

Alternative respiratory genes can improve tolerance to abiotic stresses in rice (*Oryza sativa* L.)

Vajira Rupika Wanniarachchi

Abstract

The non-phosphorylating alternative respiratory pathway (AP) for plants, which consists of two protein groups, alternative dehydrogenases (NDHs) and alternative oxidases (AOXs), has become an area of research interest due to its potential role during stress tolerance. Although the physiological functions of AOXs have been extensively studied those of NDHs are largely unclear and less is known about their responses to abiotic stresses. The main objective of this study was to investigate whether the alterations in AP genes could infer tolerance to abiotic stresses in cereal crops, using rice a model cereal. To achieve this, it was first necessary to identify all candidate genes encoding members of this pathway in rice. To explore the role of AP in stress in rice two approaches were taken; 1. Examine the expression of AP genes in different varieties of rice with varied tolerance to salt and 2. Development of transgenic lines of rice overexpressing AP genes and assess impact of growth under salt stress.

Database screening of the rice genome revealed that both AOX and NDH genes are present in rice. NDH grouped into three distinct families, NDA, NDB and NDC, as in Arabidopsis, but AOX2 type gene was absent. Co-expression of AP genes was observed in response to chemical inhibition of the cytochrome pathway of the mitochondrial electron transport chain, suggesting potential co-regulation. Analysis of promoter regions revealed that these genes share common *cis*-acting regulatory elements, previously found to be functional in Arabidopsis. However, functional analysis of them in rice is yet to be investigated.

A number of rice AP genes was co-expressed in response to salinity stress also. A comparative study of two contrasting rice cultivars, Nipponbare and Langi, further revealed

the existence of cultivar and tissue-dependent variation in gene expression. As found in other species, AOX proteins exist as homodimers in rice and the reduced (active) form was found to be predominant in mitochondria from salt-stressed tissues while the oxidized (inactive) form was more abundant in non-stressed tissues, suggesting post-translational regulation of AOX may be more important in rice than protein levels *per se*. A novel polyclonal antibody raised against NDB2 showed a higher abundance of NDB2 in salt-treated shoots than in non-treated shoots, suggesting NDB2 might be important in salinity tolerance responses. However, in contrast to shoots, corresponding bands were not detected in either salt-treated or non-treated roots. This suggests the possibility of having different post-translational regulations in shoots and roots in rice. However, this needs to be further investigated.

A study on 16 different rice cultivars revealed the existence of wide genotypic and tissue-specific variation in gene expression and tolerance mechanisms in response to salinity stress. Expression of *AOX1a* and *NDA2* in leaf tissues showed a strong positive correlation with tissue Na^+ content. But, there was no correlation between AP gene expression and shoot growth. In fact, the expression of *AOX1c* and *NDB2* in roots revealed strong negative correlation with relative growth of roots. Although it is possible that some AP components play a certain role in salinity tolerance in some cultivars, transcriptional data did not reveal any particular trend in AP gene expression in response to salinity stress amongst the cultivars tested, suggesting AP gene expression levels may not be good molecular markers for salt tolerance in rice.

Transgenic rice plants overexpressing salt-responsive *AtAOX1a* or *AtNDB2*, driven by the rice actin-1 promoter were successfully generated via *Agrobacterium*-mediated transformation. Yield performances of segregating transgenic lines under salinity stress were evaluated at panicle initiation stage, and a significant reduction of empty seed production was revealed in some transgenic lines compared to their controls, suggesting a possible increase in tolerance to high salinity. This result suggests that overexpression of heterologous stress-responsive genes could be a potential avenue for improving rice towards a particular stress tolerance, however this needs confirmation when homozygous lines have been selected.