Nitric oxide signalling in a CO$_2$-enriched environment

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Carbon dioxide is one of the main greenhouse gases contributing to global warming, with associated effects on crop yields. A paper by Du et al. in this issue of *Journal of Experimental Botany* (pages 893–904) suggests that in this scenario nitric oxide may be a pivotal signalling molecule involved in the regulation of nutrient uptake and assimilation by plants.

Atmospheric CO$_2$ concentration is predicted to rise from 370 ppm at present to 550 ppm by 2050, and simultaneously it is estimated that 50% more food will be needed to satisfy the dietary requirements of the growing global populations of humans and farm animals (Jaggard et al., 2010). In this context, understanding how CO$_2$ affects crop yields, allowing suitable improvement programmes, could be crucial in preventing potential food shortages. CO$_2$ enhances plant growth, which in turn requires a greater amount of underpinning nutrients. To facilitate nutrient uptake in CO$_2$-enriched environments plants increase lateral root formation, a process mediated by nitric oxide (NO) (Wang et al., 2013). Moreover, CO$_2$ and NO can modulate nitrate reductase (NR) activity (Jin et al., 2009; Niu et al., 2012), the first enzyme of the nitrate assimilation pathway, although their potential interplay in its control has remained unclear.

Du et al. (2016) suggest that the regulation of NR activity under high CO$_2$ levels depends on nitrate availability, with NO playing a crucial role. They show that the enzyme has dual regulation (Fig. 1): in both leaves and roots of Arabidopsis plants subjected to high CO$_2$ levels, it is inhibited at high nitrate concentration, whereas a low concentration enhances its activity. This differential regulation is mimicked using NONOate as an NO donor, and the effect of CO$_2$ on NR activity is reversed by cPTIO (an NO scavenger). Although CO$_2$ triggers NO levels in both conditions, it is always greater under high nitrate availability, probably as a result of high nitrate levels increasing NO emission from Arabidopsis plants (Frungillo et al., 2014). Given these results, NO may be the signalling molecule acting downstream of CO$_2$ to control NR activity, depending on nitrate concentration (Du et al., 2016).

**Differential regulation of nitrate reductase activity**

NO is a pivotal signalling molecule that controls a plethora of fundamental functions in animals and plants. There are different sources of its production in plants, highlighting NR and NO synthase (NOS)-like enzymatic activities (Gupta et al., 2011). The former is a well-characterized pathway (Rockel et al., 2002), while in the latter NOS-like enzymatic activity has been described using typical mammalian NOS inhibitors (Corpas et al., 2009), but genes encoding a clear homologue of animal NOS have not been identified in sequenced plant genomes.

Elevated CO$_2$ inhibits NR and increases NOS-like activity in tomato roots, suggesting that NOS rather than NR is responsible for NO production and development of lateral roots in response to high CO$_2$ levels (Wang et al., 2013). Moreover, using Arabidopsis noal plants (in which NOS-like activity is impaired) or L-NAME as a NOS inhibitor, Du et al. confirm that NOS-generated NO is responsible for controlling NR activity under high CO$_2$ concentrations.

A key unsolved issue is how CO$_2$ controls NOS activity, although this is unlikely to be straightforward since NOS-like protein has not been identified in plants. Another issue concerns how NOS-produced NO differentially regulates NR activity depending on nitrate supply under high CO$_2$ concentration. Irrespective of the source of generation, NO mainly acts through post-translational modifications of proteins. Chief among them is S-nitrosylation, the addition of an NO group to a cysteine thiol group to form S-nitrosothiols, which can modify protein functions. S-nitrosothiols are key signalling molecules as they have an essential role in fundamental...
aspects of plant biology (Yu et al., 2014). For instance, NO and/or S-nitrosylation may play a crucial role in N uptake and assimilation (Frungillo et al., 2014; Dong et al., 2015).

Recently, using nox1 and gsnor1 mutants, which have higher levels of NO and S-nitrosogluthathione (a major low molecular weight S-nitrosothiol considered to be an NO reservoir in cells), respectively, Frungillo et al. (2014) demonstrated that S-nitrosothiols inhibit whereas NO does not affect NR activity, highlighting different regulation of NR activity by these NO-derived molecules. In addition, they showed that S-nitrosylation suppresses nitrate uptake and reduction and therefore could have an impact on N assimilation (Fig. 1).

Taking this into account, Du et al. have provided a significant advance concerning regulation of NR activity under elevated CO₂, demonstrating that S-nitrosylation could be the mechanism that mediates the inhibition of NR activity under high nitrate concentration (Fig. 1). This inhibition could be a direct consequence of the high levels of NO generated in plants with high nitrate supply. By contrast, they suggest that an interaction of NO with the heme and molybdogen centres may be responsible for the enhancement of NR activity under low nitrate conditions (Du et al., 2008). Although there is no real evidence to corroborate these mechanisms, these results suggest that there is a critical NO level above which the S-nitrosylation and consequently inactivation of NR takes place.

**Improving crop yields**

Nitrogen is essential for plants, and its scarcity in soil is a common problem that affects crop yields. Effective use of N is therefore indispensable for plant growth and development and, since NR activity is crucial for N assimilation, determining the exact mechanism by which NR is modified by NO under elevated CO₂ environments is a key issue. Identification and subsequent modification of cysteine residue(s) involved in S-nitrosylation of NR is an important next step. Bearing in mind that future climate change will not only lead to higher CO₂ levels but also situations that could increase S-nitrosothiol levels, such as high temperature (Chaki et al., 2011), an NR that is not affected by S-nitrosylation may help crops cope more efficiently with changing environments.

Moreover, nitrate-based fertilizers have traditionally been used by farmers to combat insufficient N in some arable land and consequently to improve yields. However, N from these fertilizers is not fully taken up by plants, resulting in pollution and economic losses for farmers (Jaggard et al., 2010).

Based on the work of Du et al., one can speculate that in a future environment with high CO₂ levels the use of fertilizers containing low concentrations of nitrate could improve NR activity and consequently N assimilation. This could help reduce nitrogen-based pollution and at the same time improve crop yields. However, further studies confirming the impact of NOS-mediated NR modulation on N assimilation and crop yields under CO₂-enriched environments have to be addressed. Interestingly, nitrate nutrition can also improve phosphorus uptake by plants grown in phosphorus-deficient media and elevated CO₂ concentration, with NO playing a key role in controlling this process (Niu et al., 2012). Therefore, an appropriate management of nitrate supply could facilitate the uptake of different essential nutrients under high CO₂ levels. In this scenario, NOS-generated NO appears to be an essential signalling molecule involved in controlling nutrient uptake and possibly plant growth in future CO₂-enriched environments.

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**References**

Corpas FJ, Palma JM, del Río LA, Barroso JB. 2009. Evidence supporting the existence of l-arginine-dependent nitric oxide synthase activity in plants. New Phytologist 184, 9–14.

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, et al. 2011. High temperature triggers the metabolism of S-nitrosothiols in sunflower mediating a process of nitrosative stress which provokes the inhibition of ferredoxin-NADP reductase by tyrosine nitration. Plant, Cell & Environment 34, 1803–1818.

Dong F, Simon J, Rienks M, Lindermayr C, Rennenberg H. 2015. Effects of rhizospheric nitric oxide (NO) on N uptake in Fagus sylvatica seedlings depend on soil CO2 concentration, soil N availability and N source. Tree Physiology 35, 910–920.

Du S, Zhang R, Zhang P, Liu H, Yan M, Chen N, Xie H, Ke S. 2016. Elevated CO₂-induced production of nitric oxide (NO) by NO synthase differentially affects nitrate reductase activity in Arabidopsis plants under different nitrate supplies. Journal of Experimental Botany 67, 893–904.

Du S, Zhang Y, Lin X, Wang Y, Tang C. 2008. Regulation of nitrate reductase by nitric oxide in Chinese cabbage pakchoi (Brassica chinensis L.). Plant, Cell & Environment 31, 195–204.

Frungillo L, Skelly MJ, Loake GJ, Spoel SH, Salgado I. 2014. S-nitrosothiols regulate nitric oxide production and storage in plants through S-nitrosothiol considered to be an NO reservoir in cells. Nature Communications 5, 5401.

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT. 2011. On the origins of nitric oxide. Trends in Plant Science 16, 160–168.

Jaggard KW, Qi A, Ober ES. 2010. Possible changes to arable crop yields by 2050. Philosophical Transactions of the Royal Society B: Biological Sciences 365, 2835–2851.

Jin CW, Du ST, Zhang YS, Lin XY, Tang CX. 2009. Differential regulatory role of nitric oxide in mediating nitrate reductase activity in roots of tomato (Solanum lycopersicum). Annals of Botany 104, 9–17.

Niu Y, Chai R, Dong H, Wang H, Tang C, Zhang Y. 2012. Effect of elevated CO₂ on phosphorus nutrition of phosphate-deficient Arabidopsis thaliana (L.) Heynh under different nitrogen forms. Journal of Experimental Botany 64, 355–367.

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM. 2002. Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. Journal of Experimental Botany 53, 103–110.

Wang H, Xiao W, Niu Y, Jin C, Chai R, Tang C, Zhang Y. 2013. Nitric oxide enhances development of lateral roots in tomato (Solanum lycopersicum L.) under elevated carbon dioxide. Planta 237, 137–144.

Yu M, Lamattina L, Spoel SH, Loake GJ. 2014. Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytologist 202, 1142–1156.