



RESEARCH PAPER

Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation

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Received 7 April 2006; Accepted 20 June 2006

Abstract

Potassium accumulation is essential for grapevine (*Vitis vinifera* L.) growth and development, but excessive levels in berries at harvest may reduce wine quality particularly for red wines. In addition to decreasing the free acid levels, potassium also combines with tartaric acid to form largely insoluble potassium bitartrate. This precipitates during winemaking and storage, resulting in an increase in wine pH that is associated with negative impacts on wine colour, flavour, and microbiological stability. For these reasons, a better understanding of potassium transport and accumulation within the vine and berries is important for producing fruit with improved winemaking characteristics. Here two genes encoding KUP/KT/HAK-type potassium transporters that are expressed in grape berries are described. Their function as potassium transporters was demonstrated by complementation of an *Escherichia coli* mutant. The two transporters are expressed most highly in the berry skin during the first phase of berry development (pre-veraison), with similar patterns in two grapevine varieties. The timing and location of expression of these transporters are consistent with an involvement in potassium accumulation in grape berries.

Key words: Berries, grapevine, potassium transporters, *Vitis vinifera*.

Introduction

Potassium is an essential macronutrient for plant growth and development. It is often the most abundant cation in plant tissues. Potassium plays key roles in the osmoregulation of cells and has a dominant effect on the membrane potential of the cell which to a large extent determines the uptake of many different cations, anions, and sugars. In grape berries, potassium is the most abundant cation where it contributes to charge balance and may be involved in sugar transport (Lang, 1983). Considering the importance of berry potassium accumulation for wine quality, little is known about how berry potassium content changes over the developmental period or how potassium is accumulated in berries.

Understanding the dynamics and mechanisms of grape berry potassium accumulation is important because elevated levels of berry potassium can have a negative effect on wine quality by increasing berry and wine pH (Gawel *et al.*, 2000). Potassium reduces acid levels in berries and musts, and interacts with tartaric acid to form potassium bitartrate which has limited solubility. The precipitation of potassium bitartrate during winemaking significantly lowers tartaric acid levels, resulting in an increase in wine pH. Elevated pH has deleterious effects on wine quality due to a decrease in colour stability, greater susceptibility to microbiological spoilage, and potentially less satisfactory sensory characters (Sommers, 1975, 1977). Many wine grapes in Australia are grown in warm irrigated regions with high soil potassium, which favours the accumulation of elevated levels of potassium (Mpelasoka *et al.*, 2003). A solution to the problem of higher pH is to add tartaric acid during

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Abbreviations: EST, expressed sequence tag; IPTG, isopropyl- β -D-thiogalactopyranoside; ORF, open reading frame; wpf, weeks post-flowering.

winemaking to lower must and wine pH. This process is costly and may create technical complications during winemaking. The potassium transporters present in grape berries are being studied in order to understand better the mechanism of potassium transport and storage. A detailed understanding of this process is required to develop strategies for reducing the levels of potassium in berries, thus improving their suitability for winemaking and reducing the need for expensive additions of exogenous tartaric acid.

In plants, there are multiple mechanisms for potassium transport into cells. Potassium channels mediate passive low affinity potassium transport across plant cell membranes, whereas potassium carriers mediate energized high and low affinity uptake. The potassium channels in plants have been well described (Very and Sentenac, 2003). One potassium channel was recently characterized in grape berries where it was shown to be expressed pre-veraison in the pericarp (Pratelli *et al.*, 2002). In addition to potassium transport mediated by channels, there are other transporters such as the KUP/KT/HAKs which have not been well characterized in grapes. The KUP/KT/HAKs are similar to a family of potassium transporters found in *Neurospora crassa* and other fungi which function as proton/potassium symporters. These transporters are variable in function, with some being high and others being low affinity. In *Arabidopsis* and cotton, these transporters are involved in cell elongation (Rigas *et al.*, 2001; Ruan *et al.*, 2001; Elumalai *et al.*, 2002) and also high affinity potassium uptake. The biophysical function of these transporters in plant cell membranes is unknown, but they are likely to function as proton-energized transporters of potassium. These energized transporter systems could be important for the accumulation of potassium in cells where the gradient may not be favourable to passive potassium accumulation mediated by channels.

During the course of berry development, potassium may play different roles depending on developmental stage. In pre-veraison berries, cells divide and expand, during which time potassium may play an important role as an osmoticum. After veraison (a word used by viticulturists to describe the initiation of ripening which is defined in this study as the last time sample prior to an increase in soluble solids content), grape berries continue to enlarge, but presumably the cell expansion during this phase of development is driven by the increase in sugar in the cell vacuole and potassium may play a secondary role in the accumulation of sugars. To investigate the role of the KUP/KT/HAK potassium transporters in grape berry development, two cDNAs encoding putative potassium transporters belonging to this family from grape berries were isolated. Their function was tested by complementation studies in an *Escherichia coli* mutant deficient in potassium uptake, and their transcript levels were studied in relation to berry potassium accumulation.

Materials and methods

Grapevine sampling

For northern analysis, flowers (at the 50% cap fall stage), berries [from 2 weeks post-flowering (wpf) to 18 wpf], leaves at two stages of maturity (unexpanded and partly expanded), green canes, and seeds (3 wpf) from *Vitis vinifera* L. cv. Shiraz were collected from John Harvey's Slate Creek vineyard (Willunga, South Australia) in the 1996/1997 season. Berries were immediately deseeded and all tissues were frozen in liquid nitrogen and stored at -80°C until required. Shiraz root tips were obtained from canes that had been rooted in perlite. Berry soluble solids ($^{\circ}\text{Brix}$) were measured with a refractometer (model 10430; Reichert, Vienna, Austria). Frozen berries (6 wpf) were peeled and the flesh separated to provide the 'flesh' and 'peel' samples.

For real-time polymerase chain reaction (PCR) analysis and K elemental analysis, flowers (at the 50% cap fall stage) and berries (from 2 to 16 wpf, at fortnightly intervals) of *V. vinifera* L. cv. Cabernet Sauvignon were collected from John Harvey's Slate Creek vineyard in the 2003/2004 season. Biological duplicates were collected by collecting berries from adjacent rows.

RNA/DNA extraction and cDNA production

Total RNA was extracted from grapevine tissues as described by Davies and Robinson (1996). For real-time PCR analysis, total RNA was further purified using a Qiagen (Valencia, CA, USA) RNeasy column including a DNase digestion step as described by the manufacturer. First-strand cDNA for real-time PCR was made with Superscript III enzyme (Invitrogen, Carlsbad, CA, USA) by using 1 μg of purified total RNA in a reaction volume of 20 μl as described by the manufacturer with the (dT)₁₇-adaptor of Frohman *et al.* (1988). Following synthesis, the cDNA was treated with RNase H as described by the manufacturer.

DNA for Southern blot analysis was extracted from unexpanded Shiraz leaves as described by Thomas *et al.* (1993).

Analysis of potassium levels in Cabernet Sauvignon berry series

Berries that had been collected were snap-frozen, then ground to a powder under liquid nitrogen and analysed for potassium content essentially as described by Zarcinas *et al.* (1983). Approximately 0.5 g of powdered grape berry was mixed with 5 ml of concentrated nitric acid. The slurry was then heated to 140 $^{\circ}\text{C}$ with stepwise increases over 8 h. The temperature was maintained until the sample boiled down under reflux to a volume of 1 ml. The sample was allowed to cool and the residue diluted to 20 ml with water. After mixing, the sample was filtered and the solution analysed for macro- and micronutrient content by inductively coupled plasma optical emission spectroscopy (Spectro Modula).

Isolation of grapevine potassium transporter cDNAs

Two potassium transporters were isolated from distinct sources. *VvKUP1* was isolated as an expressed sequence tag (EST; accession number CF403902) from a cDNA library prepared from pre-veraison, deseeded Cabernet Sauvignon berries at development stage 32 (as defined by Coombe, 1995). The clone contained the entire open reading frame (ORF) and was fully sequenced using synthetic oligonucleotide primers and ABI BigDye technology (Applied Biosystems, Foster City, CA, USA).

A fragment containing the central portion of *VvKUP2* was isolated from 10 wpf Shiraz berry cDNA by nested PCR using redundant primers designed to the conserved regions of aligned KUP proteins. The remainder of the cDNA was obtained by 3' and then 5' rapid amplification of cDNA ends (RACE). The reconstructed clone

contained the entire ORF. For expression analysis, an entire clone was generated from 4 wpf Cabernet Sauvignon cDNA by PCR using synthetic oligonucleotide primers designed to begin and end at the start and stop codons of the ORF (forward primer, ATGGATCCTGACCATGGGAG; reverse primer, TCAGACGATATAAACCATGCCAA). The *VvKUP2* cDNA was cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) for DNA sequencing prior to subcloning into the vector pPAB404, for expression analysis in *E. coli* (see below).

Sequence analysis

Sequence analyses were carried out with software programs in the GCG Wisconsin Package Version 8 (Devereux *et al.*, 1984). Database searches were done through NCBI using the BLAST options (Altschul *et al.*, 1990). Multiple sequence alignment was done using ClustalW (Thompson *et al.*, 1994). A distance matrix was calculated by Prodist (Felsenstein, 1989) and the unrooted phylogenetic tree was produced using Neigbor (Felsenstein, 1989).

Southern and northern blot analysis

Southern blot analyses were carried out as described by Davies and Robinson (1996) except that 10 µg of genomic DNA was digested to completion with the restriction endonuclease enzymes listed in the figure legends. Probes were prepared by random priming on isolated cDNA fragments using a GigaPrime kit (Geneworks, Adelaide, Australia). The cDNA fragments used as probes for *VvKUP1* and *VvKUP2* were from the 3' ends of the cDNA clones and were 605 and 1563 bp, respectively.

Northern blot analyses using the same probes as used for the Southern blot analysis were carried out as described by Davies and Robinson (1996) except that 2.8 µg of total RNA was run in each lane of the gel.

Analysis of gene expression by real-time PCR

A template for a standard curve for the *VvKUP1* gene was created via PCR by producing a 169 bp fragment from the 3' region of the cloned and sequenced cDNA grapevine clone using the following primers: *VvKUP1*-For, TGAGCTTTGAAACATGGGAAGACT; and *VvKUP1*-Rev, TTCTTGTTACCAAGCCTTCCGG.

The melt curves for the products of these assays produced a single peak, indicating that a single species had been amplified. The products were also checked on an agarose gel. A dilution series of the purified fragment was used to create a standard curve for the estimation of gene expression in the test samples. To normalize the level of cDNA in each real-time PCR, each cDNA reaction was completed under the same conditions and ubiquitin primers were used to produce an internal standard during the real-time reactions. The primers made to a grape ubiquitin homologue (GenBank accession no. CA808925) were: *Ubi*-fwd (5'-AGTAGATGACTGGATTGGA GGT-3') and *Ubi*-rev (5'-GAGTATCAAAACAAAAGCATCG-3').

The reactions (done in triplicate) contained cDNA or purified fragment at the appropriate level, ×1 SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), and forward and reverse primers at a final concentration of 0.28 µM. The cycles were as follows: 95 °C, 8 min; 40 cycles of step 1, 95 °C, 30 s; step 2, 58 °C, 30 s; step 3, 72 °C, 30 s. Melt (50–96 °C), held 45 s on the first step and 5 s on subsequent steps. The reactions were incubated in a Corbett RotorGene RG-3000 cyclor and the data analysed with RotorGene V6 software.

Expression of the *VvKUP2* gene was also analysed by real-time PCR as described above. The primers used: *VvKUP2*-For, ATGC TTCCTGCCATTTCCACATA; and *VvKUP2*-Rev, GGTTGGCA TGGTTATATCGTCTG produced a 201 bp fragment.

Functional analysis of grapevine putative potassium transporters

The *E. coli* strain TK2420 deficient in the three potassium uptake systems (Kdp⁻, Kup⁻, Trk⁻) was used for complementation studies (Epstein *et al.*, 1993). Both *VvKUPs* were cloned into pPAB404 containing an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter (Buurman *et al.*, 1995) and then transformed into the TK2420 strain. Each *E. coli* strain containing a *VvKUP* cDNA and the strain containing a plasmid lacking an insert were grown in KML media (10 g l⁻¹ tryptone, 5 g l⁻¹ yeast extract, 10 g l⁻¹ KCl) containing 100 µg ml⁻¹ ampicillin. Cells were harvested when the OD₆₀₀ reached 1.0 and were washed four times with deionized water. For complementation tests, 10 µl of cells were resuspended in water and placed on minimal media plates (Senn *et al.*, 2001) (5 mM phosphoric acid, 0.4 mM MgSO₄, 6 µM FeSO₄, 1 mM citric acid, 1 mg l⁻¹ thiamine, 0.2% glycerol, 8 mM asparagine, 20 µM CaCl₂, and 1.5% Bactoagar) with 0.5 and 30 mM KCl. Arginine base was used to neutralize the medium to pH 7.5. Cells were grown at 37 °C for 16 h on the 5.0 mM KCl plates and for 72 h on the 0.1 mM KCl plates.

Results

Sequence comparisons

The ORFs of the two grapevine potassium transporters were of similar size, *VvKUP1* (GenBank accession no. DQ465409), 773 amino acids; *VvKUP2* (GenBank accession no. DQ465410), 793 amino acids. The sequence of the predicted proteins showed that they are both likely to be potassium transporters of the KUP/KT/HAK family but that they were distinctly different from each other, being only 45.8% identical at the amino acid level [70.6% similar as calculated by the BESTFIT program (Devereux *et al.*, 1984)]. The nearest BlastP match to the *VvKUP1* sequence was to a KUP/KT/HAK-type potassium transporter (At4g13420) from *Arabidopsis* which was 56.4% identical. The nearest match to the *VvKUP2* sequence was with a KUP/KT/HAK-type potassium transporter (AAK53759) from the common ice plant, *Mesembryanthemum crystallinum*, which was 81.8% identical. An unrooted phylogenetic tree containing all the known KUP/KT/HAK-type potassium transporters (13 in total) from *Arabidopsis*, the transporter from *M. crystallinum*, and the two putative grape transporters is shown in Fig. 1. From this, it seems that *VvKUP1* is most closely related to the AtHAK5 (At4g13420) transporter. The function of AtHAK5 has been demonstrated by mutant complementation in *E. coli* (Ahn *et al.*, 2004), yeast (Rubio *et al.*, 2000), and *in planta* (Gierth *et al.*, 2005). *VvKUP2* is most closely related to McHAK2 (AAK53759.1) whose function was demonstrated in yeast (Su *et al.*, 2002). *VvKUP2* is also closely related to AtKUP2 (At2g40540) which has also been shown to function as a potassium transporter by complementation studies in *E. coli* (Kim *et al.*, 1998) and yeast (Quintero and Blatt, 1997). Given the close sequence similarity of the above clones to genes with demonstrated potassium transporter function, it is likely that the grape homologues also function as potassium transporters.

Southern blot analysis of potassium transporters

Results of the Southern blot analysis (Fig. 2) suggest that there may be only a single gene for each of the sequences in the grape genome. There are multiple bands in the *Bam*HI and *Hind*III lanes probed with the *VvKUP2* clone, but the fragment used as a probe contains a *Hind*III site and two *Bam*HI sites (adjacent to each other) which accounts for the two bands in the *Bam*HI lane at least. Given that families of KUP/KT/HAK-type transporters are present in other species, it might be expected that there are other members of the family in grapevine but these may not be close enough to be recognized by the probes used in this study under the described hybridization conditions.

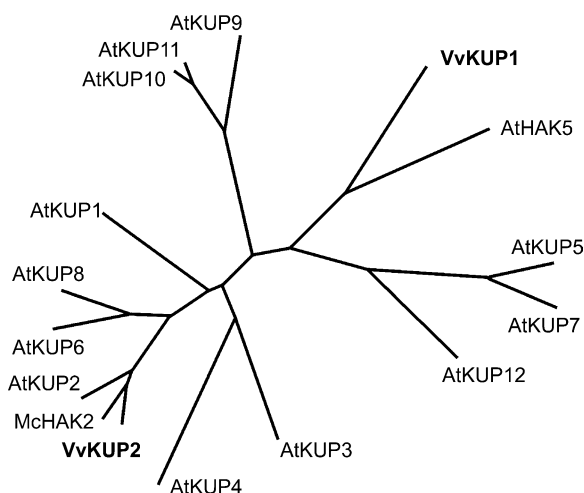


Fig. 1. Unrooted phylogenetic tree showing the relationship between the two grape transporter proteins (*VvKUP1* and *VvKUP2*), a transporter from *M. crystallinum* (*McHAK2*, AAK53759.1), and the 13 KUP/KT/HAK-like transporter proteins from *Arabidopsis* (*AtKUP1*, NP_180568.1; *AtKUP2*, NP_565936.1; *AtKUP3*, NP_186854.1; *AtKUP4*, NP_194095.2; *AtKUP5*, NP_195079.2; *AtKUP6*, NP_177187.2; *AtKUP7*, NP_568213.2; *AtKUP8*, NP_196992.1; *AtKUP9*, NP_193729.1; *AtKUP10*, NP_174397.1; *AtKUP11*, NP_181051.1; *AtKUP12*, NP_176222.2, *AtHAK5*, NP_567404.1).

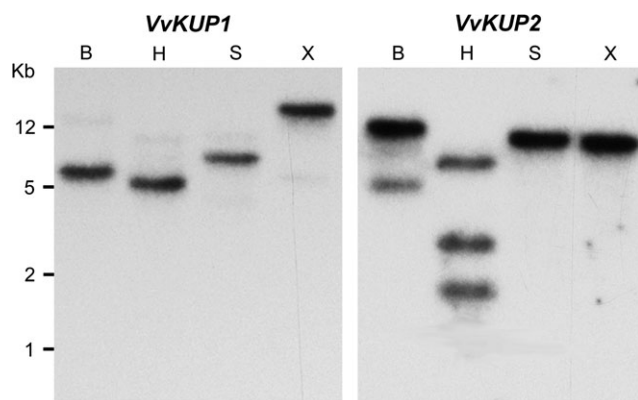


Fig. 2. Southern blot analysis of *Vitis vinifera* cv. Shiraz genomic DNA probed with radiolabelled *VvKUP1* and *VvKUP2* cDNA fragments. The endonucleases used for digestion were *Bam*HI (B), *Hind*III (H), *Sal*I (S), and *Xba*I (X).

Functional analysis of putative grape potassium transporters

To determine the function of these putative transporters, an *E. coli* mutant that was deficient in potassium uptake was complemented with the grapevine cDNAs. The two grapevine KUP/KT/HAK transporters were transformed into the mutant and plated on medium with low (0.1 mM) and high (5.0 mM) potassium. On plates containing low potassium and IPTG, the *E. coli* cells containing the grapevine KUP/KT/HAK cDNAs grew but the cells containing the empty vector did not grow (Fig. 3). This indicates that the cDNAs were able to complement the defect in potassium uptake in this mutant and therefore function as potassium transporters. On low potassium plates without IPTG, none of the strains grew, which shows that the induction of the expression of these genes in *E. coli* is required for complementation (Fig. 3). All strains grew on plates containing high levels of potassium (Fig. 3).

Potassium levels in developing grape berries

The berry weight and Brix data for the 2003/2004 Cabernet Sauvignon grape berry series show a typical pattern of berry development, i.e. two phases of rapid berry growth separated by a lag phase during which the berry weight remained roughly constant from 7 to 9 wpf (Fig. 4A). Veraison occurred at 9 wpf as indicated by the increase in

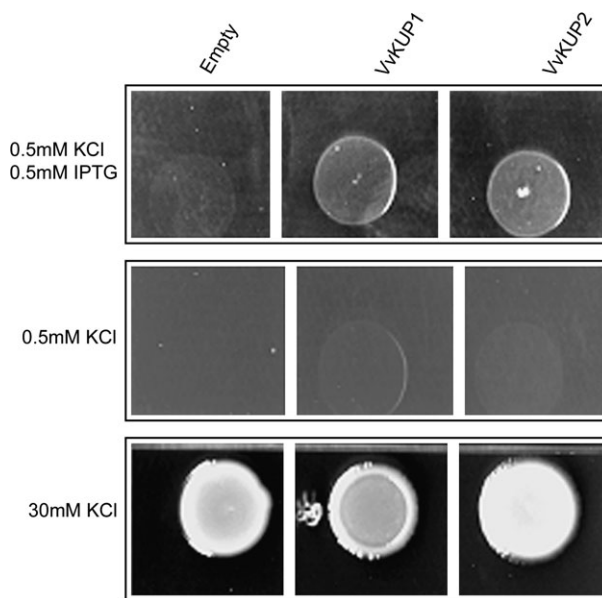


Fig. 3. Complementation of a potassium transport-deficient *E. coli* mutant (strain TK2420, Epstein *et al.*, 1993) by expression of *VvKUP1* and *VvKUP2* cDNAs cloned into the IPTG-inducible vector pPAB404 (Buurman *et al.*, 1995). Empty=the empty vector with no cDNA insert; plates in the upper panel were supplemented with a low level of potassium and expression from the vector was induced with IPTG. The middle panel medium was supplemented with low levels of potassium only and the lower panel shows growth on medium supplemented with high levels of potassium.

berry weight and Brix from that point. The concentration of potassium decreases from a maximum in 1-week-old berries until it reaches the minimum level observed at 5 wpf (Fig. 4B). Sometime between 5 and 7 wpf, the potassium concentration begins to increase and continues to increase steadily until late in development when it plateaus. This increase in potassium concentration is timed such that it begins 2 weeks (at least) prior to veraison and may coincide with the beginning of the lag phase (compare Fig. 4A, B).

When plotted on a per berry basis, the amount of K^+ per berry appears to increase in a biphasic manner until it plateaus sometime after 13 wpf when the fruit is well on the way to commercial ripeness (Fig. 4B). The phase from 9 to 13 weeks encompasses a period when the rate of increase of K^+ per berry is higher than during the early phase from 3 to 7 weeks.

Potassium transporter gene expression during grape berry development

The expression of mRNAs corresponding to the *VvKUP1* and *VvKUP2* potassium transporters was assayed in two different cultivars. Fruit of the cultivar Shiraz showed the

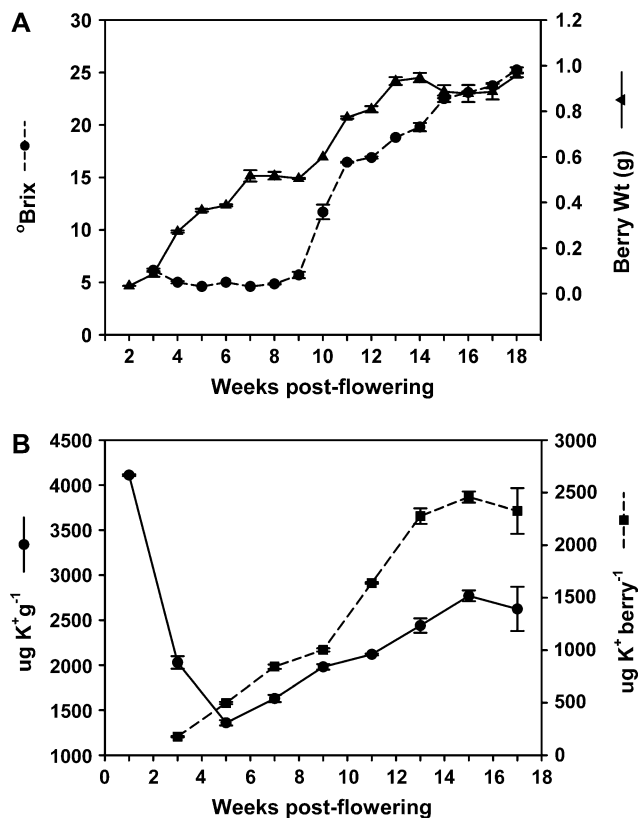


Fig. 4. Potassium levels during *Vitis vinifera* cv. Cabernet Sauvignon berry development during the 2003/2004 growing season. The bars represent the standard errors. (A) Berry development showing berry weight increase and the accumulation of total soluble solids (Brix). Veraison was at 9 wpf. (B) Accumulation of potassium plotted on a per g and a per berry basis.

expected biphasic pattern of development for the 1996/1997 season (Fig. 5A), and northern blot analysis of this developmental series showed that both transporters were expressed most highly in young berries and flowers (Fig. 5B). The *VvKUP2* transporter was also expressed in the seed and to a much lesser extent in canes (Fig. 5B). In 6 wpf berries, both genes were more highly expressed in skin than flesh.

Similar patterns of expression were found in Cabernet Sauvignon berries (2003/2004 season) using real-time PCR (Fig. 6). The expression of the *VvKUP1* gene in particular is elevated, prior to veraison, at the beginning of the lag phase (Fig. 6A). The expression of both genes throughout berry development is largely consistent with the accumulation of potassium throughout most of berry development (compare Figs 4B and 6).

Discussion

While potassium is essential for grapevine and grape berry development, excess accumulation may reduce the quality of fruit destined for wine production. Potassium levels in grape berries may be affected by a number of factors

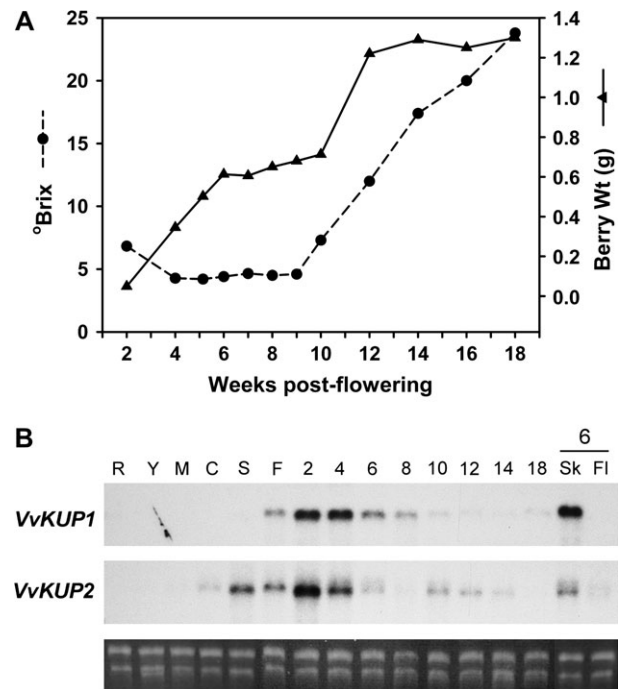


Fig. 5. *VvKUP1* and *VvKUP2* gene expression during *Vitis vinifera* cv. Shiraz berry development during the 1996/1997 growing season. (A) Development showing berry weight increase and the accumulation of total soluble solids. Veraison was at 9 wpf. The bars represent the standard errors. (B) Northern blot analysis of *VvKUP1* and *VvKUP2* transcript levels. R, root; Y, young leaves; M, medium-sized leaf; C, green cane; S, seed, F, flower; Sk, skin at 6 wpf; Fl, flesh at 6 wpf. The other tracks contain a berry series from 2 wpf through to 18 wpf. The lower panel shows RNA loading levels as visualized by ethidium bromide staining.

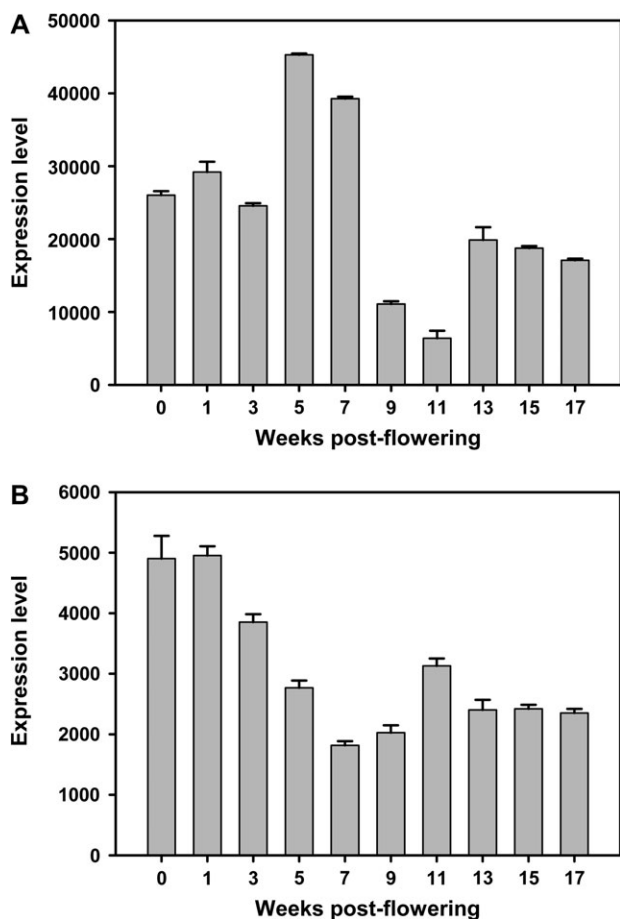


Fig. 6. *VvKUP1* and *VvKUP2* gene expression during *Vitis vinifera* cv. Cabernet Sauvignon berry development during the 2003/2004 growing season as determined by real-time PCR analysis. The bars represent the standard errors. (A) *VvKUP1*. (B) *VvKUP2*.

including the level of potassium in the soil, the variety (and rootstock), and viticultural practices (Mpelasoka *et al.*, 2003). However, little is known about how potassium is taken up from the soil, transported within the vine, and accumulated within the berry. Knowledge of these processes is crucial for developing strategies to reduce grape berry potassium accumulation and thereby improving fruit and wine pH. To understand better how potassium is accumulated in grape berries, two potassium transporters from the KUP/KT/HAK family (Maser, 2001) *VvKUP1* and *VvKUP2* were isolated from *V. vinifera* berries. Two lines of evidence indicate that they are involved in potassium transport. First, they both share sequence homology with other proteins previously shown to transport potassium (Santa-Maria *et al.*, 1997; Fu and Luan, 1998; Kim *et al.*, 1998; Rubio *et al.*, 2000; Elumalai *et al.*, 2002) and, secondly, both complement an *E. coli* mutant deficient in potassium transport.

The simple banding pattern in the Southern blot assays (Fig. 2) showed that the two *VvKUP* transporters described here are likely to be single genes, or at least to be of low

copy number. As *Arabidopsis* has at least 13 transporters of this type (Fig. 1), it is expected that the grapevine genes also belong to a multigene family. The other members of this multigene family must be sufficiently divergent from *VvKUP1* and *VvKUP2* not to be detectable by Southern blot analysis under the conditions used. Indeed a search of the grape ESTs (<http://www.ncbi.nih.gov/Database/>) indicates that there are other putative *VvKUP* transporters whose expression and function are yet to be investigated. Other types of potassium transporters have also been shown to be expressed in grape berries. A Shaker-type potassium channel has been cloned from grapevine (Pratelli *et al.*, 2002). It is expressed at low levels in a range of tissues including pre-veraison berries. These authors showed that this channel is expressed in stomatal guard cells pre-veraison and may play a role in the control of transpiration and water movement in berries.

Both *VvKUP1* and *VvKUP2* were most highly expressed in reproductive tissues (berries, flowers, and seeds) in Shiraz, and indeed were predominantly expressed in berry skins (Fig. 5B). Low levels of *VvKUP2* but not *VvKUP1* transcripts were detected in green canes of Shiraz (Fig. 5B). This indicates that the promoters of these two genes are likely to contain elements that direct expression to the same tissues and have regulatory features that are involved in directing expression to the berry. In grape berries, the skins contain as much as four times higher concentrations of potassium as compared with the flesh (Coombe, 1987; Storey, 1987). Accumulation of potassium in skin cells may require specialized molecular mechanisms of potassium transport and it is possible that the *VvKUP* transporters that are expressed primarily in the skin may function in facilitating potassium accumulation. Interestingly, some of the proteins most structurally related to *VvKUP2* (Fig. 1), including *AtKUP2*, have been shown to be most highly expressed in siliques (Ahn, 2004) which might be considered analogous in some ways to the grapevine berry. The similarities in structure and expression between transporters may suggest that an evolutionarily distinct group of potassium transporters has a particular role in fruit development. Overall, little is known about how fruits accumulate potassium which is essential for development and also an important factor in human nutrition.

The patterns of expression of the two genes were broadly similar in the berry series of the two cultivars tested (Figs 5B, 6). Some differences might be expected due to variation between cultivars and due to seasonal effects. Expression of these potassium transporters was generally highest pre-veraison. Higher levels of expression pre-veraison were also found with a grapevine potassium channel gene (Pratelli, 2002). The higher transcript abundance in berries pre-veraison suggests that these *VvKUP* transporters play a role, either in uptake into the berries or in compartmentation of potassium into the skin cells during this period. The decrease in concentration ($\mu\text{g K g}^{-1}$ berry) during the

period from 1 to 5 wpf was most likely due to rapid berry expansion with little initial potassium uptake. The amount of potassium per berry steadily increases until late in ripening (Fig. 4B). Based on data presented in Fig. 4B and other work (Mpelasoka, 2003), there appears to be a close relationship between berry growth, potassium uptake, and the timing of veraison. In the Cabernet Sauvignon fruit assayed, there was a linear increase of potassium on a concentration basis between 5 and 15 wpf (Fig. 4B). Potassium levels and the amount per berry plateau when growth ceases (Fig. 4B). As has been shown, the concentration of potassium varies with berry age but the distribution between the various tissues and cell types may also vary as development proceeds. Based on the expression levels of these two *VvKUP* transporter genes and the accumulation of potassium per berry, it appears that these two transporters are involved in uptake pre-veraison. The transcript levels of both transporters are lower post-veraison than pre-veraison (Figs 5B, 6), but significant levels of expression were detected. These transporters may therefore continue to contribute to potassium homeostasis throughout berry development. Other mechanisms may also be involved post-veraison. If berries are primarily fed by the phloem after veraison (Tyerman *et al.*, 2004), then it is likely that a mechanism largely involved in phloem unloading such as a potassium channel (Deeken, 2002) would be the important mechanism for potassium loading into berries post-veraison.

Although the data presented herein strongly indicate that the two genes described encode proteins that function in the grape berry as potassium transporters, their role during grape berry development is not clear. There are a number of possible roles for potassium during berry development (Mpelasoka *et al.*, 2003). In many tissues, potassium accumulation drives tissue expansion, but this may not be the case throughout grape berry development because of the large accumulation of sugars after veraison. Sugar accumulation does not occur during the pre-veraison stage, but the fruits are still sinks for carbohydrate and are expanding rapidly due to both cell division and cell expansion. During the pre-veraison phase, it is likely that potassium is driving expansion. The rapid increase in the levels of glucose and fructose in the berry vacuole is likely to be driving both cell and fruit expansion that occurs post-veraison (ripening). In other plant tissues, potassium influx has been shown to be associated with expansion. Cotton fibre cell expansion has been shown to be co-ordinated with changes in the expression of a sucrose transporter, a potassium transporter, and an expansin gene, together with changes to the exclusion size of plasmodesmata (Ruan *et al.*, 2001). A similar process might be suggested for grape berries where potassium influx, together with the accumulation of sugars that occurs after veraison, may combine to help the turgor-driven berry expansion. After veraison, *VvKUP* transporter gene expression is down-regulated and, although other

potassium transport mechanisms may operate during this period, the relative importance of the two transporters described in this work will, in part, be contingent upon their turnover rates.

The development of transgenic plants with altered levels of *VvKUP* gene expression and perhaps altered levels of other potassium transporter genes that contribute to potassium accumulation will provide a method for testing hypotheses regarding the contribution of different potassium transport mechanisms to grape berry potassium accumulation and development.

Acknowledgements

This work was supported in part by the Cooperative Research Centre for Viticulture (CRCV) and the Grape and Wine Research and Development Corporation (GWRDC). We are grateful to Chalk Hill Wines for the provision of grapevine samples.

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