Dynamics of glutathione during *Cyclamen persicum* somatic embryogenesis

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Introduction

The endogenous glutathione redox state is defined as the ratio of the concentration of the reduced form (GSH) and sum of the concentrations of the oxidized and the reduced form (GSH + GSSG) (BELMONTE ET AL. 2006). Several studies have reported on the crucial role of a high redox state of glutathione in the early stages of somatic embryo development. Moreover, some authors suggest that the formation of abnormal embryos can be correlated with low levels of GSSG in the late maturation phase.

The aims of this study were to quantify the contents of endogenous GSH, GSSG and cysteine (one of the GSH precursors) during differentiation of somatic embryos of *Cyclamen persicum* in order to better understand the role of somatic embryogenesis and glutathione redox state.

Material and Methods

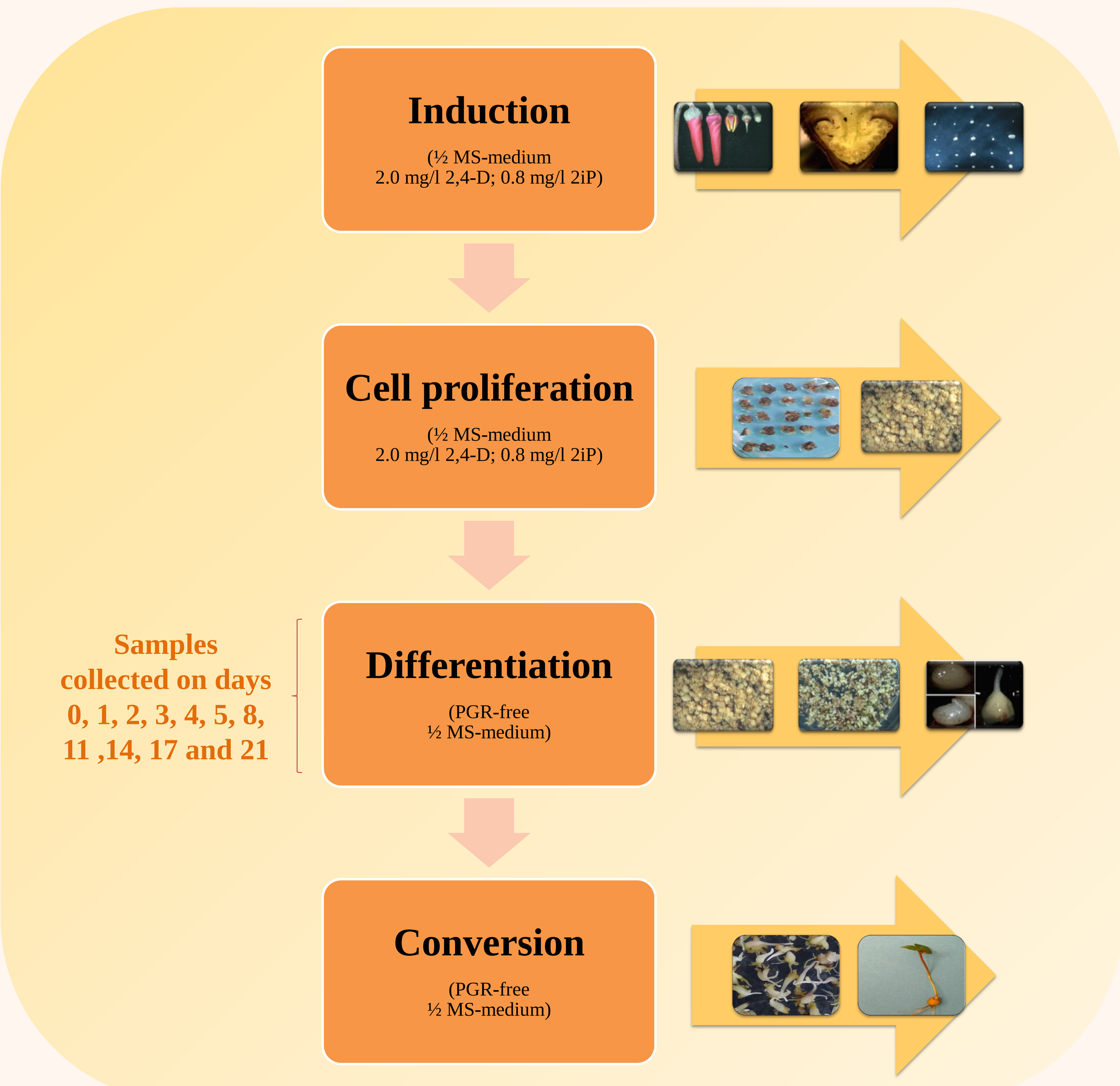

Fig. 1) Schematic representation of *Cyclamen persicum* somatic embryogenesis (WINKELMANN 2010).

Tissue was collected after the transfer of embryogenic cells to the plant growth regulator (PGR) free differentiation medium (Fig. 1). Samples were taken from cultures in liquid medium and cells cultured on solid medium. GSH, GSSG and cysteine were extracted using 5% metaphosphoric acid (MPA) and determined by HPLC coupled to ESI-TOF MS following the method described by RELLÁN-ÁLVAREZ ET AL. (2006) with modifications.

Results

The results show a high variation between the repetitions of the experiment which correlates with the variation in the formation of somatic embryos in different experiments. After 24 hours a peak in GSH and cysteine concentration was observed (Fig. 2). Thereafter, the content of cystein remained stable, while GSH increased slightly over time.

GSSG was detected in lower concentrations than GSH and was observed to increase until day 8 (Fig. 2). Further repetitions are in progress to support the findings and to correlate the results to the number and quality of the developing somatic embryos.

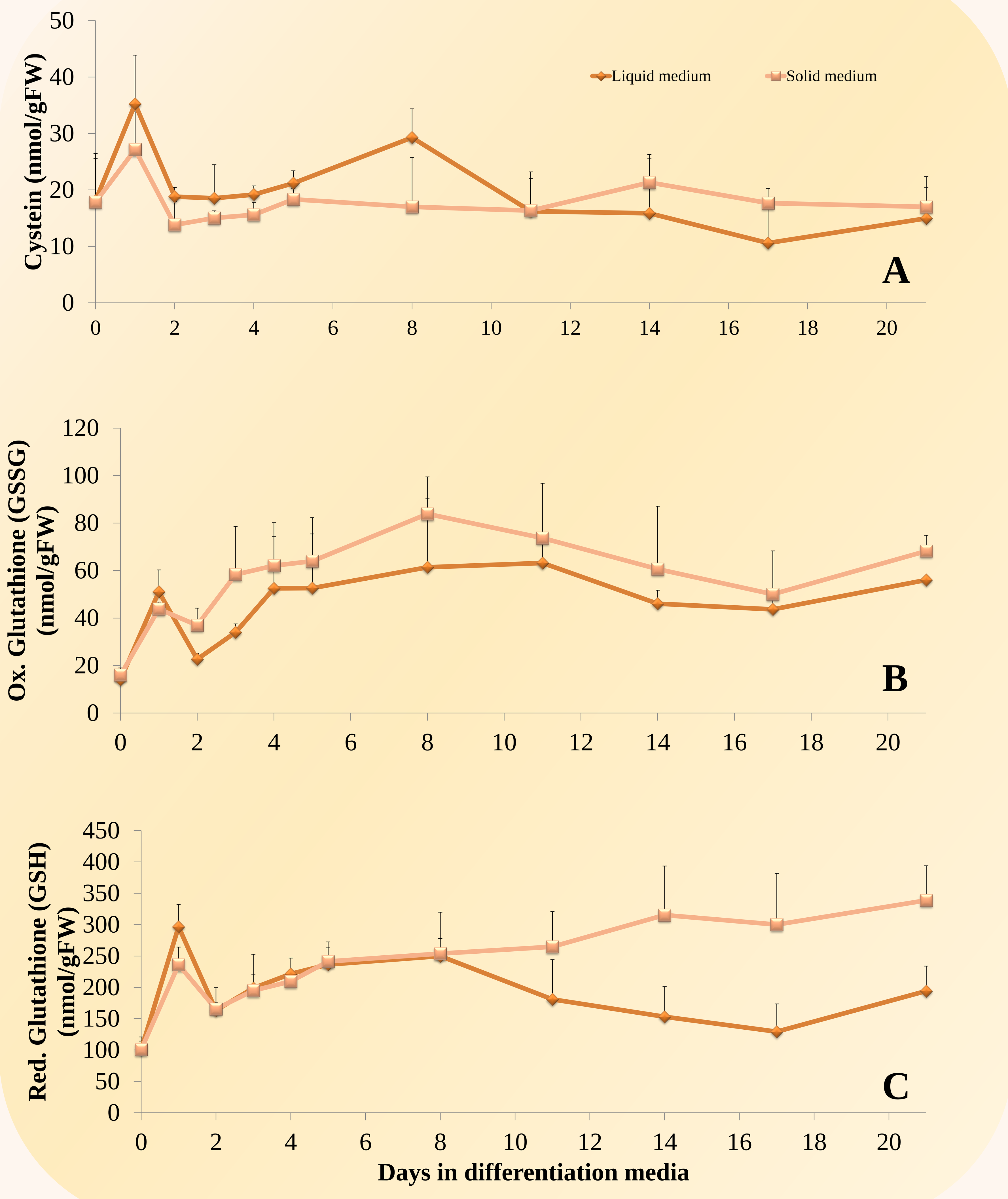

Fig. 2) Dynamics of cystein (A); GSSG (B) and GSH (C) during *Cyclamen persicum* somatic embryogenesis . n=2 (Bars represent standard deviation).

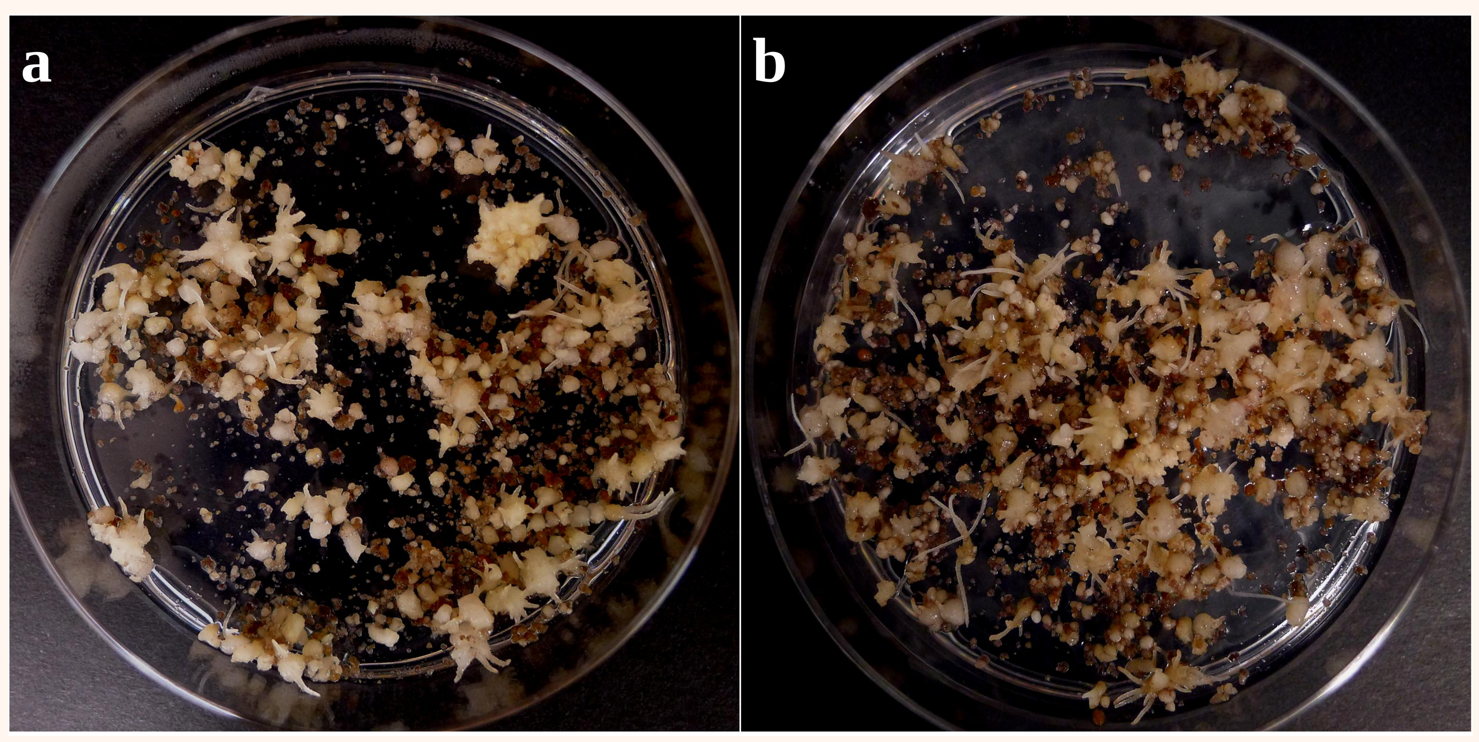

Fig. 3) Aspects of *Cyclamen persicum* somatic embryos differentiated after 28 days: a) somatic embryos cultivated in liquid culture for 14 days, after transfer to solid medium b) somatic embryos cultivated in solid medium (6 cm Petri dishes).

Outlook

* The abnormal embryos (Fig. 3) may be a result of the oxidative processes during the somatic embryogenesis. Experiments are planned to supply GSH or GSSG in different stages of embryo development to support a normal development of SE.

* Localization of reactive oxygen species and their influence on the oxidation of proteins that could modulate the development/maturation of somatic embryos of *Cyclamen persicum* is planned.

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