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Alternative oxidase regulation in roots of *Vigna unguiculata* cultivars differing in drought/salt tolerance

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Summary

The alternative oxidase (Aox) was studied at different levels (transcript, protein and capacity) in response to an osmotic shock applied to roots of cowpea (*Vigna unguiculata*). Two cultivars of *V. unguiculata* were used, Vita 3 and Vita 5, tolerant and sensitive to drought/saline stress respectively. The seedlings (17-day-old) were grown in hydroponic conditions and submitted to NaCl (100 and 200 mM) or 200.67 g L^{-1} PEG 6000 (iso-osmotic condition to 100 mM NaCl). The *VuAox1* and *VuAox2a* mRNA were not detected in either cultivar under all tested conditions while the *VuAox2b* gene was differently expressed. In the tolerant cultivar (Vita 3), the expression of *VuAox2b* gene was stimulated by an osmotic stress induced by PEG which was associated with a higher amount and capacity of the Aox protein. In the same cultivar, this gene was under-expressed in salt stress conditions with poor effect on the protein level. In the sensitive cultivar (Vita 5), the transcript level of the *VuAox2b* was unchanged in response to PEG treatment, even though the protein and the capacity tended to increase. Upon salt stress, the *VuAox2b* gene was over-expressed. At 100 mM NaCl, this *VuAox2b* gene over-expression led to a higher amount and capacity of Aox. This effect was reduced at 200 mM NaCl. Overall, these

Abbreviations: Aox, alternative oxidase; DTT, dithiothreitol; PEG, polyethylene glycol; PG, *n*-propylgallate; pyr, pyruvate; ROS, reactive oxygen species

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results suggest complex mechanisms (transcriptional, translational and post-translational) for Aox regulation in response to osmotic stress.

Introduction

Vigna unguiculata (Cowpea) is a leguminous plant known to offer good nutritional properties based on its seed protein content and to be both responsive to favourable growing conditions and one of the most widely adapted culture to drought, high temperatures, and other abiotic stress (Ehlers and Hall, 1997). In response to salinity or drought, different cultivars have been characterised according to their level of tolerance like Vita 3, considered as tolerant while Vita 5 is recognised as a sensitive cultivar (Fernandes de Melo et al., 1994).

High salinity and drought are common stress conditions that adversely affect plant growth and crop production (Xiong et al., 2002). In both cases, the ability of plants to take up water is reduced and this quickly causes reductions in growth rate, along with a cascade of metabolic changes (Munns, 2002). Various biochemical and physiological responses are induced in order to help the plant to survive (Seki et al., 2003). Among these changes, salinity and drought generate reactive oxygen species (ROS) including superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) (Hasegawa et al., 2000). ROS, products of altered chloroplast and mitochondrial metabolism during stress, cause oxidative damage to different cellular components including membrane lipids, proteins and nucleic acids (Haliwell and Gutteridge, 1986). The alleviation of this oxidative damage could provide enhanced plant resistance to salt stress (Apse and Blumwald, 2002). As it is well known, plant mitochondria possess a specific electron pathway, cyanide resistant, mediated by the alternative oxidase (Aox). This enzyme branches from the main respiratory chain at the level of ubiquinone. Its activity in vitro is dependent on substrate level, ubiquinone concentration as well as its redox poise, on redox state of Aox and α-keto acid level, mainly pyruvate (pyr) (Siedow and Umbach, 2000). Indeed, it would be expected that a higher concentration of Aox protein would result in a higher Aox activity. However, there is no direct correlation between Aox protein abundance and its activity or engagement in respiration (Juszczuk and Rychter, 2003). The role of Aox in non-thermogenic plants remains unclear but, in the last few years, it has been consistently attributed to lower mitochondrial ROS formation in plant cells (Purvis, 1997; Maxwell et al., 1999; Robson and Vanlerberghe, 2002).

In higher plants, a small Aox gene family exists. The multigene family encoding Aox consists of two discrete subfamilies: *Aox1*-type and *Aox2*-type genes (Whelan et al., 1996). In both groups, *Aox* genes are differentially expressed in response to environmental, developmental, and other cell signals (Finnegan et al., 1997; Saisho et al., 1997, 2001; McCabe et al., 1998; Considine et al., 2001; Saika et al., 2002). The functioning of Aox2-type proteins would be linked to developmental processes, while the Aox1-type proteins would be preferentially induced in stress conditions (Considine et al., 2002). However, very recently, the dramatic response of Aox2 to a specific subset of treatment conditions also indicated a role for Aox2 in stress responses (Clifton et al., 2005).

Previously, two *Aox2*-type genes were identified in *V. unguiculata* and these genes are orthologous to soybean Aox genes *2a* and *2b* (Costa et al., 2004). In this paper, the expression of the *Aox* genes, and the amount and capacity of Aox protein were studied in roots of Vita 3 and Vita 5 cultivars in relation to plant response towards unfavourable conditions (salt and PEG stress).

Materials and methods

Plant material and stress conditions

Vita 3 and Vita 5 cowpea seeds (*V. unguiculata* (L.) Walp) were obtained from the seed bank of the Departamento de Fitotecnia, Universidade Federal do Ceará. Fortaleza, Ceará, Brasil. Seeds were surface sterilised for 5 min in 0.5% (w/v) CaOCl, rinsed with water, and germinated in the dark, at 25 °C, on filter paper soaked with distilled water. After 3 days, the seedlings were transferred to hydroponics systems and transported to a controlled growth chamber with a light intensity of 200 $\mu E\,m^{-2}\,s^{-1}$ at leaf level, a 14 h photoperiod, temperatures of 24 °C (day) and 20 °C (night) and 70% relative humidity. The seedlings were grown in Knop medium (1.44 $g\,L^{-1}$ $Ca(NO_3)_2$, 0.25 $g\,L^{-1}$ KNO_3, 0.25 $g\,L^{-1}$ KH_2PO_4 and 0.246 $g\,L^{-1}$ $MgSO_4 \cdot 7H_2O$) with micronutrients (65.7 $mg\,L^{-1}$ FeEDTA,