



Peroxidase zymograms of sweet potato (*Ipomoea batatas* (L.) Lam.) grown under hydroponic culture

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Abstract. Stability of peroxidase zymogram under hydroponic culture was studied to test whether nitrogen and potassium had any effect on zymogram patterns. Although no significant change was observed in most major band patterns on leaves and fibrous roots in different N, K treatments, some minor variations in relative band intensity were noted. At least two cathodic bands and nineteen anodic bands were identified from this study. Peroxidase activity indicated in the zymogram is much more obvious in fibrous roots than in leaves and storage roots. Varietal differences can be distinguished from the relative band intensity of some specific zones on the zymograms of fibrous roots and leaves. Furthermore, young storage roots of cultivar Tainong 66 has an extra band, easily distinguished from Tainong 67. However, relative peroxidase band number in matured tissues were usually fewer and become ambiguous. Tissues sampled from soil culture showed no significant difference from hydroponic culture in most qualitative band patterns. Thus, the utilization of the peroxidase zymogram as a genetic marker in sweet potato for physiological and genetic correlation studies between the zymogram patterns and agronomic characters should be feasible. The hydroponic cultural system used in this study also provided a useful tool for the study of the relationship between peroxidase activity and root initiation, which is believed to be difficult in soil cultural system.

Key words: Fibrous root; Isozyme; Leaf; Nitrogen; Potassium; Varietal variation.

Introduction

Sweet Potato [*Ipomoea batatas* (L.) Lam.] is an autohexaploid ($2n=6x=90$) species (cf. Nishiyama, 1982) and is mostly propagated asexually as a cultivated vegetable. Genomic complexity and self-incompatibility have limited genetic studies. In the past decade, biochemical genetic markers via isozyme and protein electrophoresis have been available for most economically important crop species (Tanksley and Orton, 1983). However, little information is available on isozyme studies in sweet potato.

Peroxidases (E.C.1.11.1.7) are believed to occur among many higher plants and are mostly tissue specific (Bosch *et al.*, 1987). Pao and Morgan (1988) sum-

marized the possible roles and functions of peroxidase, including IAA (indole-3-acetic acid) oxidation, lignin synthesis, and detoxification of H_2O_2 and organic peroxides. Peroxidase activity also tends to change in the course of root and shoot initiation, leaf formation, callus growth, senescence, and internode elongation (Pao and Morgan, 1988). Furthermore, increase in peroxidase activity when plants are infected by pathogens has been noted by several researchers (Uritani and Stahmann, 1961; Novacky and Hampton, 1968; Seevers *et al.*, 1971). Felder (1976) pointed out that peroxidases are able to utilize peroxide to oxidize a variety of hydrogen donors such as phenolics, cytochrome-C, and nitrite. Differential expression of peroxidase in various growth and differentiation stages also have been documented (Warner and Upadhyya, 1968; Tanksley and

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idase zymogram analysis. Since 4 weeks after transplant, samples were collected at two weeks interval. Samples were soaked in gel buffer overnight and ground by a glass rod. The crude extract was obtained by centrifuging (13,000 rpm) at 4°C for 10 minutes. The extracts were then loaded into gels by using 3 mm × 3 mm Whatman No. 3 filter papers as wicks to absorb the extract and inserted into the gels. For each treatment at least 3 replications were conducted. The electrode buffer (pH 8.3) is 0.19M boric acid and 0.04M lithium hydroxide. The extraction buffer and gel buffer (pH 8.3) were a mixture of one part electrode buffer and 9 parts 0.05M Trizma base and 0.007M citric acid (Buffer C of Shields *et al.*, 1983).

Horizontal gel electrophoresis, similar to Chen *et al.* (1989), was performed in a 4°C cold chamber. The gel composition was 9% polyacrylamide mixed with 2% starch. Protein concentrations tend to vary from sample to sample; therefore, the ratio of relative tissue weight to extraction buffer (volume) was used to keep the comparison more reasonable. On average, the ratio of tissue weight to extraction buffer was 400 mg/250 µl for leaf tissue and 400 mg/200 µl for fibrous root and young storage roots. The gel was ran at 160V for 9 hours. After a run of one hour, the wicks were removed. Gels were stained by soaking in acetate buffer (pH 5.0) for 15 minutes at 10°C. 1% o-dianisidine (dissolved in 95% alcohol) was then added to a final concentration 0.1%. Three drops of 30% H₂O₂ were added into the stain buffer and incubated at room temperature until the appearance of enzyme activity (Yu, 1988).

Leaves and storage roots sampled from two field locations with minimum cultural management served as the control to distinguish the difference between soil culture and hydroponic culture.

Results and Discussion

Treatments of 400 ppm N and K were eliminated from this analysis due to the poor survival rate under hydroponic culture. Both anodal and cathodal bands were observed in leaves and fibrous roots. The anodal bands are more complex than the cathodal bands. At least 19 anodal bands were observed, but only two cathodal bands were identifiable (Fig. 1). The two cathodal bands are obvious in fibrous root tissue of the two cultivars especially in Tainong 66. However, the C1

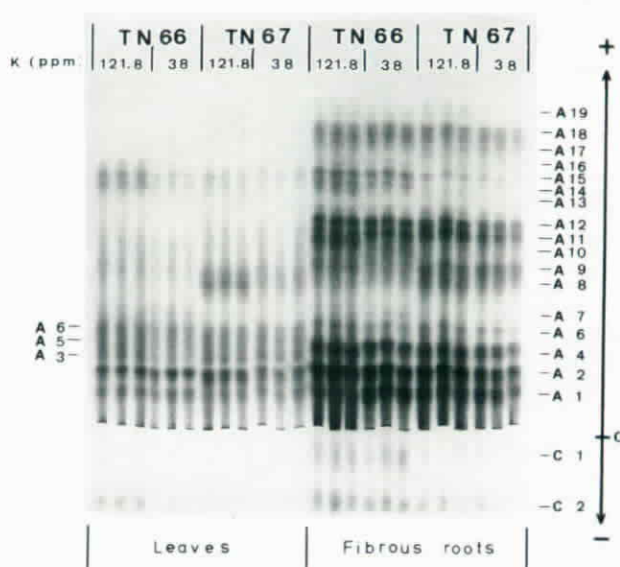


Fig. 1. Peroxidase zymograms of leaves and fibrous roots of two cultivars sampled 55 days after transplant with two levels of potassium treatment under hydroponic culture; each line representing a replication and the amount of extract applied to each line is the same.

band is generally missing in the leaf and young storage root tissue (Table 2 and Fig. 1 and 2). Lack of a C2 band was also occasionally observed in leaves and young storage roots. Differences between the two cultivars were not significant in leaf, fibrous roots and all nutrient application studies. Nevertheless, some minor variations do occur. The major difference in zymogram patterns between leaf and fibrous roots is the presence of a cluster of three anodal bands (A3, A5 and A6) in leaf as compared to the presence of Bands A4 and A6 in the fibrous roots. From Band A8 to A12, most bands are either unclear or very faint in the leaf tissue, although the fibrous roots exhibit a more clear pattern. Bands A17 to A19 were either missing or very weak in the leaves. Band A19 was also not found in young storage root tissue. Only Bands A2, A6, A11, A15, A17 and A18 were commonly found in young storage roots of both cultivars (Fig. 2). Band patterns of young storage root in Tainong 66 can be distinguished from Tainong 67 by an extra anodal band right below Band A6 (Fig. 2). The mobility of this extra band is very close to Band A5 in leaf band patterns. Band A3 was also visible in young storage roots of Tainong 66 from the soil culture but not from the hydroponic culture.

Table 2. Band distribution of peroxidase zymograms from various tissues of two sweet potato cultivars

Band No.	Cultivar					
	Tainong 66			Tainong 67		
	Leaf	Fibrous root	Storage root	Leaf	Fibrous root	Storage root
C 2	+	+	—	±	+	—
C 1	—	+	—	—	±	—
A 1	+	+	—	+	+	—
A 2	+	+	+	+	+	+
A 3	+	—	—	+	—	—
A 4	—	+	—	—	+	—
A 5	+	—	+	+	—	—
A 6	+	+	+	+	+	+
A 7	+	+	—	—	±	—
A 8	—	+	—	+	+	—
A 9	±	+	—	+	+	—
A10	—	+	—	—	—	—
A11	—	+	+	—	+	+
A12	—	+	—	—	+	—
A13	—	+	—	—	±	—
A14	+	+	—	±	±	±
A15	+	+	+	+	+	±
A16	+	+	—	±	±	—
A17	—	+	+	±	+	+
A18	—	+	+	±	+	+
A19	—	+	—	—	+	—

*“+”: Presence; “±”: Faint band; “—”: Null band.

Leaf peroxidase zymograms show no significant variation except in the relative intensity in Band A8 - A9 and A13 - A16 zones between two cultivars. As indicated in Table 2, Tainong 67 had either weak or missing bands in A10, A13, A14 and A16 in comparison with the fibrous roots of Tainong 66. Major band patterns in leaves and fibrous roots are generally not affected by the application of nutrient solutions. However, some minor variations were noted. In nitrogen application treatment, relative band intensity in the fibrous roots tended to increase as the amount of N rise in culture for both cultivars when samples were collected 55 days after transplant. Bands A8 and A9 had a more dense color at high N level. Nevertheless, the leaf tissue was not affected by this treatment. Potassium level also affected the intensity of A8 and A9 bands on both leaf and fibrous roots (Fig. 1). Bands A8 to A15 seemed much more sensitive to N and P nutrients than the other zones.

Occasionally, samples from different growth

stages during the culture did have interactions with nutrient treatments on peroxidase band intensity. For example, both Tainong 66 and Tainong 67 had a relative dense color at the A13 to A15 zone 55 days after transplant at higher nitrogen and potassium levels. Nitrogen is important for the shoot and leaf development and eventually effects the yield of storage roots. Potassium is also known to play an important role in storage root development. In this study, although nutrient application did not show significant effects on major band patterns and relative band intensity, some specific bands, such as the appearance of A10 band in Tainong 66, seemed to have positive correlation with nutrient application. Since peroxidases are believed to be associated with IAA oxidases, which are known to have an effect on storage root initiation and development of sweet potato, the use of peroxidase isozyme as markers for the study of IAA oxidase and storage initiation might be possible. From this study, the number of peroxidase bands in leaf tissue is generally not as great

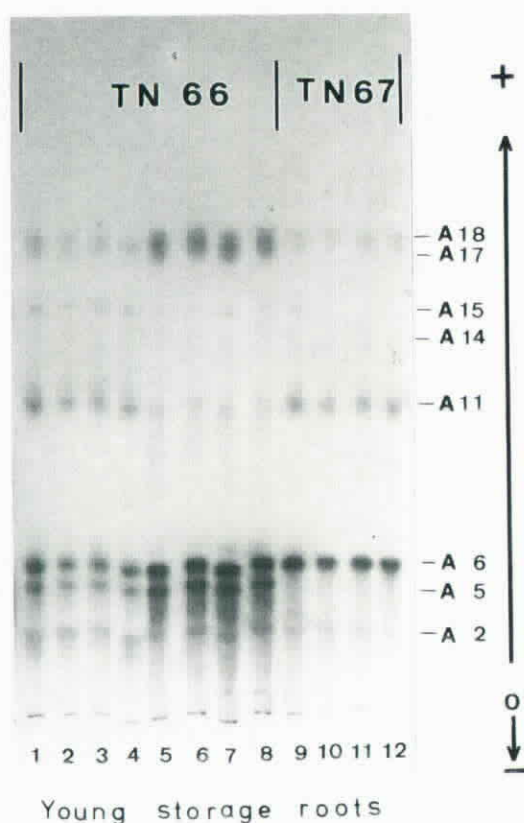


Fig. 2. Peroxidase zymograms of young storage roots from hydroponic and soil culture. Sample No. 1-4: hydroponic culture; Sample No. 5-12: soil culture; each line representing a replication and the amount of extract applied to each line is the same.

as in fibrous roots, but most band patterns were about the same. As storage roots developed, only 5 to 8 bands remained (Fig. 2). The relative band intensity of most bands is not affected by different N and K treatments. Varietal difference in response to different N, K treatments and some tissue specific bands were noted (Figs. 1 and 2). Furthermore, in comparison with other isozymes such as α , β amylase, polyphenol oxidase and phosphorylase studied in our laboratory (unpublished data), peroxidase zymograms are more stable one.

Environmental factors such as temperature, photoperiod and location are reported to vary zymogram patterns (Fieldes *et al.*, 1987; Warner and Upadhyaya, 1968; Cox and Worrall, 1987). Pao and Morgan (1988) reported that total peroxidase activity is not consistent with age in sorgham. No genotype variation was noted in their study, however, the isozyme complement underwent mainly quantitative change during

development. Nevertheless, Grosso *et al.* (1987) indicated genotypic, tissue specific, and developmental variation in peroxidase activity of *Phaseolus vulgaris*. From this study, quantitative variation in peroxidase band intensity is the most frequent variations. No significant variation in major peroxidase bands was noted, however, some minor variations based on variety, tissue specificity, cultural status, and nutrient level did occur. The assay system might provide a useful tool for developing physiological and genetic markers for developmental studies on storage root initiation of sweet potato.

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水耕甘藷之過氧化酶酶譜分析

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利用水耕液培養法探究兩主要肥料要素氮與鉀之濃度是否對甘藷過氧化酶之酶譜產生影響，結果顯示雖然一般甘藷之葉及鬚根其過氧化酶酶譜之主要帶型並無因氮肥及鉀肥施用量之不同而有明顯之改變，然一小部份之變異確實存在，尤其是在條帶顏色之相對深度上。由本實驗材料分析中發現甘藷過氧化酶酶譜至少有兩條陰向條帶及十九條陽向條帶可被鑑定出，且因所分析組織之不同而有異，一般而言其鬚根所產生之條帶比由葉子或儲存根之組織所產生之條帶為多，品種間之差異性可從葉或鬚根其特定條帶之相對深度及帶型判定。兩參試品種中，台農 66 號之幼儲藏根更明顯地比台農 67 號多出一條帶。由田間土壤栽培得之植株取樣所得之酶譜亦與水耕培養分析者十分接近，然較成熟之儲藏根及葉子其過氧化酶之條帶較少。此一測試結果顯示甘藷過氧化酶酶譜應可用來作品種鑑定及其他農藝生理、遺傳性狀間相互關係之探討。此外，所用之水耕系統並可進一步提供過氧化酶等與根形成關係探討之工具。