Transcriptomic and metabolic analyses of 35S:S523DNR tobacco plants

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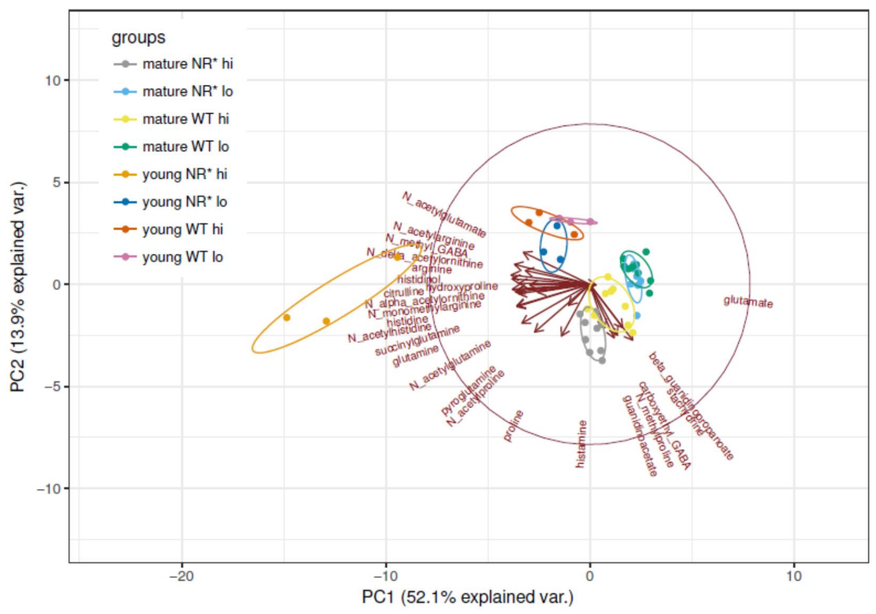
Introduction

Among several target genes tested to reduce nitrate accumulation in Burley tobacco, we found that overexpressing a deregulated form of nitrate reductase (NR) under the control of a constitutive promoter resulted in a strong reduction of the free leaf nitrate pool associated with a concomitant increase of N-accumulation into amino acids. Under both conditions, phytotron/greenhouse and field environment, 35S:S523DNR tobacco plants exhibited similar phenotypes, thereby suggesting that robust changes occurs within the whole plant cellular processes. To understand the mechanism resulting in such an elevated N-assimilation into amino acids which is due to a constitutively active nitrate reductase, 35S:S523DNR (NR*) and WT tobacco plants were grown in a phytotron under two different nitrate fertilization regimes. Transcriptomic and metabolomic analyses were performed on young and mature plants to identify major changes occurring in 35S:S523DNR plants compared to control plants. The ultimate area of such application being in frame of identifying background tobacco or *Nicotiana* species more adapted to polypeptide or protein synthesis.

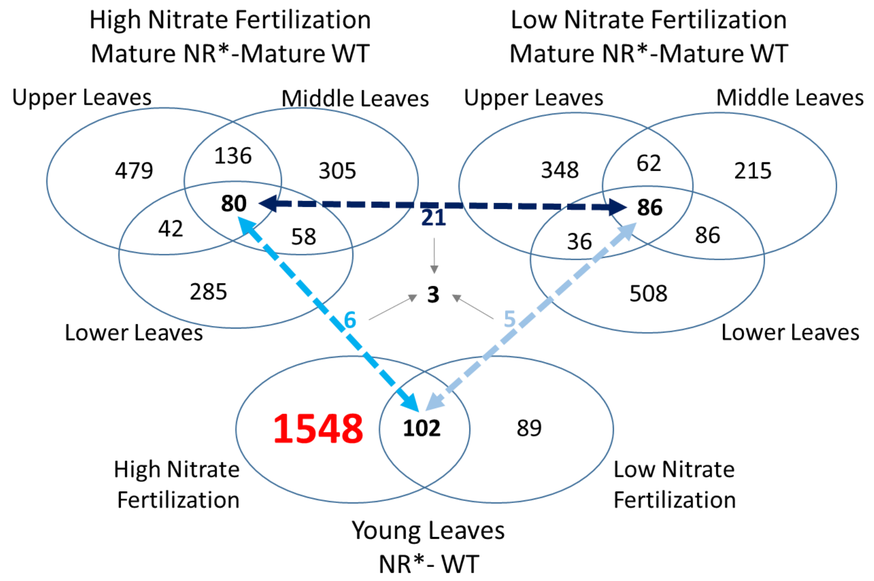
Results

Metabolic analyses in mature and young leaves of 35S:S523DNR compared to WT tobacco plants fertilized with low and high nitrate regimes



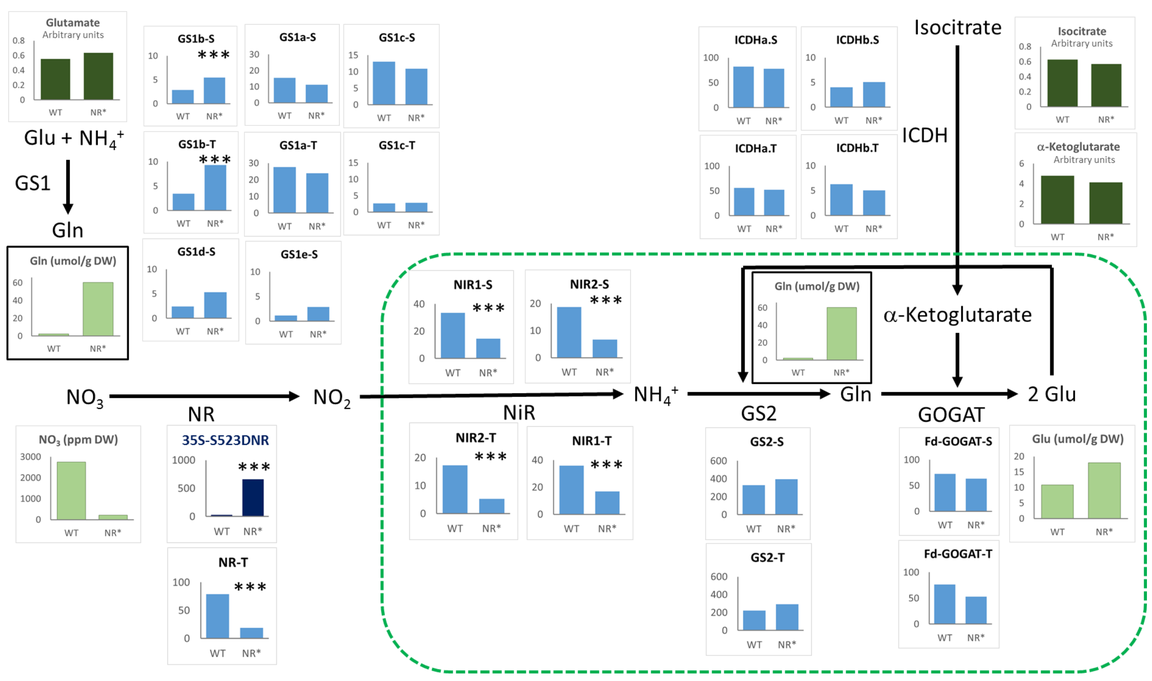
Principal component analysis (PCA) reveals that the concentrations of some metabolites of the glutamate pathway are increased in young leaves of 35S:S523DNR tobacco plants compared to WT plants, consistent with high activity within the nitrate assimilation pathway. *Various samples are plotted along its first 2 components. The red arrows represent the relative contribution of each metabolite to these components.*

Up-regulated genes in mature and young leaves of 35S:S523DNR tobacco plants compared to WT plants fertilized with low and high nitrate regimes



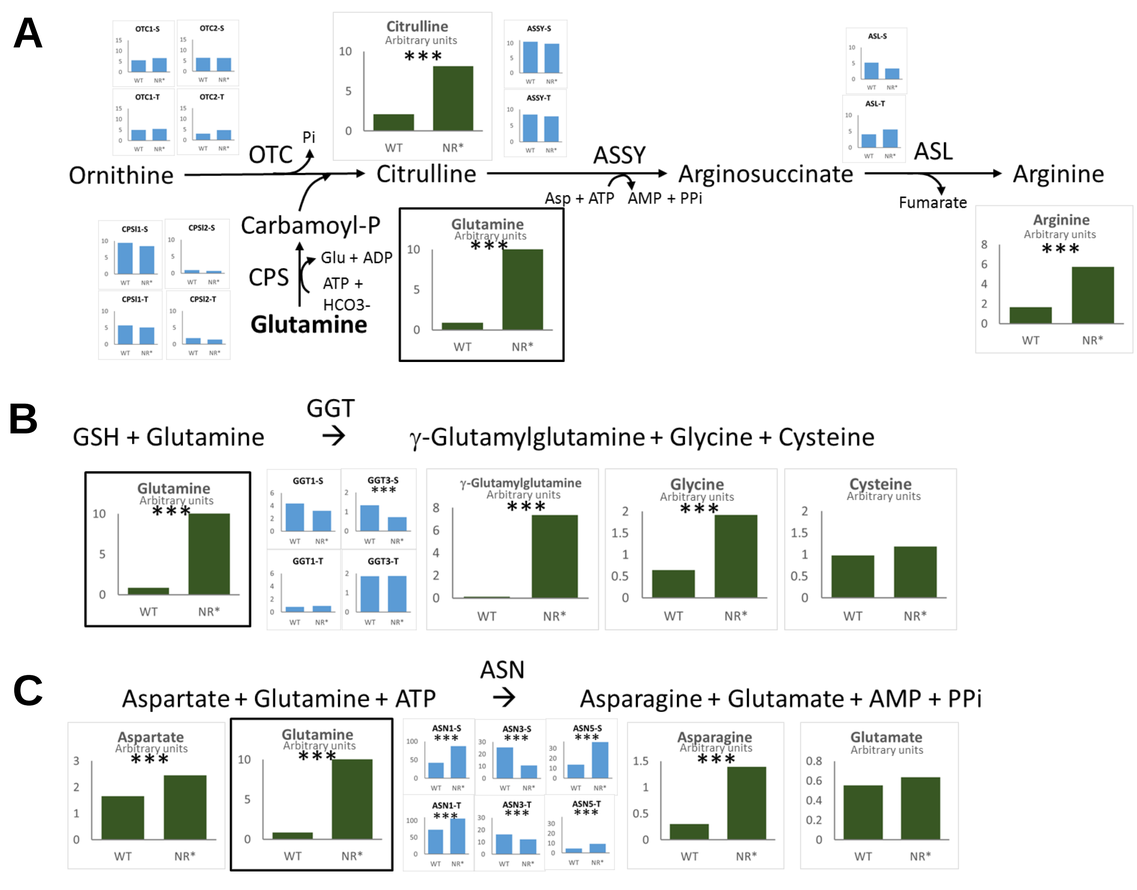
The highest number of up-regulated genes (1548) was found in young leaves of 35S:S523DNR tobacco plants compared to WT plants, attesting to high gene activitiy driven by the presence of deregulated nitrate reductase (NR*) under high nitrate fertilization (RNA-seq data; transcripts>2x; P<0.05).

Nitrate assimilation pathway: gene regulation and metabolite changes in young leaves of 35S:S523DNR tobacco plants under high nitrate fertilization



Up-regulation of 35S:S523DNR resulted in a strong assimilation of nitrate and accumulation of glutamine in young leaves. In addition, nitrite reductase gene copies (*NIR1-S, NIR1-T*, *NIR2-S, NIR2-T*) and cytosolic glutamine synthetase (*GS1b-S*, *GS1b-T)* were down- and up- regulated, respectively. *RNA-seq expression data are in FPKM.*

Gene regulation and metabolite flux changes in three glutamine-dependent pathways



The concomitant arginine (A) and γ-glutamylglutamine (B) flux increase is related to the massive glutamine accumulation and not to gene up-regulation as depicted in the corresponding pathways.
Both glutamine accumulation and activation of *ASN1-5* copies may contribute to the flux increase of asparagine (C).

Conclusion

The strongest gene activities supporting metabolic changes in 35S:S523DNR tobacco plants occured in young leaves under high fertilization rate. As expected, the major impact was observed on the N-assimilation into glutamine without effect on the key chloroplastic enzyme GS2 and GOGAT. The massive increase of glutamine contributed to the flux increase of arginine, γ-glutamylglutamine and asparagine without major transcriptomic contribution.

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