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REVIEW: PART OF A SPECIAL ISSUE ON PLANT IMMUNITY

Moving nitrogen to the centre of plant defence against pathogens

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• **Background** Plants require nitrogen (N) for growth, development and defence against abiotic and biotic stresses. The extensive use of artificial N fertilizers has played an important role in the Green Revolution. N assimilation can involve a reductase series ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$) followed by transamination to form amino acids. Given its widespread use, the agricultural impact of N nutrition on disease development has been extensively examined.
• **Scope** When a pathogen first comes into contact with a host, it is usually nutrient starved such that rapid assimilation of host nutrients is essential for successful pathogenesis. Equally, the host may reallocate its nutrients to defence responses or away from the site of attempted infection. Exogenous application of N fertilizer can, therefore, shift the balance in favour of the host or pathogen. In line with this, increasing N has been reported either to increase or to decrease plant resistance to pathogens, which reflects differences in the infection strategies of discrete pathogens. Beyond considering only N content, the use of NO_3^- or NH_4^+ fertilizers affects the outcome of plant–pathogen interactions. NO_3^- feeding augments hypersensitive response- (HR) mediated resistance, while ammonium nutrition can compromise defence. Metabolically, NO_3^- enhances production of polyamines such as spermine and spermidine, which are established defence signals, with NH_4^+ nutrition leading to increased γ-aminobutyric acid (GABA) levels which may be a nutrient source for the pathogen. Within the defensive N economy, the roles of nitric oxide must also be considered. This is mostly generated from NO_2^- by nitrate reductase and is elicited by both pathogen-associated microbial patterns and gene-for-gene-mediated defences. Nitric oxide (NO) production and associated defences are therefore NO_3^- dependent and are compromised by NH_4^+.
• **Conclusion** This review demonstrates how N content and form plays an essential role in defensive primary and secondary metabolism and NO-mediated events.

Key words: Nitric oxide, nitrate, ammonium, *Pseudomonas*, nitrate reductase, polyamines, plant defence.

INTRODUCTION

Plant and crop biologists considering the role of nitrogen (N) have, quite correctly, concentrated on its key role in driving growth, development and yield. Whilst mechanisms of N uptake and assimilation have been the focus of many studies (for example, Liu *et al*., 2015), away from NO the importance of N in plant defence against pathogens has received limited attention. While not ignored by plant scientists, the importance of N in plant defence against pathogens has not, in our opinion, achieved the prominence that it deserves. In this review, we outline how N uptake and metabolism provide the building blocks for plant defence against pathogens, or mobilizing N away from the invader to influence virulence or symptom development. Additionally, N drives the generation of nitric oxide (NO), an important defence signal. Given the importance of N fertilizers in agriculture, the effects of N arising from interplay between soil, plant genotype and pathogen require investigation.

NITROGEN ASSIMILATION

Nitrogen is taken-up in two different ways depending on when and whether it exists in NO_3^- or NH_4^+ forms. Three families of transporters (NRT1, NRT2 and CLC) have been linked to uptake and translocation of nitrate in plants. Long-distance NO_3^- transport is regulated by transporters such as AtNRT1.5 and AtNRT1.8 which, in Arabidopsis, are involved in loading and unloading into the root stele or from the shoot vasculature (Dechorgnat *et al*., 2011). NH_4^+ uptake is carried out by plasma membrane-located AMT/MEP/Rh transporters (Khademi *et al*., 2004).

After take-up by roots, NO_3^- is first reduced to NO_2^- by cytosolic nitrate reductase (cNR) where NAD(P)H is used as electron donor and further plastidal nitrite reductase reduces nitrite to ammonium. In contrast, NH_4^+ is taken up by roots and transported into plastids, then further assimilated to glutamate by glutamine and glutamate synthase (Crawford and Forde, 2002). NO_3^- assimilation is a more energetically demanding process compared with NH_4^+, as reduction to NO_2^- requires one NADH and a further three NADPH equivalents for further reduction to NH_4^+ in the plastid (Noctor and Foyer, 1998).

NH_4^+ is assimilated into amino acids via the glutamate synthase/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle. A range of aminotransferases will transfer the amino group from glutamate to catalyse the formation of amino acids. A notable example is asparagine synthetase (AS) which forms asparagine and glutamate and which, with glutamine synthetase (GS), plays an important role in N assimilation (Lam *et al*., 1996). The link with 2-oxoglutarate (2-OG) integrates N

assimilation with the bioenergetic tricarboxylic acid (TCA) cycle. Within amino acid biosynthetic pathways, 2-OG plays an important role in providing the required carbon skeletons.

NITROGEN FERTILIZATION AND PATHOGENICITY

The interplay for N between host and pathogen may be dramatically affected by agricultural practices which depend on extensive application of synthetic N fertilizer. The increase in the application of N fertilizers over the past few decades has been a major factor in improving crop productivity (Kant *et al*., 2011). However, such fertilizer use can have conflicting effects on plant–pathogen interactions. Although N input increases plant defence, it also increases the availability of N compounds for exploitation by pathogens (Tavernier *et al*., 2007), and overapplication of nitrogen fertilizers has been shown to enhance disease development (Solomon *et al*., 2003). It has been suggested that increased N supply causes greater disease susceptibility through changes in canopy structure that can provide an environment favourable for pathogen growth. However, in yellow rust (caused by *Puccinia striiformis*), leaf N content rather than canopy structure was important for sustaining epidemics on winter wheat (Neumann *et al*., 2004). Increased supply of N to the plant led to higher spore production by the powdery mildew fungus *Oidium lycopersicum*, and increased leaf colonization by the bacterium *Pseudomonas syringae* pv tomato suggested that increased leaf N caused greater susceptibility to these pathogens. N fertilization has been shown to increase levels of powdery mildew attack on cereals in the field, and experiments carried out on seedlings of six different barley cultivars showed a positive correlation between N application and powdery mildew disease severity (Jensen and Munk, 1997). A further link between host and pathogen N was noted by Robert *et al*. (2002) who correlated spore production by the rust fungus *Puccinia triticina* in wheat seedlings. The number of spores was 70 % less in the low N plants but the percentage of N in the spores was higher than in the leaves, suggesting that the pathogen is highly efficient at taking up N from the host and indeed the effectiveness of this mechanism(s) would be important for virulence.

NITROGEN USE BY PATHOGENS

Any consideration of the role of N in plant demand must also assess the requirements of the pathogen. The N required by fungal pathogens for growth comes entirely from plant sources such as NO_3^-, NH_4^+ and amino acids, the exact combination of which will vary depending on the part of the plant being infected. Some reports suggest that N sources in the plant are limiting, and that N starvation controls pathogenicity genes and may be a cue for disease development (Thomma *et al*., 2006; López-Berges *et al*., 2010). However, other studies suggest that there is a plentiful supply of N for fungal pathogen growth (Solomon *et al*., 2003; Pageau *et al*., 2006; Tavernier *et al*., 2007; Walters and Bingham, 2007). A large number of amino acids have been shown to be present in the apoplast of tomato leaves, with some of them, such as glutamine, glutamate, alanine and γ-aminobutyric acid (GABA) at millimolar concentrations, sufficient to support pathogen growth during the early stages of infection (Solomon and Oliver, 2001). Interestingly, these authors observed an increase in the amino acid concentration between 7 and 14 d after infection which was correlated with increased fungal biomass in the leaf. This may suggest a long-term requirement for N nutrition from the host. This could be extracted through increased protease activity, possibly due to the induction of an extracellular serine protease P69B (Solomon and Oliver, 2001). An additional source of host N nutrition is GABA produced by the irreversible conversion of glutamate by glutamate decarboxylase. It is usually found in plants at low concentrations but levels rise in response to a number of stresses, such as low temperature, heat shock and drought (Forde and Lea, 2007). Solomon and Oliver (2001) showed that *Cladosporium fulvum* was able to utilize GABA as an N source, with fungal growth on GABA similar to that when grown on aspartate or glutamate, suggesting its potential as an efficient N source for pathogen growth.

ROLE OF NITROGEN NUTRITION IN PLANT DEFENCE

Direct contributions by N to defensive metabolites

Against the trends noted above, other studies have noted that increased N resulted in increased host resistance; for example, in the interactions of *Fusarium oxysporum* f. sp. *lycopersici* and *Botrytis cinerea* with tomato plants which showed increased resistance with greater N application. Such results suggest specific and complex pathogen–N interactions (Hoffland *et al*., 2000). Increased N can contribute to every level of plant defence; constitutive or induced. Plants reconfigure their primary and secondary metabolism in response to pathogen infection, and these are also influenced by N (Ward *et al*., 2010). The nutrition regime will impact on the patterns of amino acid biosynthesis to affect gene expression, including those of defence genes. N levels have been shown to affect the production of constitutive defences based on alkaloids (Stout *et al*., 1998) and (poly) phenolics under different N regimes (Johnson *et al*., 1987).

Other studies have focused on establishing how ‘expensive’ the production of constitutive defences is or how N could be distributed against the competing demands of growth and defence (Herms and Mattson, 1992; Huot *et al*., 2014). Metabolite ‘hot spots’ for competition include phenylalanine-derived phenolics and N-containing cyanogenic glycosides (Goodger *et al*., 2007). Models suggesting how interacting plant defence hormones act to establish the relative balance between the plant growth vs. defence requirements have been recently proposed (Huot *et al*., 2014). In the context of such considerations, the role of the soil should not be ignored. Biological mineralization of organic material can influence dynamics of N availability. For instance, ammonification of organic forms of N and further nitrification alter the availability of different N forms to the plant during its growth. These dynamic changes in N status can become extreme with changes in pH (Fu *et al*., 1987), soil humidity and oxygen levels (Smith *et al*., 1998).

Beyond altered availability of N, the form of N can affect disease development and plant resistance. For instance, symptoms of black root rot (*Rhizoctonia solani*) of sugar beets (Afanasiev and Carlson, 1942) or *Fusarium* wilt on tomato (Borrero *et al*., 2012) were reported to increase following NH_4^+

nutrition. In a recent metabolomic study, we have found that NH_4^+ nutrition enhances the content of apoplastic sugar and amino acids as well as GABA, thereby increasing the availability of nutrients to the invading pathogen; in this case *P. syringae* (Gupta *et al*., 2013). Conversely, under NO_3^- nutrition, increased resistance to *P. syringae* was observed. Leaving aside NO_3^--mediated impacts on NO generation (considered below), other defence-associated features were also augmented. One was the increase in polyamine levels, i.e. putrescine, spermidine and spermine production (Gupta *et al*., 2013). Polyamines are known to increase plant resistance; for example, increasing in barley (*Hordeum vulgare*) during the hypersensitive response (HR)-associated resistance response against the powdery mildew fungus *B. graminis* f. sp. *hordei* (Cowley and Walters, 2002*a*, *b*). Polyamines are now well established as signal molecules influencing the cell cycle, DNA and protein synthesis, as well as programmed cell death (Tiburcio *et al*., 2014). Polyamines are oxidatively deaminated by a series of amine oxidases to produce hydrogen peroxide (H_2O_2), and this has been suggested to contribute to oxidative cross-linking of cell walls to reduce pathogen ingress. H_2O_2 has been shown to be required for polysaccharide–protein cross-linking and lignification for penetration defence. Furthermore, aminoaldehydes and 1,3-diaminopropane from polyamine oxidation are involved in secondary metabolite synthesis and abiotic stress tolerance (Cona *et al*., 2006). Polyamines are also involved in systemic resistance. For instance, methyl jasmonate- (MJ) induced systemic resistance in powdery mildew infection in barley was accompanied by increased production of putrescine and spermidine along with other defence-related metabolites (Walters *et al*., 2002).

Nitrogen mobilization as a defence strategy

The efficient remobilization of N from leaves during grain filling is important to enable the plant to meet the high demands of the growing seed, and in cereals such as barley and wheat, flag leaf senescence correlates with grain N content. However, N remobilization can also occur prematurely in response to many environmental factors including pathogen attack (Masclaux-Daubresse *et al*., 2010). In this context, it is relevant that Olea and colleagues (2004) showed that during infection of tomato by the bacterial speck pathogen *P. syringae* pv. tomato, aspartate synthetase (AS) expression increased in the phloem of the main and secondary veins of the leaf. As the product of the AS-dependent catalysis of glutamine and aspartate is aspargine, which is preferred for energy storage and transport in some species due to the high C/N ratio, such vascular AS expression would reduce the availability of important nutrients to the pathogen.

The levels of enzymes involved in nitrogen assimilation such as NR and glutamine synthetase 2 (GS2) decrease with leaf age, whereas levels of glutamate dehydrogenase (GDH) and glutamine synthetase 1 (GS1) increase. The expression and activity of these latter two enzymes can therefore be used as senescence markers. Interestingly, following infection of tobacco leaves with viruses, and virulent and avirulent strains of the bacterial pathogen *P. syringae*, the use of fungal elicitors and application of phytohormones such as salicylic acid (SA) showed alterations in GS1 and GDH activity similar to senescence. Most notably there was an overall decrease in GS activity and an increase in GS1 and GDH expression, the exact response varying and depending on the individual interaction (Pageau *et al*., 2006). Similar effects on senescence markers were also observed by Tavernier *et al*., (2007) when looking at the compatible interaction of the hemibiotrophic pathogen *Colletotrichum lindemuthianum* with *Phaseolus vulgaris*. It was noted that GS1 expression was greater with infection by the avirulent strains of *P. syringae* whereas GHD expression was more associated with cell death occurring as a result of both resistance responses and disease development. Taking all of these observations together, it is possible to hypothesize that GS1 acts like a metabolic defence gene, remobilizing N away from the infection site in a scorched-earth defence mechanism that has been referred to as 'slash and burn' (Pageau *et al*., 2006; Tavernier *et al*., 2007).

Nitric oxide in plant defence

The production of NO is a feature of microbially catalysed oxidoreductive reactions occurring between NO_3^- and NH_4^+ during soil N cycling. Plants possess a number of distinct pathways for production of NO, located in different cellular compartments and with activation dependent on physiological, developmental and stress conditions (Gupta, 2011). Cytosolic NR, mitochondrial nitrite NO reductase, plasma membrane nitrite NO reductase and xanthine oxido-reductase are reductive pathways. Nitric oxide synthase-like enzyme (NOS-like)-, polyamine- and hydroxylamine-mediated pathways are oxidative in nature. Central to all of these mechanisms is a dependence on the relative availability of N assimilation products (see below). NO has a specific role in each compartment, possibly interacting with local signal events. For example, NO has recently been shown to modulate mitochondrial alternative oxidase and aconitase activities, thereby helping the plant in shift from primary metabolism towards amino acid biosynthesis (Cvetkovska and Vanlerberghe, 2012*a*, *b*; Gupta *et al*., 2012*a*, *b*). In the case of peroxisomal NO production, this has been shown to inhibit catalase and glyoxylate oxidase activity; enzymes which play roles in β-oxidation and in the detoxification of reactive oxygen species (ROS) (Ortega-Galisteo *et al*., 2012). Although a number of such enzyme activities have been linked to disease resistance, further investigation is necessary (Chern *et al*., 2013).

Nitric oxide appears to play important roles in many facets of plant defence. The first report came from Noritake *et al*. (1996), who reported that NO plays a role in accumulation of phytoalexin biosynthesis. Later, the role of NO in driving cell death during the HR was defined in plants (Durner *et al*., 1998; Delledonne *et al*., 2001, 1998). These authors and many others, including ourselves (Mur *et al*., 2005), have shown that the rate of NO production influences the kinetics of HR formation. Mechanistically, this is likely to involve an interaction with ROS which is also central to forming the HR (Delledonne *et al*., 1998; Torres *et al*., 2002; Yun *et al*., 2011). Due to its high diffusibility and lipophilic nature, NO can cross plant membranes and react with superoxide O_2^-, leading to the generation of peroxynitrite ($ONOO^-$). Excess NO can also react with O_2^-, leading to accumulation of less toxic NO_2, N_2O_3 and N_2O_4.

The NO–ROS interactions are also important in downstream signalling pathways. Nitrosylation is another mechanism where reversible reaction of NO with the thiol groups of reduced cysteine residues plays a role in activation and inactivation of specific protein functions (Gupta, 2011). Due to the high content of iron and thiols, mitochondrial NO can nitrosylate many proteins in mitochondria. For example, the *P. syringae*-derived elicitor harpin induced mitochondrial NO production and nitrosylation of the photorespiratory mitochondrial glycine decarboxylase complex (GDC). Since NADH is needed for redox control, nitrosylation leads to inhibition of GDC, which can affect overall redox balance and promote cell death (Palmieri *et al*., 2010).

Nitric oxide also plays a role in defence responses induced by pathogen-associated molecular patterns (PAMPs) (Zeidler *et al*., 2004). As a signalling component of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Delledonne *et al*., 1998), NO can be considered part of the Zig–Zag model of plant defence (Jones and Dangle, 2006). Additionally, recognized PAMPs located on *P. syringae* pv. tomato were demonstrated to elicit NO-mediated stomatal closure and thereby reduce the penetration efficiency of the pathogen. The bacterial virulence factor coronatine has been shown to block this effect, thus allowing entry into the sub-stomatal chamber (Melotto *et al*., 2006). NO has also been shown to influence the formation of papillae, which are cell wall appositions produced in response to fungal pathogens and central to many non-host defence mechanisms (Prats *et al*., 2005). Taking all of these points together, N availability, together with subsequent NO generation, will have a wide-ranging impact on the ability of plants to withstand microbial attack. In terms of both NO production elicited via PTI and ETI, one of the most important downstream effects is the initiation of SA biosynthesis (Durner *et al*., 1998). SA plays a central role in both localized and systemic resistance to infection, and NO is now known to be an integral component of the signalling pathway (Mur *et al*., 2013). Extensive bioinformatic analysis of NO-responsive promoters in arabidopsis in response to infection found that *cis*-elements linked to SA responsiveness were prominent (Palmieri *et al*., 2008). Further, NO is known to nitrosylate key cysteines on TGA-class transcription factors to aid in the initiation of SA-dependent gene expression (Lindermayr and Durner, 2009; Lindermayr *et al*., 2010; Gupta, 2011; Mur *et al*., 2013; Yu *et al*., 2014). This can be countered through nitrosylation of A NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEIN1 (NPR1) leading to oligomerization of NPR1 within the cytoplasm to reduce TGA activation (Tada *et al*., 2008; Lindermayr *et al*., 2010). On the other hand, Després and colleagues (2003) reported that TGA1 relies on the oxidation state of cysteine residues to mediate the interaction with NPR. Another key SA protein that is nitrosylated is the SA-binding protein 3 (SABP3) which has carbonic anhydrase activity (Wang *et al*., 2009). Silencing SABP3 gene expression suppressed a HR elicited by *P. syringae* pv. tomato (Slaymaker *et al*., 2002).

It is important not to forget the interacting pathogen, where NO can also play a role in host invasion. NO has been shown to be generated in the appressoria of *Blumeria graminis* and *Magnaporthe oryzae* to aid penetration of the host (Prats *et al*., 2008; Samalova *et al*., 2013). In the case of *Phytophthora cryptogea*, the virulence factor/elicitor cryptogein aids pathogenesis by promoting host cell death via NO generation (Lamotte *et al*., 2004). Thus, not only N nutrition, but the relative availability of N to drive either host or pathogen NO generation is a relevant consideration.

Role of N nutrition in NO generation during defence against pathogens

The NR pathway catalyses the reduction of nitrate to nitrite using NADH as electron donor [$NAD(P)H + 3H_2O^+ + 2NO_2^- \rightarrow NAD^+ + 2NO + 5H_2O$]. It is now well established that NO_3^- and NO_2^- play a role in increased NR activity and NO emissions (Gupta *et al*., 2005; Planchet *et al*., 2005). In an important recent development, it was shown that *S*-nitrosothiol (SNO) signalling regulates both nitrate uptake and reduction, to fine-tune nitrate homeostasis (Frungillo *et al*., 2014). It is therefore possible that defence-associated NO could influence this homeostatic mechanism to favour N diversion either away from the infection site or towards defensive metabolism.

NH_4^+ nutrition is also of importance, as this leads to reduced levels of NO via suppression of NR activity (Planchet *et al*., 2005; Gupta *et al*., 2013). The NR NO-generating mechanism has been shown to be central to the HR in response to avirulent *Pseudomonas* interactions with the host (Melotto *et al*., 2006; Gupta *et al*., 2013; Vitor *et al*., 2013). Gupta and colleagues (2013) also reported that during compatible interaction between tobacco and *P. syringie* pv. *tabaci* the NR NO-generated mechanism increased plant resistance. A similar trend of response was observed in *Verticillium dahliae* infection in arabidopsis (Shi and Li, 2008). Yamamoto-Katou *et al*. (2006) showed that silencing NR leads to reduced levels of NO in response to elicitin. These authors concluded that mitochondrial NO plays a role in defence. Mitochondria produce NO from complex III and IV of mitochondrial electron transport. This process requires relatively low oxygen levels (Gupta *et al*., 2005), a situation that could arise due to defence responses depleting local oxygen levels within tissues. The implication of all of these studies is that the strength of NO generation and, thus, the efficacy of the defence response, is strongly dependent on the relative availability of NO_3^-/ NO_2^-.

A parallel consideration must be NO generation during plant defence via a NOS-like pathway (Delledonne *et al*., 1998; Wendehenne *et al*., 2001) Despite having no homologue in higher plants, it was shown that NOS-like activity is responsible for NO during plant response to various pathogen infection and resistance responses (Chaki *et al*., 2009; Yoshioka *et al*., 2009). However, evidence for this is mainly based on the use of pharmacological NOS inhibitors such as arginine analogues; PBITU [*S*,*S*′-1,3-phenylene-bis(1,2-ethanediyl)-bis-isothiourea], L-NAME (NG-nitro-L-arginine methyl ester), L-NMMA (NG-monomethyl-L-arginine), L-NIL [N6-(1-iminoethyl)-L-lysine)] or AET [2-(2-aminoethyl) isothiourea] (Gaupels *et al*., 2011). This stated, direct assays of NOS-like activity have suggested an involvement in disease resistance to the necrotrophic pathogen *Botrytis cinerea* in *Nicotiana benthamiana* (Asai and Yoshioka, 2009), and cryptogein-induced cell death (Besson-Bard *et al*., 2008). Whatever the exact nature of this NOS-like activity, its dependence on arginine retains the link with N assimilation, NO and defence.

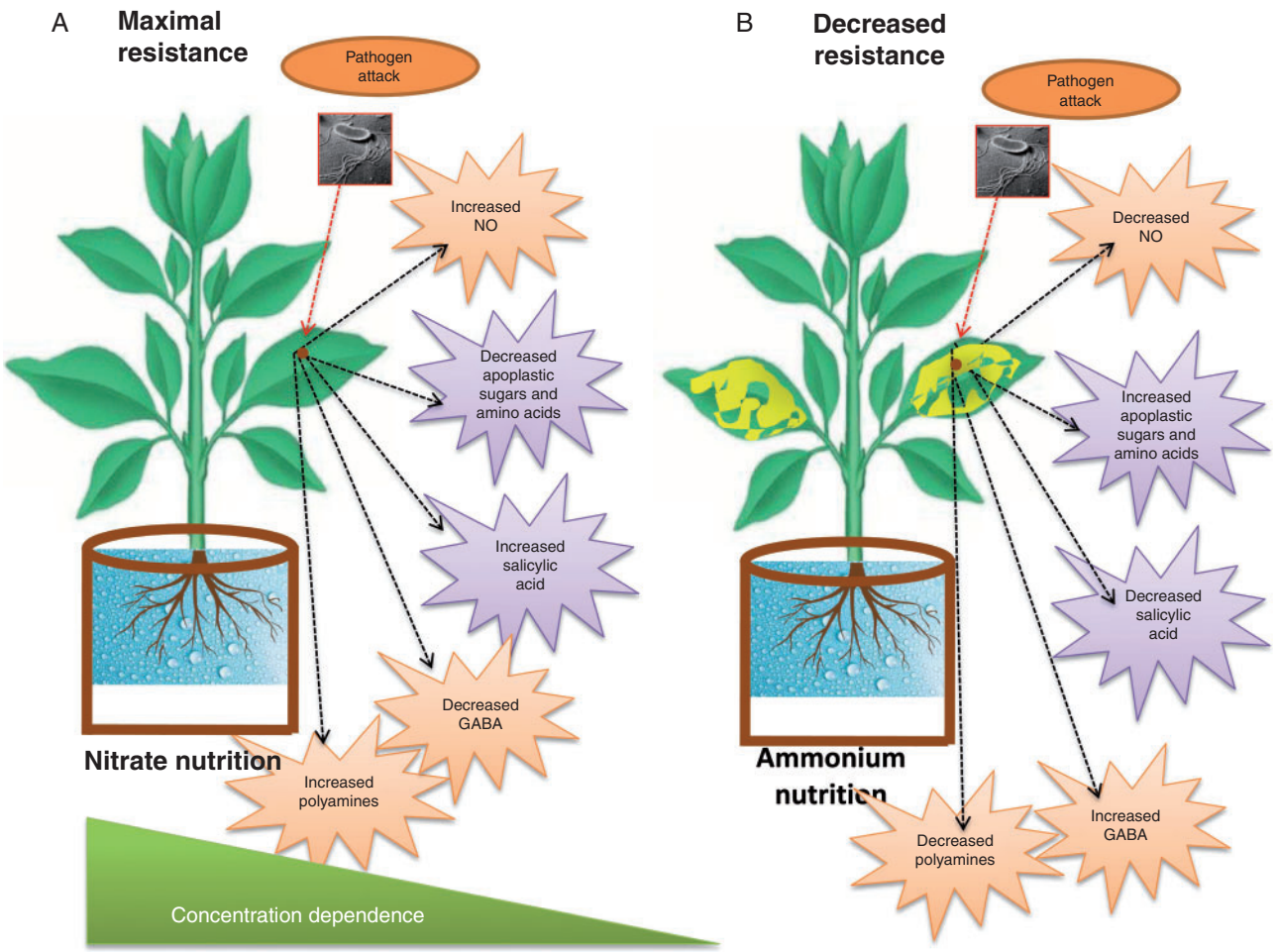

FIG. 1 Effect of NO_3^- vs. NH_4^+ on plant resistance to pathogen infection. Growth on NO_3^- nutrition leads to increased levels of NO, SA, PR gene expression, induction of the polyamine pathway, a decrease in apoplastic sugars and amino acids, and an overall increase in plant resistance in a concentration-dependent manner. Growth on NH_4^+ nutrition leads to increased levels of apoplastic sugars and amino acids, reduced levels of SA and PR gene expression, induction of GABA biosynthesis and reduced plant defence response.

A similar indirect link with N is implicit in the polyamine-mediated NO pathway (Tun *et al.*, 2006). As in the NOS-mediated NO pathway, in the polyamine NO pathway arginine acts as a substrate for production of spermine and spermidine to produce NO. Polyamine generation requires arginine to act as a substrate for the enzyme arginine decarboxylase. Arginine levels can be determined by modulation of arginase, leading to altered NO production (Flores *et al.*, 2008). Normal levels of NO were returned by providing spermine to the plants, suggesting that the polyamine synthesis from arginine is involved in the production of NO. The mechanisms through which polyamines mediate NO generation are not completely known. However, polyamines clearly play a role in plant defence (Walters, 2003), with increased spermine in tobacco leading to increased resistance against *Pseudomonas tabaci* and also the hemibiotrophic oomycete *Phytophthora parasitica* var. *nicotiana* (Moschou *et al.*, 2009) and potentiating defence against *Pseudomonas viridiflava* in arabidopsis (Gonzalez *et al.*, 2011). Defence mechanisms involving polyamines may include ROS production and cell death as well as cell wall reinforcement mechanisms (Walters, 2003).

CONCLUDING REMARKS

Far from considering N assimilation as only providing the building blocks of primary metabolism, this review has suggested that its appropriate concentration and form is essential for effective plant defence. N influences both constitutive and induced defences, as an element in key signal molecules such as NO and polyamines. We have highlighted how resistance based upon PTI, ETI or the mobilization of nutrients away from the site of infection ('slash and burn') could all be compromised under different N conditions. The potential importance of these observations cannot be overstated, and breeding programmes focusing on resistance need also to consider interactions under different N regimes. Continued advances in the development of disease-resistant crop plants requires further understanding of how N, NO and polyamines contribute to PTI and ETI, together with elucidation of mechanisms involved in movement of N resources away from the site of infection. Understanding of how different pathogens, with different infection strategies, respond to N levels to influence nutrition and pathogenicity is also of fundamental importance in this context.

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