

SDS-PAGE detection of a specific marker in 'Fécua Branca' cassava cultivar, *Manihot esculenta* Crantz (Euphorbiaceae)

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ABSTRACT. Proteins from young unexpanded leaves of seven cassava cultivars *Manihot esculenta*, Crantz (Euphorbiaceae) were investigated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The comparison was made through the protein patterns obtained, and their relative amounts determined in plants leaves infected or not with the bacteria *Xanthomonas axonopodis* pv. *manihotis*. The electrophoretic protein pattern obtained from the investigated cultivars ("Fibra", "Fécua Branca", "Branca de Santa Catarina", "Verdinha", "IAC-12", "IAC-13", and "IAC-14") showed a polypeptide subunit present exclusively in the "Fécua Branca" cultivar, with a molecular weight of 93.5 kDa. Also, protein fractions were more intensely stained in young leaves of *M. esculenta* plants infected with the bacteria. SDS-PAGE did not detect specific markers for pathogenesis, but the 93.5 kDa protein fraction can be used as a molecular marker for the "Fécua Branca" cultivar.

Key words: cassava cultivars, proteins, SDS-PAGE, cassava bacterial blight (CBB).

RESUMO. Detecção por SDS-PAGE de um marcador específico no cultivar de mandioca, Fécua Branca, *Manihot esculenta* Crantz (Euphorbiaceae). As proteínas de folhas jovens não expandidas de sete cultivares de mandioca, *Manihot esculenta*, Crantz (Euphorbiaceae) foram investigadas neste trabalho, através da técnica de eletroforese em gel de poliácridamida com dodecil sulfato de sódio (SDS-PAGE). As comparações foram feitas pelo padrão de proteínas obtido e as suas quantidades relativas em folhas de plantas de *M. esculenta* infectadas e não infectadas pela bactéria *Xanthomonas axonopodis* pv. *manihotis*. O padrão eletroforético das proteínas extraídas das cultivares investigadas ("Fibra", "Fécua Branca", "Branca de Santa Catarina", "Verdinha", "IAC-12", "IAC-13", and "IAC-14") mostrou uma subunidade polipeptídica com peso molecular diferente (93.5 kDa) exclusivamente presente na cultivar "Fécua Branca". As frações de proteínas nas folhas de plantas de *M. esculenta* infectadas pela bactéria apareceram mais intensamente coradas. A técnica de SDS-PAGE não detectou marcadores específicos relacionados com o processo de infecção, mas a fração protéica 93.5 kDa pode ser usada como marcador molecular da cultivar "Fécua Branca".

Palavras-chave: cultivares de mandioca, proteínas, SDS-PAGE, patogênese em mandioca.

Introduction

Cassava (*Manihot esculenta* Crantz) is the fourth most important carbohydrate source for human consumption in the tropics, after rice, sugar, and maize (Bellotti *et al.*, 1999). Cassava roots are mainly used for consumption in natura and for flour and starch production. Cassava leaves have also been evaluated as a protein source for human and/or domestic animal consumption (Peluzio *et al.*, 1998).

Vegetative propagation has been the cassava reproduction predominant form (Nassar, 1992; Olsen and Schaal, 1999). This means of reproduction perpetuates superior genetic combinations, but it also favors viral and bacterial diseases accumulation, which reduces productivity and may lead to the extinction of superior genotypes. The material losses mainly due to the causal agent of cassava bacterial blight (*Xanthomonas axonopodis* pv. *manihotis* Dye) have been reported (Verdier *et al.*, 1998).

Although cassava is propagated almost exclusively by stem cuttings, high levels of polymorphism have been found in *M. esculenta* germplasm from African, Colombian, and Brazilian varieties (Lefèvre and Charrier, 1973a, b; Chavarriaga-Aguierre *et al.*, 1999; Resende *et al.*, 2000). Isozymes have been analyzed to show genetic polymorphism and are used as molecular markers to discriminate and cluster 'unnamed' *M. esculenta* cultivars (Machado *et al.*, 2000). The allele frequencies for the various isozyme loci has been found to be different among cassava cultivars; however, none of the allele products acts as specific marker for specific cultivars (Resende *et al.*, 2000).

In the present study, we investigated the use of proteins, after separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), to detect pathogenesis markers, or specific markers for cassava cultivars.

Material and methods

The *M. esculenta* plants, originated from cultivars collected in the State of Parana northwestern ("Fibra") and southwestern ("Fécua Branca" and "Verdinha") regions, and from cultivars produced at the Agronomic Institute of Campinas ("Branca de Santa Catarina", "IAC 12", "IAC 13", and "IAC 14"), State of São Paulo. These cultivars have been maintained by vegetative propagation for six years and are used as material supply in production programs (Gonçalves-Vidigal *et al.*, 1997); they are planted yearly at the State University of Maringá (UEM) Iguatemi Experimental Farm.

Samples "Fibra", "Fécua Branca", "Branca de Santa Catarina", "Verdinha", "IAC-12", "IAC-13", and "IAC-14" cultivars were planted in plastic bags (volume = 5 L), containing a mix of red soil (Paleudult), sand, and organic material at 3:1:1 proportion (v/v), previously sterilized with methyl bromide. Plant growth occurred in an acclimated chamber, at 18–32°C. Ammonium sulphate and potassium chloride (1:3) were applied at 15-day intervals, for 18 weeks.

Xanthomonas axonopodis pv. *Manihotis*, cultivated in culture medium containing 1% peptone, 0.5% yeast extract, 1% NaCl, and 1.5% agar (Henrifarma), pH 7.5, was used to infect five plants at each cassava cultivar. The bacterial solution (OD = 0.5; λ = 600 nm) was prepared with twice-distilled water and applied, with a syringe, in the leaves lower epidermis surface, to induce a lesion. After 7 days, the typical water-soaked angular leaf spots and exudate production below the terminal buds showed on infected plants, indicating pathogenesis. After two

weeks, samples of young unexpanded leaves, from the top of infected and non-infected plants, were collected and used for electrophoretic analysis.

The resolution polyacrylamide gel (15%) was prepared using 9.32 mL of 30% acrylamide and 0.10% bis-acrylamide dissolved in 5.4 mL of 1.5 M Tris-HCl, pH 8.8, 108.3 μ L 20% SDS, 5.95 mL twice-distilled water, 108.3 μ L 10% ammonium persulfate, and 4.3 μ L TEMED. The stack gel was prepared using 1.9 mL of 10% acrylamide and 0.5% bis-acrylamide dissolved in 1.9 mL of 1.5 M Tris-HCl, pH 6.8, 18.8 μ L 20% SDS, 18.8 mL twice-distilled water, 30 μ L 10% ammonium persulfate, and 1.5 μ L TEMED. In the electrode chambers we used 0.01 M Tris-glycine, pH 8.3, and 500 mg SDS.

Samples of young unexpanded leaves (10–20 mm in length) from each infected and non-infected *M. esculenta* plant were individually homogenized with a glass rod in an Eppendorf tube using 80 μ L of 1.0 M phosphate buffer, pH 7.0, 5% PVP-40, 0.01 M dithiothreitol (DTT), 10 mM sodium metabisulfite, 50 mM ascorbic acid, 1.0 mM EDTA, and 0.5% β -mercaptoethanol solution. After homogenization, the samples were centrifuged at 25,000 rpm for 30 minutes at 4 °C in a Sorvall 3K30 centrifuge and the supernatant of each sample was used for analysis. Protein concentration was determined by the Bradford (1976) method and 30 μ g of protein from each sample was submitted to electrophoresis. The running conditions were 2 hours at 80 V, and 2–3 hours at 120 V. Protein molecular weight standards (Gibco BRL, Grand Island, NJ) were electrophoresed with the samples of *M. esculenta* leaves and used to determine the relative molecular weight of cassava proteins.

After electrophoresis, the gels were incubated for 12 hours with 50 mL ethanol, 12 mL acetic acid, 75 μ L formaldehyde, and 39 mL twice-distilled water. After fixation, the gel was washed three times with 50% ethanol (20 min each time), once with 3% sodium thiosulfate for 1 min, and three times with twice-distilled water (20 min each time). The gels were stained with 250 mg silver nitrate, 75 μ L formaldehyde, and 100 mL twice-distilled water. Then, they were washed three times with twice-distilled water and the proteins were visualized, using 6 g sodium carbonate, 2 mL sodium thiosulfate, and 50 μ L formaldehyde dissolved in 100 mL of twice-distilled water. The proteins were fixed with 50 mL ethanol, 75 μ L formaldehyde, and 38 mL twice-distilled water.

Polyacrylamide gels were dried as described by Pereira *et al.* (2001). The gels were kept at room temperature for 1 h in a mixture of 7.5% acetic acid and 10% glycerol embedded in 5% gelatin, placed between two sheets of wet cellophane paper

stretched on an embroidering hoop, and left to dry for 24-48 h.

Results and discussion

A large number of proteins (33 polypeptide subunits) was detected in the young unexpanded leaves of *M. esculenta* cultivars. Extracted leaf proteins electrophoretic patterns showed that the main protein subunits were concentrated between 14.5 and 230 kDa. A careful analysis of the gels led to the identification of a polypeptide subunit with different molecular weight (93.5 kDa), present exclusively in the "Fécua Branca" cultivar (Figure 1).

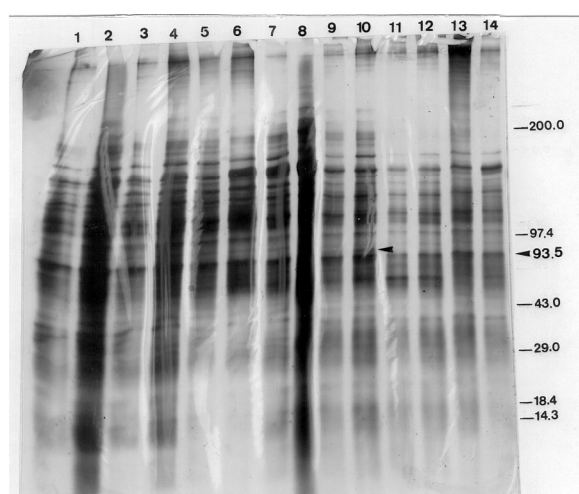


Figure 1. Protein electrophoretic patterns in young unexpanded leaves from the cultivars "Fibra" (lines 1-2), "Branca de Santa Catarina" (lines 3-4), "IAC-12" (lines 5-6), "IAC-13" (lines 7-8), "Fécua Branca" (lines 9-10), "Verdinha" (lines 11-12), and "IAC-14" (lines 13-14) of *Manihot esculenta*, obtained by SDS-PAGE. Samples 2, 4, 6, 8, 10, 12, and 14 show proteins more intensely stained in leaves of *M. esculenta* plants infected with *Xanthomonas axonopodis* pv. *manihotis*. The specific 93.5 kDa protein was exclusively present in the "Fécua Branca" cultivar (lines 9-10). Protein molecular weight standards (Gibco BRL, Grand Island, NJ) are indicated on the right side of the polyacrylamide gel

A biochemical marker in the "Fécua Branca" cultivar is very important, since the cassava cultivars evaluation in the Northwestern region of Parana State showed that this cultivar has the highest root yielding, high root dry matter contents, and high bacterial resistance (Vidigal-Filho *et al.*, 2000). Furthermore, biochemical markers are also important because there is some disagreement when botanical and agronomic characteristics are applied to discriminate closely related cultivars of *M. esculenta* (Leitão Filho, 1971; Viégas, 1976; Silva, 1979). The present study showed that the protein fraction of 93.5 kDa can be identified in this important "Fécua Branca" cultivar and then, used in

combination with botanical and agronomic parameters to certify the cultivated or newly derived plants genetic makeup.

No specific protein was detected in leaves of *M. esculenta* plants infected with *X. axonopodis* pv. *manihotis*. However, the electrophoretic patterns obtained showed that the protein fractions were more intensely stained in the young leaves of *M. esculenta* plants infected by the bacterium (Figure 1). The more intensely stained proteins suggest a quantitative difference and are an indication of a differential gene expression in young unexpanded leaves of cassava infected with *X. axonopodis* pv. *manihotis*. An increase in synthesis of the enzymes peroxidases, phenylalanine ammonia lyase, glutamic dehydrogenase, and ascorbic acid oxidase, has been reported to occur in rice leaves infected with *X. oryzae* bacteria (Rao and Nayudu, 1979), and qualitative and quantitative differences in the esterase isozymes have been reported for young unexpanded leaves of cassava plants infected with *X. axonopodis* pv. *manihotis* (Pereira *et al.*, 2001). The protein fractions differential expression verified in the leaves of cassava does not necessarily indicates large biochemical genetic difference, but should be a consequence of physiological and biochemical reactions in the infected plant.

The SDS-PAGE preparation for protein study in young unexpanded leaves of *M. esculenta* does not detect specific pathogenesis markers, but its relative simplicity and low cost, in contrast to other molecular markers, especially recommend the latter technique as an effective tool to detect a specific marker (protein 93.5 kDa) in the "Fécua Branca" cassava cultivar.

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