VAUGHN AND LULAI: SUBERIN STAINS
COMPARISON OF FLUORESCENT STAINS FOR THE DETECTION OF SUBERIN IN POTATO PERIDERM

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Abstract

Several fluorescent staining procedures to detect suberin in normal periderm of potato were assessed. Among the stains tested, treatment with the alkaloid fluorochrome berberine followed by counterstaining with crystal violet appears to be the most sensitive combination. Crystal violet increased contrast by quenching lignin fluorescence which is also present in the periderm cell walls. This technique may be useful for studying the role of suberin in disease resistance and wound healing of potato tubers and other tissues.

Compendio

Se determinaron varios procedimientos de tinción fluorescente para detectar suberina en el periderma normal de papa. Entre las tinciones probadas, el tratamiento con el alcaloide berberina fluoricromada seguido por una tinción con cristal violeta parece ser la combinación más sensítiiva. El cristal violeta incrementó el contraste por la extinción de la fluorescencia de la lignina que se encuentra también presente en las paredes de las células del periderma. Esta técnica puede ser útil para el estudio del papel de la suberina en la resistencia a las enfermedades y en la cicatrización de heridas en los tubérculos y otros tejidos.

Introduction

The periderm of the potato (Solanum tuberosum L.) tuber is a specialized layer of cells arising from a cambium layer, the phellogen, and serves to prevent water loss from the underlying storage parenchymal cells as well as prevent the entry of pathogens (11). The periderm in mature tubers is composed of 6-10 layers of cells with suberized cell walls (11). Suberin is a

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Mention of company or trade names does not imply endorsement by the United States Department of Agriculture.

Accepted for publication: February 4, 1991.

ADDITIONAL KEY WORDS: Aniline blue, berberine, crystal violet, lignin.
polymer composed of phenolic and aliphatic subunits present in periderms and other plant tissues (8). Suberin is also deposited in walls of living cells underneath wounded tissue (1, 12). Suberin is extremely resistant to attack by potato pathogens because they appear to be unable to effectively degrade mature suberin enzymatically (9).

Several procedures have been routinely used to stain suberin for microscopical examination in various plant tissues (2, 7, 10), but we have found the sensitivity and/or specificity of these techniques to be relatively low. Fluorescent techniques are generally more sensitive than white light techniques due to the increased contrast generated against the dark background. The alkaloid berberine has been shown to be effective in localizing suberin and lignin in fresh sections of plant tissue (4). Aniline blue was used to quench nonspecific fluorescence (fluorescence in tissue not containing lignin or suberin) while providing a fluorochrome for callose (4). We have found that the stain crystal violet, when applied to sections obtained from fixed, embedded tissue following berberine staining, quenched lignin fluorescence as well as nonspecific berberine staining. In this paper we compare this technique with several other techniques that have been used to localize suberin in potato periderm.

**Materials and Methods**

Potato tubers of the cultivar Norchip were grown at the Red River Valley Potato Research Farm near Grand Forks, North Dakota. Tubers were hand harvested and allowed to suberize at 20 C for 2 weeks. Three tubers free of any evident disease were sampled, and pieces of tissue (2 X 10 X 10 mm) containing the periderm layer were excised, fixed in FAA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin (Paraplast Plus) (2). Serial sections 5 μm thick were cut on a rotary microtome and affixed to glass slides using Haupt's adhesive (2). Paraffin was removed from the sections with xylene, and the sections were rinsed in two changes of 95% ethanol to remove any xylene, and air-dried. Slides were either untreated for observance of autofluorescence or stained in either (a) aqueous 0.1% (w/v) berberine-HCl, 15 min, followed by aqueous 0.5% (w/v) aniline blue, 15 min; (b) aqueous 0.1% (w/v) berberine-HCl, 15 min, followed by aqueous 0.5% (w/v) crystal violet, 1 min; (c) a saturated solution of Sudan III in 70% ethanol, 30 min; or (d) a saturated solution of phloroglucinol in 20% HCl, 5 min. Slides were rinsed twice with either 70% ethanol (slides stained with Sudan III) or distilled water (all other treatments) and dried on a warming plate at 40 C. After drying, a drop of immersion oil and a coverslip were placed over the sections. Sections were viewed with a Zeiss Universal microscope using excitation filter BP 450-490 (450-490 nm peak emission), chromatic beam splitter FT 510 (510 nm) and barrier filter LP 520 (520 nm). Pictures of representative sections were