FLUORESCENCE IMAGING FOR EVALUATION OF WATER AVAILABILITY TO PLANTS

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Abstract: The aim of this study is to investigate the applicability of fluorescence imaging for dynamic aspects of soil-water availability to plants. Rhodamine B solution was used as a fluorophore and its transport through the plants was monitored. Mainly the fluorophore optimal concentration and image acquisition parameters were investigated.

Key Words: fluorophores, plants, imaging

INTRODUCTION

The proper circumstances are necessary for plant growth, which enhances the slopes stability by mechanical support of plant roots, and improve the aboveground biomass by the presence of water in soil. Therefore, it is essential to identify the appropriate materials improving the water-holding capacity of soil (Yang et al. 2014).

Water shortage is commonly related to increased level of reactive oxygen species, which affects nearly all plant functions. High temperatures and irradiation and low water accessibility during the growing season result in stress perception (Farzi et al. 2017).

Silica-based granules as well as hydrogels are two types of the most commonly used water-retention additives. Hydrogels are conventionally polyacrylamide gels in a crystalline form, which are capable of absorbing water up to several hundred-times of their own weight. SiO_2-based additives, on the other hand, are usually enriched with carbon components or cellulose and therefore they are able to adhere to soil particles. This effect leads to increase of the surface area followed by adsorption of water or other nutrients (Farrell et al. 2013).

In this study, for the first time, the applicability of real-time monitoring of solution transport by fluorescence *in vivo* imaging system was investigated. The aim of this study was to determine if the fluorescence imaging technique may serve for characterization of water-retention additives and for observation of the water availability.

MATERIAL AND METHODS

Preparation of experimental plant model

Sunflower (*Helianthus annuus*) used as an experimental plant was cultivated by procedure as described in previous work (Vaneckova et al. 2016). In first experiments, leaves of 2 weeks old plant were cut, washed with water and immersed in 2 ml tube containing water solution of rhodamine B.

Similarly, whole plants (with roots) were first washed and then placed in the 5 ml tube containing the fluorophore. All the plant models were fixed in the tube with parafilm.

Fluorescence imaging

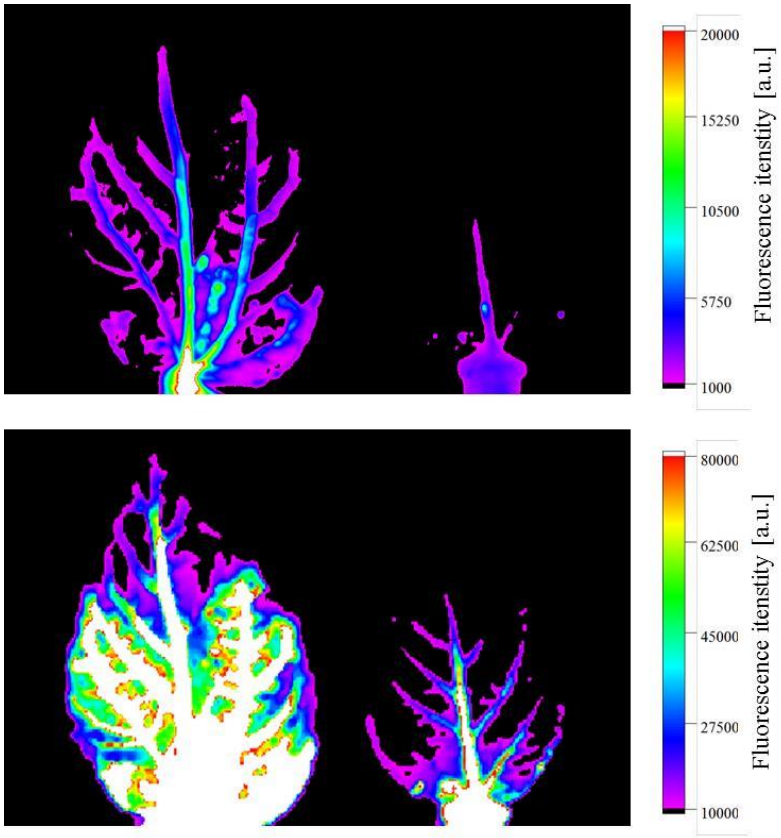
The fluorescent images were captured by *In Vivo* Xtreme Imaging System (Bruker, MA, USA). Rhodamine B in plants was examined at excitation wavelength λ_{ex} 550 nm and emission wavelength λ_{em} 600 nm. The parameters of imaging were set as follows: f-stop 1.1, field of view 19 × 19 cm; exposure time and binning were manually optimized to maximize detection efficiency. Fluorescence intensity in the regions of interest was quantified by Bruker Molecular Imaging Software.

RESULTS AND DISCUSSION

Imaging parameters optimization

As a first step, imaging parameters were optimized. Under the exposure time 2 s and binning 1×1 (Figure 1, top), we were able to visualize rhodamine B in concentration of 1×10^{-7} M (Figure 1, Top left), whereas transport of rhodamine in concentration of 1×10^{-7} M (Figure 1, Top right) cannot be clearly seen in the plant. However, increased exposure time and binning enabled to expand the detection capability. As shown in Figure 1 Bottom, concentration as low as 1×10^{-8} M can be observed under acquisition parameters: exposure time 10 s and binning 4 × 4.

Figure 1 Fluorescence images of sunflower leaves immersed in rhodamine B solution (1×10^{-7} M – left, 1×10^{-8} M – right), excitation wavelength 550 nm, emission wavelength 600 nm. Top – exposition time 2 seconds, binning 1×1, Bottom – exposition time 10 seconds, binning 4×4



Time dependence

Based on the results obtained using the leaves, the whole-plant experiments were carried out. As shown in Figure 2, the sunflower plant was immersed in the rhodamine B solution and images were taken at specific time-intervals (0, 4, and 8 hours). In comparison with control experiment (immersion in pure water), significant increase in fluorescence intensity was observed in dependence on time of treatment. The proposed method is enabling not only to monitor the extremely low amounts of fluid being transported into the plant, but also real-time visualization of the distribution within the plant (Figure 3). Moreover, it is possible to quantify the fluorescent signal in the particular point of the plant and therefore to determine the time required to deliver the solution to the specific plant part (Figure 4).

Figure 2 Photograph of sunflower plant immersed in rhodamine solution

Figure 3 Whole plant imaging in 4-hour time intervals. Top row – rhodamine 1×10^{-7}M (in water), bottom row – control (water).

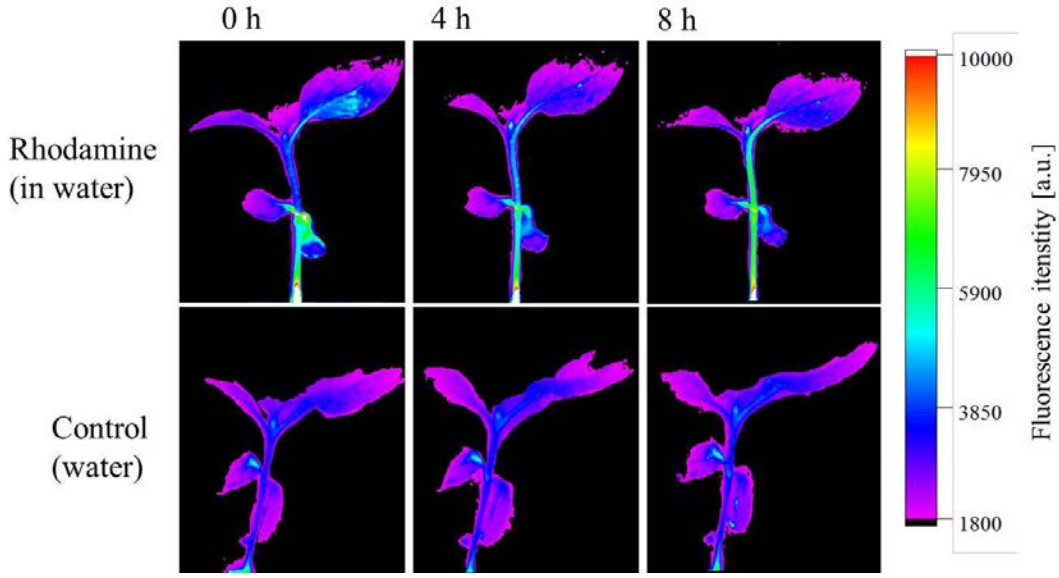
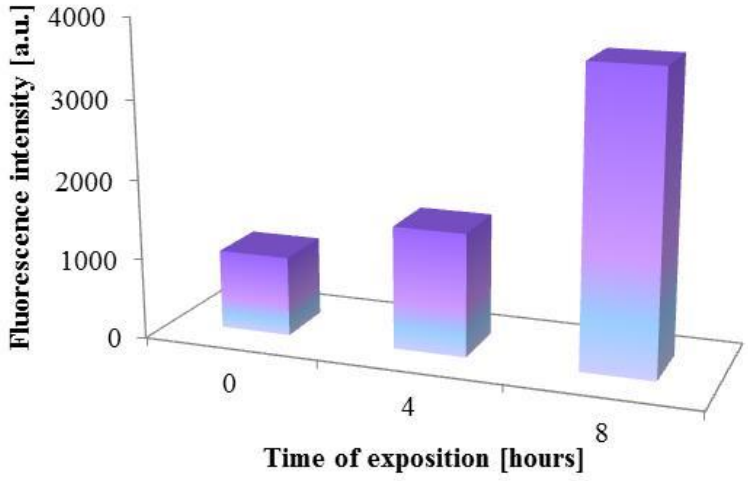


Figure 4 Quantification of fluorescence in the sunflower stem (mean intensity, control signal subtracted)



Even though, the presence of the rhodamine B might potentially influence the uptake of the fluid from the water-retention compound due to the toxicity to the plant cells, the sensitivity of the fluorescence imaging enables the use of extremely low concentrations (submicromalar). As shown elsewhere (Tan et al. 2014), the toxic effect due to the reactive oxygen species was observed in thousand-time higher concentrations (submilimolar). Therefore, it is believed that the influence of root cell damage on the fluid transport is eliminated.

CONCLUSION

Based on the results, the fluorescence imaging is demonstrated to be capable of monitoring and evaluate the ability of the water-retention additive to deliver water to the plant. Using the submicromolar concentrations of fluorophores (e.g., rhodamine B), it is possible to quantify the effectivity of water-supply systems in long-term real-time experiments.

In future, the water-retention additives will be soaked by the solution of rhodamine B and their ability of water supplementation will be investigated.

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