



RESEARCH PAPER

Genetic mapping of natural variation in potassium concentrations in shoots of *Arabidopsis thaliana*

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Abstract

Naturally-occurring variation in K⁺ concentrations between plant genotypes is potentially exploitable in a number of ways, including altering the relationship between K⁺ accumulation and growth, enhancing salinity resistance, or improving forage quality. However, achieving these requires greater insight into the genetic basis of the variation in tissue K⁺ concentrations. To this end, K⁺ concentrations were measured in the shoots of 70 *Arabidopsis thaliana* accessions and a Cape Verdi Island/Landsberg *erecta* recombinant inbred line (RIL) population. The shoot K⁺ concentrations expressed on the basis of fresh matter (KFM) or dry matter (KDM) were both broadly and normally distributed as was the shoot dry matter content per unit fresh weight (DMC). Using the data from the RILs, four quantitative trait loci (QTL) were identified for KFM and three for KDM. These were located on chromosomes 2, 3, 4, and 5. Two of the QTLs for KFM overlapped with those for KDM. None of these QTLs overlapped with those for fresh weight or dry weight, but the QTL for KDM located on chromosome 3 overlapped with one for DMC. *In silico* analysis was used to identify known or putative K⁺ and cation transporter genes whose loci overlapped with the QTLs. In most cases, multiple genes were identified and the possible role of their gene products in determining shoot K⁺ concentrations is discussed.

Key words: Accession, *Arabidopsis*, mapping, naturally-occurring variation, potassium, quantitative trait loci.

Introduction

Potassium is one of the major essential elements required by plants and is present in cells exclusively as its monovalent ion. It plays a major osmotic role in both the vacuole and cytosol, and also activates key enzymes in the cytosol (Leigh and Wyn Jones, 1984). Optimal growth, as measured by fresh or dry weight, occurs when K⁺ is present in shoots at concentrations of at least 1–2% in dry matter (Leigh and Wyn Jones, 1984), although most plants accumulate K⁺ to much higher concentrations (Leigh and Johnston, 1983; Thompson *et al.*, 1997; Broadley *et al.*, 2004). These supra-optimal concentrations reflect the large proportion of total K⁺ that is located in the vacuole while growth limitations at lower concentrations are thought to indicate the point at which K⁺-dependent processes in the cytosol are compromised (Leigh and Wyn Jones, 1984; Walker *et al.*, 1996, 1998). Accumulation of K⁺ to concentrations in excess of those needed for maximal growth is termed luxury uptake and is agronomically wasteful because it results in a decrease in the amount of dry matter produced per unit amount of K⁺ absorbed (K⁺-use efficiency; Marschner, 1995). Optimal concentrations of K⁺ in crops are usually maintained by the addition of fertilizers, but it might be possible to breed more K⁺-efficient crops if the genes determining K⁺ concentrations in plants were known.

Membrane transport processes mediate K⁺ uptake by roots, secretion into the xylem, and distribution within the shoot, including partitioning between cytosol and vacuole. These processes can be active or passive depending on the prevailing conditions (Maathuis and Sanders, 1996; Schachtman, 2000; Tester and Leigh, 2001; Véry and Sentenac, 2003). Passive uptake of K⁺ into roots is

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mediated by voltage-gated, inwardly rectifying K⁺-selective channels such as AKT1 located on the plasma membrane of root cells (Lagarde *et al.*, 1996; Hirsch *et al.*, 1998; Kim *et al.*, 1998; Broadley *et al.*, 2001; Gierth *et al.*, 2005). At low external concentrations, uptake into roots is via H⁺/K⁺-linked symports (Maathuis and Sanders, 1996) some of which are encoded by members of the HUP/HAK gene family (Tester and Leigh, 2001; Véry and Sentenac, 2003). Although some K⁺ is partitioned into root cell vacuoles, possibly via TPK (formerly KCO)-type channels located on the tonoplast (Czempinski *et al.*, 1997, 2002; Schönknecht *et al.*, 2002; Bihler *et al.*, 2005), the majority is transported to the xylem. Secretion into the xylem of *Arabidopsis* involves SKOR, an outward-rectifying K⁺-selective channel located in the plasma membrane of stelar cells (Gaymard *et al.*, 1998). The processes governing uptake and redistribution of K⁺ in leaves are less well understood than those in roots, but a number of K⁺ channels and K⁺/H⁺ symports are expressed in shoots (Véry and Sentenac, 2003). The number of genes involved in uptake and distribution of K⁺ in plants plus the large gene families that encode K⁺ and cation transporters (Mäser *et al.*, 2001) make it difficult to identify genes that are particularly important in determining K⁺ concentrations in the shoots of plants. Examining natural variation of K⁺ concentrations in plants and attempting to identify genes for this trait through quantitative trait loci (QTL) analysis and gene mapping is one approach to addressing this problem.

Genotype-dependent variation of K⁺ concentrations in shoots has been reported in forage grasses (Reeder *et al.*, 1986; Saiga *et al.*, 1992; Slepser *et al.*, 1977; Vogel *et al.*, 1989), wheat (Zhang *et al.*, 1999), and maize (Harada *et al.*, 2001). In forage crops, the genotypic variation in K⁺ concentrations is important for identifying lines that will reduce the occurrence of hypocalcaemia (milk fever) and hypomagnesaemia (grass tetany) in dairy cows, syndromes that are related to the mineral balance of the plants (Grunes *et al.*, 1970; Goff and Horst, 1997). For the other crops, the research is aimed at identifying the basis of K⁺-use efficiency or increasing the discrimination of K⁺ over Na⁺ for salinity resistance (Maathuis and Amtmann, 1999). QTL analyses for variation in shoot K⁺ concentrations have been conducted in rice (Wu *et al.*, 1998; Koyama *et al.*, 2001; Lin *et al.*, 2004; Ren *et al.*, 2005), sugar beet (Schneider *et al.*, 2002), and *Miscanthus* (Atienza *et al.*, 2003). These studies have demonstrated the presence of loci affecting K⁺ concentrations although the identity of the underlying genes remains largely unknown. In rice, SKC1, a QTL affecting K⁺ concentrations under saline conditions, was shown to be coincident with *OsHKT8*, a Na⁺ transporter (Ren *et al.*, 2005). A QTL analysis has been undertaken for Cs⁺ accumulation in *Arabidopsis* (Payne *et al.*, 2004).

In the work reported here, naturally occurring variation in K⁺ concentrations in shoots of different accession of *Arabidopsis* was investigated and QTL analysis was

undertaken using recombinant inbred lines (RILs). The roles of candidate K⁺ and cation transporter genes which coincide with the QTLs are discussed.

Materials and methods

Plant material

The seeds of 70 *Arabidopsis thaliana* accessions were obtained from either the Sendai Arabidopsis Seed Stock Center [source of J44(Bur-0), J69(Edi-0), J71(Eil-0), J84(For-1), J117(Ka-0), J121(Kil-0), J125(Kn-0), J129(Kr-0), J134(Le-0), J237(Tu-1)], the Nottingham Arabidopsis Stock Centre [NASC; supplied N945(An-1), N955(Bay-0), N963(Bd-0), N965(Be-0), N969(Bl-1), N997(Bs-1), N1003(Bsch-0), N1007(Bu-0), N1073(Chi-0), N1085(Co-1), N1149(Est-0), N1153(Et-0), N1155(Fe-1), N1181(Ga-0), N1185(Gd-1), N1193(Gie-0), N1195(Gö-0), N1199(Gr-1), N1219(Ha-0), N1229(HI-0), N1237(Hs-0), N1239(In-0), N1241(Is-0), N1337(Lip-0), N1345(Lm-2), N1347(Lö-1), N1353(Lu-1), N1357(Ma-0), N1365(Me-0), N1371(Mnz-0), N1375(Mrk-0), N1377(Ms-0), N1391(Nd-0), N1393(Nie-0), N1433(Or-0), N1435(Ove-0), N1437(Oy-0), N1453(Pf-0), N1455(Pi-0), N1469(Pn-0), N1483(Rd-0), N1491(Rsch-0), N1493(Ri-0), N1525(Si-0), N1535(St-0), N1549(Ta-0), N1589(Wc-1), N1595(Wil-1)], or the *Arabidopsis* Biological Resource Center [provided CS6624(Bla-12), CS6651(Bu-20), CS6719(Ge-2), CS6738(HI-2), CS6775(Li-5), CS6850(Rsch-4), CS6855(Sf-1), CS6859(Sg-2), CS6869(Ts-2), CS6872(Ts-6), CS6873(Ts-7), CS6882(Uk-4)]. The seeds of RILs derived from the parental accessions Cape Verdi Islands and Landsberg *erecta* (Cvi/Ler; Alonso-Blanco *et al.*, 1998) were obtained from NASC.

Arabidopsis growth and K⁺ measurements

Arabidopsis plants were grown hydroponically on a rock wool bed (L=250 mm, W=200 mm, H=30 mm) placed in a tray with drain holes and moistened with 0.8 l of nutrient solution (1.5 mM KH₂PO₄, 0.25 mM K₂HPO₄, 1.5 mM MgSO₄, 7.5 mM Ca(NO₃)₂, 67 µM Na₂EDTA, 9 µM FeSO₄, 10 µM MnSO₄, 30 µM H₃BO₄, 1 µM ZnSO₄, 1 µM CuSO₄, 0.02 µM (NH₄)₆Mo₇O₂₄, and 0.1 µM CoSO₄). Ten seeds for each line were placed 11 mm apart in rows separated by 20 mm. The plants were grown for 2 weeks in a greenhouse at 22 °C with 16 h of light supplemented with artificial illumination as needed. To prevent depletion of nutrients, the rock wool was washed with 1.5 l of the nutrient solution at 6, 4, and 2 d before sampling. The experiment was repeated twice for the accessions and three times for the Cvi/Ler RILs.

The shoots of six to ten plants of each accession or RIL were harvested, weighed fresh and dried at 80 °C for 2 d. After reweighing, the dried plants were extracted with 1.5 ml of 0.1 M HCl for 2 h at room temperature in a 2 ml microtube. An aliquot of the extract was diluted with 0.1 M HCl and K⁺ concentration was determined using a Hitachi Model Z8100 atomic absorption spectrophotometer, (Hitachi High Technologies Corporation, Tokyo). Mean K⁺ concentrations derived from all replicate experiments are reported and were used in QTL analyses.

Statistical analysis and QTL mapping

The sets of data for accessions and RILs were included in an analysis of variance model (ANOVA) to determine the genotypic effects and repetition factors. The broad sense heritability was calculated by one-way ANOVA. A multiple regression model was used to estimate the genetic effect of QTLs and the contribution of each QTL to the total genotypic variance. Two-way interactions among QTLs for each trait were tested by ANOVA using two corresponding molecular markers

as fixed factors and the trait as a dependent variable; no interaction was found among QTLs at a significance level of $P \leq 0.05$. Statistical analysis was performed using the `aov()`, `cor.test()` and `lm()` functions of the R 2.0 statistical software package (<http://www.r-project.org/>).

QTL analysis was conducted as described in Harada *et al.* (2004). Data for 241 molecular markers for the Cvi/Ler RILs with an average spacing of approximately 2 cM were obtained from NASC (<http://nasc.nott.ac.uk/>). The genetic distance between the markers was calculated using MAPMAKER 3.0 (Lander *et al.*, 1987). To locate a QTL, a simplified type of composite interval mapping was conducted using MQTL (Tinker and Mather, 1995). The likelihood ratio described by Haley and Knott (1992) was used for the test statistic (TS) by MQTL. The TS threshold for QTL declaration was determined for each genome by a permutation test with 2000 replications to keep the probability of a type I error below 5% (Tinker and Mather, 1995). Candidate genes for each QTL were screened *in silico* by comparing the position of the QTL with the loci of genes encoding putative K^+ and cation transporters (<http://www.arabidopsis.org/servlets/mapper>).

Results

Variation in shoot K^+ concentrations

Potassium concentrations in *Arabidopsis* shoots were expressed relative to either the weight of fresh matter (KFM) or dry matter (KDM) and both parameters were broadly distributed both in the 70 accessions and the 98 Cvi/Ler RILs (Fig. 1). The range of variation for these parameters and for dry matter content per unit fresh weight (DMC) was similar in both the accessions and the RILs, being about 2-fold for KFM and KDM and 1.5-fold for DMC (Table 1). Plant growth expressed as weight of fresh matter (FM) or

dry matter (DM) also showed broad distributions and the range of variation for these parameters was larger than for KDM, KFM, and DMC. With the exception of FM in the RILs, all parameters were normally distributed in both the accessions and the RILs. The phenotypic differences between Cvi and Ler (parental genotypes of the RILs) for each of the phenotypic parameters were: KFM, $21.8 \mu\text{mol K}^+ \text{g}^{-1}$; KDM, $0.27 \text{mmol K}^+ \text{g}^{-1}$; FM, 1.2mg plant^{-1} ; DM, $0.28 \text{mg plant}^{-1}$; DMC, 1.3mg g^{-1} . For KFM and KDM these differences were about twice the standard deviations measured on the RILs.

Broad sense heritability, which indicates the ratio of genetic variance to the total variance, ranged from 0.373 to 0.573 for KFM and KDM in the accessions and RILs (Table 1). The genotypic effects were significant for all five traits in the RILs and accessions, except for KDM and DMC in the accessions (Table 1). Although the broad sense heritability for KDM was not low (0.467), the heritability does not always parallel the significance of genetic effects. The less significant effects in the accessions might have resulted from the interaction between genotype and environments because the accessions have a wider genetic background. There was some variation for all traits between replicate experiments (data not shown) indicating that some aspects of the growth conditions were affecting K^+ concentrations, but these were not investigated further. Significant correlations ($P < 0.001$) were observed between FM and DM, between KFM and KDM, and between KFM and DMC in both the accessions and RILs (Table 2). Plant growth, measured as FM or DM, was highly correlated with

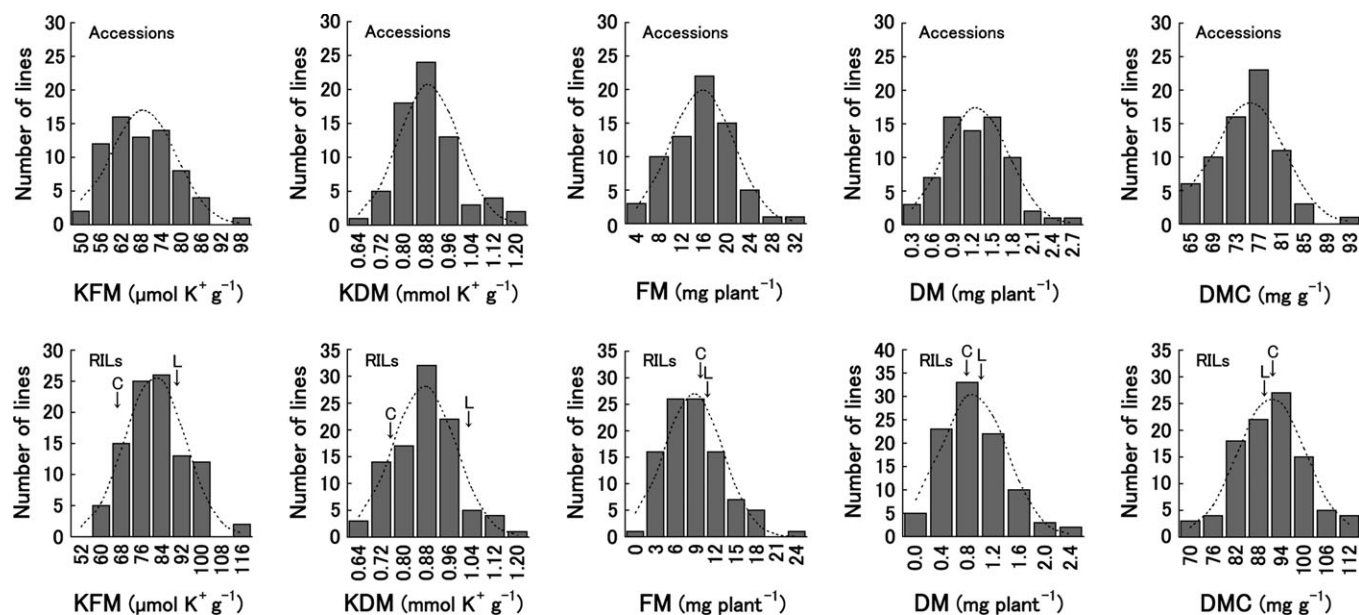


Fig. 1. Frequency distributions of K^+ concentrations (KFM and KDM), weights of fresh (FM) and dry (DM) matter, and dry matter content (DMC) of shoots of *Arabidopsis* accessions (upper panels; $n=70$) and Cvi/Ler RILs (lower panels; $n=98$). The units for the parameters are: KFM, $\mu\text{mol g}^{-1}$ FM; KDM, mmol g^{-1} DM; FM, DM, mg plant^{-1} ; DMC, mg DM g^{-1} FM. Values on the x -axis are the lower limit of the concentration for each bar. Arrows in the lower panels indicate the values of the parameters in the Cvi (C) or Ler (L) parental lines of the RILs. Normal curves for the distributions are indicated by the dashed lines.

Table 1. Basic statistical parameters for K⁺ concentrations, dry matter content and plant growth for 70 *Arabidopsis* accessions and 98 *Cvi/Ler* RILs

Lines	Trait ^a	Mean ± SD	Minimum	Maximum	Genetic effect ^b	Heritability
Accessions	KFM	72 ± 10	54	100	193*	0.373
	KDM	0.93 ± 0.11	0.71	1.24	0.022	0.467
	FM	17.6 ± 5.5	6.7	33.2	60.7*	0.264
	DM	1.41 ± 0.47	0.48	2.77	0.452*	0.244
	DMC	77 ± 6	66	95	0.627	0.303
RILs	KFM	85 ± 12	65	121	433***	0.573
	KDM	0.91 ± 0.11	0.67	1.20	0.037***	0.474
	FM	10.1 ± 4.2	1.9	24.3	36.5***	0.559
	DM	1.09 ± 0.50	0.17	2.73	0.497***	0.743
	DMC	94 ± 9	74	116	0.238***	0.657

^a KFM, K⁺ concentration in fresh matter (μmol g⁻¹); KDM, K⁺ concentration in dry matter (mmol g⁻¹), FM, fresh matter (mg plant⁻¹), DM, dry matter (mg plant⁻¹), DMC, dry matter content (mg DM g⁻¹ FM).

^b Variance associated with the genotype effect by 2-way ANOVA and its significance, **P* < 0.05; ****P* < 0.001.

Table 2. Correlation coefficients between K⁺ concentrations, dry matter content and plant growth in shoots of *Arabidopsis* accessions (n=70) and *Cvi/Ler* RILs (n=98)

Values above the diagonal line are for the RILs and those below for the accessions. Significance: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

	KFM ^a	KDM ^b	FM ^c	DM ^d	DMC ^e
KFM ^a		0.710***	0.156	0.244*	0.506***
KDM ^b	0.824***		0.133	0.134	-0.225*
FM ^c	0.415***	0.272*		0.977***	-0.092
DM ^d	0.499***	0.288*	0.985***		0.097
DMC ^e	0.510***	-0.043	0.242*	0.364**	

^a KFM, K⁺ concentration in fresh matter.

^b KDM, K⁺ concentration in dry matter.

^c FM, fresh matter.

^d DM, dry matter.

^e DMC, dry matter content.

KFM and KDM in the accessions, but these relationships were absent or less significant in the RILs.

Genetic mapping

To identify the genetic loci involved in the variation in shoot K⁺ concentrations, QTL analysis was conducted using the *Cvi/Ler* RILs. The TS and threshold for QTL declaration were calculated by MQTL for all traits (Fig. 2). Four QTLs for KFM and 3 QTLs each for KDM and DMC were identified and these were distributed between all the chromosomes except chromosome 1 (Fig. 2; Table 3). Two QTLs for FM and one for DM were identified on chromosome 5. The weak peak of KFM found in chromosome 4 at 53 cM was disregarded because the genetic effect of the peak was not significant when tested by a multiple regression analysis. The weak peaks for DMC, FM, and KDM found at 106, 66, and 118 cM, respectively, on chromosome 5 were disregarded for the same reason. The remaining QTLs were named using the trait abbreviation suffixed with the number of the chromosome and an ordering number (Table 3).

The genetic effect and percentage variance explained by the QTLs were calculated using a multiple regression model instead of MQTL because MQTL cannot estimate the genetic effect for multiple QTLs. The genetic markers used for this regression analysis are indicated in Fig. 2. Individual QTLs explained from 2.7% to 15.4% of the total phenotypic variation of the traits (Table 3). No interaction between the QTLs was found by 2-way ANOVA. KDM3.1 and KFM5.2 explain 9.0% and 13.0% of the total variance for their traits, respectively, and these are major QTLs for K⁺ concentrations in *Arabidopsis* shoots. KFM4.1 and KDM4.1 were coincident as were KFM5.2 and KDM5.1. KDM3.1 coincided with a QTL for DMC. In general, the TS peaks of the QTLs for KFM were larger than those for KDM. DM5.1, the major and only QTL for DM overlapped with FM5.1. DM5.1 has an opposite effect to QTLs for KFM and KDM (Table 3) even though KFM was positively correlated with DM (Table 2). The single QTLs identified for DM and FM did not overlap with any of those for KFM or KDM. Three QTLs were found for DMC even though there was only a small difference in this parameter between the parental lines (Fig. 1). The genetic effect of DMC3.1 is positive while that of the other two was negative. Despite the significant correlation between DMC and KFM in the RILs (Table 2), the QTLs for DMC did not overlap with those for KFM.

Discussion

The K⁺ concentration in shoots of plants is affected by multiple processes including uptake and efflux by roots, storage in root cell vacuoles, loading into the xylem, uptake and storage in leaf cells, and recirculation to the roots via the phloem. Multiple transporters of varying specificity for K⁺ may be involved in each of these steps (Véry and Sentenac, 2003) so the number of genes that can potentially influence natural variation in K⁺ concentrations in shoots is large. The presence of QTLs for K⁺ accumulation has

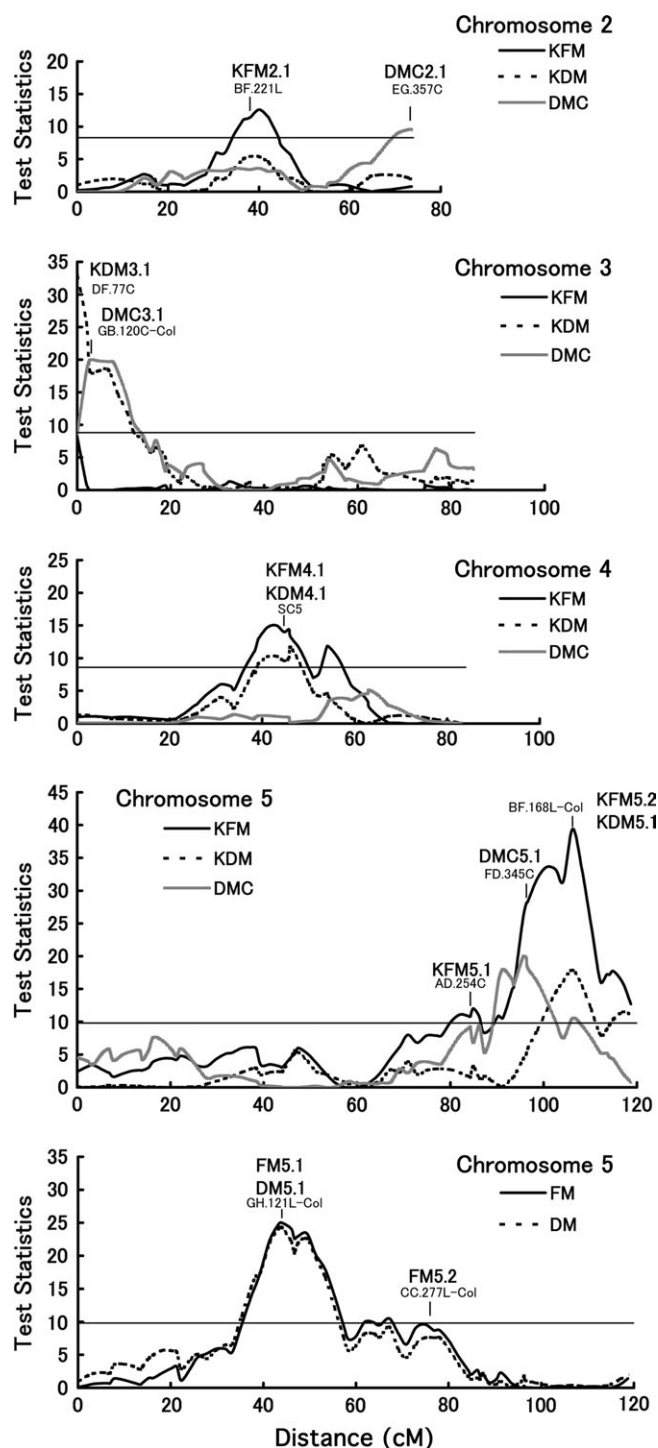


Fig. 2. Location of QTLs for K⁺ concentrations (KFM or KDM), weights of fresh (FM) and dry matter (DM), and dry matter content (DMC) of the Cvi/Ler RILs. Test statistics were calculated using the computer program MQTL and the threshold test statistics for QTL declaration (horizontal lines) were determined by a permutation test with 2000 replications to keep the probability of a type I error below 5% (Tinker and Mather, 1995). The name of QTL and positions of markers flanking the QTLs are shown.

already been reported for rice (Wu *et al.*, 1998; Koyama *et al.*, 2001; Lin *et al.*, 2004; Ren *et al.*, 2005), sugar beet (Schneider *et al.*, 2002), and *Miscanthus* (Atienza *et al.*, 2003). In *Arabidopsis*, seven QTLs for K⁺ concentrations in seeds were reported by Vreugdenhil *et al.* (2004) using the same population of Cvi/Ler RILs employed here. This population yielded two major and five minor QTLs for K⁺ concentrations in shoots (Fig. 2) and only one of these, KDM3.1, overlaps with any of the QTLs reported by Vreugdenhil *et al.* (2004). Thus different gene products must be involved in determining K⁺ levels in different tissues, consistent with the tissue-specific expression shown by different K⁺ transporters (Véry and Sentenac, 2003). Two QTLs for shoot Cs⁺ concentration have been reported on chromosomes 1 and 5 using the same RILs (Payne *et al.*, 2004). KFM5.2 is coincident with one of these QTLs for Cs⁺ accumulation and both have a negative effect. This suggests that the genes responsible for KFM5.2 might also mediate Cs⁺ accumulation. This cross comparison demonstrates the benefits of using the same RILs for different QTL analyses.

Most of the K⁺ in plant cells is located in the vacuole where its role is osmotic (Leigh and Wyn Jones, 1984). Electrical neutrality is maintained by accumulation of organic or inorganic anions and there may be close relationships between the concentrations of particular anions and K⁺. For instance, a significant positive correlation was found between nitrate and K⁺ concentrations in 21 silage corn varieties grown with high applications of N fertilizer (Harada *et al.*, 2001). In the present study, nitrate was the anion supplied at the largest concentration, but no correlation was observed between nitrate and K⁺ concentrations in either the accessions or the RILs (data not shown). Though a major QTL for nitrate accumulation (NA3) was found at the position of KFM5.1 (Harada *et al.*, 2004), NA3 has an opposite QTL effect to KFM5.1. These results suggest that genetic controls for nitrate and K⁺ concentrations are independent.

Potassium is not metabolized in plant cells and hence the gene products involved in K⁺ accumulation regulate intra- and intercellular membrane transport, not assimilatory pathways for this ion. Therefore *in silico* screening to identify candidate genes for the QTLs was limited to comparing the position of each QTL with the loci of genes encoding known or putative K⁺ transporters (Table 4).

KFM2.1 is close to the loci of *AtAKT1*, *AtAKT6*, *AtCNGC6*, and *AtCNGC14*. AKT1 and AKT6 are members of a family of K⁺-specific voltage-gated ion channels while the CNGCs encode cyclic-nucleotide-gated cation channels (Véry and Sentenac, 2003). The role of the *AtAKT6* gene product has not yet been determined so its potential role in regulating K⁺ concentrations cannot be assessed. *AtAKT1* encodes a voltage-gated, inwardly rectifying K⁺-selective channel that is localized in the plasma membrane of root epidermal and cortical cells and leaf mesophyll cells. It is

Table 3. QTL for K⁺ concentrations, dry matter content and plant growth of Cvi/Ler RILs

QTL ^a	Chromosome	Position (cM)	Marker ^b	QTL effect ^c	Test statistic	PVE ^d (%)
KFM2.1	2	40	BF.221L	($\mu\text{mol g}^{-1}$) -5.6	12.6	4.0
KFM4.1	4	43	SC5	-8.9	15.0	5.5
KFM5.1	5	82	AD.254C	-4.8	11.1	2.7
KFM5.2	5	106	BF.168L-Col	-12.0	39.4	13.0
KDM3.1	3	0	DF.77C	(mmol g^{-1}) -0.12	33.3	9.0
KDM4.1	4	43	SC5	-0.07	10.3	3.1
KDM5.1	5	106	BF.168L-Col	-0.09	17.9	5.1
FM5.1	5	44	GH.121L-Col	(mg plant^{-1}) 3.1	24.4	11.5
FM5.2	5	76	CC.277L-Col	1.7	9.8	4.2
DM5.1	5	44	GH.121L-Col	(mg plant^{-1}) 0.36	25.5	15.4
DMC2.1	2	73	EG.375C	(mg DM g^{-1}) -4.7	9.6	3.4
DMC3.1	3	5	GB.120C-Col	4.1	19.6	4.8
DMC5.1	5	96	FD.345C	-6.6	19.9	8.7

^a QTL were identified using MQTL (Tinker and Mather 1995).

^b Mapping data for DNA markers in RIL population were obtained from NASC.

^c The QTL effects are expressed as Ler standard.

^d Percentage variance explained by each QTL. This was estimated using a multiple regression model. The sum of the individual PVE values for the QTLs for each physiological parameter yield the total variance explained by those QTLs.

Table 4. Candidate K⁺ and cation transporter genes with loci that coincide with the QTLs for K⁺ concentrations

QTL (and position in cM)	Candidate genes and positions in cM ^a
KFM2.1 (40)	<i>AtAKT1</i> (51), <i>AtAKT6</i> (47), <i>AtCNG6</i> (40), <i>AtCNGC14</i> (40)
KFM4.1 (43)	<i>ATHAK5</i> (46)
KFM5.1 (82)	<i>AtKAT1</i> (97), <i>AtTPK2</i> (98), <i>AtKCO3</i> (98)
KFM5.2 (106)	<i>AtKTPK1</i> (112), <i>AtCNGC1</i> (109), <i>AtCNGC5</i> (113), <i>AtKEA5</i> (109), <i>AtNHX3</i> (112)
KDM3.1 (0)	<i>AtSKOR</i> (7)

^a Positions are those in the AGI (Arabidopsis Genome Initiative) map (<http://www.arabidopsis.org/servlets/mapper>).

responsible for passive K⁺ uptake in roots (Lagarde *et al.*, 1996; Dennison *et al.*, 2001; Véry and Sentenac, 2003; Gierth *et al.*, 2005) and plants with disrupted *AKT1* have lower K⁺ concentrations than the wild type and do not grow as well (Hirsch *et al.*, 1998; Broadley *et al.*, 2001). CNGC probably act as general cation transport pathways (Demidchik *et al.*, 2002) and thus they may influence K⁺ concentrations by promoting the uptake of cations that replace K⁺ in its vacuolar role (Leigh and Wyn Jones, 1984).

KDM3.1 overlaps with the locus of *AtSKOR* which encodes another voltage-gated, K⁺-selective channel. *AtSKOR* is expressed in root stelar cells and the SKOR protein is involved in secretion of K⁺ into the xylem for transport to the shoot. Plants in which *AtSKOR* is disrupted by T-DNA insertion have a lower shoot K⁺ concentration than the wild type (Gaymard *et al.*, 1998). *AtSKOR* also overlaps with one of the QTLs for K⁺ concentrations in *Arabidopsis* seeds (Vreugdenhil *et al.*, 2004).

KFM5.1 is close to *AtKAT1* which is mainly expressed in stomates, etiolated hypocotyls, and flower stalks, and is induced by auxin in auxin-sensitive tissues with rapid cell elongation (Véry and Sentenac, 2003; Philippar *et al.*, 2004). This QTL also overlaps with *AtTPK2* (formerly *AtKCO2*; see Becker *et al.*, 2004) encoding a two-pore, four-transmembrane domain K⁺ channel (Mäser *et al.*, 2001) and *AtKCO3* encoding a one-pore, two-transmembrane domain channel similar to animal inward-rectifying K⁺ channels (Véry and Sentenac (2003). *TPK* genes were thought to encode Ca²⁺-regulated, outward-rectifying K⁺ channels, but evidence is now emerging that the gene products are more functionally diverse (Becker *et al.*, 2004; Bihler *et al.*, 2005). KFM5.2 overlaps with the locus of *AtTPK1* (formerly *KCO1*) which has been investigated in some detail. It encodes a two-pore K⁺ channel that is located on the tonoplast and is expressed in a number of tissues including roots and leaves (Czempinski *et al.*, 1997, 2002; Schachtman, 2000). Knockouts in *TKP1* have significantly decreased SV-channel activity in their tonoplast (Schönknecht *et al.*, 2002), but the gene may not encode the channel (Bihler *et al.*, 2005). Two genes for CNGCs (*AtCNGC1* and *AtCNGC5*), one for a putative K⁺ efflux antiporter (*AtKEA5*) and one for a Na⁺/H⁺ antiporter (*AtNHX3*) also correspond to KFM5.2 (Table 4). Their role in determining shoot K⁺ concentrations is likely to be through influencing the uptake of other cations to replace K⁺ in the vacuole. The K⁺ transporter gene, *AtHAK5* corresponds to KFM4.1. This gene is a member of the *AtKUP/HAK/KT* gene family and encodes a high affinity K⁺ transporter. *AtHAK5* expression is substantially up-regulated in *Arabidopsis* roots and older leaves in response

to K⁺-deprivation (Ahn *et al.*, 2004; Armengaud *et al.*, 2004; Hampton *et al.*, 2004; Gierth *et al.*, 2005).

Interestingly most of the K⁺ transport genes that coincided with QTLs overlapped with the TS peaks for KFM rather than KDM. This may be because the osmotic role of K⁺ is dependent on the concentration of K⁺ in tissue water (Leigh and Johnston, 1983) and so will be more closely related to K⁺ in fresh matter than dry matter.

This study indicates that control of K⁺ concentrations in the shoots of *Arabidopsis* involves multiple gene products. Unravelling whether all genes are important or just one dominates each QTL will require more detailed studies.

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