Functional characterization of VvCAX3: a grapevine cation/H^+ exchanger that transports Ca^{2+} and other cations

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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 (Ca^{2+}) 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 Ca^{2+} 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 topologic 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**).

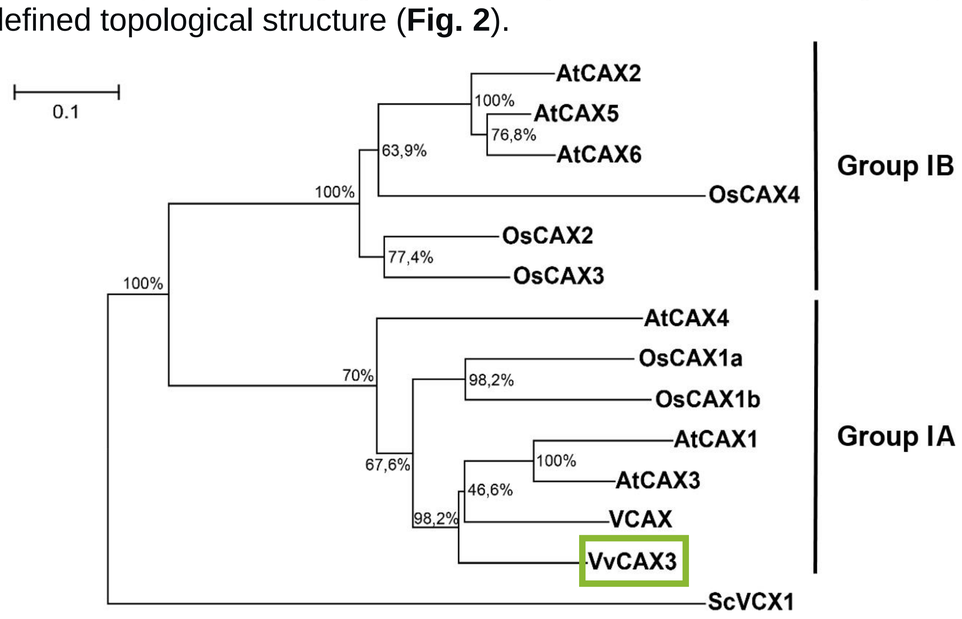


Fig.1 – Phylogenetic relationship between VvCAX3 and other CAX proteins from *Arabidopis thaliana* (AtCAX1, AtCAX2, AtCAX3, AtCAX4, AtCAX5, AtCAX6), *Oryza sativa* (Os CAX1a, OsCAX1b, OsCAX2, OsCAX3, OsCAX4), *Saccharomyces cerevisiae* (ScVCX1) and *Vigna radiata* (VCAX).

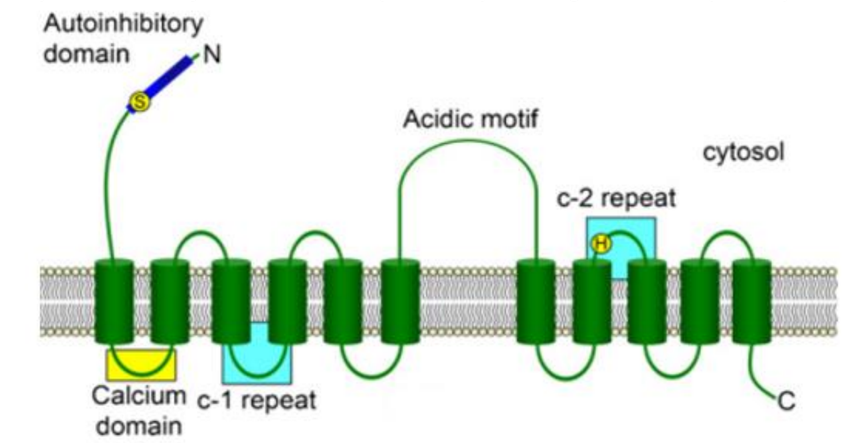


Fig.2 –Proposed topological model of plant CAX proteins (Adapted from [3]).

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** (***VvsCAX3***).

Functional complementation of a yeast mutant for Ca^{2+} transport

The functional characterization of VvCAX3 was performed following heterologous expression on a yeast strain characterized for its high sensitivity to Ca^{2+} (**Fig. 3, Fig. 4**).

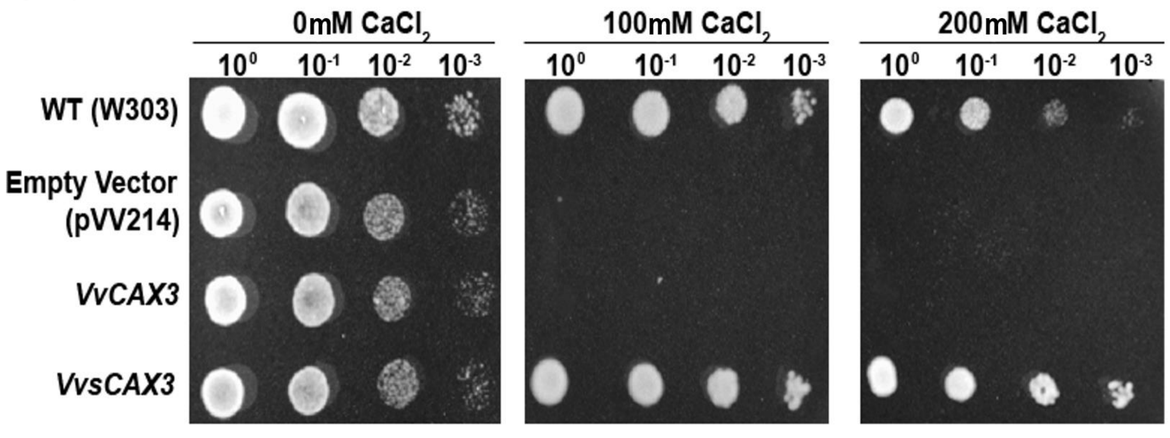


Fig.3 – Complementation assays of *S. cerevisiae* K667 strain by *VvCAX3* and the truncated *VvsCAX3*. Cells were transformed with the vector pVV214 alone (empty vector) or with the same vector carrying *VvCAX3* or *VvsCAX3*. Wild-type strain (WT; W303) was used as positive control. Several fold dilutions were platted as drops in YPD medium supplemented with $CaCl_2$ (0, 100, 200 mM), and growth was analyzed after 2 days.

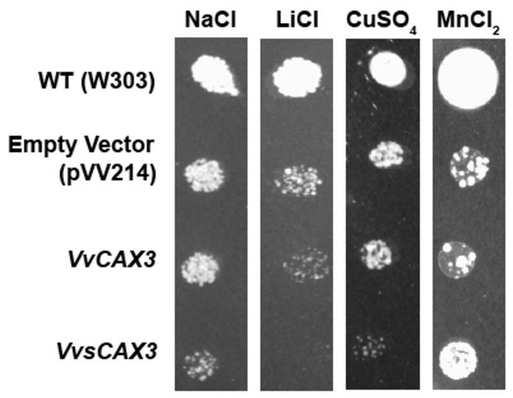
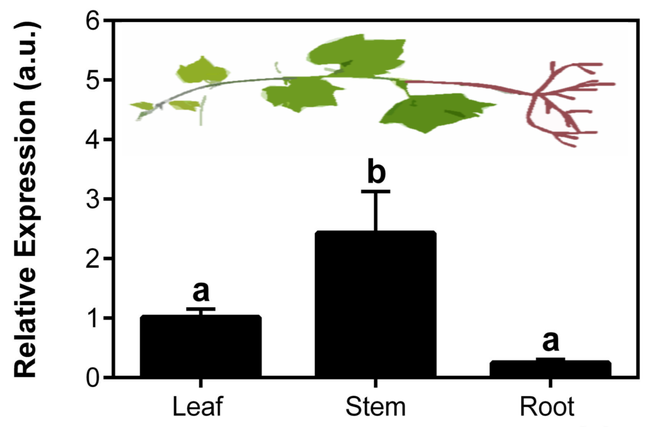


Fig. 4 – Growth assays of *S. cerevisiae* K667 strain carrying *VvCAX3*, the truncated *VvsCAX3*, or the empty vector. Wild-type strain (WT; W303) was used as positive control. Cells were platted as drops in YPD medium supplemented with NaCl (500 mM), LiCl (50 mM), $CuSO_4$ (6 mM) and $MnCl_2$ (750 µM), for 2 days.

Expression of VvCAX3 in grapevine

To assess the involvement of VvCAX3 in grapevine calcium dynamics, its expression was studied by real-time PCR in different plant organs, berry developmental stages and upon elicitation with specific compounds.

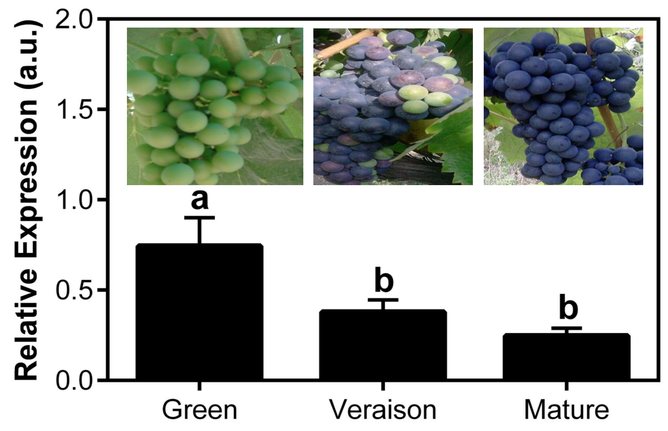


The highest expression of *VvCAX3* was detected in the grapevine **stems** followed by the leaves and the roots (**Fig. 5**).

Fig.5 - Expression of *VvCAX3* by real-time PCR in grapevine organs cv. “Trincadeira”.

Transcript levels were higher in the **green stage** of berry development, followed by veraison and mature stages (**Fig. 6**), consistent with the pattern of calcium accumulation in fruits [4].

Fig.6 - Expression of *VvCAX3* throughout grape berry development cv. “Vinhão”, by real-time PCR.



VvCAX3 expression increased in grape cell suspensions (CSB) upon elicitation with Ca^{2+} (100 mM), Na^+ (100 mM) and methyl jasmonate (Meja, 20 µM) (**Fig. 7**). In contrast, Mn^{2+} (5 mM) and sucrose (150mM) caused an apparent reduction in transcript levels.

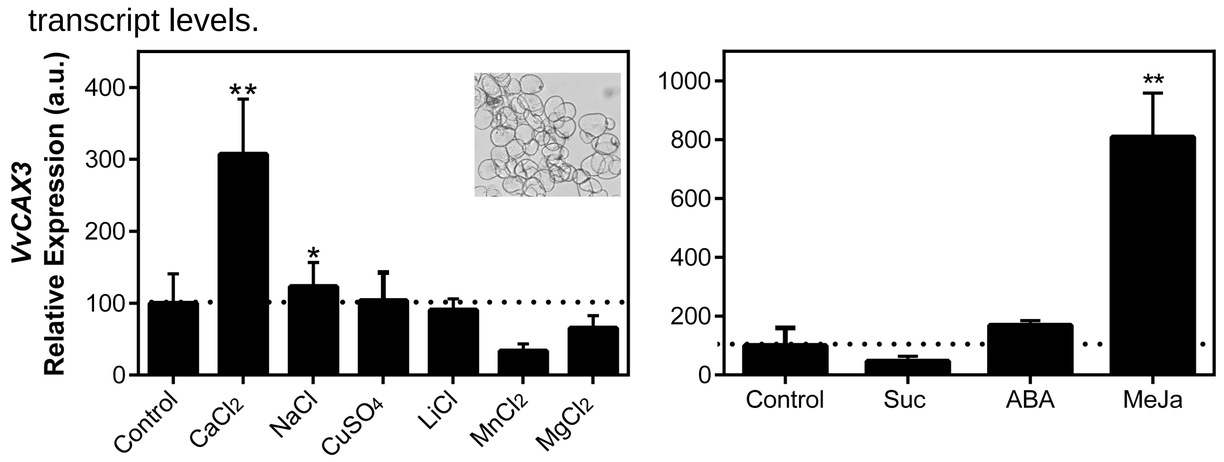


Fig.7- Expression of *VvCAX3* in grape cell suspensions (CSB – Cabernet Sauvignon Berry), by real-time PCR. Cells were elicitated for 12 hours with: 100 mM $CaCl_2$, 100 mM NaCl, 150 µM $CuCl_2$, 75 mM LiCl, 5 mM $MnCl_2$, 100 mM $MgCl_2$, 150 mM sucrose, 20 µM abscisic acid (ABA) and 20 µM methyl jasmonate (MeJa).

CONCLUDING REMARKS

- VvsCAX3 lacking the CAX autoinhibitory domain was able to restore the growth defect of the yeast strain K667 at high Ca^{2+} levels, validating the role of the protein in Ca^{2+} transport.
- Likewise, VvsCAX3 restored the growth defect of the yeast strain at high Mn^{2+} levels, but increased its sensitivity for Na^+, Li^+ and Cu^{2+}, 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 Ca^{2+} and Na^+, further supporting its involvement in the homeostasis of calcium and other cations in grapevine.

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