Scientia Horticulturae 224 (2017) 215–218

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

A rapid and effective method for observation of suberized cell layers in potato tuber skin

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ARTICLE INFO

Keywords:
Fluorescence observation
Potato (*Solanum tuberosum* L.) tuber
Potato common scab
Microtuber

ABSTRACT

The suberization of surface cells is important for potato tubers for water and mineral metabolisms, and disease resistance during growth and development. Observation of structural changes of suberized cell layers can provide the most constructive logical evidence for related research. A quick and effective method to observe suberized cell layer in potato tuber skin is reported in this paper. This improved method removed the common fixation steps and reduced staining time to only 5–10 min. The process to analyze a tuber sample takes less than 30 min. Various types of potato tubers, including mature and immature tubers, PCS (potato common scab) pathogen infected tuber and microtubers were used in this study. Qualified observations were obtained and are presented. The method provides an alternative for research related to tuber skin studies, such as potato tuber disease development, maturation, and storage quality.

1. Introduction

Suberin is a complex polyester composed of poly-functional, long-chain fatty acids (suberin acids) and glycerol found in many plants (Graça, 2015). The suberization of surface cells is important for potato tubers to control water pumping in and out, maintain mineral metabolism and provide protection against pathogen infection during tuber growth. It is also a spontaneous developmental phenomenon in potato tuber maturation. The potato is widely used in research focused on studies of suberin biosynthesis (Vishwanath et al., 2015; Landgraf et al., 2014), skin composition (Company-Arumí et al., 2016), transpiration (Schreiber et al., 2005) and degradation by pathogens (Beaulieu et al., 2016). However, observation of structural changes in suberized cell layers under various stress conditions is less frequently reported.

Due to high complex genome and relatively long genetic transformation cycle of potatoes, researchers studying suberin turned to *Arabidopsis thaliana* for a period of time (Domergue et al., 2010; Panikashvili et al., 2010). However, since *Arabidopsis thaliana* plants are too small for morphological observations and they do not produce tubers, potatoes are the preferred model plant for suberin related studies. As Ranathunge et al. (2011) pointed out that there are many gaps and unanswered questions in suberin research, the increasing availability of molecular tools for model systems will help to fill these gaps in the future. With potatoes as the experimental material, researchers began to use gene silencing (Verdaguer et al., 2016), mutations (Thangavel et al., 2016) and other new technologies for more in-depth analysis of suberin.

Potatoes are widely cultivated around the world and, unfortunately, are prone to many difficult to control diseases (e.g. potato common scab). Field management measures, such as chemical control, biocontrol, fertilizer, irrigation, crop rotation, alteration of planting dates, or soil pH manipulation are not fully effective. More and more evidence shows a clear positive relationship between suberized cell layers and disease resistance in potatoes (Padilla-Reynaud et al., 2015; Boher et al., 2013). Morphological observation of suberized cell layers is, therefore, becoming a valuable tool to study potato tubers − pathogens interaction. The structural change observation of suberized cell layers can provide the most logical evidence for related research. Earlier, Brundrett et al. (1988, 1991) reported a systematic suberin staining procedure with Berberine-Aniline Blue, Sudan Red 7B and Fluoral yellow 088 in plants. Lulai and Morgan (1992) introduced Neutral Red (NR) as a sensitive cytofluorochrome histochemical probing for the hydrophobic domain of suberin in the potato periderm. Lux et al. (2005) improved a formula of staining and clearing solutions based on the previous reports. In order to obtain higher quality observation results, SEM (scanning electron microscopy) and TEM (transmission electron microscopy) techniques have also been applied for suberin morphological observations (Serra et al., 2009; Franke et al., 2005).

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http://dx.doi.org/10.1016/j.scienta.2017.06.024
Received 26 January 2017; Received in revised form 15 May 2017; Accepted 14 June 2017
Available online 11 July 2017

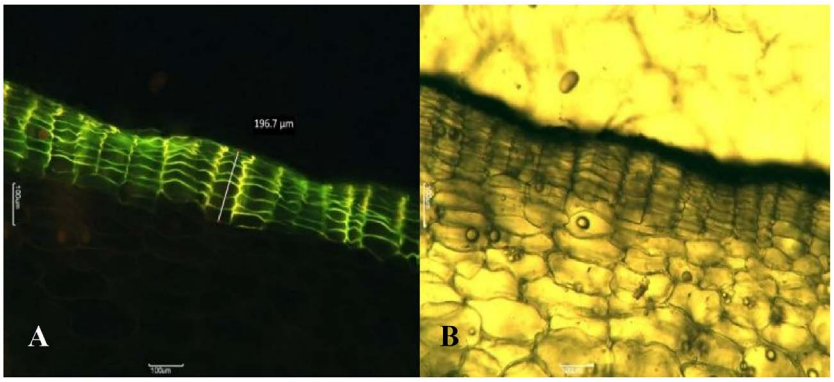

Fig. 1. Cross-sections of a field grown mature potato tuber (*Solanum tuberosum* L. cv. Bintje). (A) Under fluorescence microscope and (B) the same image under the bright field. Scale bars = 100 µm.

Although most of these methods can achieve satisfactory results, they are often time consuming, require more reagents, and have complicated technical steps. Particularly, they cannot be used for observation of large numbers of tubers in a short time. Since suberin biosynthesis in cells is continuously changing during growth, transportation and storage or pathogen infection processes of potatoes, without real-time observation and analysis of suberin the experimental results cannot reflect the real changes of the biological processes. At the same time, there is a certain degree of variation in different tuber parts of potatoes, therefore, a large number of observations in a short time are required to reduce sample variations.

A quick and effective method for observation of suberized cell layer in potato tuber skins is reported in this paper. This improved method removed the common fixation step, which normally takes overnight, and reduced staining time from 2 h to 5–10 min. This newly developed method takes only less than 30 min to analyze a potato sample from the beginning of free-hand sectioning to the completion of the photographing to obtain high-quality photographs for further analysis.

2. Materials and methods

2.1. Potato materials

Potato (*Solanum tuberosum* L.) varieties used in this study included cultivars 'Bintje', 'Green Mountain' and 'Goldrush'. Cultivar 'Bintje' (Munstersen × Fransen) was registered in Canada in 1979 (registration number 1933); cv. 'Goldrush' (ND450-3Russ × Lemhi Russet) was registered in Canada in 1999 (registration number 4905); cv. 'Green Mountain' (Dunmore × Excelsior) was registered in Canada in 1923 (registration number P-11) (http://www.inspection.gc.ca/plants/potatoes/potato-varieties/eng/1299172436155/1299172577580). Tubers from different sources and under different stages of development were used in this study, including field grown mature potato tubers (170 days after planting), field grown immature potato tubers (80 days after planting), PCS pathogen infected tuber harvest from Dalhousie University experimental field; and microtubers generated from tissue culture techniques.

2.2. Observations of suberin layer in tuber skins

The solutions required in this analysis included the fluorescent staining solution TBO containing 0.05% (w/v) Toluidine Blue O (BioShop catalogue number TOL250 https://www.bioshopcanada.com/secure/detail.asp?Pin=TOL250) dissolved in 0.1 M sodium acetate (pH 4.5), NR solution containing 0.1% (w/v) Neutral Red (> 90%, BioShop catalogue number NTR002; https://www.bioshopcanada.com/secure/detail.asp?Pin=NTR002) dissolved in 0.1 M potassium phosphate (pH 6.5), and the clearing solution containing lactic acid (85% lactic acid, BioShop catalogue number LAC660; https://www.bioshopcanada.com/secure/detail.asp?Pin=LAC660) and ddH_2O water at 1:1 (v/v) ratio.

Potato tubers are first washed with water and dried carefully on paper towel. A small portion of the skin tissue (approximately 5 mm wide x 10 mm long x 3 mm thick) from a tuber was cut off using a surgical blade. It was then hand-sliced by cross section as thin as possible using a two-side razor blade. The resulting section was about 5 mm x 3 mm. After cutting 20–30 slices, they are carefully transferred into a 1.5 mL Eppendorf tube by forceps. Immediately after, 1 mL TBO solution was added into the Eppendorf tube and kept for 5–10 min in complete darkness to stain the sections. Following this, the TBO solution was removed and slices were thoroughly washed two/three times with ddH_2O. Slices were then stained with the NR solution for 1–3 min and subsequently washed two/three times with ddH_2O. Finally, the slices were washed once with the cleaning solution for a few seconds and then again two/three times with ddH_2O.

Slices were placed on a glass slide and observed under a fluorescent microscope (Leica DM IRB) using the excitation filter at 450–490 nm. Images were recorded using a CCD colour camera system (Moticam 2500, 5.0 M Pixel) with the software program (Motic images plus 2.0). Cell layer was determined based on the images and the thickness of the suberized cell layers was measured using the same software based on the scale bar of the image as a standard.

3. Results

The method described in this study allowed us to observe clearly the suberized cells under the fluorescent microscope. Fig. 1 demonstrated the suberized cell layers. The method also allowed us to count the number of suberized cell layers. There are 8 suberized cell layers. The thickness of the suberized cell layers is 196.7 µm.

With the same method, tubers of other field grown varieties and microtubers were also analyzed (Fig. 2). The results confirmed that this method could be applied to the analysis of all kinds of potato tubers, field tubers, tissue cultured microtubers and potato common scab (PCS) infected tubers included (Fig. 2). Besides potatoes, we have used this method to detect suberized cells in other plants, including carrot root, melon stem, ginger rhizome, and green beans pod (data not shown). We are confident that with careful adjustment of the procedure, the method could be applied to all plants in general.

Most importantly, the entire procedure to process one tuber requires only 20–30 min, which means a person can easily analyze over a dozen of tubers during a regular working day. The possibility to evaluate a larger number of tubers per day allows us to perform fast tests when tuber maturation process is still ongoing. Fig. 3 demonstrated the progress of suberized cell layers in cv. 'Bintje' during 14 days of storage after harvested from a field trial.

Based on our observations, we found that in some varieties (example 'Goldrush'), the suberized cells were well packed. In such cases, the number of layers was high, but the thickness of the layer was not. Therefore, we believe that a suberized skin should be characterized by