed, ascorbic acid was omitted from the extracting solution.

Duplicate gels containing total soluble protein were incubated in 10% TCA for 30 min, after which they were stained with 0·2% Coomassie blue (3 drops) and stored in the dark for about 70 hr. Peroxidase gels were incubated for 1 hr in solution containing equal parts of 0·006% H_2O_2 and saturated benzidine solution. A similar solution, except for being buffered with sodium acetate–acetic acid saturated with EDTA, was used for incubating Fe containing protein gels. Polyphenol oxidase isozymes were visualized by placing gels in 0·008 M 3,4-dihydroxyphenylalanine (DOPA) for 1 hr, while ascorbic acid oxidase bands were visualized by placing gels in a solution of 200 ppm ascorbic acid (neutralized with NaOH) in 0·1 M citrate-P buffer with pH 4·5.

[4] D. M. TAYLOR, P. W. MORGAN, H. E. JOHAM and J. V. AMIN, *Plant Physiol.* **43**, 243 (1968).
[5] D. J. DURZAN, *Can. J. Botany* **44**, 359 (1966).
[6] P. F. SMITH, *Ann. Rev. Plant Physiol.* **13**, 81 (1962).