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Distinctive Potassium-Accumulation Capability of Alligatorweed (*Alternanthera philoxeroides*) Links to High-Affinity Potassium Transport Facilitated by K^+-Uptake Systems

Zhi-zhong Song and Yan-hua Su*

Alligatorweed is well known for its potassium (K^+)-accumulating capabilities and its strong resistance to undesired growth conditions. The results of this study revealed properties of K^+ accumulation and its contribution to drought stress in alligatorweed. In addition, we attempted to characterize the molecular mechanisms of K^+ accumulation in this plant. Alligatorweed plants showed a consistent increase in biomass in response to external K^+ concentrations, ranging from micromolar levels up to 50 mmol L^{-1}; K^+ was also accumulated accordingly in the plants. The stem was the most K^+-accumulating organ, accumulating up to 13% of the K^+. Moreover, this K^+ superaccumulation caused improved resistance to drought stress. The apparent K^+ uptake by the roots showed a typical high-affinity property, and the Michaelis constant increased at higher rates of plant K^+ in the starting materials. Furthermore, three putative, K^+-uptake transporter complementary DNAs (cDNAs) were isolated from alligatorweed (*ApKUP1*, *ApKUP2*, and *ApKUP3*, respectively) using degenerated primers and rapid amplification of cDNA end techniques. The expression of *ApKUP1* and *ApKUP3* was predominately localized to the leaves, whereas *ApKUP2* was expressed throughout the entire plant. The expression of *ApKUP1* and *ApKUP3* was stimulated in the stems and roots when K^+ was depleted from the external medium. Moreover, *ApKUP3* expression was enhanced in the stem in response to abscisic acid treatment and drought stress. In conclusion, our findings provide further insight into the mechanisms of K^+ accumulation linked to K^+ uptake in alligatorweed.
Nomenclature: Alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb.
Key words: Aquatic weed, K^+ transporter, drought-resistance, molecular link.

As the most-abundant cation in the cytoplasm of the cell, potassium (K^+) plays a crucial role in the growth and development of plants (Kochian and Lucas 1988; Maathuis and Sanders 1996; Lebaudy et al. 2007). In addition, K^+ contributes to improving plant tolerance to various environmental stresses, such as drought (Benlloch-González et al. 2008; Li et al. 2011; Tiwari et al. 1998), salt (Maathuis and Amtmann 1999; Mian et al. 2011), and cold (Rai et al. 2008).

To meet typical growth demands, a sufficient amount of K^+ must be absorbed effectively from the soil solution via the plant roots and then be distributed and accumulated throughout the different parts of the plant (Gaymard et al. 1998; Gierth, and Mäser 2007; Lebaudy et al. 2007; Ward et al. 2009). The transport of K^+ in plants is believed to involve two major uptake systems: the high-affinity system, which is effective at transporting submillimolar concentrations of external K^+, and the low-affinity system, which acts within the millimolar range (Epstein et al. 1963; Kochian and Lucas 1988).

Many transport systems that facilitate high- or low-affinity K^+ transport have been identified in plants (Gierth and Mäser 2007; Lebaudy et al. 2007; Véry and Sentenac 2003; Ward et al. 2009). The low-affinity systems mainly involve K^+ channels (Fernando et al. 1990; Glass 1976, 1983; Véry and Sentenac 2003) that primarily mediate the K^+ flow between the apoplasts and the cytosol, where K^+ concentrations are normally maintained at levels exceeding tens of millimolars. However, their function is not exclusive because some K^+ channels, such as AtAKT1, have also been shown to contribute directly to root K^+ acquisition from medium that contains several micromoles of K^+ (Hirsch et al. 1998). High-affinity systems in plant roots are potentially major components of the K^+ uptake from the soil, where the K^+ concentration is normally in the submillimolar range. Three major transporter families, including KT/HAK/KUP, TRK/HKT and the cation/proton antiporters (CPA), have been identified in plants (Gierth and Mäser 2007; Lebaudy et al. 2007; Mäser et al. 2001; Véry and Sentenac 2003). The KT/HAK/KUP transporters have attracted extensive research interest. The first plant member of this family was identified in barley (*Hordeum spp.*)(Santa-Maria et al. 1997). Subsequent studies have provided a comprehensive insight into KT/HAK/KUP-mediated, high-affinity K^+ transport in barley (Rubio et al. 2000; Vallejo et al. 2005). Consistent efforts were made to characterize the actions of this family of transporters in a variety of plant species, including mouse-ear cress [*Arabidopsis thaliana* (L.) Heynh.](Fu and Luan 1998), cayenne pepper (*Capsicum annuum* L.)(Martinez-Cordero et al. 2004), crystalline iceplant (*Mesembryanthemum crystallinum* L.)(Su et al. 2002), Japanese birdsfoot trefoil (*Lotus corniculatus* L. var. *japonicus*)(Desbrosses et al. 2004), wine grape (*Vitis vinifera* L.)(Davies et al. 2006), tomato (*Solanum lycopersicum* L. var. *lycopersicum*)(Nieves-Cordones et al. 2007; Wang et al. 2002), and slender seagrass [*Cymodocea nodosa* (Ucria) Aschers.](Garciadeblas et al. 2002), and so forth.

In plants, the regulation of the plant water status and drought adaptation, therefore, depends on the activity and number of ion channels and osmolite transporters. The phytohormone abscisic acid (ABA) regulates many stress-related processes (Becker et al. 2003). During drought or salt stress, rising ABA levels regulate guard-cell ion transport to promote stomatal closure and thereby reduce transpiration, which would result in a loss of cellular K^+ level (Becker et al. 2003; Wolf et al. 2006).

Alligatorweed is an invasive, aquatic weed that displays a rather aggressive increase in biomass. The plant is able to survive many environmental stresses, and it is difficult to

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control its spread. Although alligatorweed is well known for its strong capacity for K^+ accumulation, even at extremely low, external K^+ concentrations, e.g., 0.2 μmol L^{-1} (Peng and Hu 1986; Xie and Ni 1987), there are only a few studies concerning K^+ uptake in alligatorweed. Nevertheless, alligatorweed is an excellent model for understanding the mechanisms of K^+ acquisition and accumulation and for pursuing further insights into its molecular basis and regulatory networks. Currently, features of K^+ accumulation and distribution, especially the molecular mechanisms, remain largely unknown.

In this work, we evaluated the biological response of alligatorweed to various external K^+ conditions (ranging from 0.01 to 50 mmol L^{-1}), examined the properties of its K^+ accumulation and uptake kinetics, and subsequently estimated its improved drought-resistance capability in relation to K^+ accumulation. We also attempted to trace the molecular basis of alligatorweed superior K^+ accumulation by cloning three putative *KUP* complementary DNAs (cDNAs) and to investigate their expression profiles in response to different K^+ supply, drought, and ABA conditions. The aim of this study was to provide further insight into the physiological and molecular mechanisms of K^+ accumulation in alligatorweed.

Materials and Methods

Plant Culture. Naturally grown alligatorweed plants were obtained from the Pipa Lake (located a short distance from the Dr. Sun Yat-sen Mausoleum, Nanjing, China) and were adapted in a laboratory greenhouse for 1 mo in one-quarter strength Murashige–Skoog (MS) liquid medium, made according to protocols (Murashige and Skoog 1962). Branches with identical stem diameters and identical node and leaf numbers were collected and transferred into one-quarter MS liquid medium. These plants were further incubated in the dark to induce root growth and obtain individual, vertical plantlets for further experiments. The greenhouse was maintained under a 12/12 h day/night light cycle and a 28/23C day/night temperature cycle. The nutrient solution was refreshed every other day.

Physiological Studies. Fully developed plantlets with identical sizes were subjected to treatments for 7 d in one-quarter MS-based nutrient solution. For K^+ treatments, the KNO_3 and KH_2PO_4 in the MS media were replaced with sodium salts, and final K^+ concentrations were obtained upon the addition of KCl to the desired amount of 0.01, 0.1, 1, 5, 10, or 50 mmol L^{-1}. Compared with the control conditions (1 mmol L^{-1} K^+), drought stress was simulated upon the supplement of polyethylene glycol (PEG) 6000 to a final concentration of 15% (w/v), by adding 150 g of PEG 6000 into per liter of media supplied with 1 or 10 mmol L^{-1} of KCl (+K), respectively. For the ABA treatments, the +K solutions were supplemented with ABA at 500 μmol L^{-1}. Each treatment was performed in triplicate, and the experiments were repeated at least twice. Samples were photographed and collected to obtain additional measurements. To obtain the fresh weight, seedlings were rinsed in distilled water, soaked, and then weighed. The roots were scanned with an Epson (Long Beach, CA) Rhizo scanner (Rhizo 2004b), and data were acquired with Epson WinRHIZO software. To obtain the dry weight of the biomass, seedlings were separated into roots, stems, and leaves and were dried in an oven at 105 C for 30 min, followed by baking at 70 C for 48 h. Dried samples were ground into a fine powder and were fully digested with HNO_3–$HClO_4$ (Lu 2000). The K^+ contents were measured using flame atomic absorption spectrometry.

K^+ Uptake Kinetics. Seedlings were precultured in one-quarter MS solutions containing 0.02, 1.0, or 20 mmol L^{-1} KCl for 7 d to achieve gradients in plant K^+ accumulation. Seedlings that were similar in size and appearance were then equilibrated in one-quarter MS media, supplemented with zero K^+ (K^+ replaced with Na^+) for 48 h. For the uptake experiments, 10 seedlings were rinsed with a 0.2 mmol L^{-1} $CaCl_2$ solution and subsequently transferred into a light-protected flask containing 100 ml of absorption solution, which consisted of 0.2 mmol L^{-1} $CaSO_4$ and KCl at the desired concentration. A pilot time course was conducted to determine the K^+ uptake in 1 mmol L^{-1} K^+ absorption solution by removing 1 ml of medium at each desired time point. The K^+ uptake in response to the external K^+ concentrations was measured at the maximal time point (90 min of incubation). The roots were rinsed three times in 0.2 mmol L^{-1} $CaSO_4$, left wet on soaked paper towels, and weighed. The K^+ concentration in the absorption solution was measured using flame atomic absorption spectrometry. The K^+ uptake in the roots was calculated according to the depletion of the K^+ concentration in a fixed volume of absorption solution.

Molecular Analysis. General nucleotide manipulation was performed according to the methods of Sambrook and Russell (2001). Total RNA was purified from the seedlings using the RNAiso Plus Kit (TaKaRa, Kyoto, Japan). The first strand cDNA was synthesized using 2 μg of total RNA for the template and oligo$(dT)_{18}$ for the anchored primer according to the protocol in the PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa).

To obtain partial cDNA fragments, degenerate primer pairs were designed according to the conserved domains of the amino acid sequences from known plant KUPs (Table 1). Partial cDNAs were amplified using iProof High-Fidelity Polymerase (Bio-Rad Laboratories, Hercules, CA) and the first-strand cDNA for the template. The polymerase chain reaction (PCR) products were purified using the High Pure PCR Product Purification Kit (Roche, Molecular Diagnostics, Pleasanton, CA) and sequenced. Genomic DNA was isolated with the Universal Genomic DNA Extraction Kit (Version 3.0; TaKaRa).

Rapid Amplification of cDNA Ends (RACE) and Generation of Full-Length cDNAs. Gene-specific primers (GSPs) were designed according to the partial cDNA fragments obtained above. Cloning of the three- or five-primer end of the *KUP* gene cDNAs was performed using the SMARTer RACE cDNA Amplification Kit (BD Biosciences Clontech, San Jose, CA). The three- (or five-) primer end nested PCR was performed with two-round amplifications using the universal primer mix–gene-specific primer (UPM/GSP_{out}), and the nested universal primer–gene-specific primer (NUP/GSP_{in}) PCR fragments were purified, propagated in the pGEM-T vector (Promega, Madison, WI), and sequenced. Full-length cDNAs were amplified using pairs of the open reading frame (ORF) amplification primers obtained from RACE (Table 1) and sequenced.

Table 1. Primers used for cDNA cloning.[a,b]

Purpose	Gene or primer name	Primer (5′–primer end to 3′–primer end)
Partial cDNA cloning	ApKUP1	[F]: CYWGYATGGTKATYGGKGATGG
		[R]: CCATACCKSRCRAYGCASCGG
	ApKUP2	[F]: CAGGHDCTGAAGCHATGTTTGC
		[R]: CCATACCKSRCRAYGCASCGG
	ApKUP3	[F]: GCRTWYCAGAGYYTYGGYGTGGT
		[R]: GAWAYGCAGCYTGRCCCATRTA
3′–Primer end RACE nested PCR amplification	ApKUP1	[out]: GTTTGCTGATTTAGGACACTTC
		[in]: GTTTGCTGATTTAGGACACTTC
	ApKUP2	[out]: GGTTCCCATTATGTTGTCCCTTG
		[in]: GGTTCCCATTATGTTGTCCCTTG
	ApKUP12	[out]: CTCTTGGTGGCTGTGTTTTGTG
		[in]: CTGTTGTGTTCCCGTGCCTTCT
5′–Primer end RACE nested PCR amplification	ApKUP1	[out]: GGATGGATGTCTTTGGGAGGTGTTC
		[in]: CTACAACACTATGGCACTCATCGAG
	ApKUP2	[out]:TGATTGGGAATGCATATGGTCTGG
		[in]: GCCAATCTCTAGGATGCTTCCCAAG
	ApKUP3	[out]: GAGCTGATGACAATGGTGAAGGTGG
		[in]: CCAAAGCCTTGGAGTAGTATATGGAG
ORF amplification	ApKUP1	[F]: ATGGATGCTCATGTTAATTGTTGGG
		[R]: TTAAACAACATAAACCATTCCGAC
	ApKUP2	[F]: ATGGATACTCAGCCCGAGTCTGAC
		[R]: CTAGACATAGTATATCATCCCAACT
	ApKUP3	[F]: ATGTTAAAGAAATTAGGGAAGCAT
		[R]: TTACACATAAAATACTTGTCCGAC

[a] Abbreviations: cDNA, complementary DNA; RACE, rapid amplification of cDNA end; PCR, polymerase chain reaction; ORF, open reading frame.
[b] Degenerate primers used in this work were endowed with $Y = C$ or T; $W = A$ or T; $K = G$ or T; $S = G$ or C; $R = A$ or G; $H = A$, C, or T; $D = A$, G, or T; $M = A$ or C; $N = A$, C, G, or T; $V = A$, C, or G; and $B = C$, G, or T.

Reverse-Transcription (RT)-PCR Analyses. To determine tissue-specific expression, seedlings were grown in one-quarter MS media supplied with 1 mmol L^{-1} of KCl (+K) for a week before treatments. For the −K treatments, 1 mmol L^{-1} of KCl was omitted from the growth media. For the ABA treatments, the +K solutions were supplemented with ABA at 500 μmol L^{-1}. For PEG treatments, the +K solutions were supplemented with PEG 6000 at 15% (w/v), by adding 150 g PEG 6000 L^{-1} media supplied with 1 mmol L^{-1} of KCl (+K). All treatments were incubated for 48 h before examination. Total RNA was purified from the treated seedlings using the RNAiso Plus Kit (TaKaRa). The first-strand cDNA was synthesized from 2 μg of total RNA using the PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa). Semiquantitative RT-PCR was performed using GSP pairs (Table 2). *Actin* was amplified as an internal control.

Statistical and Graphical Analyses. For all experiments, data were statistically analyzed using the SPSS 13.0 program (SPSS, Chicago, IL). The same letters indicate no significant differences; different letters mean significantly different at $P < 0.01$ using the *t* test.

Results and Discussion

Distinct Biological Responses of Alligatorweed to External K^+. Potassium is one of the major nutrients required for plant growth. In agriculture, the application of K^+ fertilizers efficiently promotes crop yield and improves crop quality. Many plants require a reasonable range of K^+ concentrations in the roots because higher concentrations might inhibit growth (e.g., > 20 mmol L^{-1} K^+ accumulated close to the fertilization site). Unlike many plants, the growth of alligatorweed exhibited a consistent gain in biomass with increasing K^+ concentrations from 10 μmol L^{-1} to 50 mmol L^{-1} (Figure 1a). Measurements of the fresh weight revealed that maximal growth was achieved at the highest concentration of K^+ supply (50 mmol L^{-1}; Figure 1b). Compared with the leaves and roots, the highest amount of K^+ was accumulated in the stem, which stored >13% K^+ in dry weight (Figure 1c). Consistent with the growth response in alligatorweed, K^+ accumulation reinforced with increasing K^+ concentrations in the growth media (Figure 1c), implying that alligatorweed is a K^+-accumulating plant. Moreover, both the total root length (Figure 1d) and root surface area (Figure 1e) of roots were increased in response to the ameliorated K^+ supply, which possibly secured the

Table 2. Primer pairs used for reverse-transcription–polymerase chain reaction experiments.

Gene	Primer (5′–primer end to 3′–primer end)	Amplicon size
		bp
ApKUP1	[F]: GCATCAGGTTTAGCGGTGATGAC	520
	[R]: CCAGCCCACCGATCCTATCGATG	
ApKUP2	[F]: GGAATGCATATGGTCTGGCTTGT	520
	[R]: CCAAGACCATATCGTATGTATCG	
ApKUP3	[F]: CTATGACAGTGTGCCAGGTGGAT	508
	[R]: CCTGGAACTATGGAAGTGTTCTC	
Actin	[F]: CATGTTTGAGACCTTCAACACCCC	510
	[R]: GTAGTCTCATGGATACCCGCAGCT	

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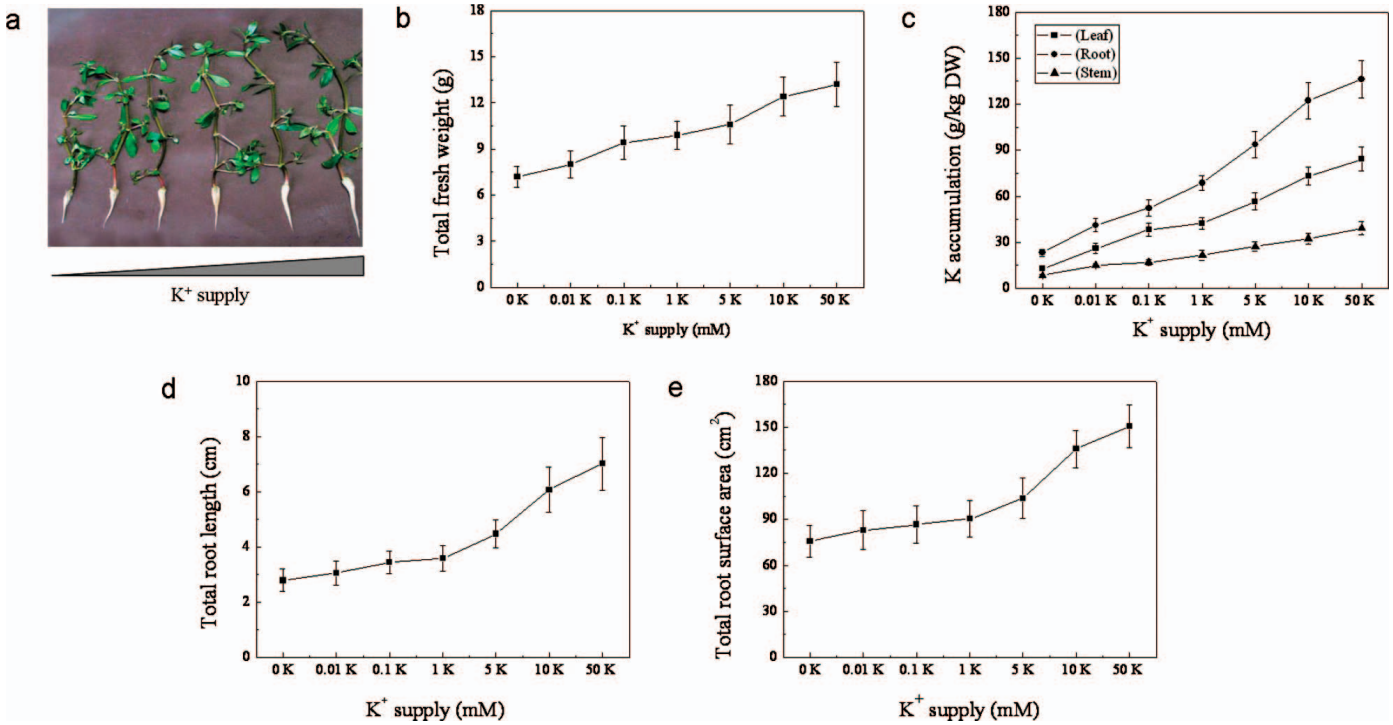

Figure 1. Biological response of alligatorweed to external K^+ supply. (a–e) Seedlings were grown in a one-quarter Murashige–Skoog (MS) solution supplemented with 0.01, 0.1, 1, 5, 10, or 50 mmol L^{-1} KCl. The values presented are means ± SE, $n = 12$.

powerful K^+ accumulation via plant roots and then sufficiently transported it into the aboveground plant components and also partially explained the consistent growth and tissue K^+ accumulation.

Compared with other plants, K^+-accumulation plants, such as alligatorweed, possess stronger capabilities for accumulating K^+ from extremely limited sources (Peng and Hu 1986; Xie and Ni 1987). Surprisingly, in this study, we observed an abnormal response of alligatorweed in favor of extremely high concentrations of K^+, e.g., 50 mmol L^{-1}, accompanied by both consistent growth and tissue K^+ accumulation at the whole-plant level. Thus, despite its reputation as a widely spread, difficult-to-control aquatic weed, alligatorweed is an excellent model for dissecting the molecular mechanisms of K^+ super-accumulation associated with resistance to abiotic stresses.

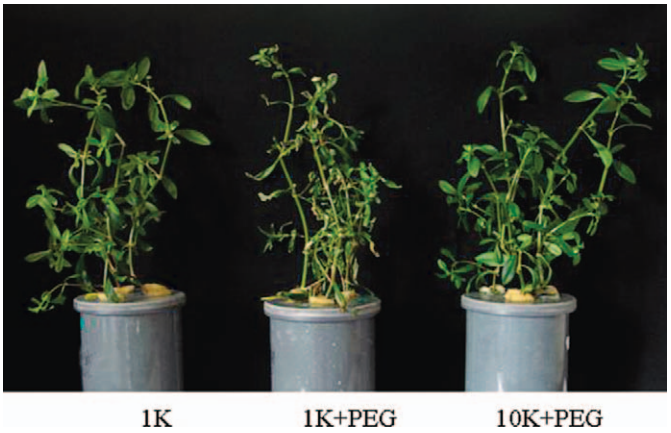

Figure 2. Elevated K^+ accumulation rescues damage from drought stress in alligatorweed plants. Seedlings were treated for 7 d before examination. Abbreviations: 1 K or 10 K, 1 or 10 mmol L^{-1} K^+; +PEG, PEG 6000 at 15% (w/v). Values are presented as means ± SE, $n = 12$.

Elevated K^+ Accumulation Abolishes Damages from Drought Stress. Compared with control conditions (1 K), the addition of 15% (w/v) PEG 6000 (1 K + PEG) caused severe damage (approximately 32% loss of total fresh biomass), whereas the application of 10 mmol K^+ (10 K + PEG) rescued the plant from such damages, resulting in an approximately 21% increase in total fresh biomass compared with the 1-K control (Figure 2 and Table 3). In addition, elevated K^+ concentrations (10 K + PEG conditions) also significantly enhanced the internal K^+ accumulation (approximately 38% at the whole-plant level) under drought stress (Table 3). These findings again support the key role of K^+ in improving plant tolerance to drought stresses (Benlloch-González et al. 2008; Li et al. 2011; Tiwari et al. 1998).

Kinetics of K^+ Uptake in the Roots of Alligatorweed. Time-course experiments were performed for 120 min after absorption, with the maximal uptake at 90 min (Figure 3a). Under these experimental conditions, the K^+ uptake in alligatorweed roots showed a saturable response to external K^+ in the 0.01 to 2.0 mmol L^{-1} range (Figure 3b). In response to the elevated plant K^+ concentrations achieved from preculture conditions, the rate of K^+ absorption decreased from approximately 30 μmol g^{-1} fresh weight per hour for roots precultured under 0.02 mmol L^{-1} K^+ to 7.8 μmol g^{-1} fresh weight per hour under 20 mmol L^{-1} K^+ (Figure 3b and Table 3). In contrast, the K_m values increased (3.2, 5.3, and 87.6 μmol L^{-1} for roots precultured under 0.02, 2, or 20 mmol L^{-1}, respectively), i.e., the affinity to K^+ was reduced (Figure 3b; Table 4), indicating that, when exposed

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Table 3. Whole-plant level fresh weight and K^+ accumulation analysis under drought stress.[a,b]

Treatments	Total fresh wt	K^+ accumulation
	g	g kg^{-1} dry wt
1 K (control)	9.7 ± 0.9 b	42.3 ± 3.9 a
1 K + PEG	6.4 ± 0.7 a	51.9 ± 5.3 b
10 K + PEG	10.3 ± 1.1 b	72.5 ± 5.8 c
1 K + ABA	9.9 ± 1.0 b	50.4 ± 4.6 b

[a] Abbreviations: K^+, potassium; 1 K, 1 mmol L^{-1} K^+; PEG, polyethylene glycol 6000 at 15% (w/v); 10 K, 10 mmol L^{-1} K^+; ABA, abscisic acid.
[b] Data are given as means ± SE from three independent experiments. Different letters indicate significance at $P < 0.01$ using the *t* test.

Table 4. Parameters analyses of the K^+ uptake kinetics.[a,b]

K^+ preculture level	K_m	V_{max}
mmol L^{-1}		μmol g^{-1} FW h^{-1}
0.02	3.2 ± 1.1 a	30.2 ± 3.8 c
2	5.3 ± 1.8 a	15.4 ± 2.2 b
20	87.6 ± 5.7 b	7.8 ± 4.4 a

[a] Abbreviations: K^+, potassium; K_m, Michaelis constant; V_{max}, maximum velocity.
[b] Data are given as means ± SE from three independent experiments. Different letters indicate significance at $0.01 < P < 0.05$ using the *t* test.

to conditions of sufficient K^+ supply, the low-affinity systems were involved in maintaining "luxury uptake" of external- K^+. Nevertheless, the apparent root K^+ uptake predominantly involved the high-affinity systems. However, the affinity for K^+ decreased with increasing K^+ concentrations (Figure 3), indicating that multiple affinity systems might contribute to alligatorweed K^+ absorption and transport.

Cloning of New *KUP* Genes from Alligatorweed. Using RACE techniques with degenerate primers derived from conserved domains of known plant *KUP*s, we successfully cloned three full-length putative *KUP* cDNAs from alligatorweed, designated *ApKUP1-3* (GenBank JN635515, JN635516, and JN635519, respectively)(Figure 4a). Homology analyses revealed that the three new members belong to the KT/HAK/KUP transporter family. Functional domains (Figure 4b) were predicted using the Pfam software (http://pfam.janelia.org/search, accessed: November 2011). The deduced amino acid sequences of the *ApKUP*s shared an overall identity of 57% (Figure 4b).

Since the identification of the first high-affinity plant KT/HAK/KUP system in barley (Santa-Maria et al. 1997), consistent efforts have been made to expand our knowledge to more plant species and to characterize the association between these high-affinity transporters and the properties of K^+ acquisition in plant roots. In this study, we successfully cloned three new members of the KT/HAK/KUP family from the K^+ superaccumulating alligatorweed plant and made it possible to examine the molecular basis of superior K^+ accumulation in alligatorweed.

Expression Analysis of *ApKUP* Genes. RT-PCR analyses revealed that, under controlled conditions, *ApKUP1* and *ApKUP3* were primarily localized to the leaves and, to a much lesser extent, the stems (Figure 5a), whereas *ApKUP2* was evenly distributed throughout the entire plant (Figure 5a). Upon K^+ starvation, the expression of *ApKUP1* and *ApKUP3* was largely induced in the stems and roots (Figure. 5b), whereas *ApKUP2* expression remained unaffected (data not shown). Moreover, drought stress and ABA treatments also stimulated *ApKUP3* expression in the stem (Figure 5c). These results indicate that when the K^+ supply is sufficient, *ApKUP2* might contribute to root absorption and K^+ accumulation during normal growth conditions at the whole-plant level, whereas the expression of *ApKUP1* and *ApKUP3* is enhanced under conditions of stress, such as K^+ deprivation and drought, which indicates that enhanced K^+ accumulation occurs under abiotic stress conditions. In addition, under drought stress, the enhanced transcripts of *ApKUP1* and *ApKUP3* might partially explain the obviously increased K^+ accumulation in tested organs (Table 3).

ABA stimulates anion channels and outward-rectifying K^+ channels in the physically isolated guard cells and causes the loss of cellular K^+ (Becker et al. 2003; Wolf et al. 2006). Although 500 μmol L^{-1} ABA treatments had little effect on alligatorweed plant growth and fresh weight gain, ABA treatments specifically induced the expression of K^+ transporter *ApKUP3* transcript in stems, which may partially explain the increased *ApKUP* activities and cellular K^+ accumulation (Figure 5c and Table 3). These results imply that ABA may have a role in K^+ transportation and distribution in two distinct ways that are still unknown in alligatorweed.

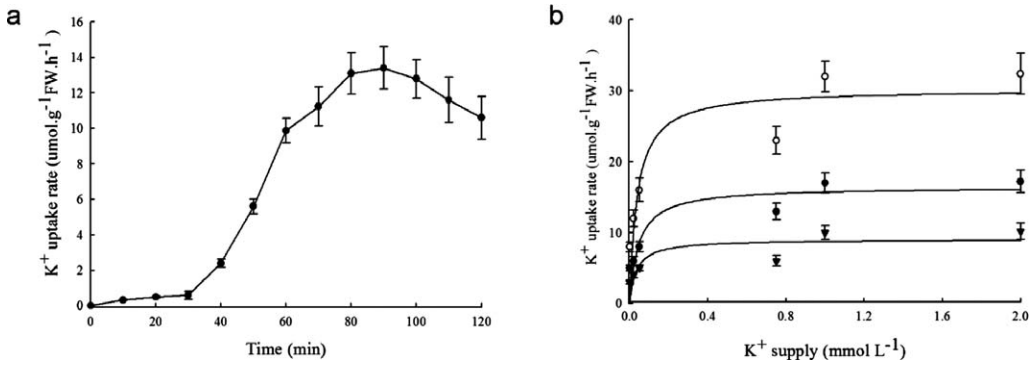

Figure 3. Kinetics of K^+ uptake in alligatorweed roots. (a) A pilot time course of K^+ uptake. (b) The rate of K^+ uptake in the roots as determined at the optimal time point (90 min). Plants were precultured in a one-quarter Murashige–Skoog (MS) solution supplemented with ○, 0.02 mmol L^{-1}; ●, 2 mmol L^{-1}; or ▼ 20 mmol L^{-1} K^+. Values represent means ± SE, $n = 13$. The experiment was repeated three times.

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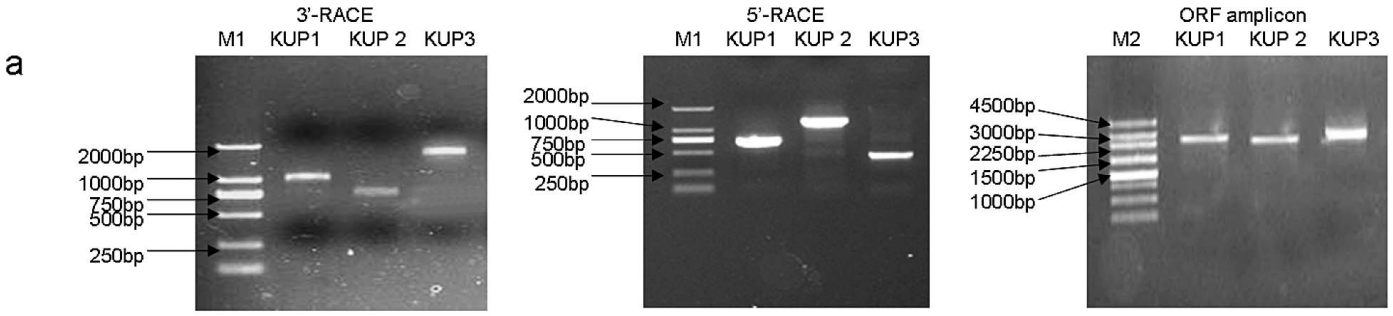

b

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ApKUP1   ....................................MEAHVNCWGSNNNKDKSWRGVML.LAYQSLGVVYGDLSISPLYVFKSTFA   49
ApKUP2   ..............................MDTQFESDDENETEPRISPSKLTWLNMSRTLLLAYQSVGVVYGDLSTSPLYVYTSTFK   58
ApKUP3   MLKKLGKHSNILKSASMASISEGTENRGSMWDLLQKIDQFMEEEAGQIKNAYRQKRISKLLILRLAFQSLGVVYGDLGTSPLYVFYNTFP   90
Consensus                                                            l la qs gvvygdl  splyv   tf

ApKUP1   EDIKHSETNEEIFGVISIVFWTLTLIPLFKYVFIVLRADDNGEGGTFALYSLICRHAKVSLLPNRQASDEVSTYKMEHPPETTSTSKVK   139
ApKUP2   GKRSMQLSPEEIFGAFSLIFWSLTLIPLIKYAFFVLSADDNGEGGTFALYSLICRHAKFSLLPNQQAADEELSAYRYGHRSPTSASSPIK   148
ApKUP3   HGVKDSD...DVVGAISLIIYSLTIVPLIKYVFIVCRANDNGQGGTFALYSLICRHANVNTIPNRHRTDEELITTYSRFRFHEDSFAAKTR   177
Consensus             g  s     ltl pl ky f v  a dng ggtfalysl crha     pn    de    y

ApKUP1   MVIEKHKCIHTALLILVILGTQMVVGLGLLTPAISVFTAVSGLESLMSHENHQYAVVFITCFILVCLFALQHYGTHRVGFIFAFILILWL   229
ApKUP2   RFLDKHKKIRIALLVFVLIGAQMVIGLGVLTPAISVLSSVTGLQVAEKKINEG.ELMILACVILVCLFALQHGCTHKVAFLFAFIVFIWL   237
ApKUP3   QWIPKHSSRKNALILIVIVGTQMVIGLGILTPAISVLSASGGIKVDHPKVSND.IVVIVAVVILVGLFSLQHYGTDKVGWIFAFIVLIWF   266
Consensus  l kh     all  vl g  mv gdg ltpaisv     g                    ilv lf lqh gt  v   fapi   w

ApKUP1   ICVSGIGLYNILHWNPRVVQAISPYYMYKFLKKTIIGGWMSIGGVLLCITGSEAMFADLGHFSHMAIQIAFTFIVYPTLILAYMGQAAYL   319
ApKUP2   FSIFGIGLYNVIYWNPKIVQAFSPHYIIKYFRETCKDGWISIGRILLCITGTEAMFADIGHFTAASVKIAFTCVVYPCLVVQYIGQAAFL   327
ApKUP3   IVIGGIGLYNIWKYDSSVIRAFSPVYIYRYLKNGGKDGWTSIGGILLSITGTFALFADLAHFFVLAVQIAFTVIVFPCLILAYSGQAAYL   356
Consensus    g g yn          a sp y          gw slg  ll  tg ea fad  hf       aft  v p l   y gqaa l

ApKUP1   SMHHQKNDKISFYLSVPEKVKWPVILVAILASVVGSQAIISGAFSIINQSQSIGCFPRVKVVHTSLKIHGQIYIPEINWILMVLCIAVTI   409
ApKUP2   .TKNLSSIEHSFYLSIPEFVYWPVFVIATLAAIVGSQAIITATFSIVKQCQSIGCFPRVKVVHTSKHIYGQIYIPEINWILMIITLGVTI   416
ApKUP3   .VNHQLDVVDAFYHSIPDSIYWPVFVVATLAAIVASQATISATFSIIKQCALALGCFPRVKVVHTSKNFLGQVYIPDINWILMVICIAVTA   445
Consensus          fy s p    wpv   a la  v sqa i   fsi  q   lgcfprvkvvhts    gq yip inwilm l   vt

ApKUP1   GFRDTKHLGNASGLAVMTVMLVTTCLMSIVIVLCWHKPPIIAICFLLFFGSIEILYFSASIVKFAEGAWFPLLIALILMTIMFVWHYATI   499
ApKUP2   GFRDTTMIGNAYGLACISVMLITTCLNTIVLTFVWQKG.ALAIPFLIFFGFIEGVYLSSAFIKVPQGGWVPLMLSIVIVLVMYVWHYGTR   505
ApKUP3   GFRNPNQIGNAYGTAVVIVMIATTFLMIIIMILVWRWHWILVIIFTAISILVFLTYFSAVIFKVDQGGWVPLVIAAAFILVMVVWHYGNV   535
Consensusgfr     gna g a   vml tt lm l    w        f      e  y s   k   g w p         m vwhy

ApKUP1   KKYEFDLHNKVSLEWILALGPSLGVSRVEGICMVFTDLTCGIAANFSFFVTNLPAMFKILIFVCVKYVFVPYIFPAERMLISRVGPFAHR   589
ApKUP2   KKYNFDLHNKVSLKWILCLGPSLGIVRVEGICLIYSELAVGVEAIFSHFVTNLPAHFKVLVFVCVKSVFVPHVSPEERFLIGRICPRPYR   595
ApKUP3   KRYEFEMHSKVSMAWILCLGPSLGLVRVEGICLVYTELASGVESIFSHFITNLPAIFSVVVFVCVKYLFVPYTVEEERFLVKRIGPKTFH   625
Consensusk y f  h kvs  w l lgpslg  rvpgig     l  g p  fs f tnlpa h    fvcvk  pv      er l  r  p

ApKUP1   SYRCIIRYGYRDVHQDIDSFESQLAAKLNEFIRFLWFQNHTEGALDSQHLETRCHEPNDCRLAVMETIRYPHEPPYEIELNPQFVSVSIG   679
ApKUP2   MYRCIVRYGYKDIARDLGEFEDLLIKSVAEFIQMEAVEPQFIGSLSSSYLGRMAVISTRTIQCSSSLIVSEHDLS.DINEITQ....SSR   680
ApKUP3   MFRCVARYGYKDLHKKDEDFEEKLFHNLSIFVRLESMMEGCTDSEEYSLYGQQTAESRDCLILNGNTMS.......EFL...........   697
Consensus  rc  rygy d       fe  l     f

ApKUP1   IPIVESMRDIIEMK..PVKFVKFIDESWIGDEKEAQMLQFLDLLWEAQQAGSAFILGHSHVRAKQGSSFFKKLAINFGYNFIRRNCFGFD   767
ApKUP2   ALTIQSLRSVYEDESVQIFRRRVRFQIPQSPAMDFEVRDFLDLIEAKEAGVAYIMGHSYVKARRSSSFFKKLAIDIGYSFIRRNCFGFS   770
ApKUP3   .PIVESRDSIVPAN..SPIRYINIGPGSSRHTSSINEIDFLFLNSCRDAGVVHILGNTVIKARKDSSIYKKIAVDYIYAFLRKICEEHS   784
Consensus  t  s           r                 el  l    ag   i g    a   ss  kk a    y flr  cr

ApKUP1   VAIKVEPVSLLEVGMVYV                                                                          785
ApKUP2   VAINIEHHSLIEVGMIYY                                                                          788
ApKUP3   VIENVEHESLLNVGQVFY                                                                          802
Consensusv    p  sl  vg
```

Figure 4. Cloning and sequence analysis of three new *ApKUP*s. (a) Rapid amplification of complementary DNA (cDNA) end cloning and full-length cDNA amplification of *ApKUP*s (*ApKUP1*, 2361 bp; *ApKUP2*, 2370 bp; *ApKUP3*, 2412 bp; M1, DL2000 marker; M2, 250-bp marker). (b) Deduced amino acid sequences for the three new *ApKUP*s.

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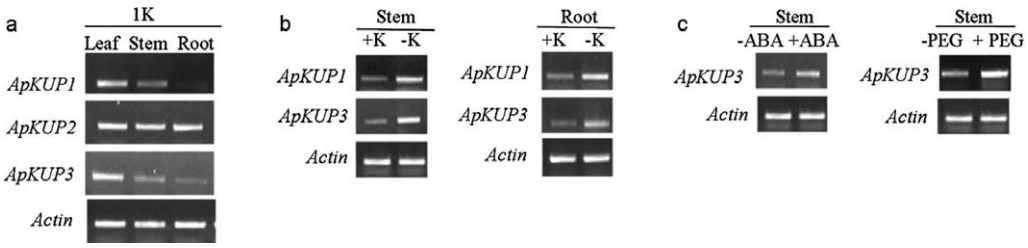

Figure 5. Expression profiles of *ApKUP*s using semiquantitative reverse-transcription–polymerase chain reaction analysis. All treatments were incubated for 48 h before examination.

In conclusion, we report a distinguishing K^+ super-accumulation and consequent benefit of resistance against drought stress in alligatorweed. As a preliminary attempt to characterize the molecular mechanisms underlying the high-affinity K^+ acquisition in the roots, we successfully cloned three new members of the KT/HAK/KUP family. The use of expression analyses allowed us to assess the involvement of these genes in promoting K^+ accumulation against abiotic stresses. Further research using electrophysiological strategy, yeast complementation, and insertion mutagenesis are required to explore the molecular mechanisms with the intent of providing a link between K^+-accumulation in relation to stress-resistance capabilities in alligatorweed. Hopefully, we will be able to provide genetic strategies for management of this weed.

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