INTRODUCTION

Grapevines are economically one of the most important fruit species worldwide. Thus, it is essential for winegrowers to guarantee fruit quality upon adverse climate conditions, including heavy rains before harvest that cause severe skin cracking and fruit spoilage. Calcium (Ca²⁺) is beneficial to the fruit integrity, and thus for quality, due to its key structural and signaling roles, acting as osmoticum within vacuoles, as strengthening agent in cell walls, and as secondary messenger for a large number of abiotic stress responses [1]. In fact, a close relationship has been demonstrated between increased tomato fruit integrity, increased Ca²⁺ levels and increased activity of CAX-type cation/H⁺ exchangers (CAXs), that appear to predominantly reside on the vacuole [2]. Therefore, the identification and characterization of grapevine CAX transporters is a landmark towards understanding calcium dynamics in the grape berry.

RESULTS

Phylogenetic and topological analysis of VvCAX3
Prospection of the grapevine genome allowed the identification of VvCAX3, a putative calcium-H⁺ antiporter belonging to the family of cation-H⁺ exchangers (Fig. 1), with a well defined topological structure (Fig. 2).

![Diagram of VvCAX3](image)

Fig.1 – Phylogenetic relationship between VvCAX3 and other CAX proteins from Arabidopsis thaliana (AtCAX1, AtCAX2, AtCAX4, AtCAX5, AtCAX6), Oryza sativa (Os CAX1a, OsCAX1b, OsCAX2, OsCAX3), Oryza sativa (ScVvCAX1) and Vigna radiata (VCAX).

![Diagram of VvCAX3](image)

Fig.2 – Proposed topological model of plant CAX proteins (Adapted from [3]).

Considering the predicted topological model, two forms of VvCAX3 were cloned: the whole gene (VvCAX3) and a truncated form without the codons encoding the autoinhibitory domain (VvCAX3-2).

Functional complementation of a yeast mutant for Ca²⁺ transport

The functional characterization of VvCAX3 was performed following heterologous expression on a yeast strain characterized for its high sensitivity to Ca²⁺ (Fig. 3, Fig. 4).

![Yeast complementation assay](image)

Fig.3 – Complementation assays of S. cerevisiae K667 strain by VvCAX3 and the truncated VvCAX3. Cells were transformed with the vector pVS214 alone (empty vector) or with the same vector carrying VvCAX3 or VvCAX3-2. Wild-type strain (WT, W303) was used as positive control. Several fold dilutions were plated as drops in YPD medium supplemented with CaCl₂ (0, 100, 200 mM) and growth was analyzed after 2 days.

CONCLUDING REMARKS

- VvCAX3 lacking the CAX autoinhibitory domain was able to restore the growth defect of the yeast strain K667 at high Ca²⁺ levels, validating the role of the protein in Ca²⁺ transport.
- Likewise, VvCAX3 restored the growth defect of the yeast strain at high Mn²⁺ levels, but increased its sensitivity for Na⁺, Li⁺ and Cu⁺, suggesting its additional involvement in the transport of these cations.
- VvCAX3 transcripts were detected in all grapevine organs, and expression decreased gradually during grape berry development, in accordance to the pattern of calcium accumulation in the fruit.
- VvCAX3 expression was upregulated by Ca²⁺ and Na⁺, further supporting its involvement in the homeostasis of calcium and other cations in grapevine.