Animation and Image Analysis for Evaluation of Soybean (Glycine max L. Merrill.) Somatic Embryo Growth

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Introduction

Recent advances in machine vision technology have resulted in an increase in the use of image analysis in different areas of cell biology. Image analysis has been used to study in vitro-grown animal cells using light microscopy (Gauthier et al., 1992) and the movement and compartmentalization of proteins in plant cells using confocal microscopy (Haseloff and Siemering,

1998).

In the area of plant developmental biology, image analysis has been utilized with tissue culture systems to collect information on tissue growth and development. Smith et al. (1989) were the first to use image analysis as a non-invasive method to evaluate growth of in vitro cultures of some woody, vegetables and grass plants. Computer vision techniques were later used to follow the kinetics of somatic embryo formation in suspension cultures of carrot (Cazzulino et al., 1990; Ibaraki et al., 1996). In order to select coffee somatic embryos with high germination efficiency, Ling et al. (1996) used machine vision to first establish the relationship between the elongation coefficient and the growth aspect ratio of mature embryos and then determine the ability of those embryos to germinate. Using computer analysis to evaluate size, shape and color-related measurements from images of sweet potato somatic embryos (during torpedo and cotyledonary-stage), Padmanabhan et al. (1998a) identified germination-competent and non-competent embryo types and then compared these characteristics with the embryo anatomy using histological analysis (Padmanabhan et al.,1998b). These reports demonstrated the potential of image analysis for embryo growth studies. However, to our knowledge, there are no reports on the use of image analysis to evaluate growth of somatic embryos from their earliest developmental stage or using computer animation to better understand embryo development and growth over time. For most analysis of plant biological systems, evaluations are still based on human vision and results can often be highly variable and very subjective. This makes comparisons difficult with qualitative data from different laboratories.

The objective of this work was to demonstrate the capability of characterizing somatic embryo development using image analysis and animation techniques.

Materials and Methods

Biological material:

Proliferative embryogenic tissue of soybean (Glycine max L. Merrill. cv. Jack) was initiated and maintained as described by Santarem et al. (1997). Briefly, somatic embryos were initiated from immature cotyledons, cultured on a medium containing 40 mgL-1 2,4-D. Embryogenic tissues were subcultured

and maintained on a medium contain 20 mgL-1 2,4-D. For embryo development, embryogenic tissues were transferred to a medium containing MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1968), 6% maltose, 0.5% activated charcoal, pH 5.7 and 0.2% Gelrite.

Environmental conditions:

Developing embryos were placed under the following two different environmental conditions: 23°C with low light intensity (12 'mol'm-2's-1) and 27°C with high light intensity (38 'mol'm-2's-1). A replicate for both environments was made.

Acquisition system:

Digital images of somatic embryos growing under the above conditions were taken every 24 hrs using a 24 bit RGB digital camera (Leica DC 100), mounted on a Leica LZMFIII stereomicroscope using a stroboscopic illuminator (Fostec ACE, USA). Images were captured using DC100 2.31 software (Leica Microscopy Systems, Germany).

Image Analysis:

Images of 18 clusters of developing embryos were collected every 24 hrs and analyzed using Visilog 5.1 software (Noesis S.A., 1997). Area (square mm), color (based on CIE chromaticity diagram (Norman et al., 1991)) and other morphological characteristics were evaluated.

Three grains of sterile sand were placed on the media around the clusters of developing embryos and used as fixed points for registration referencing during embryo growth. After the images were collected, they were rotated using Visilog (Noesis S.A., 1997), using the first image (day 0) as a reference to rotate the images for the next days (from day 1 to 12) using the Visilog warp tool (Noesis S.A., 1997). Image background of rotated images was subtracted and replaced using Adobe Photoshop 5.0.

For the animation in figure 1, each frame contains the image of a representative developing embryo cluster from each treatment as well as a graph showing the mean of the two dimensional surface area of the cluster.

For animation purposes, the images were processed as described above and were placed together using the SMIL language in Real Player (www.real.com).

Statistical analysis:

A completely randomize design was used. Two-dimensional surface area measurements, obtained using the Visilog analysis tool, were analyzed using SAS. The General Linear Model was used and the means by treatment were grouped (SAS Institute Inc., 1990).

Results and Discussion

The embryo images, collected daily, showed obvious differences in growth within the two environments tested. This difference became clear after 6 days (P= 0.05), where embryos grown at 27°C developed more rapidly than those cultured at 23°C. Embryo morphology was revealed in excellent detail and data on growth was clearly reflected in the animation. Side-by-side comparisons of the animation (Fig. 1) showed more consistent and synchronous development of embryos at 23°C compared to embryos at 27°C. Although use of the higher temperature apparently increased the growth rate of the somatic embryos, those grown at 23°C displayed much higher germination frequencies after further embryo development and desiccation (unpublished data).

Figure 1. Real Player animation of embryo cluster growth. Download RealPlayer.

Detailed analysis of the images suggests that the color of the globular embryos may be correlated with embryo differentiation and further growth. Since perceived embryo color may depend on the monitor's characteristics, absolute color designation is given in the CIE 1931 (x, y) chromaticity diagram (CIE, 1931), which is the standard for quantitatively specifying color. The (x, y) coordinate following each named color indicates the exact location of the color on the CIE chromaticity diagram. As can be observed with this animation, pale yellow (0.38, 0.36) globular embryos appear to have a low capacity of differentiation and the few than do differentiate show very

abnormal morphology and delayed growth. Pale green (0.32, 0.62) globular embryos showed the highest differentiation rate. Changes in embryo color over time is also noticeable. A high percent of globular embryos at 23°C turned translucent green (0.24, 0.36) after 4 days and then dark green (0.02, 0.68) within 6 - 7 days. At one week of development, the number of globular embryos with a dark green color was higher at 23°C than at 27°C. During early stages of embryo development, color may be a useful parameter for estimation of embryo quality.

As opposed to end point image analysis to measure embryo growth in other reports, the continuous monitoring of the embryo development allows us to "backtrack" developing embryos to determine exactly what tissue types give rise to the highest quality embryos. To start and stop the animation click on the play and pause buttons.

Animation and image analysis show tremendous potential for increasing our understanding of plant developmental processes (see also http://sunflower.bio.indiana.edu/~rhangart/plantmotion/moovie_frames/plantmoovies.html. With the proper reporter gene, image analysis could be further exploited to gather information on comparative gene expression over time. The expression of fluorescent proteins driven by tissue specific promoters, could be potentially followed during specific stages of the embryo development. As image collection instrumentation and image analysis software improve, data collection and analysis will be tremendously simplified using animation and image analysis.

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