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CHARACTERIZING SWEET POTATO GERM PLASM, USING PAGE ON ROOTS GROWN IN VITRO

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Introduction

Characterization is a major aspect of germ plasm conservation. General plant germ plasm collections are now estimated to aggregate several millions of accessions because of extensive duplications (Williams 1989). The maintenance of such duplicates is expensive and could be avoided by characterizing the genotypes before they are incorporated into collections. Nowadays, beside field characterization (extremely important from the breeder's point of view), sophisticated methods based on fragmenting DNA molecules by restriction enzymes (RFLPs) have been developed to identify precisely each genotype. Nevertheless, methods involving polyacrylamide gel electrophoresis (PAGE) of proteins and isoenzymes are still a fast and practical tool for germ plasm characterization, especially to identify duplicates and mislabelled materials. Isoenzyme electrophoresis provides a description that is relatively unaffected by the environment and is economical and simple to use.

We aimed to develop a technique that would help characterize and identify sweet potato germ plasm. We expected this technique to be useful in manipulating germ plasm banks, and in helping identify duplicates and group similar genotypes before field evaluation.

Methods

The adventitious roots (length 0.30-0.40 cm; diameter 0.10-0.15 cm) of seven genotypes were cultured at 25 ^{o}C and under a 16-h light photoperiod. The nutrient medium was half-strength MS salts (Murashige and Skoog 1962), supplemented with calcium pantothenate (2 mg/L), gibberellic acid (20 mg/L), ascorbic acid (100 mg/L), calcium nitrate (100 mg/L), L-arginine HCl (100 mg/L), putrescine HCl (20 mg/L), and sucrose (3%).

Four roots were taken from each culture and homogenized, at a rate of 1:2 (w/v), with a buffer containing 50 mM Na_2PO_4 (pH 7.0) and 6 mM dithiothreitol (Shields et al. 1983). The homogenate was centrifuged at 13,000 *g* for 10 min at 5 ^{o}C, and 20 µL of the supernatant was located in the gel.

A high pH non-dissociating, discontinuous buffer system was used for the polyacrylamide gels as described by Davis and Orstein (1964). The resolving gel mixture was Tris-HCl (pH 8.8)

with 10% polyacrylamide, and the stacking gel mixture was Tris-HCl (pH 6.8) with 2.5% polyacrylamide. The reservoir buffer was Tris-glycine (pH 8.3).

Electrophoresis was carried out in a cooled Protean-II vertical slab apparatus connected to an electrophoresis power supply ATTA-AE-3105 with 500 V-500 mA capacity.

The gels were stained for esterase isoenzymes (Kahler and Allard 1970) and the zymograms analysed visually for relative electrophoretic mobility of bands, band quality, and stability and repeatability of results.

Results

The seven genotypes tested were heterogeneous, showing good genotype specificity. The band with Rf = 0.66 was common to all genotypes; Rf = 70 was common to six genotypes (but not to TIB-10). Other bands were common to more than one genotype, but each showed a unique array of bands. The zymograms showed stability in the patterns, with no differences among them during 1 month of culture. After that, however, changes occurred in the Rfs of the faster bands in 'Brondal', 'TIB-10', and 'Rose Centennial', which became slightly slower.

Discussion

Ortega (1987) developed techniques for characterizing sweet potato genotypes, based on the electrophoretic patterns of proteins and isoenzymes of the roots. Similarly Huaman and De la Fuente (1988) mentioned research conducted by Stegemann at the GTZ in Germany, using storage root proteins and esterases for verifying duplicates and characterization. These methods are, without doubt, effective and can be excellent alternatives when root cultures are not possible. However, thickened roots are necessary for using the technique. The choice of *in vitro* root cultures or *in vivo* thickened roots depends on the availability of plant material.

Esterase isoenzymes from sweet potato roots grown *in vitro* proved to be an adequate solution for the fast identification of genotypes, as the zymograms showed a specific array of bands for each of the seven genotypes that could be repeated from 7- to 30-day cultures.

The many advantages in using root cultures for characterization are:

(1) Roots are easily available throughout most of the life cycle of the sweet potato plants;
(2) Root cultures are quickly and easily produced, and do not require complicated techniques;

(3) Because only roots are used, the plants can be maintained without damaging their variability as characterization goes on;
(4) Because the zymograms showed stability among cultures that were 1 week to 1 month old, there is no hurry to use donor cultures and electrophoresis can be programmed over one month, using the same cultures;
(5) Because the plants do not need to be cultured in soil, the method is relatively cheap;
(6) Because cultures are kept in small containers in the laboratory, space is saved during culture and analysis;
(7) The genotypes are protected against environmental stresses, pests, and diseases.

Despite these advantages, isoenzymes are limited in the level of polymorphism and number of loci that can be detected. Thus, electrophoresis should be used in a manner that complements those standard methods of characterization that involve quantitative and qualitative descriptors (Bernatsky and Tanksley 1989; Ramírez et al. 1987; Simpson and Withers 1986).

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