Using Green Fluorescent Protein (GFP) Reporter Genes in RNAlater™ Fixed Tissue

Anna-Lisa Paul\textsuperscript{1} and Robert J. Ferl\textsuperscript{1,2}

\textsuperscript{1}Department of Horticultural Sciences and \textsuperscript{2}Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32611 USA

Plants in an orbital environment experience conditions that are distinctly unlike the earth-bound environments that have directed their evolution on Earth. This presents a unique opportunity to examine biological responses, particularly those involved in integrating gravity as a force shaping biological systems. One means of measuring these adaptive responses is to monitor the expression of genes that allow survival in those peculiar environments. In a recent series of spaceflight experiments conducted on the International Space Station (ISS) we utilized Arabidopsis thaliana (Arabidopsis) plants engineered with specific gene promoters driving to Green Fluorescent Protein (GFP). These biological sensor plants are referred to as TAGES, an acronym for Transgenic Arabidopsis Gene Expression System. One component of the TAGES Spaceflight experiment was the use of several different GFP reporter gene constructions to record tissue-specific changes in the patterns of gene expression in real time. Another component was the evaluation of genome-wide changes in these plants, a goal that required the use of the nucleic acid preservative RNAlater™ (Ambion).

The utility of GFP gene reporters is well established (Haseloff & Amos, 1995; Manak et al., 2002; Paul et al., 2003; Paul et al., 2004; Sheen et al., 1995). The application to spaceflight is especially appealing as gene expression data can be collected telemetrically, thereby placing minimal demands on crew time.

The use of RNAlater™ also offered the opportunity to evaluate the preservation of GFP for postflight analysis. In a series of preliminary experiments prior to launch, the TAGES plants were utilized in ground-based studies as part of a Payload Verification Test (PVT) to test the operations and science before the experiment was launched. One aspect that was evaluated was the responsiveness of the biosensor plants to environmental stress and the efficacy of using RNAlater™ to preserve evidence of GFP expression long after the stress response was incurred, in addition to its general use as an effective means to preserve the integrity of RNA.

In the PVT, plates of TAGES plates were prepared with two or three genotypes and a variety of methods used to collect GFP expression data. Figure 1 shows GFP reporter gene plants grown vertically on nutrient agar plates. The TAGES ISS experiments utilized a variety of plant lines engineered to report on several aspects of the spaceflight environment, and among the plants shown in Figure 1, the left-hand side of each plate contains Adh::GFP plants, which respond to reduced levels of oxygen. The plants on the far right-hand side of the plate are used as positive controls (CaMV35s::GFP) and continuously express GFP throughout their cells. In the experiment shown

Figure 1. 100mm\textsuperscript{2} Petri plate containing TAGES seedlings from the Payload Verification Test. The photograph on the left (A) was taken in white light, (B) shows the same plate photographed through a 510nm long pass filter in 488nm blue light with an Illumatool™ device. The native red fluorescence of chlorophyll is prominent in the Adh::GFP plants on the left side of the plate, and intense GFP expression can be seen in the positive control plants (CaMV35s::GFP) on the right side of the plate. The image in C was captured during PVT with the GFP Imaging System that is currently installed aboard the ISS.
in Figure 1A and 1B, the roots of the plants were covered with a thin layer of solid agar, which reduced the flow of oxygen to those tissues. As seen from the faint green glow in the roots of those plants, that thin blanket of agar was sufficient to induce the sensitive Adh::GFP reporter gene. Figure 1A was photographed with a standard digital camera in white light, and that same camera was used to photograph 1B through an Illumatool™ (Light Tools Research) GFP imaging device. The image in Figure 1C was captured by the TAGES GFP Imaging System (GIS) developed by Kennedy Space Center, which was later launched on STS-129 and installed on the ISS for the subsequent TAGES spaceflight experiments.

Although the resolution of the GIS is excellent for general patterns of gene expression, it was our hope that RNALater™-preserved material returned from the ISS could be utilized to capture higher resolution tissue-specific data. Figure 2 shows images of a representative plant returned from orbit in TAGES experiment 2B, which was launched on STS-131, and spent several weeks on the ISS as part of the APEX-TAGES payload. The plants were grown for 12 days on the ISS, and then harvested to RNALater™ in a Kennedy Space Center Fixation Tube (KFT) (Ferl et al., 2011). The KFT was allowed to equilibrate at ambient temperature for 12-18 hours, and then placed in the MELFI freezer on the ISS. The samples were recovered on the return flight of STS-132, photographed and then prepared for subsequent biochemical assays (analyses in progress). The RNALater™ fixed plant in Figure 2 is a constitutive GFP line (CaMV35s::GFP). This line was used as a positive control for GFP expression in the imaging portion of the TAGES experiment. Figure 2A shows a white light image 2A and 2B provides the corresponding fluorescent image of the plant. 2C shows a tangle of roots from the collection of harvested material before the individual plants were separated. The GFP expressing root can be seen clearly on the backgrounds of the non-GFP roots.

Ground-based experiments were also conducted to calibrate GFP reporter gene utility for gene expression assays in RNALater™ fixed material. Figure 3 shows the hypocotyl-root junction of Adh::GFP plants. The top row shows un-stressed plants, while the bottom row shows the pattern of GFP expression in hypoxic plants (flooding for 48 hours). Panels 3A and 3D are white light image, 3B and 3E are the corresponding fluorescent microscope images, and panels 3C and 3F are fluorescent microscope images of the same regions of plants after preservation in RNALater™. The exposure times for 3B and 3E is 5 seconds (Olympus S2X12, GFP long-pass filter); however, since preservation in RNALater™ diminishes the fluorescence of both chlorophyll (red) and GFP (green), the exposure time for 3C and 3F was extended to 10 seconds. In 3C the autofluorescence of plant cell wall is noticeable, and in 3F it can be seen that the GFP fluorescence that was masked by chlorophyll in the hypocotyl can now be seen with better clarity. The phenomenon of diminished GFP fluorescence in RNALater™ preserved samples has also been demonstrated in flow cytometry applications (Zaitoun et al., 2010).
Cell and subcellular resolution of GFP is possible in RNAlater™ preserved tissue. Figure 4 shows the fluorescent microscopy used to collect subcellular data from a plant preserved in RNAlater™. The white light photograph in 4A shows the surface of the leaf, venation and a trichome. Figure 4B shows the same section of the leaf under fluorescent illumination to visualize GFP expression and 4C shows the same section after preservation in RNAlater™. Again, an extended exposure time was required to obtain similar signal strengths as seen in fresh tissue. In this case, the image in 4C was also subjected to post-capture processing. Minor adjustments of brightness and contrast can enhance the visualization of GFP. A comparison of the positions of the guard cells fluorescing in 4B compared to those in 4C confirm that the green fluorescence. Fluorescent microscopy shows that GFP is distributed primarily among the guard cells of the epidermis, and is especially prominent in the nuclei of the guard cells and the trichome.

RNAAlater™ preservation does not seem to be an impediment to at least a few other standard methods of subcellular labeling techniques. Figure 5 provides examples of DAPI nuclear staining (Figures 5A, 5B, 5C) and calcofluor white cell wall staining.

In conclusion, plants containing GFP reporter genes can still be evaluated after being preserved in RNAlater™. This method of preserving GFP analyses may be important in situations where sophisticated imaging technology is not immediately available on orbit. It does appear that the level of GFP visualization is lessened in RNAlater™, but that the location of the signal is maintained even at subcellular levels. In addition, it is clear that plant material preserved in RNAlater™ can also be effectively used for other traditional sub-cellular labeling techniques. Given that RNAlater™ is a well-documented on-orbit fixative with a repeated and extensive deployment history within KFTs, it is likely that RNAlater™ could serve space biology science well beyond its originally intended purposes of nucleic acid preservation.

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REFERENCES


