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Phosphoregulation of the photorespiratory enzyme, glycolate oxidase, in response to light and CO₂ content in *Arabidopsis thaliana*

Younes Dellero¹, Edouard Boex-Fontvieille¹, Sophie Massot¹, Valérie Flesch¹, Françoise Gilard^{1,2}, Marlène Davanture³, Michel Zivy³, Guillaume Tcherkez^{1,2}, Michael Hodges¹, Mathieu Jossier^{1*}

¹ Institut de Biologie des Plantes, Saclay Plant Sciences, Université Paris Sud, 91405 Orsay cedex, France

² Plateforme Métabolisme Métabolome, IFR87 La Plante et son Environnement, Institut de Biologie des Plantes, Université Paris Sud, 91405 Orsay cedex, France

³ INRA, Plateforme d'Analyse Protéomique de Paris Sud Ouest (PAPPSO), 91190 Gif-sur-Yvette, France

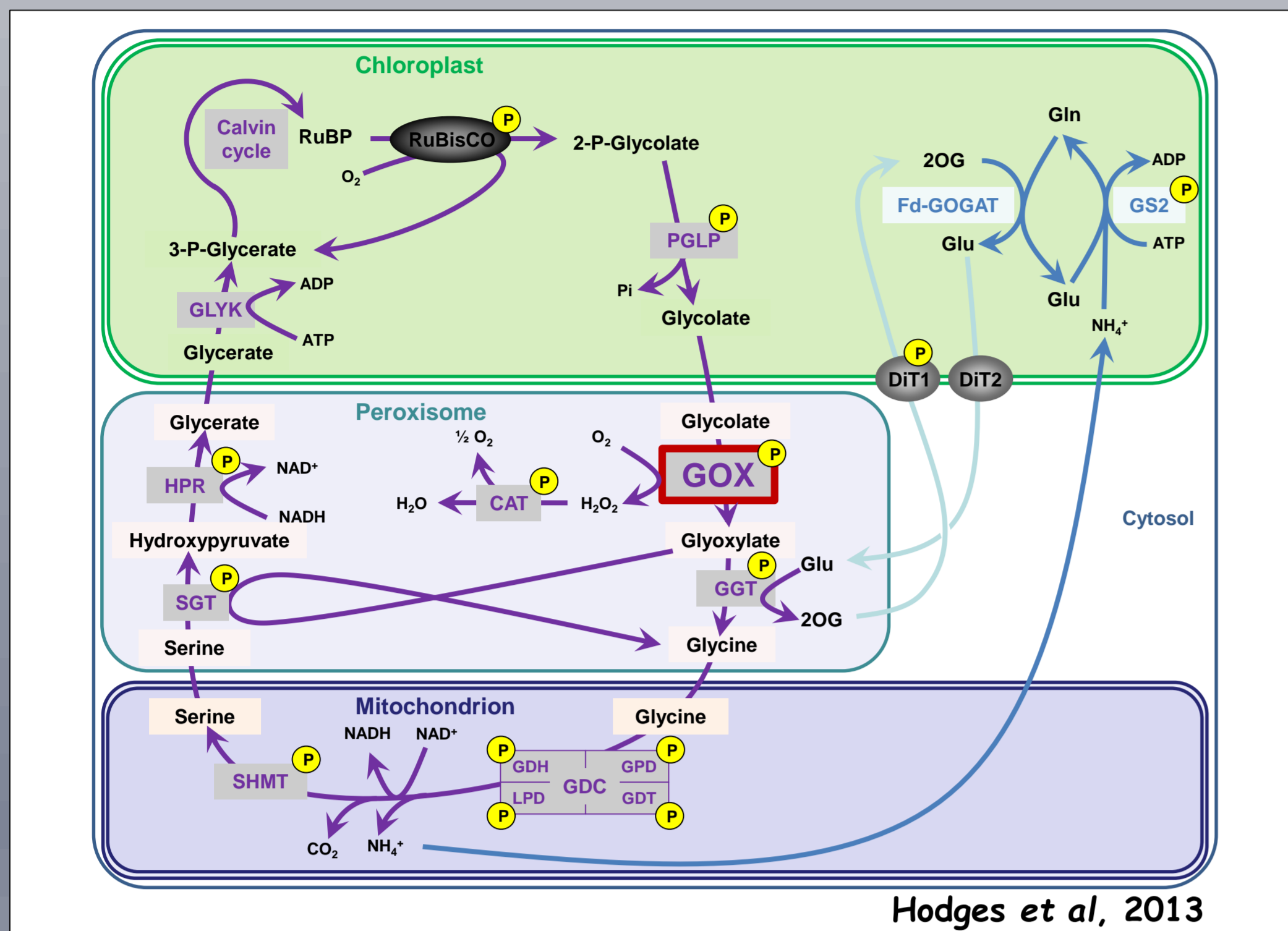
*Corresponding author : mathieu.jossier@u-psud.fr

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Phosphoregulation of photorespiration : an unexplored domain

Phosphorylated enzymes of the photorespiratory cycle according to the PhosPhAt database.



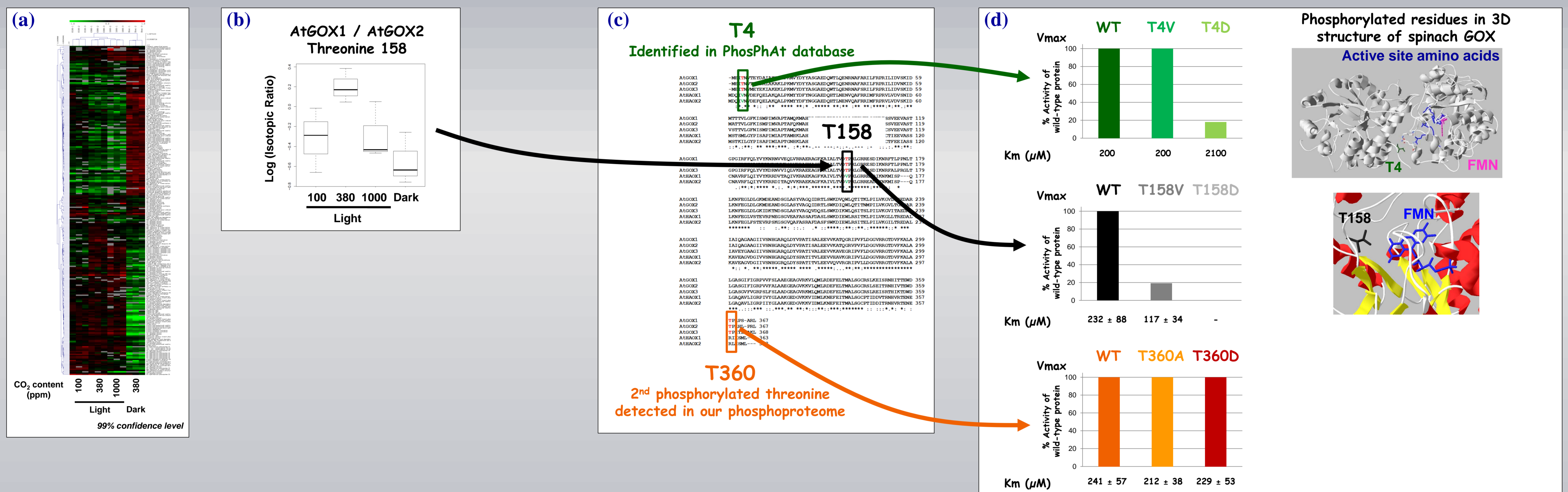
Hodges et al, 2013

- Photorespiration is an essential process for all oxygenic photosynthetic organisms (Bauwe *et al.*, 2010). It allows the recycling of the toxic metabolite 2-phosphoglycolate produced by Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) oxygenase activity. The photorespiratory cycle has often been described as 'wasteful' to plant productivity as it leads to the production of CO₂ and ammonia that must be re-assimilated.
- It is of paramount importance for plants to be capable of modifying their metabolism in response to rapidly changing environmental conditions such as light/dark transitions and changing CO₂ content. Although little is known about photorespiratory cycle regulation, the PhosPhAt database indicates that many photorespiratory enzymes can be phosphorylated, thus suggesting that regulation by protein phosphorylation is a possible mechanism.
- We present results of a quantitative phosphoproteome analysis revealing that amongst the 8 photorespiratory enzymes, the glycolate oxidase (GOX) is differentially phosphorylated in response to light/dark transition and to altered photorespiratory conditions. We examined each GOX phosphorylation site identified in our phosphoproteome analysis (T158 and T360) and in PhosPhAt (T4) using mutated recombinant GOX proteins to evaluate the impact of each phosphorylation events on enzymatic parameters.
- It should be noted that *Arabidopsis* rosette leaves contains two photorespiratory GOX genes: GOX1 (At3g14420) & GOX2 (At3g14415).

Mutation at T4 or T158 modifies GOX kinetic parameters

A quantitative phosphoproteome revealed a differentially phosphorylated residue of GOX at T158.

Mimic a phosphorylation (T4D and T158D) or suppress it (T158V) leads to the inhibition of recombinant GOX1 and GOX2 activity *in vitro*.

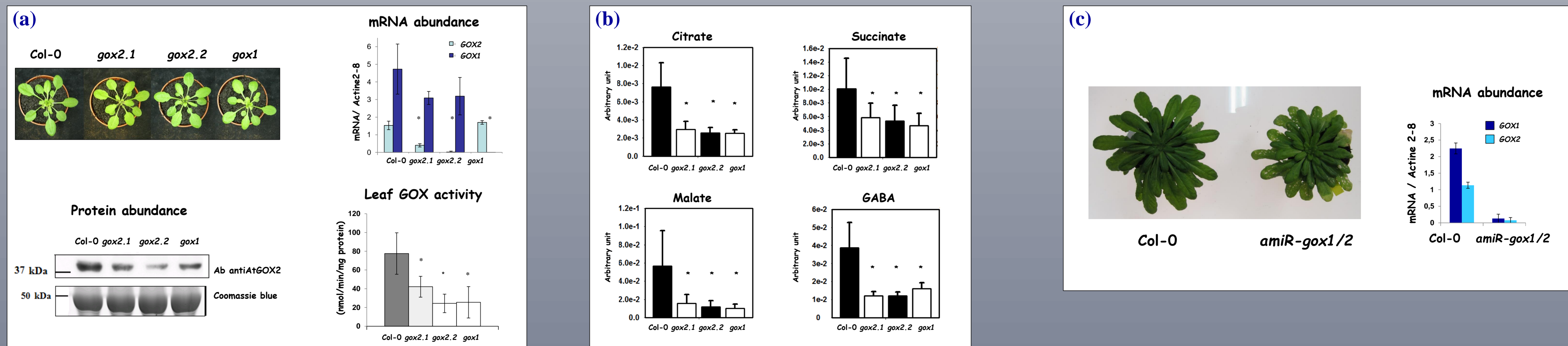


A global quantitative phosphoproteomics analysis was undertaken using leaf extracts from *Arabidopsis thaliana* plants subjected to a 4 h light-dark transition or a 4 h light either at 100ppm, 380ppm or 1000ppm CO₂ in 21% O₂ to modify the photorespiration rate (oxygenation-to-carboxylation ratio). 264 phosphopeptides showed a significant change in their phosphorylation status between the different conditions (99% confidence level) (a). One of these phosphopeptides corresponded to the photorespiratory enzyme GOX (T158 of AtGOX1 and AtGOX2) (b). Mutation of T158, T360 (a 2nd phosphorylated residue identified in our phosphoproteome) and T4 (present in the PhosPhAt database) (c) either to a valine or an alanine (to suppress phosphorylation) or to an aspartic acid (to mimic a phosphorylation) modified the kinetic parameters of recombinant AtGOX1 and AtGOX2 (d).

atgox1 and atgox2 are affected in organic acid content but show no growth phenotype in air

Even if atgox1 and atgox2 do not show a developmental phenotype, several organic acids of the Krebs cycle are less abundant.

While amiR-gox1/2 plants with almost no GOX activity exhibit a "photorespiratory" phenotype in air.



To investigate the consequences of a reduced GOX activity on leaf photorespiration and primary metabolism, T-DNA insertion mutants of AtGOX1 and AtGOX2 exhibiting different GOX activities, protein and mRNA levels (a) were subjected to GC-MS metabolomic analyses. Our data show a strong decrease of several organic acid contents in atgox1 and atgox2 rosette leaves (b). The knock-down of both GOX genes in amiR-gox1/2 plants show a "photorespiratory" phenotype in a normal atmosphere (c) although they grow normally in air containing 3000ppm CO₂. This mutant will be used as a tool to study the consequence of GOX phosphorylation in planta by introducing mutated forms of maize GOX1 into the amiRNA line.

Conclusions

Our phosphoproteome analysis revealed that photorespiratory GOX can be differentially phosphorylated with respect to CO₂ content and light/dark transition. GOX phosphorylation-mimic mutants at T4 and T158 have modified enzymatic parameters that indicate that GOX phosphorylation could regulate photorespiratory flux. Further work will be carried out to understand *in planta* the role of these phosphorylation events and their impact on plant metabolism including the Krebs cycle to limit the carbon and nitrogen loss.