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Commentary

Nitric oxide, nitrate reductase and UV-B tolerance

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Land plants rely on light for photosynthesis and have evolved leaves as light-capturing organs. As an undesired corollary, there is also a considerable absorption of damaging light, in particular ultraviolet B (UV-B) radiation (280–320 nm). In modern times, exposure to UV-B radiation is increasing progressively, due to thinning of the protective ozone layer by human activities, for example the production of halones such as chlorofluorocarbon compounds (CFCs). In the stratosphere, UV-mediated activation of CFCs generates halogen radicals, which initiate a chain reaction of ozone depletion (Andrady et al. 2010). UV-B radiation is dangerous because it impairs genome stability by damaging nucleic acids (Ries et al. 2000), such that UV-B-resistant *Arabidopsis* mutant *uvi1 (UV-B insensitive 1*) displays increased genome stability (Tanaka et al. 2002). The impact of UV light on genes and genomes is one of the driving forces of evolution (Tuteja et al. 2001), and plants have evolved various strategies to moderate this stress. Since UV damage often involves the production of free radicals such as reactive oxygen species (ROS) and nitric oxide (NO) (Tossi et al. 2009, Zhang et al. 2011), it is not surprising that the counteracting plant defence systems include various antioxidants such as ascorbate and glutathione (Jansen et al. 1998). While thickening of the leaf surface has been shown to be important at the morphological level (Jenkins 2009, Hayashi et al. 2011), most studies have focused on the production of UV-B reflecting and absorbing 'sun-block' chemicals. Flavonoids, as a prominent example, are phenylpropanoid compounds that can efficiently absorb light in the wavelength range between 240 and 545 nm. The role of flavonoids in preventing cellular damage through light irradiation is well established and they accumulate in the vacuoles of epidermal cells in order to minimize the absorption of UV within the mesophyll (Agati et al. 2009).

Recently, various studies have pointed to the involvement of NO in response to UV radiation (An et al. 2005, Qu et al. 2006), which appears to act through abscisic acid-mediated steps (Tossi et al. 2009). Mechanisms of NO action in UV protection are now emerging, with a very recent study showing an effect by inducing chalcone synthase (*CHS*) and chalcone isomerase (*CHI*) genes linked to polyphenylpropanoid production (Tossi et al. 2011). An elegant study by Zhang et al. (2011) shows a link between nitrate reductase (NR)-mediated NO production and UV-B-induced flavonoid production (Figure 1).

During the last decade enormous progress has been made in research on NO in plants, which has been shown to influence various physiological and developmental processes (reviewed in Gupta et al. 2011*a*, Mur et al. 2006). One of the hottest, and most controversial, topics in NO research is NO synthesis. Plants can produce NO through either oxidative or reductive pathways. The most well established is the NAD(P)H-linked reduction of nitrite by cytosolic NR under normoxic conditions. For efficient NO production, NR requires low concentrations of nitrate and high concentrations of nitrite because NR has a two times higher affinity for nitrite in comparison to nitrate (Rockel et al. 2002). NR-dependent NO increases during hypoxia due to a decrease in pH (Rockel et al. 2002) and is also affected by post-translational modification (Lillo et al. 2004).

The existence of a plasma membrane-located nitrite NO reductase has also been demonstrated. Under low oxygen conditions, plant mitochondria can produce NO from nitrite at the site of complex III, IV and AOX (alternative oxidase) (Gupta 2011, Gupta and Igamberdiev 2011). As an alternative, oxidative pathways are based on a nitric oxide synthase (NOS)-like enzyme and hydroxylamine-mediated NO and polyamine-mediated NO are oxidative pathways (Gupta et al. 2011*a*). In all oxidative NO production pathways, L-arginine acts as a substrate or intermediate. However, the molecular bases of these biochemically defined pathways remain to be elucidated. Among the oxidative pathways, the NOS-like enzyme has been linked

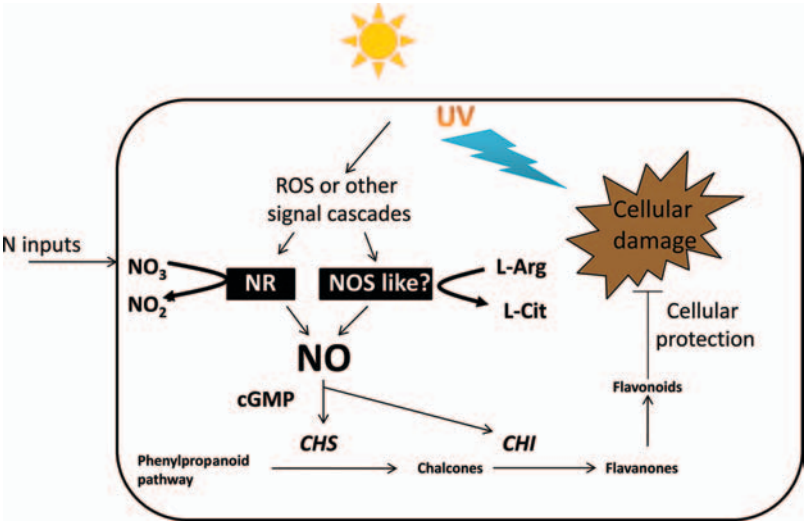

Figure 1. Scheme showing the role of NR or NOS derived NO in counteracting UV-B and that exposure of plants to UV-B increases ROS. The produced ROS or other signal cascades induce NR or NOS activity, which leads to NO production. The produced NO alone, or together with cyclic GMP (cGMP), induces the up-regulation of chalcone synthase (*CHS*) and chalcone isomerase (*CHI*) genes linked to polyphenylpropanoid production. The derived flavonoids protect the plants from damage caused by UV-B radiation.

to NO production during various stress and developmental conditions (Corpas et al. 2004). Various NOS-like enzymes were characterized in plants, but their molecular basis is still doubtful in higher plants (Moreau et al. 2008). It should also be acknowledged that NR is the mostly widely acknowledged source of NO, the mechanism of which is well understood and has been proven genetically. Thus, NR-dependent NO plays a role in programmed cell death in *Nicotiana benthamiana* (Yamamoto et al. 2003), floral transition (Seligman et al. 2008), cold stress (Gupta et al. 2011*a*, 2011*b*), osmotic stress (Kolbert et al. 2010), crassulacean acid metabolism development (Freschi et al. 2010) and stomatal function (Neill et al. 2003). Often the role of NO is proved through the use of NR gene (*nia*) genetic mutant or suppressed lines.

In this issue, Zhang et al. (2011) have proved that NO derived from NR plays a role in conferring UV stress tolerance. Direct association between *Nia* gene expression, NR activity, NO emission and nitrite content was measured when confirming the role of NR in plants. UV-B radiation stimulated NR activity and *NIA1* gene expression in silver birch (*Betula pendula*). By using tungstate, which binds to the molybdenum binding site of NR, and glutamine, which is a feedback NR inhibitor, the authors confirmed that NR activity and NO emission are required for flavonoid accumulation in silver birch and NO scavenger cPTIO abolished NO. In contrast, Zhang et al. (2011) failed to show any effect of the mammalian NOS inhibitors NG-nitro-L-Arg methyl ester (L-NAME) and *S*,*S*′-1,3-phenylene-bis(1,2-ethanediyl)-bis-isothiourea (PBITU) on UV-B-induced NO generation. Thus, NOS-like activity is not responsible for NO emission in this context. This contrasts with the work of Hao et al. (2009) and Tossi et al. (2009) in maize and *Ginkgo biloba*, which suggested a role for an NOS-like source for NO during UV-B stress. This inconsistency could indicate that different species utilize different NO sources for similar roles. Differences may also be due to inaccuracies in NO measurement (reviewed by Mur et al. 2011); it should be noted that the haemoglobin assay used by Zhang et al. (2011) is a technically difficult approach, but produces highly accurate and highly specific measurements.

A number of points arise from the role of NR in NO generation in UV-B. Firstly, it will be important in assessing how far NO is important for UV tolerance. Thus, for example, *Arabidopsis nia1* mutants could be fully assessed for their responses, and relative sensitivity, to UV-B. Should NO emerge as a vital mediator of tolerance to UV-B, its NR dependence could indicate that environmental N status could affect plant fitness areas and biodiversity in certain areas, for example the sub-polar regions, where ozone thinning is most prominent. Further, this could suggest targets for improving crop stress tolerance should UV-B radiation occur in more temperate regions. Nitrogen use efficiency is already a major target for crop breeding, but it could also influence tolerance to UV-B stress (Hirel et al. 2007). More widely, it is a very important task to unlock the enzymatic sources of NO emission in various stress and environmental conditions. These would suggest genetically modified approaches that could provide more responsive NO-generating plants following the imposition of stress. Alternatively, suitable alleles of the NO-generating complex could, within a germplasm population, be identified for exploitation in crop breeding. Thus, by targeting NR as a source of UV-B-induced NO production, Zhang et al. (2011) have provided a major advance that could make the derivation of UV-B-resistant crops an important step closer.

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