A rapid and efficient measure of GSH/GSSG ratio in perennial plant tissue using luminescence method based on GSH/GSSG-Glo™ Assay Promega kit

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Recently more and more studies identified the relationship between oxidative metabolism and plant developmental processes. We get interested in the involvement of ROS metabolism in the control of plant architecture, in addition to the well identified nutrient and hormone factors. It is well described in the literature that variations in ROS metabolism are key event in cell signaling (del Río, 2015; Mittler, 2017). As an example, some authors pointed out the correlation between the cell cycle progression and the ratio of reduced and oxidized glutathione (GSH/GSSG) in plant development (Diaz Vivancos *et al.,* 2010). The widely used method to access this ratio is based on colorimetric reaction between DTNB (5,5-dithio-bis-(2-nitrobenzoic acid)) and GSH measured by photospectrometry (412nm). However, this method is rather complex and suffers from interactions with many colored compounds present in sample extracts at this wavelength, particularly in lignified perennial plants. In our study, we improved the method in order to avoid these problems based on luminescence quantification. For this purpose, we adapted the GSH/GSSG-Glo ™ kit from Promega to plant tissues. The main modification of the manufacturer protocol consist in the addition of an acid extraction followed by a neutralization steps prior resuming with standard Promega kit protocol. In our hands, we routinely achieved rapid yet precise measurements of GSH and GSSG contents and easy GSH/GSSG ratio determination. It is worth noting that this procedure overcomes the issues which otherwise are likely to occur due to the high content of colored compounds and of polyphenols in plant tissues that interfere with photospectrometer measurements. The improvement of the initial protocol with the acid extraction was critical to obtain realistic and repeatable measurements of GSH/GSSG ratio in perennial plants, that are of great importance in plant development studies.

Key words: GSH and GSSG assay, GSH/GSSG ratio, perennial plant tissue, protocol improvement