**SUMMARY DESCRIPTION OF THE PROJECT OBJECTIVES**

Nitric oxide (NO) is a highly inducible molecule and over accumulated during stress responses, such as drought, cold and pathogen infection. Several key developmental processes within a plant life cycle have been reported to be signalled by this gaseous molecule, and among them seed germination, de-etiolation, gravitropic response or root growth are well characterized.

The importance of NO as a plant growth and stress regulator is emerging considerably, despite the current knowledge about its signalling pathway is still limited. Therefore, the identification and characterization at the molecular level of NO targets is essential to get a deeper insight into this pathway.

This is a summary description of the project objectives:

# 1. Genetic dissection of NO responses in root growth

2. Characterization of NO cell biology and transcriptomic profiling of NO responses.

3. Direct identification of S-nitrosylated proteins.

**DESCRIPTION OF THE WORK PERFORMED**

1. *Genetic dissection of NO responses in root growth*

Analysis of the cell number and cell size in the root meristem reveals that NO application reduces root meristem size. The total number of cells between the quiescent centre (QC) and the start of the rapid elongation in the cortex layer is significantly changed as the inflection point on the cell length curve marking the transition into the rapid elongation zone occurs around cell numbers 17, 33, 28 in SNP (NO-donor), cPTIO (NO-scavenger) and control respectively. It is noticeable that analysis of *cue1/nox1* mutant showed similar results to those obtained after SNP treatment with an inflection point around cell numbers 17-18. Interestingly, significant differences in cell sizes in the apical root meristem were also detected at this stage (cells 1-10 and cells 11-20). NO scavenged by cPTIO partially blocked the action of the NO specific releasing compound SNAP, clearly establishing that NO is a contributing element in the promotion of cell elongation. Hence, in the initial phases of root growth after germination, NO reduces root meristem size by promoting cell elongation in the root meristem and therefore decreasing the number of dividing cells. Remarkably, long-term treatments with NO-donor SNP (up to 5 days) almost abolished the pool of dividing cells by enhancing cell elongation in all cell types of the root meristem. Using the *CycB1;1:GUSDB* reporter, marking cells in the G2 stage of the cell cycle, we have compelling evidence that over-accumulation of NO decreases the pool of dividing cells.

1. *Characterization of NO cell biology and transcriptomic profiling of NO responses*

Moreover, microarray analysis reveals that NO induces the expression of several cell-wall remodelling enzymes (CWRs). Mining the spatiotemporal distribution of transcripts by means of the eFP browser (http://bar.utoronto.ca, Winter et al., 2007) reveals that transcripts of these genes mostly reach their highest levels throughout the root apical meristem and suggests that these enzymes could be important for cell wall loosening during the elongation of cells in the meristem. Intriguingly, NO also represses a distinct set of CWRs, most probably reflecting cell wall tightening during the inhibition of cell elongation in the differentiation zone since they are mostly expressed in that part of the root. Interestingly, NO treatment decreases cell elongation to some extent in differentiation zone.

Auxin promotes root growth by modulating gibberellin (GAs) response, and GAs are plant hormones that regulates root growth controlling both cell division and cell elongation. GAs promote etiolated growth in plants (characterized by increased hypocotyl growth) by degradation of DELLA proteins (GAI, RGA, RGL1, RGL2, RGL3). DELLA proteins act as a negative regulator of PIFs (Phytochrome interacting factors) that mediate cell elongation. NO is able to inhibit hypocotyl elongation during dark growth. Remarkably, NO-overproducer mutants (nox/cue/argah) display significantly shorter hypocotyls than the wild type. Our results suggest that GAs action reverts to some extent the NO-related inhibition of hypocotyls growth and also cell elongation in the differentiation zone. In this work, we propose that NO interacts with the GAs pathway in the regulation of hypocotyl growth in etiolated seedlings NO-treated seedlings induce the expression of DELLA repressor genes and furthermore *quadruple* and *global* *DELLA* mutants (impaired in GAs signalling) show high levels of insensitiveness to the NO-mediated inhibition of hypocotyl elongation in dark-grown plants. Taken together, these findings suggest that NO could be playing a main role in GA regulation of hypocotyl growth.

**DESCRIPTION OF THE MAIN RESULTS ACHIEVED**

1. *Nitric oxide inhibits primary root growth by affecting root apical meristem activity*

High levels of NO, released by NO donors or using NO over accumulating mutants (*cue1/nox1*), produced a decrease in the primary root length by reducing root meristem size and cell division rates (Fernández-Marcos et al. 2011, Proc Natl Acad Sci USA)

1. *Nitric oxide regulates auxin transport*

Nitric oxide modulates polar auxin transport by influencing auxin efflux transport. Recent genetic analysis indicates that polar auxin transport is impaired in plants with altered nitric oxide accumulation (Fernández-Marcos et al. 2011, Proc Natl Acad Sci USA)

1. *Nitric oxide represses auxin inducible promoters*

Increased NO accumulation in mutants, where endogenous NO levels are enhanced, depletes auxin-dependent reporter expression in the apical auxin maximum (Fernández-Marcos et al. 2011, Proc Natl Acad Sci USA)

1. *Gibberellins and nitric oxide involvement in cell elongation processes*

Combined addition of gibberellins and nitric oxide partially restores primary root growth and cell elongation in root cells (Fernández-Marcos et al., 2012, Plant Signal Behav)

EXPECTED FINAL RESULTS. POTENTIAL IMPACT AND USE

Previous reports pointed out that nitric oxide is detected in normal/basal growth conditions, presenting maxima in the root tip and the beginning of the elongation-differentiation zone. Careful examination by DAF-2DA analysis of wild-type roots reveals that NO is additionally accumulated in the stem cell niche, composed of an organizing centre (QC), which maintain stem cell identity in the neighbouring population of cells. In order to elucidate the specific role of this molecule in the stem cell niche, we are characterizing the phenotype of mutants involved in NO-synthesis or metabolism in these cell types.  Our results suggest that differentiation of columella stem cell (CSC) daughter into columella cells (CCs) is significantly promoted when the NO source is reduced, either in NO-deficient mutants or using a chemical biosynthesis inhibitor, L-NMMA. Since auxin plays a main role in QC/initial/columella cell organization, we are analysing whether the abnormal phenotypes present in these NO-related mutants were related to modifications in auxin metabolism and/or transport and the putative genetic interactions between both pathways.