Cesium Toxicity in Arabidopsis[1]

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Cesium (Cs) is chemically similar to potassium (K). However, although K is an essential element, Cs is toxic to plants. Two contrasting hypotheses to explain Cs toxicity have been proposed: (1) extracellular Cs^+ prevents K^+ uptake and, thereby, induces K starvation; and (2) intracellular Cs^+ interacts with vital K^+-binding sites in proteins, either competitively or noncompetitively, impairing their activities. We tested these hypotheses with Arabidopsis (*Arabidopsis thaliana*). Increasing the Cs concentration in the agar ($[Cs]_{agar}$) on which Arabidopsis were grown reduced shoot growth. Increasing the K concentration in the agar ($[K]_{agar}$) increased the $[Cs]_{agar}$ at which Cs toxicity was observed. However, although increasing $[Cs]_{agar}$ reduced shoot K concentration ($[K]_{shoot}$), the decrease in shoot growth appeared unrelated to $[K]_{shoot}$ per se. Furthermore, the changes in gene expression in Cs-intoxicated plants differed from those of K-starved plants, suggesting that Cs intoxication was not perceived genetically solely as K starvation. In addition to reducing $[K]_{shoot}$, increasing $[Cs]_{agar}$ also increased shoot Cs concentration ($[Cs]_{shoot}$), but shoot growth appeared unrelated to $[Cs]_{shoot}$ per se. The relationship between shoot growth and $[Cs]_{shoot}/[K]_{shoot}$ suggested that, at a nontoxic $[Cs]_{shoot}$, growth was determined by $[K]_{shoot}$ but that the growth of Cs-intoxicated plants was related to the $[Cs]_{shoot}/[K]_{shoot}$ quotient. This is consistent with Cs intoxication resulting from competition between K^+ and Cs^+ for K^+-binding sites on essential proteins.

Potassium (K) is an essential macronutrient. It is required (as K^+) at concentrations of 100 to 150 mM in the cytoplasm of plant cells to activate enzymes and stabilize protein and nucleotide structure (Leigh and Wyn Jones, 1984; Marschner, 1995). The cation Cs^+ shares similar chemical properties to K^+ and competes with K^+ for cation binding sites in proteins (Avery, 1995). Unfortunately, Cs^+ does not behave identically to K^+ and inhibits the activity of many K^+-activated enzymes (Avery, 1995). Consequently, Cs^+ is potentially toxic to plants. In addition, Cs^+ inhibits the inward-rectifying K channels in the plasma membranes of plant cells (White, 1997; White and Broadley, 2000), including the major inward-rectifying K channels involved in plant K nutrition (e.g. AtAKT1 in Arabidopsis [*Arabidopsis thaliana*]). Excessive Cs^+ in the rhizosphere could, therefore, induce K starvation in plants. The growth of a number of plant species, including bean, tomato, Arabidopsis, and rice, is inhibited by Cs^+ concentrations in the rhizosphere exceeding 200 μM (Cline and Hungate, 1960; Kordan, 1987; Sheahan et al., 1993; Hasegawa, 1996; White and Broadley, 2000). The symptoms of Cs intoxication can be reversed, however, by supplying more K. This is consistent with the hypothesis that Cs is toxic because it interferes with K uptake and/or K biochemistry.

Natural soil Cs concentrations are generally low and nontoxic to plants. The stable isotope ^{133}Cs occurs naturally in the aluminosilicate mineral pollucite and may reach concentrations of 25 $\mu g\ g^{-1}$ dry soil (White and Broadley, 2000). Only in areas containing Cs-rich pollucite ores, such as those found in southeastern Manitoba (Teertstra et al., 1992), might Cs cause environmental toxicity. However, two radioisotopes of Cs (^{134}Cs and ^{137}Cs) are of environmental concern due to their emissions of harmful β and γ radiation, relatively long half-lives, and rapid incorporation into biological systems (White and Broadley, 2000). These isotopes arise from the manufacture and testing of thermonuclear weapons and from intentional and unintentional discharges from nuclear installations. They enter the terrestrial food chain through plants, and their presence in foodstuffs impacts upon both health and commerce. Plant roots take up Cs from the soil solution as the monovalent cation (Cs^+), which is transported symplastically to the xylem. Theoretical models suggest that, in K-replete plants, Cs influx to root cells is predominantly through voltage-insensitive cation channels (VICCs), with "high-affinity" K^+/H^+ symporters transporting the remainder (White and Broadley, 2000). In Arabidopsis, members of the *AtCNGC* and *AtGLR* gene families are thought to encode VICCs (White and Broadley, 2000; Demidchik et al., 2002; White et al., 2002), and members of the

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AtKUP/AtHAK gene family encode the K^+/H^+ symporters. However, since plant K-status regulates the expression of genes encoding several cyclic nucleotide gated channels (CNGCs), glutamate receptors (GLRs), and K^+ uptake permeases (KUPs; Kim et al., 1998; Maathuis et al., 2003; Ahn et al., 2004), it is possible that the relative contributions of VICCs and KUPs are reversed in K-deficient plants (White et al., 2004).

Here we investigate the mechanism(s) of Cs toxicity in Arabidopsis. We consider three hypotheses: (1) Cs inhibits plant growth because it reduces K^+ uptake and causes K starvation; (2) intracellular Cs is toxic per se, perhaps due to irreversible binding to essential K-dependent proteins; and (3) Cs^+ competes with K^+ for essential biochemical functions and, therefore, Cs toxicity is related to the $[Cs]_{shoot}/[K]_{shoot}$ quotient. We conclude that Cs toxicity is determined by the $[Cs]_{shoot}/[K]_{shoot}$ quotient. However, we did observe that the expression of many (but not all) genes was altered similarly in response to both K starvation and Cs intoxication. Thus, although Cs intoxication was not perceived genetically solely as K starvation, some Cs-induced K deficiency may be evident. We also note a significant increase in the expression of the gene encoding the H^+/Cs^+ symporter AtHAK5 in K-starved plants. This resulted in an increased Cs^+ influx to K-starved plants and characteristic changes in its pharmacology.

RESULTS

K Dependence of Shoot Growth

The Arabidopsis accession Wassilewskija (Ws2) was grown for 21 d on agar containing a complete mineral supplement with K concentrations ($[K]_{agar}$) between 0.5 and 20,000 μM (Fig. 1). The relationships between (A) shoot fresh weight (FW) and (B) mean shoot K concentration ($[K]_{shoot}$) and $[K]_{agar}$ fitted the equation of a rectangular hyperbola (Table I). Shoot FW increased with increasing $[K]_{agar}$ up to a maximum value (Fig. 1A). At the highest $[K]_{agar}$ assayed (20 mM), shoot FW was 20.0 ± 4.5 mg ($n = 6$ experiments). The critical $[K]_{agar}$, at which shoot FW was 90% of its value at 20 mM $[K]_{agar}$, was 9.9 mM. The $[K]_{shoot}$ also increased with increasing $[K]_{agar}$ up to a maximum value (Fig. 1B). This approximated 220 μmol g^{-1} FW (Table I). The $[K]_{agar}$ at which the $[K]_{shoot}$ was half-maximal was 0.93 mM. This value is similar to the K_m of the low affinity mechanism for K^+ uptake into plants (Epstein, 1972). The relationship between shoot FW and $[K]_{shoot}$ was almost linear (Fig. 1C). However, neither shoot FW nor $[K]_{shoot}$ can increase infinitely, and data from plants grown at the highest $[K]_{agar}$ cluster around the same coordinates. The increase in shoot FW as a function of $[K]_{shoot}$ was about 0.09 g^2 $mmol^{-1}$.

K Alleviates the Inhibition of Shoot Growth by Cs

To determine the effects of Cs concentration in the agar ($[Cs]_{agar}$) on growth, plants were grown for 21 d on

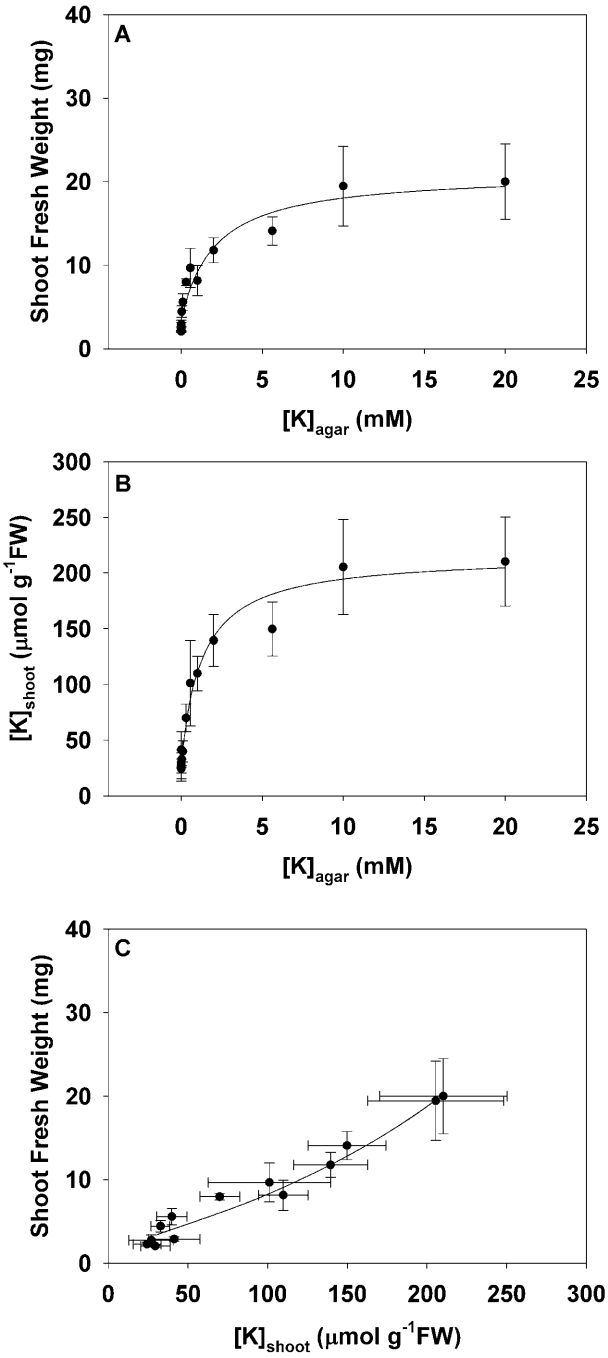

Figure 1. The relationships between shoot FW versus the K concentration in the agar ($[K]_{agar}$; A), shoot K concentration ($[K]_{shoot}$) versus $[K]_{agar}$ (B), and shoot FW versus $[K]_{shoot}$ (C) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing K concentrations between 0.0005 and 20 mM. All data represent means ± SE from at least three replicate experiments. Rectangular hyperbolae were fitted to the relationships between shoot FW and $[K]_{shoot}$ versus $[K]_{agar}$ and the relationship between shoot FW and $[K]_{shoot}$ was derived from these. The parameters for all equations are given in Table I.

Table I. *Parameters for the regression lines of Figures 1, 2, and 3*

The relationships between shoot FW (g) and shoot K concentration ($[K]_{shoot}$, μmol g^{-1} FW) versus the K concentration in agar ($[K]_{agar}$, μM; Fig. 1) were fitted to a rectangular hyperbola: $Y = A+B/(1+DX)$. The relationships between shoot FW (g) versus Cs concentration in the agar ($[Cs]_{agar}$, μM; Fig. 2) were fitted to the equation of a logistic sigmoidal curve: $FW = C/(1 + \exp(-B(\log[Cs]_{agar} - Ka_{50})))$. The relationships between shoot Cs concentration ($[Cs]_{shoot}$, nmol g^{-1}FW) versus $[Cs]_{agar}$ (Fig. 3) were fitted to the equation for a straight line: $[Cs]_{shoot} = K[Cs]_{agar}$. Data are expressed as means ± SE for n data.

Relationship		Parameter (units)	Mean ± SE
Shoot FW versus $[K]_{agar}$	Fig. 1A $n = 72$	D (M^{-1})	484 ± 238
		B (mg)	−17.6 ± 2.3
		A (mg)	21.0 ± 2.3
$[K]_{shoot}$ versus $[K]_{agar}$	Fig. 1B $n = 70$	D (M^{-1})	764 ± 353
		B (μmol g^{-1}FW)	−185 ± 21.0
		A (μmol g^{-1}FW)	216 ± 20.2
Shoot FW versus $[Cs]_{agar}$ at 2 mM $[K]_{agar}$	Fig. 2 $n = 80$	B (log μM)$^{-1}$	−8.25 ± 1.7
		C (mg)	14.98 ± 0.52
		Ka_{50} (log μM)	2.76 ± 0.028
Shoot FW versus $[Cs]_{agar}$ at 20 mM $[K]_{agar}$	Fig. 2 $n = 64$	B (log μM)$^{-1}$	−9.35 ± 3.2
		C (mg)	19.4 ± 0.78
		Ka_{50} (log μM)	3.26 ± 0.038
$[Cs]_{shoot}$ versus $[Cs]_{agar}$ at 2 mM $[K]_{agar}$	Fig. 3 $n = 51$	K (L kg^{-1}FW)	16.4 ± 0.49
$[Cs]_{shoot}$ versus $[Cs]_{agar}$ at 20 mM $[K]_{agar}$	Fig. 3 $n = 40$	K (L kg^{-1}FW)	6.3 ± 0.19

agar containing a complete mineral supplement plus ^{134}Cs-labeled $[Cs]_{agar}$ between 0 and 10 mM. Since the toxicity of Cs in the rhizosphere has been shown to depend on the rhizosphere K concentration (Kordan, 1987), this experiment was performed at $[K]_{agar}$ of 2 mM and 20 mM. The shoot FW, mean shoot Cs concentration ($[Cs]_{shoot}$), and $[K]_{shoot}$ were determined on bulked shoot material. However, since it was impractical to determine $[Cs]_{shoot}$ from ^{134}Cs content and $[K]_{shoot}$ from inductively coupled plasma optical emission spectrometry (ICP-OES) simultaneously, experiments to determine $[Cs]_{shoot}$ and $[K]_{shoot}$ were performed separately. There were no differences in shoot FW between plants grown in the presence or absence of ^{134}Cs (data not shown). Plants were grown at each $[K]_{agar}$-$[Cs]_{agar}$ combination at least eight times.

Arabidopsis grown at low $[Cs]_{agar}$ at a $[K]_{agar}$ of 20 mM had greater shoot FWs than those of plants grown in the absence of Cs (Fig. 2). The reason for this is unknown. There was a gradual decline in shoot FW as $[Cs]_{agar}$ was increased above 0.3 mM, in the presence of 2 mM $[K]_{agar}$, or above 1 mM, in the presence of 20 mM $[K]_{agar}$. The relationship between shoot FW and $[Cs]_{agar}$ fitted the equation of a logistic sigmoidal curve. The minimal shoot FW in the presence of high $[Cs]_{agar}$ was close to zero. The maximal shoot FW of plants grown in the presence of 20 mM K was generally greater than that of plants grown in the presence of 2 mM K (Parameter C in Table I). The $[Cs]_{agar}$ at which shoot FW was half-maximal (Ka_{50}), which is a measure of the tolerance of a plant to Cs in the rhizosphere, was significantly lower for plants grown with 2 mM $[K]_{agar}$ than for plants grown with 20 mM $[K]_{agar}$ ($P < 0.001$). The rate of change of shoot FW as the $[Cs]_{agar}$ was increased (Parameter B in Table I) did not differ significantly ($P > 0.05$) when plants were grown in the presence of 2 mM or 20 mM $[K]_{agar}$.

K Reduces Cs Accumulation in Shoots

The shoot Cs concentration ($[Cs]_{shoot}$) increased linearly with increasing $[Cs]_{agar}$ up to an apparent maximum $[Cs]_{shoot}$ (Fig. 3). At the maximal $[Cs]_{shoot}$, shoot FW was minimal. When assayed at a specific $[Cs]_{agar}$, the $[Cs]_{shoot}$ was higher with 2 mM $[K]_{agar}$ than with 20 mM $[K]_{agar}$, and the rate of increase in $[Cs]_{shoot}$ with $[Cs]_{agar}$ (Parameter K in Table I) differed significantly ($P < 0.001$) between plants grown with 2 mM and 20 mM $[K]_{agar}$. Thus increasing rhizosphere K concentration reduces the accumulation of Cs in the shoot.

Cs Reduces K Accumulation in Shoots

Increasing the $[Cs]_{agar}$ reduced $[K]_{shoot}$ (Fig. 4A). In general, $[K]_{shoot}$ was smaller in plants grown in the presence of 2 mM $[K]_{agar}$ than those grown in the presence of 20 mM $[K]_{agar}$ at the same $[Cs]_{agar}$. Intriguingly, the $[K]_{shoot}$ of plants grown at low $[Cs]_{agar}$ (<0.01 mM $[Cs]_{agar}$ at 2 mM $[K]_{agar}$, <2 mM $[Cs]_{agar}$ at 20 mM $[K]_{agar}$) were greater than those grown in the absence of Cs. When $[Cs]_{agar}$ was increased beyond these concentrations, $[K]_{shoot}$ declined.

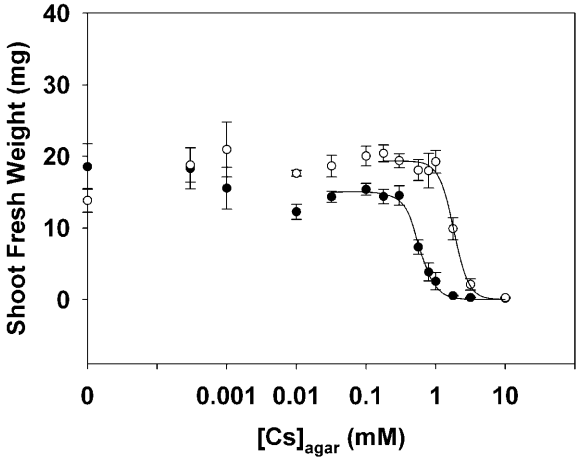

Figure 2. The relationships between shoot FW versus the Cs concentration in the agar ($[Cs]_{agar}$) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing Cs concentrations up to 10 mM and K concentrations of either 2 mM (●) or 20 mM (○). All data represent means ± SE from at least eight replicate experiments. A logistic sigmoidal curve was fitted to the relationships between shoot FW versus $[Cs]_{agar}$. The parameters for these equations are given in Table I.

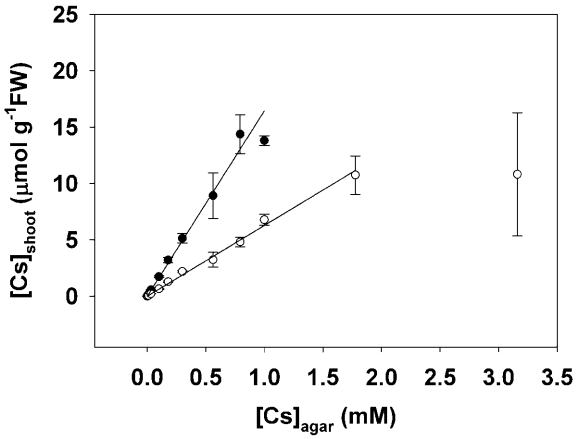

Figure 3. The relationship between the shoot Cs concentration ($[Cs]_{shoot}$) versus the Cs concentration in the agar ($[Cs]_{agar}$) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing Cs concentrations from 0.0003 to 10 mM and K concentrations of either 2 mM (●) or 20 mM (○). All data represent means ± SE from at least three replicate experiments. A linear regression was fitted to the relationship between $[Cs]_{shoot}$ versus $[Cs]_{agar}$. The parameters for all equations are given in Table I. Data for $[Cs]_{shoot}$ of plants with shoot FW less than 10% of the maximal were not included in these regressions.

Since $[Cs]_{shoot}$ increased (Fig. 3) and $[K]_{shoot}$ decreased (Fig. 4A) with increasing $[Cs]_{agar}$, it follows that the $[Cs]_{shoot}/[K]_{shoot}$ quotient increased as $[Cs]_{agar}$ increased (Fig. 4B). As expected, $[Cs]_{shoot}/[K]_{shoot}$ was greater in plants grown in the presence of 2 mM $[K]_{agar}$ than those grown in the presence of 20 mM $[K]_{agar}$ at the same $[Cs]_{agar}$. The $[Cs]_{shoot}$ approximated one-fortieth that of $[K]_{shoot}$ when grown in the presence of 0.2 mM $[Cs]_{agar}$ and 2 mM $[K]_{agar}$ and one-twentieth that of $[K]_{shoot}$ when grown in the presence of 2 mM $[Cs]_{agar}$ and 20 mM $[K]_{agar}$. The rate of increase in $[Cs]_{shoot}/[K]_{shoot}$ with increasing $[Cs]_{agar}$ was greater in plants grown with 2 mM $[K]_{agar}$ than those grown with 20 mM $[K]_{agar}$ (Fig. 4B). The relationship between $[Cs]_{shoot}/[K]_{shoot}$ versus $[Cs]_{agar}$ in the presence of 2 mM $[K]_{agar}$ also appeared to be biphasic, the rate of increase in $[Cs]_{shoot}/[K]_{shoot}$ with increasing $[Cs]_{agar}$ increasing abruptly at $[Cs]_{agar}$ greater than 0.5 mM. This might indicate a change in the complement of transporters contributing to the uptake of monovalent cations as $[Cs]_{agar}$ was increased, but other interpretations are possible.

Three Hypotheses to Explain Cs Toxicity in Arabidopsis

From the data presented above, three hypotheses might be considered to explain Cs toxicity in Arabidopsis. The first is that $[Cs]_{agar}$ reduces shoot FW because it causes K starvation by lowering $[K]_{shoot}$ (Fig. 4A). The second is that $[Cs]_{shoot}$ is toxic per se (Avery, 1995). The third is that Cs^+ competes with K^+ for essential biochemical functions, and Cs toxicity is related to the $[Cs]_{shoot}/[K]_{shoot}$ quotient. These hypotheses were tested.

Cs Toxicity Is Not Caused Solely by K Starvation

When assayed in the absence of Cs, a significant, positive, linear relationship between shoot FW and $[K]_{shoot}$ was observed (r^2 = 0.968; Fig. 1C). However, when plants were grown in the presence of Cs, positive linear relationships between shoot FW and $[K]_{shoot}$ were less significant (r^2 = 0.449 with 2 mM $[K]_{agar}$ and r^2 = 0.511 with 20 mM $[K]_{agar}$; Fig. 5). This implies that the reduction in shoot FW by toxic $[Cs]_{agar}$ is unlikely to be caused by K starvation alone.

The Relationship between Shoot Cs Concentration and Shoot FW Depends on Shoot K Concentration

Shoot FW decreased as $[Cs]_{shoot}$ increased in the presence of either 2 mM or 20 mM K (Fig. 6A). The exact

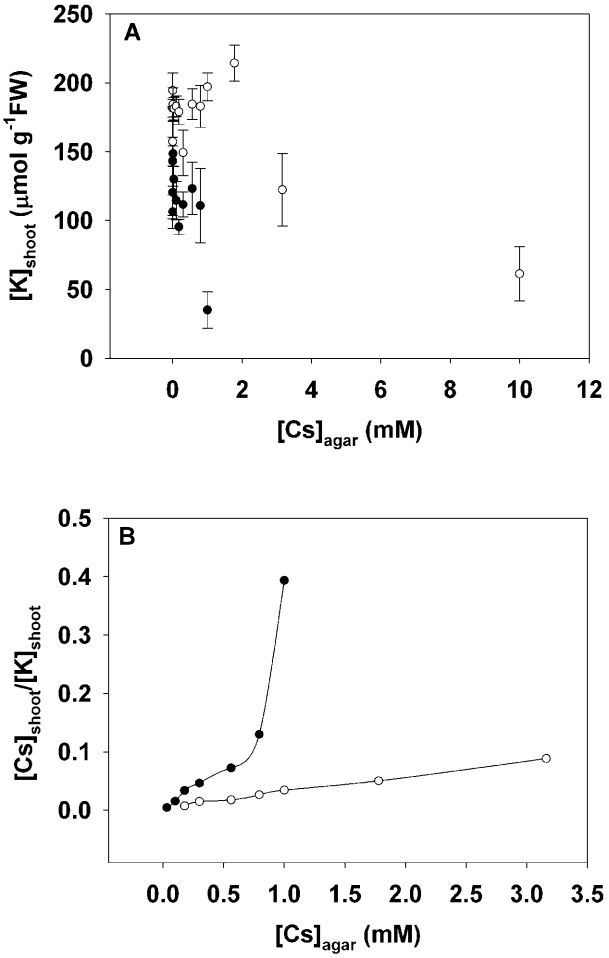

Figure 4. The relationships between the shoot K concentration ($[K]_{shoot}$) versus the Cs concentration in the agar ($[Cs]_{agar}$; A) and the quotient of Cs to K concentrations in the shoot ($[Cs]_{shoot}/[K]_{shoot}$) versus the Cs concentration in the agar ($[Cs]_{agar}$; B) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing up to 10 mM Cs and either 2 mM (●) or 20 mM (○) K. All data represent means ± SE from at least three replicate experiments. Data for $[K]_{shoot}$ below 20 μmol g^{-1} FW were excluded from this figure.

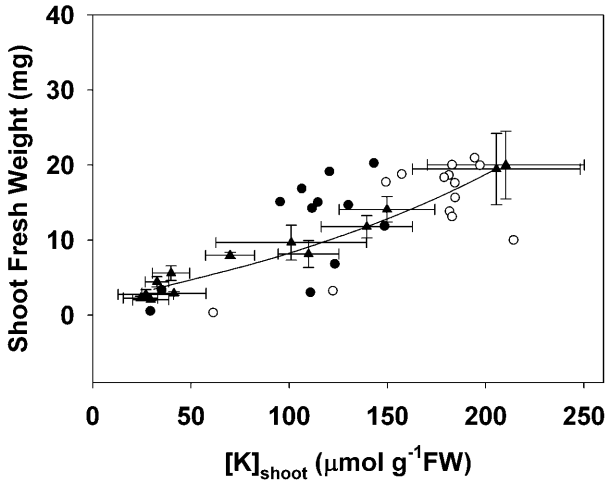

Figure 5. The relationship between shoot FW versus the K concentration in the shoot ($[K]_{shoot}$) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing between 0.0005 and 20 mM K in the absence of Cs (▲), or containing between 0.0003 and 10 mM Cs and 2 mM (●) or 20 mM (○) K. All data represent means from at least three replicate experiments. The SEs for data on plants grown in the absence of Cs are shown for comparison. The line represents the relationship between shoot FW and $[K]_{shoot}$ in the absence of Cs derived from the rectangular hyperbolae fitted to the relationships between shoot FW versus $[K]_{agar}$ and $[K]_{shoot}$ versus $[K]_{agar}$ (Fig. 1). Parameters for all fitted equations are given in Table I.

relationship between shoot FW and $[Cs]_{shoot}$ is derived from the logistic sigmoidal relationships between shoot FW versus $[Cs]_{agar}$ (Fig. 2) and the linear relationships between $[Cs]_{shoot}$ versus $[Cs]_{agar}$ (Fig. 3). It resembles a logistic sigmoidal curve. The shoot FWs of seedlings grown in the presence of 20 mM K were greater than those grown in the presence of 2 mM K at the same $[Cs]_{shoot}$ when plants were alive. This implies that the absolute shoot FW does not depend solely on $[Cs]_{shoot}$. The $[Cs]_{shoot}$ at which shoot FW was half-maximal (Ks_{50}), which is a measure of the tissue Cs tolerance of a plant, was lower for plants grown with 2 mM $[K]_{agar}$ than for plants grown with 20 mM $[K]_{agar}$. When grown in the presence of 2 mM $[K]_{agar}$, the Ks_{50} was 9,570 ± 285 μmol g^{-1} FW, and when grown in the presence of 20 mM $[K]_{agar}$, the Ks_{50} was 11,440 ± 352 μmol g^{-1} FW. The observation that plants grown at 20 mM $[K]_{agar}$ had a greater shoot FW at the same $[Cs]_{shoot}$ than those grown at 2 mM $[K]_{agar}$ may be related to a protective effect of increased $[K]_{shoot}$ (Figs. 1 and 4). Indeed, when shoot FW was expressed as a percentage of the value obtained in the absence of $[Cs]_{agar}$, the effect of $[Cs]_{shoot}$ on (maximal) shoot FW was similar irrespective of the $[K]_{agar}$ (Fig. 6B). This suggests that an interaction between Cs and K might determine shoot FW.

To investigate the effects of interactions between Cs and K on shoot FW, the relationship between shoot FW and the $[Cs]_{shoot}/[K]_{shoot}$ quotient was determined (Fig. 7). At a low $[Cs]_{shoot}/[K]_{shoot}$, the shoot FWs of plants grown in the presence of 20 mM K were greater than those grown in the presence of 2 mM K. This implies that when $[Cs]_{shoot}$ does not affect growth, shoot FW is determined primarily by $[K]_{shoot}$. However, the shoot FW of plants grown at 2 mM K or 20 mM K decreased with increasing $[Cs]_{shoot}/[K]_{shoot}$ with the same relationship when $[Cs]_{shoot}$ becomes toxic. This suggests that the $[Cs]_{shoot}/[K]_{shoot}$ quotient determines the reduction in shoot FW, probably because Cs^+ cannot replace K^+ in its biochemical functions and competes for its binding sites. Thus, Cs toxicity might be perceived by a plant cell as K deficiency.

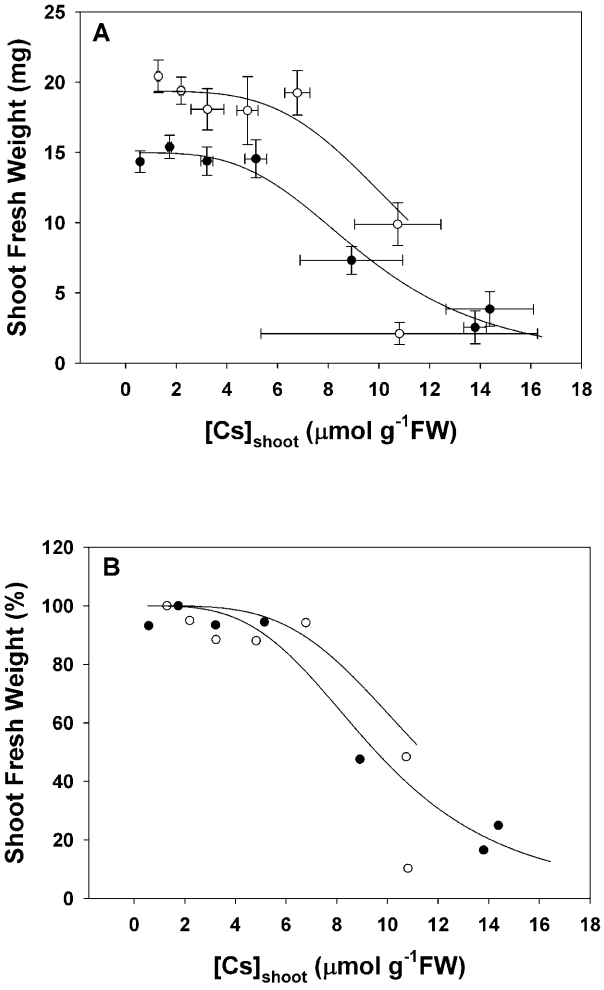

Figure 6. The relationships between shoot FW versus the Cs concentration in the shoot ($[Cs]_{shoot}$; A) and between shoot FW, expressed as a percentage of the value obtained in the absence of $[Cs]_{agar}$, versus $[Cs]_{shoot}$ (B) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing between 0.0003 and 10 mM Cs and 2 mM (●) or 20 mM (○) K. All data represent means ± SE from at least three replicate experiments. The line represents the relationship between shoot FW and $[Cs]_{shoot}$ derived from the logistic sigmoidal curve fitted to the relationships between shoot FW versus $[Cs]_{agar}$ (Fig. 2) and the linear regression fitted to the relationships between $[Cs]_{shoot}$ versus $[Cs]_{agar}$ (Fig. 3). Parameters for all fitted equations are given in Table I. Data for $[Cs]_{shoot}$ of plants with shoot FW less than 10% of the maximal were not plotted.

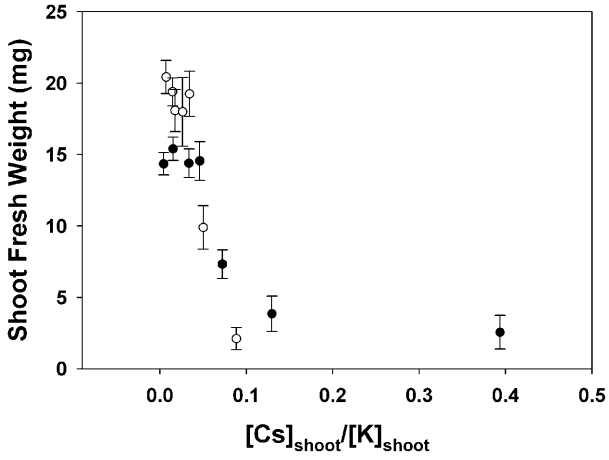

Figure 7. The relationship between shoot FW versus the quotient of Cs to K concentrations in the shoot ($[Cs]_{shoot}/[K]_{shoot}$) for Arabidopsis accession Ws2 grown for 21 d on mineral-replete agar containing between 0.0003 and 10 mM Cs and 2 mM (●) or 20 mM (○) K. All data represent means ± SE from at least three replicate experiments. Data for $[Cs]_{shoot}$ of plants with shoot FW less than 10% of the maximal and for $[K]_{shoot}$ below 20 μmol g^{-1} FW were not plotted.

Transcriptional Profiles of K-Replete, K-Starved, and Cs-Intoxicated Plants

To test the hypothesis that Cs toxicity might be perceived by a plant cell as K deficiency, the transcriptional profiles of K-replete, K-starved, and Cs-intoxicated plants were compared. It was assumed that if Cs intoxication were perceived as K deficiency, then the transcriptional profiles of these two treatments would be identical. However, it is obvious that the transcriptional profiles of Arabidopsis plants subjected to K starvation or Cs toxicity will change with the magnitude and/or duration of stress. Therefore, plants were harvested 7 d after the imposition of these stresses when tissue K concentration had declined (compare with Table I; Hammond et al., 2003) or tissue Cs concentration had become toxic (C.R. Hampton, unpublished data).

When compared with K-replete plants, the expression of 1,349 genes differed significantly ($P < 0.05$) in roots and the expression of 3,972 genes differed significantly ($P < 0.05$) in shoots of K-starved plants. The 50 statistically most significant changes in gene expression (based on *P* values) in K-starved roots and shoots are shown in Table II. It is noteworthy that many genes involved in defense responses, and also numerous transcription factors, are among these genes. In addition, the expression of the K-transporter gene *AtHAK5/AtPOT5* was increased 9-fold in Arabidopsis roots by K starvation (Table II). This transporter is likely to catalyze the uptake of both K^+ and Cs^+ (White et al., 2004). The expression of several genes encoding other transporters that might contribute to Cs^+ fluxes was also significantly greater ($P < 0.05$) in roots of K-starved plants than in roots of K-replete plants. These included *AtGLR1.2* and *AtGLR1.3*. However, no other *AtKUP*, *AtCNGC*, or *AtGLR* gene showed differential expression in roots of K-starved and K-replete plants. In shoots of K-starved plants, the expression of *AtHAK5/AtPOT5*, *AtKUP9*, *AtCNCG1*, *AtCNGC13*, *AtGLR1.2*, *AtGLR1.3*, and *AtGLR1.4* was significantly higher ($P < 0.05$) and the expression of *AtKUP2*, *AtKUP3*, *AtKUP5*, and *AtKUP8* was significantly lower ($P < 0.05$) than in shoots of K-replete plants.

Several, but not all, of the genes whose expression responded to K starvation showed similar changes in expression upon Cs intoxication. When compared with K-replete plants, the expression of 964 genes differed significantly ($P < 0.05$) in roots and the expression of 2,551 genes differed significantly in shoots ($P < 0.05$) of Cs-intoxicated plants. The expression of 22% (211) of the genes whose expression was altered ($P < 0.05$) in roots by Cs intoxication was also altered by K starvation. Assuming that the expression of 24,000 genes was being assayed on the ATH1 array, if the genetic responses to Cs intoxication and K starvation in roots were independent, only 54 differentially expressed genes would be expected to be common to both stresses [(964/24,000) × (1,349/24,000) × 24,000 = 54]. Thus, the transcriptional responses in Arabidopsis roots to both Cs intoxication and K starvation do not appear to be independent phenomena ($\chi^2_{1df} = 501$). The expression of 43% (1,098) of the genes whose expression was altered ($P < 0.05$) in shoots by Cs intoxication was also altered by K starvation. Again, if the genetic responses to Cs intoxication and K starvation in shoots were independent, only 422 differentially expressed genes would be expected to be common to both [(2,551/24,000) × (3,972/24,000) × 24,000 = 422]. Thus, the transcriptional responses in Arabidopsis shoots to both Cs intoxication and K starvation do not appear to be independent phenomena ($\chi^2_{1df} = 1{,}451$). These observations are consistent with Cs intoxication being perceived, in part, as K deficiency. Interestingly, four (At2g021020, At1g19610, At2g46600, and At2g46750) of the 50 statistically most significant changes in gene expression in Cs-intoxicated roots and two (At5g26340 and At1g65690) of the 50 statistically most significant changes in gene expression in Cs-intoxicated shoots were also in the 50 statistically most significant changes in gene expression upon K starvation (compare Tables II and III). The expression of several genes encoding transporters that might contribute to Cs^+ fluxes was increased significantly ($P < 0.05$) in Cs-intoxicated plants. The expression of genes encoding AtGLR1.2 and AtGLR1.3 was increased in roots of Cs-intoxicated plants. In shoots of Cs-intoxicated plants, the expression of *AtKUP6*, *AtCNCG1*, *AtCNGC11*, *AtCNGC12*, *AtCNGC13*, *AtCNGC20*, and *AtGLR1.3* was increased significantly ($P < 0.05$).

The Pharmacology of Cs Influx Changes with Plant K Status

The transcriptional profiling of Arabidopsis roots suggests that the complement of transport proteins

Table II. *The 50 most significant genes expressed differentially in shoots and roots of K-starved compared to K-replete plants*

In three replicate experiments, Arabidopsis (Ws2) were grown on MS-agar for 14 d before being transferred to a hydroponics system where plants were grown in MS-solutions containing or lacking K to generate K-replete or K-starved plants. Shoots and roots were harvested after a further 7 d growth and total RNA extracted. Total RNA was then used to probe Affymetrix Arabidopsis ATH1 (22K) GeneChips.

K Starved									
Shoots					Roots				
AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]	AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]
At3g60420	Expressed protein	1.37E-08	8.0	0.1	At1g12780	UDP-Glc 4-epimerase	1.95E-05	2.6	0.11
At4g18280	Gly-rich cell wall protein	2.79E-07	23.5	1.1	At3g12900	Oxidoreductase	5.45E-05	7.1	0.77
At1g16510	Auxin-induced protein family	3.70E-07	11.0	0.4	At2g46600	Calcium-binding protein	5.53E-05	2.4	0.11
At2g23120	Expressed protein	4.30E-07	7.7	0.3	At4g18950	Protein kinase	6.07E-05	2.6	0.14
At5g10695	Expressed protein	7.09E-07	4.2	0.1	At3g12820	Myb family transcription factor	6.11E-05	3.5	0.25
At3g27210	Expressed protein	8.07E-07	4.2	0.1	At5g38820	Amino acid transporter family	8.37E-05	2.5	0.14
At4g05020	NADH dehydrogenase	9.05E-07	5.8	0.2	At1g25560	AP2 domain transcription factor	9.64E-05	2.9	0.20
At2g41010	Expressed protein	1.11E-06	4.1	0.1	At1g16510	Auxin-induced protein family	1.33E-04	2.9	0.21
At1g01560	Mitogen-activated protein kinase (MPK11)	1.64E-06	4.3	0.1	At4g33666	Expressed protein	1.38E-04	3.3	0.27
At5g26340	Hexose transporter	1.86E-06	4.0	0.1	At4g19200	Gly/Pro-rich protein	1.40E-04	2.5	0.16
At1g78410	Expressed protein	1.88E-06	10.0	0.5	At1g09070	C2 domain-containing protein	1.56E-04	2.0	0.10
At5g19875	Expressed protein	1.98E-06	5.9	0.2	At4g13420	K transporter (HAK5/POT5)	1.95E-04	9.0	1.53
At1g43910	AAA-type ATPase family	2.08E-06	6.0	0.3	At5g06270	B-type cyclin	2.10E-04	2.1	0.12
At1g13340	Expressed protein	2.23E-06	7.0	0.1	At4g15610	Membrane protein	2.47E-04	2.1	0.12
At5g52750	Heavy-metal-associated domain-containing protein	2.23E-06	5.2	0.2	At4g22070	WRKY transcription factor 31 (WRKY31)	2.78E-04	2.0	0.12
At1g65690	Expressed protein	2.88E-06	8.2	0.5	At4g29190	Zinc finger transcription factor	2.84E-04	2.1	0.13
At5g18310	Expressed protein	2.99E-06	3.8	0.1	At1g23020	Ferric-chelate reductase	2.93E-04	2.2	0.14
At5g06320	Harpin-induced protein 1 family (NDR1/HIN1-like protein 3)	3.01E-06	3.6	0.1	At2g02120	Plant defensin protein (PDF2.1 family)	3.02E-04	2.6	0.22
At4g23880	Hypothetical protein	3.11E-06	4.8	0.2	At5g01720	F-box protein family (FBL3)	3.02E-04	2.7	0.22
At2g37970	Expressed protein	4.11E-06	4.8	0.2	At4g01250	WRKY family transcription factor	3.11E-04	2.7	0.22
At1g74020	Strictosidine synthase family	4.14E-06	5.3	0.3	At1g76410	RING zinc finger protein	3.35E-04	2.5	0.20
At2g17500	Auxin efflux carrier protein family	4.49E-06	5.0	0.2	At1g63720	Expressed protein	3.51E-04	2.3	0.17
At3g01830	Calmodulin-related protein	5.07E-06	14.6	0.5	At5g02780	In2-1 protein	3.55E-04	2.6	0.23
At5g17860	Cation exchanger (CAX7)	5.22E-06	6.6	0.4	At3g19580	Zinc finger protein	3.56E-04	2.0	0.12
At2g30550	Lipase (class 3) famil	5.93E-06	3.4	0.1	At2g46750	FAD-linked oxidoreductase family	3.65E-04	2.7	0.23
At1g21520	Expressed protein	6.54E-06	7.9	0.5	At2g30670	Short-chain dehydrogenase /reductase family protein	3.66E-04	2.2	0.16

(*Table continues on following page.*)

Table II. (*Continued from previous page.*)

K Starved									
Shoots					Roots				
AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]	AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]
At1g19020	Expressed protein	7.11E-06	8.6	0.6	At4g19810	Glycosyl hydrolase family 18	3.71E-04	1.8	0.10
At1g28370	Ethylene responsive element binding factor 11 (EREBP11)	7.90E-06	4.1	0.2	At1g80440	Kelch repeat containing F-box protein family	3.73E-04	2.4	0.19
At3g51860	Cation exchanger (CAX3)	7.96E-06	16.4	1.6	At5g22630	Prephenate dehydratase family	3.84E-04	1.9	0.10
At1g09070	C2 domain-containing protein	8.39E-06	5.6	0.3	At1g68840	AP2 domain protein RAP2.8 (RAV2)	3.84E-04	1.8	0.10
At1g75270	Dehydroascorbate reductase	9.70E-06	2.8	0.1	At1g80590	WRKY family transcription factor	4.21E-04	2.1	0.15
At1g18210	Calcium-binding protein	1.04E-05	5.3	0.3	At1g19610	Plant defensin protein (PDF1.4 family)	4.26E-04	2.3	0.18
At3g63380	Calcium-transporting ATPase	1.13E-05	7.8	0.3	At3g13610	Oxidoreductase	4.33E-04	1.9	0.12
At2g31980	Cys proteinase inhibitor B (cystatin B)	1.22E-05	6.4	0.4	At2g30660	3-Hydroxyisobutyryl-Coenzyme A hydrolase (CHY1)	4.46E-04	1.9	0.11
At1g75170	Sec14 cytosolic factor family	1.34E-05	3.7	0.2	At1g64660	Methionine/cystathionine gamma lyase	4.49E-04	2.4	0.20
At2g39710	Expressed protein	1.37E-05	3.2	0.1	At3g51860	Cation exchanger (CAX3)	4.56E-04	2.0	0.13
At3g48890	Cytochrome b5 domain-containing protein	1.46E-05	2.6	0.1	At5g05410	DRE-binding protein (DREB2A)	5.56E-04	2.2	0.17
At2g28710	C2H2-type zinc finger protein	1.48E-05	7.5	0.3	At5g59820	Zinc finger protein Zat12	5.56E-04	2.1	0.16
At3g44860	Methyltransferase-related	1.51E-05	4.1	0.2	At1g56160	Myb family transcription factor	6.46E-04	5.5	0.24
At3g47480	Calcium-binding EF-hand family protein	1.54E-05	11.8	1.2	At1g21000	Expressed protein	6.81E-04	1.8	0.11
At4g09030	Arabinogalactan-protein (AGP10)	1.56E-05	4.7	0.3	At4g23420	Oxidoreductase famly	6.90E-04	2.3	0.21
At3g46620	Expressed protein	1.65E-05	2.8	0.1	At3g45300	Isovaleryl-CoA-dehydrogenase precursor (IVD)	7.48E-04	1.8	0.11
At1g68620	Expressed protein	1.71E-05	6.7	0.3	At3g61930	Expressed protein	7.67E-04	4.0	0.61
At5g22630	Prephenate dehydratase family	1.76E-05	3.9	0.2	At3g11930	Ethylene-responsive protein	7.73E-04	1.7	0.10
At4g23610	Expressed protein	1.77E-05	4.4	0.3	At5g63600	Flavonol synthase	8.43E-04	1.7	0.10
At2g37750	Expressed protein	1.77E-05	19.2	0.2	At3g10980	Expressed protein	8.51E-04	1.7	0.10
At2g20145	Disease resistance protein (TIR class)	1.87E-05	4.6	0.1	At5g27920	F-box protein family	8.81E-04	2.1	0.18
At1g51780	Auxin conjugate hydrolase/IAA-amino acid hydrolase (ILL5)	1.89E-05	4.3	0.3	At2g18050	Histone H1	9.63E-04	2.6	0.29
At5g27920	F-box protein family	1.90E-05	3.4	0.2	At1g74760	Hypothetical protein	9.78E-04	2.2	0.21
At4g21830	Expressed protein	1.92E-05	18.5	0.2	At3g61890	Homeobox-Leu zipper protein ATHB-12	9.98E-04	2.6	0.28

[a]AGI numbers. [b]Brief descriptions of the transcript identified by BLAST searches. [c]Statistical significance (*P* value) of differential gene expression in plants starved of K. [d]Fold change of transcript abundance in plants starved of K, relative to K-replete plants. [e]The associated SE for that fold change (footnote d) based on three replicate experiments.

Table III. *The 50 most significant genes expressed differentially in shoots and roots of Cs-intoxicated compared to nonintoxicated plants*

In three replicate experiments, Arabidopsis (Ws2) were grown on MS-agar for 14 d before being transferred to a hydroponics system where plants were grown in MS-solutions lacking or containing 2 mM Cs to generate nonintoxicated or Cs-intoxicated plants. Shoots and roots were harvested after a further 7 d growth and total RNA extracted. Total RNA was then used to probe Affymetrix Arabidopsis ATH1 (22K) GeneChips.

Cs Intoxicated									
Shoots					Roots				
AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]	AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]
At4g11650	Osmotin-like protein (OSM34)	9.51E-06	4.6	0.11	At5g38910	Germin-like protein	4.15E-05	5.1	0.43
At2g04160	Subtilisin-like Ser protease AIR3	1.58E-05	2.5	0.09	At1g22890	Hypothetical protein	6.40E-05	2.3	0.11
At4g11280	1-Aminocyclopropane-1-Carboxylate synthase 6 (ACS6)	2.73E-05	2.4	0.09	At2g43570	Glycosyl hydrolase family 19 (chitinase)	2.17E-04	2.0	0.11
At2g39330	Jacalin lectin family	3.14E-05	2.6	0.12	At3g51600	Nonspecific lipid transfer protein 5 (LTP 5)	2.26E-04	2.7	0.21
At1g72830	CCAAT-binding factor B subunit	3.46E-05	2.3	0.09	At2g02120	Plant defensin protein (PDF2.1 family)	2.34E-04	2.0	0.11
At4g16660	Heat shock protein hsp70	4.74E-05	2.1	0.08	At4g17030	Expansin-related protein 1 precursor (At-EXPR1)	2.35E-04	2.3	0.15
At4g39830	L-Ascorbate oxidase	5.08E-05	2.4	0.12	At2g30000	Expressed protein	4.20E-04	1.9	0.11
At3g14990	Putative 4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein	6.73E-05	2.6	0.15	AtCg00750	Ribosomal protein S11	4.80E-04	2.1	0.15
At2g47800	Glutathione-conjugate transporter AtMRP4	7.53E-05	2.2	0.10	At2g02850	Plantacyanin	4.86E-04	1.8	0.11
At5g26920	Calmodulin-binding protein	8.25E-05	2.9	0.19	At1g19610	Plant defensin protein (PDF1.4 family)	5.07E-04	2.3	0.18
At5g54100	Expressed protein	9.36E-05	2.1	0.10	At5g44580	Expressed protein	5.11E-04	1.7	0.09
At3g10500	Expressed protein	1.09E-04	2.3	0.13	At5g09570	Expressed protein	5.80E-04	2.7	0.18
At3g03470	Cytochrome P450	1.12E-04	2.5	0.15	At3g09440	Heat shock protein hsc70-3	6.09E-04	1.8	0.11
At2g40140	CCCH-type zinc finger protein	1.22E-04	2.1	0.10	At1g32940	Subtilisin-like Ser protease	7.26E-04	1.7	0.10
At5g48180	Kelch repeats protein family	1.29E-04	2.2	0.12	At3g11580	DNA-binding protein	7.66E-04	1.6	0.09
At3g05970	AMP-binding protein	1.31E-04	1.9	0.08	At5g57230	Hypothetical protein	7.94E-04	1.7	0.09
At4g11850	Phospholipase D-gamma	1.45E-04	2.2	0.12	At3g47480	Calcium-binding EF-hand family protein	8.15E-04	2.8	0.33
At2g38470	WRKY transcription factor	1.47E-04	2.3	0.14	At4g32520	Gly hydroxymethyl transferase	8.28E-04	1.6	0.09
At3g06860	Fatty acid multifunctional protein (AtMFP2)	1.56E-04	1.9	0.09	At1g74590	Glutathione transferase	9.55E-04	1.9	0.14
At4g02940	Oxidoreductase	1.57E-04	2.0	0.11	At2g46680	Homeobox-Leu zipper protein ATHB-7	9.69E-04	1.8	0.11
At4g34135	Glucosyltransferase	1.77E-04	2.4	0.16	At5g26340	Hexose transporter	9.77E-04	1.7	0.10
At3g09350	Expressed protein	1.82E-04	2.5	0.17	At1g76520	Auxin efflux carrier protein	9.99E-04	2.3	0.22
At5g13750	Transporter-related protein	1.82E-04	2.6	0.18	At2g29530	Small zinc finger-related protein (TIM10)	0.001196	1.6	0.09
At1g77920	bZIP transcription factor, bZIP50	1.84E-04	2.3	0.14	At1g72890	Disease resistance protein (TIR-NBS class)	0.001209	2.0	0.16
At2g14610	Pathogenesis-related protein 1 (PR-1)	1.88E-04	2.5	0.17	At3g55090	ABC transporter	0.001297	2.3	0.25
At4g36640	Sec14 cytosolic factor family	1.93E-04	2.2	0.13	At4g29340	Profilin 3	0.001311	1.6	0.09

(*Table continues on following page.*)

Table III. (*Continued from previous page.*)

Cs Intoxicated									
Shoots					Roots				
AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]	AGI[a]	Name[b]	*P* Value[c]	Fold Change[d]	SE[e]
At2g16060	Class 1 nonsymbiotic hemoglobin (AHB1)	2.03E-04	2.3	0.14	At4g30270	Xyloglucan endotransglycosylase (meri5B)	0.001365	1.8	0.13
At4g37640	Calcium-transporting ATPase 2	2.19E-04	1.9	0.09	At2g22430	Homeobox-Leu zipper protein ATHB-6	0.001383	1.7	0.11
At5g54840	SGP1 monomeric G-protein	2.20E-04	2.0	0.11	At3g47780	ABC transporter	0.001422	2.6	0.31
At5g41790	Myosin heavy chain-related protein	2.27E-04	2.0	0.11	At3g49120	Peroxidase	0.001545	2.8	0.38
At2g38860	(YLS5) proteasel (pfpI)-like protein	2.34E-04	2.5	0.18	At2g46600	Calcium-binding protein	0.001563	1.6	0.09
At2g18680	Expressed protein	2.39E-04	2.6	0.12	At3g55740	Pro transporter 2 (ProT2)	0.001595	1.8	0.15
At1g56120	Wall-associated kinase 2	2.50E-04	2.6	0.21	At3g29575	Expressed protein	0.001601	1.6	0.10
At1g65690	Expressed protein	2.63E-04	4.3	0.51	At4g34710	Arg decarboxylase SPE2	0.001605	2.6	0.34
At3g18830	Mannitol transporter	2.66E-04	2.2	0.15	At3g28510	Expressed protein	0.001608	2.5	0.30
At4g34200	D-3-Phosphoglycerate dehydrogenase	2.70E-04	2.2	0.14	At3g09390	Metallothionein-related protein	0.00165	2.5	0.30
At2g46680	Homeobox-Leu zipper protein (ATHB-7)	2.75E-04	3.5	0.37	At1g13930	Expressed protein	0.001665	1.6	0.09
At3g13940	Expressed protein	2.78E-04	1.9	0.10	At3g47470	Light-harvesting chlorophyll a/b-binding protein (CAB-4)	0.001716	3.0	0.44
At4g12280	Copper amine oxidase like protein	3.20E-04	2.5	0.20	At2g25510	Expressed protein	0.001775	3.8	0.70
At3g52430	Phytoalexin-deficient 4 protein (pad4)	3.27E-04	1.9	0.11	At4g31470	Pathogenesis-related protein	0.001962	1.6	0.11
At3g50910	Expressed protein	3.29E-04	2.1	0.14	At1g68620	Expressed protein	0.002074	2.1	0.22
At5g42050	Expressed protein	3.32E-04	2.1	0.14	At2g18690	Expressed protein	0.002099	2.4	0.31
At1g71330	ABC transporter	3.34E-04	3.7	0.42	At5g41280	Hypothetical protein	0.002135	1.7	0.12
At2g17040	No apical meristem (NAM) protein family	3.38E-04	2.0	0.12	At2g46750	FAD-linked oxidoreductase family	0.002152	2.0	0.20
At3g09440	Heat shock protein hsc70-3	3.40E-04	2.0	0.12	At3g17030	Expressed protein	0.002168	1.5	0.09
At5g26340	Hexose transporter	3.41E-04	3.7	0.43	At3g25830	Myrcene/ocimene synthase	0.002198	1.6	0.11
At4g31500	Cytochrome P450 83B1	3.43E-04	1.7	0.08	At2g38210	SOR1-related	0.002274	1.8	0.15
At5g62620	Galactosyltransferase family	3.61E-04	1.9	0.10	At1g64600	Expressed protein	0.002305	1.7	0.12
At1g12650	Expressed protein	3.71E-04	2.7	0.15	At5g16010	3-Oxo-5-alpha-steroid 4-dehydrogenase	0.002306	2.6	0.36
At3g08720	Ribosomal-protein S6 kinase (ATPK19)	3.78E-04	2.4	0.18	At5g57300	UbiE/COQ5 methyltransferase family	0.002317	1.6	0.11

[a]AGI numbers. [b]Brief descriptions of the transcript identified by BLAST searches. [c]Statistical significance (*P* value) of differential gene expression in Cs-intoxicated plants. [d]Fold change of transcript abundance in Cs-intoxicated plants, relative to nonintoxicated plants. [e]The associated SE for that fold change (footnote d) based on three replicate experiments.

able to catalyze Cs^+ influx to plants changes with plant K-status. In K-replete plants, Cs influx is likely to be dominated by VICCs, such as those encoded by *CNGC*s and *GLR*s (White and Broadley, 2000). However, in K-deficient plants, KUPs may contribute significantly to Cs^+ influx (Table II; White et al., 2004). To test this hypothesis, advantage can be taken of the different pharmacologies of VICCs and KUPs. VICCs are inhibited by Ca^{2+} and lanthanides (White, 1997; White and Broadley, 2000), whereas high-affinity

Table IV. *Effects of 1 mM K channel and transport inhibitors on Cs influx from a solution containing 50 μM CsCl*

Arabidopsis were grown previously in solutions containing high (2 mM), medium (100 μM), or low (0.5 μM) K concentrations.Cs influx is expressed as a percentage of that observed in the absence of inhibitors, which was 0.34 ± 0.069, 0.46 ± 0.040, 0.26 ± 0.026 μmol g^{-1} FW root for Arabidopsis grown with 0.5 μM, 100 μM, and 2 mM K, respectively. The K concentrations in the roots and shoots of Arabidopsis grown with 0.5 μM K were 5.4 ± 1.75 and 1.1 ± 0.27 μmol g^{-1} dry weight (DW), respectively. The K concentrations in the roots and shoots of Arabidopsis grown with 100 μM K were 4.8 ± 1.91 and 1.4 ± 0.07 μmol g^{-1} DW, respectively. The K concentrations in the roots and shoots of Arabidopsis grown with 2 mM K were 9.1 ± 3.37 and 3.0 ± 0.75 μmol g^{-1} DW, respectively. All data are expressed as mean ± SE ($n = 5$ experiments).

Inhibitor	Relative Influx (%)		
	Low K (0.5 μM)	Medium (100 μM)	High (2 mM)
TEA	118 ± 11	63 ± 14	89 ± 12
Ammonium	66 ± 5	57 ± 7	93 ± 35
Calcium	94 ± 21	104 ± 38	37 ± 6
Gadolinium	60 ± 7	61 ± 24	46 ± 12

K^+ transporters, such as KUPs, appear to be inhibited by ammonium (Spalding et al., 1999). As observed previously (Broadley et al., 2001), Cs influx to K-replete Arabidopsis was inhibited by Ca^{2+} and Gd^{3+} but not by tetraethylammonium (TEA^+) or ammonium (Table IV). By contrast, Cs influx to K-starved Arabidopsis was inhibited by ammonium and Gd^{3+} but not by Ca^{2+} or TEA^+. These changes in the pharmacology of Cs^+ influx, as well as the larger Cs influx to K-deficient plants than into K-replete plants (Table IV, legend), are consistent with the increased expression of *AtKUP*s encoding H^+/Cs^+ symporters in plants starved of K (Table IV).

DISCUSSION

Cs is toxic to Arabidopsis. The shoot FW of plants grown on agar decreased when $[Cs]_{agar}$ was increased above 0.3 mM in the presence of 2 mM $[K]_{agar}$ and when $[Cs]_{agar}$ was increased above 1 mM in the presence of 20 mM $[K]_{agar}$. However, since the K concentration in the soil solution is commonly in the millimolar range (Marschner, 1995) and the Cs concentration in the soil solution is generally in the low micromolar range (White and Broadley, 2000), Cs is unlikely to cause environmental toxicity in most natural environments.

The relationship between shoot FW and $[Cs]_{agar}$ fitted the equation of a logistic sigmoidal curve. The $[Cs]_{agar}$ at which shoot FW was one-half its maximal value (Ka_{50}) was greater when plants were grown at higher $[K]_{agar}$ (Table I). This might be explained if increasing $[K]_{agar}$ reduced the entry of Cs to plants and/or increased K within the plant, which protected it against cellular Cs toxicity. It was observed that increasing $[K]_{agar}$ lowered $[Cs]_{shoot}$ (Fig. 3). Thus, one mechanism whereby increasing rhizosphere K protects plants from Cs in the environment is by reducing their Cs uptake. This observation is consistent with previous studies showing that increasing rhizosphere K reduces Cs uptake (Sutcliffe, 1957; Bange and Overstreet, 1960; Handley and Overstreet, 1961) and accumulation (Menzel, 1954; Cline and Hungate, 1960; Nishita et al., 1960, 1962; Jackson et al., 1965; Jackson and Nisbet, 1990; Shaw and Bell, 1991; Shaw et al., 1992; Belli et al., 1995; Smolders et al., 1996a, 1996b; Zhu et al., 1999) by plants and provides a rationale for using K fertilization as a countermeasure in radiocesium-contaminated soils (Konoplev et al., 1993; Prister et al., 1993; Zhu et al., 2000; White et al., 2003). It was also observed that increasing $[K]_{agar}$ in the presence of Cs also increased $[K]_{shoot}$ (Fig. 4), and it is possible that this increased $[K]_{shoot}$ protected the plant against cellular Cs toxicity.

We considered three hypotheses to explain cellular Cs toxicity in Arabidopsis. These were: (1) increasing $[Cs]_{agar}$ reduced shoot FW because it inhibited K^+ uptake and caused K starvation; (2) $[Cs]_{shoot}$ was toxic per se; and (3) Cs^+ competed with K^+ for essential biochemical functions and, therefore, Cs toxicity was related to the $[Cs]_{shoot}/[K]_{shoot}$ quotient. Increasing $[Cs]_{agar}$ reduced $[K]_{shoot}$ (Fig. 4A). This is consistent with previous studies showing that increasing rhizosphere Cs reduces K uptake and accumulation in plants (Sutcliffe, 1957; Nishita et al., 1960; Maathuis and Sanders, 1996). However, increasing $[Cs]_{agar}$ also increased $[Cs]_{shoot}$ (Fig. 3) and the $[Cs]_{shoot}/[K]_{shoot}$ quotient (Fig. 4B). To differentiate between the three hypotheses to explain cellular Cs toxicity, we examined the relationships between shoot FW and $[K]_{shoot}$ (Fig. 5), $[Cs]_{shoot}$ (Fig. 6), or $[Cs]_{shoot}/[K]_{shoot}$ (Fig. 7) in detail. Since the relationships between shoot FW and $[K]_{shoot}$ obtained in the presence of Cs did not follow the same positive linear relationship between shoot FW and $[K]_{shoot}$ observed in the absence of Cs, it was concluded that Cs toxicity was unlikely to be caused by K starvation alone (Fig. 5), which is consistent with the different transcriptional profiles of K-starved and Cs-intoxicated plants (Tables II and III). The observation that the shoot FWs of seedlings grown in the presence of 20 mM K were greater than those grown in the presence of 2 mM K with the same $[Cs]_{shoot}$ (Fig. 6) suggested that Cs toxicity was not the result of $[Cs]_{shoot}$ per se but might be related to the $[Cs]_{shoot}/[K]_{shoot}$ quotient. The relationship between shoot FW and $[Cs]_{shoot}/[K]_{shoot}$ (Fig. 7) suggested that, at a nontoxic $[Cs]_{shoot}$, plant growth was determined by $[K]_{shoot}$ but that the growth of Cs-intoxicated plants was related to the $[Cs]_{shoot}/[K]_{shoot}$ quotient. This is consistent with Cs intoxication resulting from competition between K^+ and Cs^+ for binding sites on essential K^+-activated proteins, as proposed by Avery (1995).

The transcriptional profiles of K-replete, K-starved, and Cs-intoxicated plants differed. In particular, the expression of several genes encoding proteins that might catalyze Cs^+ transport across cell membranes

was altered. In K-replete plants, Cs^+ appears to be taken up largely by VICCs (White and Broadley, 2000; Broadley et al., 2001; White et al., 2003). However, when Arabidopsis are starved of K^+, the expression of several *AtKUP* genes, such as those encoding AtKUP3 and AtHAK5 (Table II; Kim et al., 1998; Maathuis et al., 2003), is increased. These AtKUPs are able to transport Cs^+ across the plasma membrane of root cells (Rubio et al., 2000; White et al., 2004), and an increase in their expression may account for the increased Cs^+ influx capacity and distinct pharmacology of Cs^+ influx in K-starved plants (Table IV). In *Escherichia coli* Cs intoxication up-regulates a high-affinity K^+ transport operon (Jung et al., 2001). Although Cs intoxication does not induce a significant increase in the expression of *AtKUP*s in roots, it is noteworthy that the expression of two *AtGLR* genes (*AtGLR1.2* and *AtGLR1.3*) was increased significantly in roots of both K-starved and Cs-intoxicated plants. It is unfortunate that the expression of genes encoding putative Cs transporters is increased in roots of Cs-intoxicated plants because this might augment the uptake of Cs leading to inexorable toxicity. An increase in the expression of genes encoding monovalent cation transporters in Cs-intoxicated plants could explain why the addition of stable Cs increased the uptake of ^{137}Cs and K in bean plants (Nishita et al., 1962; Wallace et al., 1982) and the greater $[K]_{shoot}$ in plants grown at low $[Cs]_{agar}$ (Fig. 4A).

Since the Chernobyl accident of 1986, a variety of agricultural countermeasures have been implemented to reduce the entry of radiocesium into the food chain, and this has been the most effective means of reducing the total radiation dose to the population (Alexakhin, 1993). In a recent international review of 130 countermeasures for managing radiological contamination, selective crop breeding was one of only six countermeasures considered worthy of further exploration (http://www.strategy-ec.org.uk/). The development of plants that accumulate large amounts of Cs, for phytoremediation purposes, and Cs-excluding "safe" crops is high on the agenda (White et al., 2003; Payne et al., 2004). Knowledge of the mechanisms of Cs accumulation, the interactions between Cs and K nutrition, and the causes of Cs toxicity, will inform the development of these plant phenotypes and also assist in the management of radiocesium-contaminated land. For example, our data indicate that plants with decreased VICC activity would not exclude significant amounts of Cs in K-deficient soils, just as plants with decreased KUP activity would not exclude Cs if grown on K-replete soils. Thus, the genetic responses of plants to the mineral environment should be considered when selecting crops for a particular purpose.

MATERIALS AND METHODS

Plant Material

Seeds of Arabidopsis (*Arabidopsis thaliana*) L. Heynh. accession Wassilewskija (Ws2; N1601) were obtained from the Nottingham Arabidopsis Stock Centre (NASC, Nottingham, UK). Seeds were washed in 70% (v/v) ethanol/water, rinsed in distilled water, and surface sterilized using NaOCl (1% active chlorine). Seeds were rinsed again and imbibed for 3 to 6 d in sterile distilled water at 4°C to break dormancy. Following imbibation, seeds were sown into 10 cm (length) × 10 cm (width) × 9.5 cm (depth), unvented, polycarbonate culture boxes (Sigma-Aldrich, Gillingham, UK). For the analysis of plant growth and cation content, six seeds were sown directly on 75 mL of 0.8% (w/v) sterile agar medium (Murashige and Skoog [MS] agar) containing 1% (w/v) Suc and a basal salt mix at 10% of the full-strength MS formulation (Murashige and Skoog, 1962), unless the Ca, K, Cs, or Cl concentration was varied. For the transcriptional profiling and radiocesium influx experiments, seeds were sown on perforated polycarbonate discs (diameter 91 mm, thickness 5 mm) placed over 75 mL 10% MS agar. Roots grew into the agar, but shoots remained on the opposite side of the disc. Boxes were placed in a growth room set to 24°C with 16 h light/d. Illumination was provided by a bank of 100 W 84 fluorescent tubes (Philips, Eindhoven, The Netherlands) giving a photon flux density of 45 μmol photons m^{-2} s^{-1} at plant height. Shoots were harvested 21 d after sowing for the analysis of plant growth and cation content or transferred on their polycarbonate discs to a hydroponics system situated in a Saxcil growth cabinet (S.K. Saxton, ARC Works, Cheshire, UK) after 14 d for transcriptional profiling or after 7 d for radiocesium influx experiments. In the hydroponics system, plants were supported on polycarbonate discs over 450 mL of aerated nutrient solution in a light-proof 500-mL plastic beaker. The Saxcil cabinet provided a constant temperature of 22°C and constant illumination, provided by a bank of OSRAM L 58 W/23 fluorescent tubes giving a photon flux density between 400 and 700 nm of 75 μmol photons m^{-2} s^{-1} at plant height. The relative humidity was approximately 80%. Transcriptional profiling and radiocesium influx experiments were performed on plants 21 d after germination.

Analysis of Plant Growth and Cation Content

The effect of K concentration in the agar ($[K]_{agar}$) on shoot FW and shoot K concentration ($[K]_{shoot}$) was determined in the absence and presence of Cs. In the first experiment, plants were grown on agar containing 0.5, 1, 3, 10, 30, 100, 300, 1,000, 2,000, 10,000, and 20,000 μM K. The $[K]_{agar}$ in 10% MS agar was 2 mM. To raise $[K]_{agar}$ above 2 mM, KCl was added. To reduce $[K]_{agar}$ below 2 mM, KNO_3 and KH_2PO_4 were replaced with $Ca(NO_3)_2$ and $Ca(H_2PO_4)_2$. In the second experiment, the effect of Cs concentration in the agar ($[Cs]_{agar}$) on shoot FW, shoot Cs concentration ($[Cs]_{shoot}$), and $[K]_{shoot}$ was determined in the presence of 2 mM and 20 mM K. The $[Cs]_{agar}$ was raised to 0.3, 1, 10, 100, 178, 300, 562, 794, 1,000, 1,778, 3,162, and 10,000 μM using CsCl, and the $[K]_{agar}$ was raised from 2 mM to 20 mM using KCl. The shoot FW of individual plants was determined and six shoots were bulked to determine $[K]_{shoot}$ by inductively coupled plasma optical emission spectrometry (J Y Horiba Ultima 2 ICP-OES, Jobin Yvon, Middlesex, UK). Different plants were used to determine $[K]_{shoot}$ and $[Cs]_{shoot}$. The radioisotope ^{134}Cs (Radioisotope Centre Polatom, Świerk, Poland) was used to quantify $[Cs]_{shoot}$. Plants on which $[Cs]_{shoot}$ was estimated were grown on agar spiked with ^{134}Cs at an activity of 9.165 kBq L^{-1} (4 pM ^{134}Cs). Six shoots were bulked, and their ^{134}Cs content was determined using a Wallac 1480, Wizard gamma counter (Perkin-Elmer Life Sciences, Turku, Finland).

Transcriptional Profiling

Transcriptional profiling was performed on shoot and root tissues. Plants were transferred from 10% MS agar to hydroponics 14 d after germination. In the hydroponics system, plants were fed one of three nutrient solutions (pH 5.6): (1) a basal salt mix at 10% of the full-strength MS formulation (MS solution containing 2 mM K^+) to produce K-replete plants; (2) an MS solution containing only 0.5 μM K^+, in which K salts were replaced by Ca salts, to produce K-starved plants; or (3) an MS solution plus 2 mM $CsCl_2$ to produce Cs-intoxicated plants. Plants were grown in these solutions for 7 d before plants were harvested. At each harvest, shoot and root material from 20 to 30 plants from each treatment were bulked into 1.5-mL colorless, sterile, screw-cap polypropylene tubes and snap-frozen in liquid nitrogen. Tissue samples were stored at −70°C prior to the extraction of total RNA.

Total RNA was extracted following the addition of 1 mL TRIzol reagent to tissue samples placed in liquid nitrogen according to the manufacturer's instructions (Invitrogen Life Technologies, Paisley, UK) but with the following modifications (Hammond et al., 2003): (1) After homogenization with the TRIzol reagent, the samples were centrifuged to remove any remaining plant

material. The supernatant was then transferred to a clean Eppendorf tube. (2) To aid precipitation of RNA from the aqueous phase, 0.25 mL of isopropanol and 0.25 mL of a 1.2 M NaCl solution containing 0.8 M sodium citrate were added. Samples of total RNA were purified using a Qiagen RNeasy column (Qiagen, Crawley, UK). Samples of total RNA were then sent to NASC for labeling and hybridization to Affymetrix Arabidopsis ATH1 GeneChips (Affymetrix, Santa Clara, CA). The complete set of microarray data is available from NASC (http://affymetrix.arabidopsis.info/narrays/experimentbrowse.pl).

Signal and detection call values were generated by Affymetrix Microarray Analysis Suite 5.0 software (Affymetrix). Raw data were normalized and analyzed using GeneSpring version 6.1 (Silicon Genetics, Redwood City, CA). Signal values below 20 were set to 20 to limit the number of false positive results (Hammond et al., 2003). For each sample, each signal value was normalized by dividing it by the median of all signal values above 20 of genes classified as present or marginal in that sample. To determine the transcripts that were differentially expressed in K-starved and Cs-intoxicated plants, the normalized signal value for each transcript in K-starved and Cs-intoxicated plants was divided by the normalized signal value for that transcript in the corresponding sample from K-replete plants. A cross gene error model was used in the interpretation of the data, and data were filtered using a maximum confidence level of 5% for genes whose expression was significantly different from one. The annotation of filtered genes was confirmed by cross-referencing the Arabidopsis Genome Initiative (AGI) numbers given by Affymetrix with GenBank. In addition, the target nucleotide sequences used by Affymetrix to design probes on the ATH1 GeneChip (available from http://www.affymetrix.com) were used to BLAST the GenBank sequence database to confirm the fidelity of each probe (http://www.ncbi.nlm.nih.gov/BLAST/). A similar confirmation of probe fidelity was undertaken independently by Ghassemian et al. (2001).

Radiocesium Influx Experiments

Plants used for radiocesium influx experiments were transferred from 10% MS agar to hydroponics 7 d after germination. In the hydroponics system, plants were initially fed MS solution for 7 d. Plants were then transferred for a further 7 d to one of three solutions: (1) MS solution (2 mM K), to produce K-replete plants; (2) an MS solution containing only 100 μM K^+; or (3) an MS solution containing only 0.5 μM K^+, to produce K-starved plants. In solutions (2) and (3), K salts were replaced by Ca salts. Shoots and roots of representative plants grown in each solution were harvested and weighed and their K content determined using ICP-OES. Cs influx experiments were then performed as described by Broadley et al. (2001). Briefly, polycarbonate discs supporting Arabidopsis were placed over 455 mL of an aerated, single salt solution containing 50 μM CsCl radiolabeled with 104 kBq L^{-1} ^{134}CsCl with or without 1 mM TEACl, NH_4Cl, $CaCl_2$, or $GdCl_3$. After 20 min, plants were transferred to 450 mL of a solution containing 50 μM CsCl plus 1 mM $CaCl_2$ for 2 min to remove ^{134}Cs from the root apoplast. Plant roots were blotted with tissue paper, and the shoots and roots of individual plants were separated and weighed and their Cs contents estimated from ^{134}Cs activities determined using a gamma counter.

Statistics

Each experiment described was repeated at least three times. Data were fitted to a variety of equations using GenStat for Windows (sixth edition, release 6.1.0.200, VSN International, Oxford). The significance of differences in parameters for contrasting experimental treatments was tested using parallel regression analysis. The significance of commonalities in transcriptional profiles was assessed using chi-squared analysis of the two-way contingency table.

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LITERATURE CITED

Ahn SJ, Shin R, Schachtman DP (2004) Expression of *KT/KUP* genes in Arabidopsis and the role of root hairs in K^+ uptake. Plant Physiol **134:** 1135–1145

Alexakhin RM (1993) Countermeasures in agricultural production as an effective means of mitigating the radiological consequences of the Chernobyl accident. Sci Total Environ **137:** 9–20

Avery SV (1995) Caesium accumulation by microorganisms: uptake mechanisms, cation compartmentalization and toxicity. J Ind Microbiol **14:** 76–84

Bange GGJ, Overstreet R (1960) Some observations on absorption of cesium by excised barley roots. Plant Physiol **35:** 605–608

Belli M, Sansone U, Ardiani R, Feoli E, Scimone M, Menegon S, Parente G (1995) The effect of fertilizer applications on ^{137}Cs uptake by different plant species and vegetation types. J Environ Radioact **27:** 75–89

Broadley MR, Escobar-Gutiérrez AJ, Bowen HC, Willey NJ, White PJ (2001) Influx and accumulation of Cs^+ by the *akt1* mutant of *Arabidopsis thaliana* (L.) Heynh. lacking a dominant K^+ transport system. J Exp Bot **52:** 839–844

Cline JF, Hungate FP (1960) Accumulation of potassium, cesium^{137} and rubidium^{86} in bean plants grown in nutrient solutions. Plant Physiol **35:** 826–829

Demidchik V, Davenport RJ, Tester M (2002) Nonselective cation channels in plants. Annu Rev Plant Biol **53:** 67–107

Epstein E (1972) Mineral Nutrition of Plants: Principles and Perspectives. John Wiley & Sons, New York

Ghassemian M, Waner D, Tchieu J, Gribskov M, Schroeder JI (2001) An integrated *Arabidopsis* annotation database for Affymetrix Genechip® data analysis, and tools for regulatory motif searches. Trends Plant Sci **6:** 448–449

Hammond JP, Bennett MJ, Bowen HC, Broadley MR, Eastwood DC, May ST, Rahn C, Swarup R, Woolaway KE, White PJ (2003) Changes in gene expression in Arabidopsis shoots during phosphate starvation and the potential for developing smart plants. Plant Physiol **132:** 578–596

Handley R, Overstreet R (1961) Effect of various cations upon absorption of carrier-free cesium. Plant Physiol **36:** 66–69

Hasegawa H (1996) Selection for mutants with low nitrate uptake ability in rice (*Oryza sativa*). Physiol Plant **96:** 199–204

Jackson D, Nisbet AF (1990) The effect of fertiliser treatment, soil pH and grazing on the transfer of radiocaesium to upland fell vegetation. *In* G Desmet, P Nassimbeni, M Belli, eds, Transfer of Radionuclides in Natural and Semi-Natural Environments. Elsevier Applied Science, London, pp 395–402

Jackson WA, Craig D, Hector ML (1965) Effects of various cations on cesium uptake from soils and clay suspensions. Soil Sci **99:** 345–353

Jung K, Krabusch M, Altendorf K (2001) Cs^+ induces the *kdp* operon of *Escherichia coli* by lowering the intracellular K^+ concentration. J Bacteriol **183:** 3800–3803

Kim EJ, Kwak JM, Uozumi N, Schroeder JI (1998) *AtKUP1*: an Arabidopsis gene encoding high-affinity potassium transport activity. Plant Cell **10:** 51–62

Konoplev AV, Viktorova NV, Virchenko EP, Popov VE, Bulgakov AA, Desmet GM (1993) Influence of agricultural countermeasures on the ratio of different chemical forms of radionuclides in soil solution. Sci Total Environ **137:** 147–162

Kordan HA (1987) Reversal of caesium inhibition of growth by potassium in hypocotyls of tomato seedlings (*Lycopersicon esculentum* L.). New Phytol **107:** 395–401

Leigh RA, Wyn Jones RG (1984) An hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol **97:** 1–13

Maathuis FJM, Filatov V, Herzyk P, Krijger GC, Axelsen KB, Chen S, Green BJ, Li Y, Madagan KL, Sanchez-Fernandez R, et al (2003) Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. Plant J **35:** 675–692

Maathuis FJM, Sanders D (1996) Characterization of *csi52*, a Cs^+ resistant mutant of *Arabidopsis thaliana* altered in K^+ transport. Plant J **10:** 579–589

Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press, London

Menzel RG (1954) Competitive uptake by plants of potassium, rubidium, cesium, and calcium, strontium, barium from soils. Soil Sci **77:** 419–425

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Nishita H, Dixon D, Larson KH (1962) Accumulation of Cs and K and growth of bean plants in nutrient solution and soils. Plant Soil **17:** 221–242

Nishita H, Romney EM, Alexander GV, Larson KH (1960) Influence of K and Cs on release of Cs137 from three soils. Soil Sci **89:** 167–176

Payne KA, Bowen HC, Hammond JP, Hampton CR, Lynn JR, Mead A, Swarup K, Bennett MJ, White PJ, Broadley MR (2004) Natural genetic variation in caesium (Cs) accumulation by *Arabidopsis thaliana*. New Phytol **162:** 535–548

Prister BS, Perepelyatnikov GP, Perepelyatnikova LV (1993) Countermeasures used in the Ukraine to produce forage and animal foodproducts with radionuclide levels below intervention limits after the Chernobyl accident. Sci Total Environ **137:** 183–198

Rubio F, Santa-Maria GE, Rodríguez-Navarro A (2000) Cloning of *Arabidopsis* and barley cDNAs encoding HAK potassium transporters in root and shoot cells. Physiol Plant **109:** 34–43

Shaw G, Bell JNB (1991) Competitive effects of potassium and ammonium on caesium uptake kinetics in wheat. J Environ Radioact **13:** 283–296

Shaw G, Hewamanna R, Lillywhite J, Bell JNB (1992) Radiocaesium uptake and translocation in wheat with reference to the transfer factor concept and ion competition effects. J Environ Radioact **16:** 167–180

Sheahan JJ, Ribeiro-Neto L, Sussman MR (1993) Cesium-insensitive mutants of *Arabidopsis thaliana*. Plant J **3:** 647–656

Smolders E, Kiebooms L, Buysse J, Merckx R (1996a) ^{137}Cs uptake in spring wheat (*Triticum aestivum* L. cv Tonic) at varying K supply. I. The effect in solution culture. Plant Soil **181:** 205–209

Smolders E, Kiebooms L, Buysse J, Merckx R (1996b) ^{137}Cs uptake in spring wheat (*Triticum aestivum* L cv Tonic) at varying K supply. II. A potted soil experiment. Plant Soil **181:** 211–220

Spalding EP, Hirsch RE, Lewis DR, Qi Z, Sussman MR, Lewis BD (1999) Potassium uptake supporting plant growth in the absence of AKT1 channel activity—inhibition by ammonium and stimulation by sodium. J Gen Physiol **113:** 909–918

Sutcliffe JF (1957) The selective uptake of alkali cations by red beet root tissue. J Exp Bot **8:** 36–49

Teertstra DK, Cerny P, Chapman R (1992) Compositional heterogeneity of pollucite from High Grade Dyke, Maskwa Lake, southeastern Manitoba. Can Mineral **30:** 687–697

Wallace A, Romney EM, Wood RA (1982) The role of stable cesium on plant uptake of cesium-137. Soil Sci **134:** 71–75

White P, Bowen H, Broadley M, Hampton C, Meacham M, Payne K (2004) The mechanisms of caesium uptake by plants. *In* J Inaba, H Tsukada, A Takeda, eds, Proceedings of the International Symposium on Radioecology and Environmental Dosimetry, Aomori, Japan, October 2003, pp 255–262

White PJ (1997) Cation channels in the plasma membrane of rye roots. J Exp Bot **48:** 499–514

White PJ, Bowen HC, Demidchik V, Nichols C, Davies JA (2002) Genes for calcium-permeable channels in the plasma membrane of plant root cells. Biochim Biophys Acta **1564:** 299–309

White PJ, Broadley MR (2000) Mechanisms of caesium uptake by plants. New Phytol **147:** 241–256

White PJ, Swarup K, Escobar-Gutiérrez AJ, Bowen HC, Willey NJ, Broadley MR (2003) Selecting plants to minimise radiocaesium in the food chain. Plant Soil **249:** 177–186

Zhu YG, Shaw G, Nisbet AF, Wilkins BT (1999) Effects of external potassium supply on compartmentation and flux characteristics of radiocaesium in intact spring wheat roots. Ann Bot **84:** 639–644

Zhu YG, Shaw G, Nisbet AF, Wilkins BT (2000) Effect of potassium (K) supply on the uptake of ^{137}Cs by spring wheat (*Triticum aestivum* cv. Tonic): a lysimeter study. Radiat Environ Biophys **39:** 283–290