# NADPH-generating dehydrogenases: their role in the mechanism of protection against nitro-oxidative stress induced by adverse environmental conditions

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NADPH is an essential reductive coenzyme in biosynthetic processes such as cell growth, proliferation, and detoxification in eukaryotic cells. It is required by antioxidative systems such as the ascorbate-glutathione cycle and is also necessary for the generation of superoxide radicals by plant NADPH oxidases and for the generation of nitric oxide (NO) by L-arginine-dependent nitric oxide synthase. This coenzyme is principally re-generated by a group of NADP-dehydrogenases enzymes including glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH), both belonging to the pentose phosphate pathway, the NADP-malic enzyme (NADP-ME), and NADP-isocitrate dehydrogenase (NADP-ICDH). In this study, current perspectives on these enzymes in higher plants under different stress situations are reviewed and it is also pointed out that this group of NADPH-generating dehydrogenases is a key element in supporting the mechanism of response to nitro-oxidative stress situations.

**Keywords: G6PDH, 6PGDH, ICDH, NAPDH, NADP-ME, nitric oxide, nitrosative stress, oxidative stress**
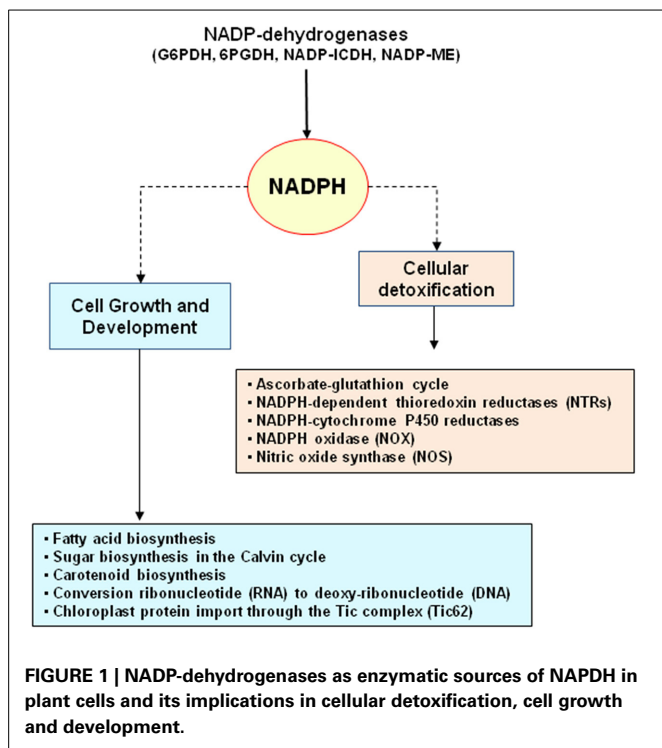
## INTRODUCTION

The supply of reducing equivalents in the form of NADPH (the reduced form of the nicotinamide adenine dinucleotide phosphate) is essential in all living organisms (Pandolfi et al., 1995; Barroso et al., 1998; Ying, 2008). Thus, NADPH is required for cell growth and proliferation which are necessary in several metabolic pathways including fatty acid biosynthesis, biosynthesis of sugars in the Calvin cycle, biosynthesis of carotenoids, conversion of ribonucleotide (RNA) to deoxy-ribonucleotide (DNA) and regulation of chloroplast protein import via the metabolic redox status of the chloroplast, specifically in the Tic62, (a component of the translocon at the inner envelope of chloroplasts, Tic complex) (Stengel et al., 2008; Kovács-Bogdán et al., 2010). NADPH is also required by NADPH-cytochrome P450 reductases (Ro et al., 2002), the generation of superoxide radicals by the NADPH oxidase (NOX) (Sagi and Fluhr, 2006) and is a necessary cofactor for the generation of nitric oxide (NO) by L-arginine-dependent nitric oxide synthase (NOS) activity (Corpas et al., 2009). NADPH is also essential by different antioxidative systems including the activity of glutathione reductase (GR), a key enzyme in the ascorbate-glutathione cycle to protect against oxidative damage (Noctor et al., 2006; Gill et al., 2013), and by

NADPH-dependent thioredoxin reductases (NTRs) in the regulation of metabolic pathways through thiol group reduction (Spinola et al., 2008; Cha et al., 2014). Curiously, in this last case it has been reported that the chloroplastic G6PDH activity can undergo a redox regulation by thioredoxin (Née et al., 2014) which suggests a complex interaction between the source of NADPH and the NTR system. In consequence, the ultimate antioxidant capacity of the cell must be determined by the availability of reducing equivalents. **Figure 1** summarizes the main pathways in plant cells where NADPH is required.

There are several enzymatic components involved in the maintenance of the pool of NADP and NADPH. NAD kinases (NADKs) catalyze the direct phosphorylation of NAD to NADP and therefore contribute to the generation of the cellular NADP pool (Pollak et al., 2007; Agledal et al., 2010). On the other hand, ferrodoxin-NADP reductase (FNR) in photosynthetic cells during the light phase is recognized as a principal source of NADPH. However, in non-photosynthetic cells during the dark phase of photosynthesis, the main enzymes capable of generating power reduction in the form of NADPH are the following: glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) (both belonging to the pentose phosphate pathway), NADP-isocitrate dehydrogenase (NADP-ICDH, EC 1.1.1.42) and NADP-malic enzyme (NADP-ME, EC 1.1.1.40), also known as NADP-malate dehydrogenase. This mini-review will focus on these groups of NADPH recycling dehydrogenases, principally in relation to their role as second lines of defense against nitro-oxidative stress.

**Abbreviations:** FNR, ferrodoxin-NADP reductase (FNR ferrodoxin-NADP reductase); G6PDH, glucose-6-phosphate dehydrogenase; GR, glutathione reductase; NADKs, NAD kinases; NADP-ICDH, NADP-isocitrate dehydrogenase; NADP-ME, NADP-malic enzyme; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NADPH oxidase; NTRs, NADPH-dependent thioredoxin reductases; ONOO⁻, peroxynitrite; 6PGDH, 6-phosphogluconate dehydrogenase; ROS, reactive oxygen species; RNS, reactive nitrogen species; Tic, The Inner envelope of Chloroplasts.

**FIGURE 1 | NADP-dehydrogenases as enzymatic sources of NAPDH in plant cells and its implications in cellular detoxification, cell growth and development.**

## SUBCELLULAR NADP-DEHYDROGENASE COMPARTMENTALIZATION AS A NADPH SUPPLY REGULATION MECHANISM

The NADPH pool is required in many processes while the contribution of each NADP-dehydrogenase in specific situations is difficult to determine. However, cell compartmentalization is required as an additional control mechanism in order to keep the NADPH supply close to the system when required, particularly given that NADPH is part of a network containing other energy-rich molecules such as NADH and ATP (Scheibe and Dietz, 2012). In addition, given that NADPH is not easily transported across membranes but, rather, operates through indirect shuttle systems, all these NADP-dehydrogenases usually have different isozymes which are localized in the different subcellular compartments. Although the localization of some of these NADP-dehydrogenases in the different organelles has been described in different plant species (Gálvez and Gadal, 1995; Corpas et al., 1998, 1999; Debnam and Emes, 1999; Hodges et al., 2003; Kruger and von Schaewen, 2003; Leterrier et al., 2007), the availability of genomes in higher plants such as *Arabidopsis thaliana* and *Oryza sativa* has facilitated a more systematic analysis of different NADP-dehydrogenases (Chi et al., 2004; Wakao and Benning, 2005; Wheeler et al., 2005).

## FUNCTION OF NADP-DEHYDROGENASES UNDER ENVIRONMENTAL STRESS CONDITIONS

Under diverse biotic and abiotic stress conditions, plants have developed a whole battery of response mechanisms in order to overcome any potential cellular damage. In many cases, these processes could be accompanied by an uncontrolled increase in reactive oxygen and nitrogen species (ROS and RNS) which might

generate nitro-oxidative stress (Corpas et al., 2007; Corpas and Barroso, 2013). As all these processes usually involve a redox response, an additional NADPH supply may be required for all the pathways using it.

To support this hypothesis, there is a body of evidence to show that, under specific stress conditions, one or more NADP-dehydrogenases are regulated at the level of activity and protein/gene expression (Valderrama et al., 2006; Liu et al., 2007, 2013; Marino et al., 2007; Mhamdi et al., 2010; Airaki et al., 2012). Moreover, the importance of some of these NADP-dehydrogenases has been confirmed by reverse genetic studies (Scharte et al., 2009; Dal Santo et al., 2012; Voll et al., 2012; Siddappaji et al., 2013).

In olive plants (*Olea europaea*) under salinity-induced nitro-oxidative stress, a general increase in the activity of the main antioxidative systems (catalase, superoxide dismutase and enzymes of the ascorbate-glutathione cycle) was accompanied by a significant increase in the activity and protein expression of G6PDH, NADP-ME, and NADP-ICDH (Valderrama et al., 2006, 2007). Similar behavior has been reported in leaves from pepper plants (*Capsicum annum*) exposed to cadmium stress which generates oxidative stress and a concomitant increase in the activity of all NADP-dehydrogenases (G6PDH, 6PGDH, NADP-ME, and NADP-ICDH) (León et al., 2002). In pepper plant leaves exposed to low temperatures (8°C) for different periods of time (1–3 d) after 24 h treatments, we observed alterations in the metabolism of ROS and RNS (an increase in lipid oxidation and protein nitration) and a general rise in the activity of the main NADPH-generating enzymes (G6PDH, 6PGDH, NADP-ME, and NADP-ICDH) which appeared to contribute to cold acclimation (Airaki et al., 2012). Arabidopsis seedlings grown under salinity conditions (100 mM NaCl) also displayed nitro-oxidative stress. Among the NADPH-generating dehydrogenases (G6PDH, 6PGDH, NADP-ME, and NADP-ICDH) analyzed under these conditions, NADP-ICDH showed maximum activity levels, mainly attributable to the root NADP-ICDH (Leterrier et al., 2012c). Another study of NADP-ICDH activity in Arabidopsis has demonstrated that this enzyme's kinetic parameters vary depending on the organ involved, being the specific activity much higher in roots than in leaves. *In vitro* analysis of NADP-ICDH activity in the presence of different ROS and RNS showed that $H_2O_2$ does not affect this activity in either organ; however, reduced glutathione (GSH) inhibited activity in leaves but not in roots. On the other hand, *S*-nitrosoglutathione, a cellular *S*-nitrosothiol used as a NO donor, and peroxynitrite ($ONOO^-$) depressed NADP-ICDH activity in leaves and roots (Leterrier et al., 2012b). Modulation of NADP-ICDH activity by RNS was also observed in pea roots (*Pisum sativum*) during natural senescence which is associated with nitro-oxidative stress since there are increases in the $ONOO^-$ levels and in the number of nitrated proteins. Thus, cytosolic NADP-ICDH activity was shown to be inhibited by nitration at Tyr392 during senescence in a process mediated by peroxynitrite (Begara-Morales et al., 2013).

Depending on the plant species involved, the organs analyzed and the intensity of stress, the response of the NAPD-dehydrogenases could also be vary. Thus, in tomato roots (*Solanum lycopersicum*) under salinity conditions (120 mM

NaCl) accompanied by oxidative stress, an overall decrease in NADPH content and the enzymatic activities of the main NADPH-generating dehydrogenases has been reported, especially NADP-ICDH activity which recorded a drastic reduction of 94% (Manai et al., 2014). This could be explained by the sensitivity of this enzyme to post-translational modification mediated by $ONOO^-$ as observed in pea roots during senescence (Begara-Morales et al., 2013). However, in *Arabidopsis thaliana* seedlings exposed to arsenic (1 mM $KH_2AsO_4$) which also generates nitro-oxidative stress based in the concomitant increase of tyrosine-nitration and lipid peroxidation, the activity of NADP-dehydrogenases (G6PDH, 6PGDH, and NADP-ICDH) did not vary significantly, suggesting that the supply of NAPDH was sufficient to withstand this stress (Leterrier et al., 2012a). Alternatively, the involvement of Arabidopsis cytosolic NADP-ICDH in leaves has been demonstrated to contribute to the maintenance of redox homeostasis under biotic stress caused by *Pseudomonas syringe* (Mhamdi et al., 2010). In the leaves of tobacco plants (*Nicotiana tabacum*), NADP-ME activity increased significantly in response to drought (Doubnerová-Hısková et al., 2014). On the other hand, in *Lotus japonicus* exposed to water stress, differential and spatially distributed nitro-oxidative stress was reported in roots and leaves. Analysis of NADP-dehydrogenase activities in roots revealed that, whereas G6PDH and NADP-ICDH activity decreased 6.5- and 1.5-fold, respectively, 6PGDH and NADP-ME increased 1.5- and 1.3-fold, respectively. However, no leaf NADP-dehydrogenase appeared to be affected, except for G6PDH which decreased by around 50% under water stress conditions (Signorelli et al., 2013). **Table 1** summarizes some examples of the response of NADP-dehydrogenases to nitro-oxidative stresses generated by different abiotic stresses.

As mentioned above, certain post-translation modifications could negatively affect activity under stress conditions although up-regulation has also been reported. For example, in *Arabidopsis*

*thaliana* under salinity (150 mM NaCl) stress conditions, the cytosolic G6PDH isozyme (G6PD6) is targeted by phosphorylation at Thr-467 whose activity increased. The important role played by this dehydrogenase was corroborated using *Arabidopsis thaliana* knockout mutants of cytosolic G6PDH (G6PD6) where the cellular redox state was altered and plants were more sensitive to salt stress (Dal Santo et al., 2012). The importance of cytosolic G6PDH in the leaves of tobacco plants (*Nicotiana tabacum*) at an early stage of defense against the *Phytophthora nicotianae* pathogen which is accompanied by oxidative burst has also been reported. This was demonstrated using a genetic approach involving over-expression of this G6PDH isozyme which improved NADPH provision for pathogen-activated NOXs at the plasma membrane during early oxidative burst (Scharte et al., 2009). In addition, these tobacco plants showed heightened resistance to drought stress. In the same way, transgenic tobacco plants over-expressing the cytosolic G6PDH from *Populus suaveolens* have enhanced cold (4°C) tolerance. Beside of the increased G6PDH activity, these transgenic plants showed lower level of lipid oxidation and higher activity of antioxidant enzymes such as superoxide dismutase and peroxidase. Moreover, these plants have activated the expression of stress-related genes. Therefore, these data clearly show the regulatory function of G6PDH during low temperature stress (Lin et al., 2013).

There are other examples of certain specific NADP-dehydrogenases being regulated at the level of activity and gene expression under diverse stress conditions. For instance, *G6PDH* mRNA expression in wheat seedlings under salt stress conditions of 150 mM NaCl reached a maximum level at 12 h of the treatment (Nemoto and Sasakuma, 2000). A similar response was observed in the expression of the *6PGDH* gene which was up-regulated in rice shoots under salt stress (150 mM NaCl) (Huang et al., 2003). By using the Arabidopsis cytosolic NADP-ICDH knockout mutant, it has been reported that the

**Table 1 | Examples of the response of NADP-dehydrogenases to nitro-oxidative stresses generated by different abiotic stresses.**

| Stress | Plant specie | Organs | Response of NADP-dehydrogenases | References |
|---|---|---|---|---|
| **Salinity** | | | | |
| NaCl (100 mM) | *Arabidopsis thaliana* | Roots | Increase activity of NADP-ICDH | Leterrier et al., 2012c |
| NaCl (120 mM) | Tomato (*Solanum lycopersicum*) | Roots | Decrease activity of NADP-ICDH | Manai et al., 2014 |
| NaCl (200 mM) | Olive (*Olea europaea*) | Leaves | Increase activity and protein expression of G6PDH, NADP-ME and NADP-ICDH | Valderrama et al., 2006 |
| **Drought** | | | | |
| Drought | Tobacco (*Nicotiana tabacum*) | Leaves | Increase activity of NADP-ME | Doubnerová-Hısková et al., 2014 |
| Drought | *Lotus japonicus* | Roots | Decrease activity of G6PDH and NADP-ICDH but increase activity of 6PGDH and NADP-ME | Signorelli et al., 2013 |
| **Heavy metal and metalloids** | | | | |
| CdCl$_2$ (0.5 mM) | Pepper (*Capsicum annum* cv Abdera) | Leaves | Increase activity of G6PDH, 6PGDH, NADP-ME and NADP-ICDH | León et al., 2002 |
| Arsenic (1 mM) | *Arabidopsis thaliana* | Seedlings | G6PDH, 6PGDH and NADP-ICDH activities do not change | Leterrier et al., 2012a |
| **Atmospheric factors** | | | | |
| Low temperature (8°C) | Pepper (*Capsicum annum*) | Leaves | Increase activity of G6PDH, 6PGDH, NADP-ME and NADP-ICDH | Airaki et al., 2012 |
| Ozone | Hybrid poplar | Leaves | Increase activity of G6PDH and NADP-ME | Dghim et al., 2013b |

loss of this isozyme function does not markedly affect the response of Arabidopsis to ozone. However, other cytosolic NADPH-producing enzymes (G6PDH and NADP-ME) showed a significant increase which contributed to maintaining the status of NADPH redox (Dghim et al., 2013a). A similar increase in G6PDH and NADP-ME has also been reported in hybrid poplar leaves in response to ozone (Dghim et al., 2013b).

## CONCLUSIONS

Together with NADH, NADPH participates in the equilibrium of cellular redox homeostasis and also maintains certain antioxidant systems such as the ascorbate-glutathione cycle and NTRs. Thus, NADP-dehydrogenase systems should be regarded as a second line of defense in order to maintain the effective functioning of the main antioxidative systems. Biochemical and genetic approaches provide a strong data basis to confirm the essential involvement of NADP-dehydrogenases in the mechanism of response to nitro-oxidative stress situations. Organ distribution and subcellular compartmentalization are regarded as additional regulatory mechanisms of these systems to ensure that the NADPH supply is at the required location. Future research will be essential to identify the specific involvement of each NADP-dehydrogenase in the different organs and cellular compartments supporting a particular pathway as all these enzymes are also involved in nitrogen and carbohydrate metabolisms.

## ACKNOWLEDGMENTS

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