GFP tracking and quantification in transgenic developing somatic embryos of soybean (*Glycine max* (L) Merrill)

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ABSTRACT

The jellyfish (*Aequorea victoria*) green fluorescent protein (GFP) gene has been successfully used as a reporter gene to evaluate the efficacy of transformation and as a marker for cellular and developmental processes in both animal and plant cells. GFP detection is very rapid and does not require destructive assays, which make GFP one of the most suitable reporter genes for tracking gene expression *in vivo* in transgenic cells, tissues, organs, and whole organisms [1, 2]. Although GFP has been recently used for promoter analysis, very little has been reported on GFP tracking and the actual quantification of GFP in living specimens. Our goal was to develop a system to track and quantitatively evaluate GFP expression over time in developing GFP-transgenic soybean somatic embryos.

The plasmids, *pGLe-mgfp5-ER* (lectin promoter [3]; *mgfp5-ER*) and *35S-mgfp5-ER* (35S promoter; *mgfp5-ER*), were introduced into proliferative embryogenic tissue of soybean [4] using particle bombardment. For GFP tracking and quantification, transgenic embryogenic tissue was placed on a medium conductive to embryo development. Thirty days after transfer, single embryos were transferred to fresh medium and digital images were acquired every 24 hrs for 21 days using a digital CCD SPOT-RT camera mounted on a Leica MZFLIII microscope equipped with a fluorescent module with the GFP plus excitation (480/40 nm) and emission (510 nm) filters. Images were processed and analyzed using Visilog® 5.1 software. Areas of interest were selected manually and GFP expression was evaluated by analyzing the intensity of the green channel of the color vision system and the area expressing GFP.

GFP expression driven by the 35S promoter was observed throughout all developmental stages of the embryos. In contrast, GFP expression driven by the lectin promoter was initially observed only on the tips and edges of the cotyledons 30 days after the embryogenic tissues were first transferred to development medium. By the middle stages of embryo development (35 – 40 days), GFP was observed in all cotyledonary tissue. During the latest stage of embryo development (45 – 60 days), GFP was observed in the cotyledons and in the upper half of the hypocotyl. Following embryo germination, the lectin driven GFP was not observed in root or shoot tissues. Evaluation of green intensity, based on gray values, shows that lectin-GFP expression gradually increases during the period of 30 to 60 days of embryo development and is highly correlated (R^2 = 0.57, P = < 0.0001) with the growth of the embryos. Intensity of GFP was not correlated (R^2 = 0.11, P = 0.0009) with changes in the green color of the embryos. Visually, the embryos turned yellow during the last stages of development. Lectin-GFP expression decreased in both area and intensity during embryo germination only in the cotyledons, but not in the hypocotyl. GFP can be used as a marker of developmental processes in plants. We have found the use of image analysis extremely useful to quantify the dynamic expression of GFP under regulatory control of different promoters.

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