

Mitochondrial Electron Transport Pathway Components

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All plants contain an alternative electron transport pathway (AP) in their mitochondria, consisting of the alternative oxidase (AOX) and type 2 NAD(P)H dehydrogenase (ND) families, that are thought to play a role in controlling oxidative stress responses at the cellular level. These alternative electron transport components have been extensively studied in plants like *Arabidopsis* and stress inducible isoforms identified, but we know very little about them in the important crop plant chickpea. Previously we demonstrated AOX activity in purified mitochondria from chickpea, identified the genes that encode the AOX isoforms and analysed their relative transcript levels. Here we do the same for NDs, and also explore the response of all AP gene transcripts to salinity stress in leaves of chickpea cultivars differing in their salinity response. A coordinated up-regulation of particular AP genes suggests that the mitochondrial alternative pathway of respiration is an important facet of the stress response in chickpea, in high Na accumulators in particular, despite high capacities for both of these activities in leaf mitochondria of non-stressed chickpeas.

Keywords: chickpea ; salinity ; abiotic stress ; alternative oxidase ; alternative NAD(P)H dehydrogenase ; mitochondria ; enzyme activity ; gene expression ; co-expression ; high Na accumulator

1. Mitochondria

Mitochondria in all higher aerobic organisms are essential for the energy status of the cell and also play key roles in oxidative stress generation and management. Plant mitochondria have a branched electron transport chain (mETC): in addition to the classical mETC consisting of large complexes that transfer electrons from intramitochondrial NADH to oxygen, coupled to proton translocation and ATP synthesis, plant mitochondria contain an alternative pathway (AP) that does not conserve energy ^{[1][2]}. The AP consists of several single-subunit type II NAD(P)H dehydrogenases (NDs) on both the outside (NDBs) and inside (NDAs and NDCs) of the inner mitochondrial membrane, and an alternative oxidase (AOX) that accepts electrons from the ubiquinol pool and competes with the cytochrome pathway ^{[3][4][5][6][7][8]}. While there has been much speculation on the role of the AP, a consensus has emerged that it plays a key role in moderating the generation of reactive oxygen species (ROS) in the mETC and affects oxidative stress signaling in plant cells through linkages with key transcription factors ^{[9][10][11][12]}. Additionally, the AP may modulate other metabolic pathways and growth, through the regulation of cellular energy status and respiratory metabolite pools ^{[7][13][14][15][16]}. Overexpression of AP components can confer enhanced tolerance of environmental stresses in plants ^{[17][18][19][20][21][22]}.

The genomic organization of AP genes, especially AOX, varies between species ^{[23][24]}. In many dicot species, exemplified by the model plant *Arabidopsis thaliana*, AOX is encoded in a small gene family consisting of several AOX1 genes and maybe one AOX2 gene, with one of the AOX1 genes the main expressed isoform, but only in the presence of stress. Monocot species, including important crop plants such as rice and wheat, lack functional AOX2 isoforms. The legume family appears to be another exception, with all species examined to date having multiple forms of AOX2, with at least one constitutively expressed and another preferentially transcribed to a high level in shoots ^[25]. In *Arabidopsis*, the NDs are also encoded in a small gene family, with two NDA genes, four NDB genes, and an NDC gene ^{[4][26]}. NDC1 and some of the NDA and NDB proteins are also found in other organelles ^[27]. The genomic structure of the ND genes in other species is less understood, although barley and rice ND genes have been identified and partially characterized ^[28].

2. Development

We have identified all NDs in the important crop legume, chickpea (*Cicer arietinum*), including five putative internal (matrix)-facing NAD(P)H dehydrogenases (*CaNDA1-4* and *CaNDC1*) and four putative external (inter-membrane space)-facing NAD(P)H dehydrogenases (*CaNDB1-4*). A splice variant of *CaNDA2*, denoted *CaNDA5*, was also expressed. We demonstrated combined ND activities in mitochondria purified from chickpea for the first time, pointing to a far greater respiratory capacity of leaves compared to roots. We have also determined the effect of salt stress on the expression of AP genes and shown that two genes in particular, *CaAOX1* and *CaNDB2* are coordinated under these conditions, as seen

in some other species [29]. There was also a marked difference in the expression of specific AP genes depending on whether cultivars accumulated high or low levels of Na in their leaves, namely up-regulation of *CaAOX1*, *CaNDB2*, *CaNDB4*, *CaNDA3* and *CaNDC1*, which may form a stress-modified mETC in tissues that accumulate Na. This information is important for our understanding of the role of mitochondria in tissue-specific functions and environmental stress responses in legumes. Given the inherently high rates of external NADH oxidation and AOX capacity in isolated mitochondria grown under control conditions, the significance of transcriptional upregulation of AP genes during stress needs to be explored at the protein and activity level, requiring the development of isoform-specific antibodies and the utilisation of existing techniques for in vivo respiratory measurements. It will also be important to determine more precisely the sub-cellular location, tissue and cell-specific patterns of expression of the multiple AOX isoforms and ND proteins in chickpea and other legumes, and how they impact plant growth and performance both in the presence and absence of stress.

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