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Importance of KUP8 for K^+ uptake in rooted plantlets of *Elaeis guineensis* under K^+ sufficient conditions

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ABSTRACT

Potassium is a major nutrient essential for plant growth and development. Acquisition of this vital element and maintenance of K^+ homeostasis are complex processes, facilitated by an array of membrane transporters including carriers and channels. Key mediators of K^+ uptake are the KT/KUP/HAK family of transporters. The oil palm (*Elaeis guineensis*), is an agriculturally important crop, but the molecular mechanisms of nutrient acquisition in this plant are poorly understood. Here we report the full sequences of three KUP transporters, *EgKUP3*, *EgKUP8* and *EgKUP11*, from oil palm, obtained by a combination of database searching, PCR and 5′ and 3′ RACE techniques. Gene expression analysis of these three transporters was conducted on oil palm rooted plantlets at 7, 14, and 21 days after treatment with a range of KNO_3 concentrations: 0.2 mM, 10 mM and 20 mM of in root and leaf tissues. The results indicated *EgKUP8* expression is significantly upregulated in root tissues under K^+ depleted conditions (0.2 mM) at 14 and 21 days. In contrast, the expression of *EgKUP3* and *EgKUP11* was not sensitive to changes in external K^+ concentration. Functional complementation using *Escherichia coli* knockout strain defective in K^+ uptake systems revealed that all EgKUPs complemented growth at 50 mM K^+ concentration while EgKUP8 was also able to complement growth at 5 mM K^+ when tested at pH 7.5. In contrast, none of the EgKUPs were able to complement growth at pH 5.5 under all external K^+ concentrations tested. These observations may suggest that although EgKUP8 is less efficient at <5 mM K^+ and low pH condition (pH 5.5), at least, when expressed heterologously in *E. coli*, the escalated gene expression observed *in-planta* under similar environmental condition may be indicative of a more complex cellular role of EgKUP8 in K^+ uptake in plants. This piece of work provides the first insights into the molecular mechanisms of mineral uptake in the oil palm and provides a number of tools for further research in this area.

1. Introduction

Potassium (K^+) is an essential plant nutrient with key roles in cell growth (Amtmann et al., 2005), enzyme activity, transcription, post-translational modification (Maathuis, 2006), transport and osmotic stress response (Osakabe et al., 2013). In the oil palm, K^+ is one of the most important elements in fertilisation and has a direct effect on yield particularly on bunch weight and bunch number (Lamade et al., 2014). It is also a very crucial macroelement in sugar transportation from autotrophic tissues (leaves) to sink tissues especially in developing reproductive organs (Lamade et al., 2014). K^+ deficiency in oil palm may affect stomata function in the leaf, making it susceptible to drought conditions (Rankine and Fairhust, 1999). It has also been shown that palms exposed to nutrient deficiency including K^+, risk higher chances of infection of bud-rot disease compared to healthy plants (Acosta and Munevar, 2003), suggesting K^+ deficiency leads to an incorrect nutrient balance and increases plant susceptibility for diseases.

Potassium is found in very high concentrations in plant cells, typically constituting between 2 and 10% of the dry cell weight (Leigh and Jones, 1984). On the contrary, the concentration of potassium readily available for plant use in soil solution is relatively low, normally ranging between 0.1 and 6 mM (Adam, 1971). The levels of K^+ in the cytoplasm are tightly regulated and maintained at a steady 100 mM (Walker et al., 1996), a concentration essential for a range of plant cell functions including enzyme activity. The vacuolar concentrations however vary widely depending on overall potassium availability to the plant. When there is an abundance of potassium, the vacuoles sequester high concentrations for release during times of deficit in order to precisely maintain the cytoplasmic levels (Walker et al., 1996).

Acquisition and homeostatic regulation of K^+ are complex processes, facilitated by an array of membrane transporters including carriers and channels (Gierth and Maser, 2007; Maathuis, 2009) which are components of high and low affinity transport systems (Epstein et al., 1963). The high affinity system is particularly important for allowing efficient uptake of K^+ at times of low nutrient availability.

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This is essential since plants often experience both long and short term K^+ deficiency (Ashley et al., 2006; Osakabe et al., 2013). Central to potassium homeostasis in many plants including barley (Walker et al., 1996; Santa-Maria et al., 1997), *Arabidopsis thaliana* (Walker et al., 1996; Quintero and Blatt, 1997), rice (Amtmann et al., 2008; Gupta et al., 2008) and tomato (Hyun et al., 2014) is the KT/KUP/HAK transporter family. The KT/HAK/KUP transporters comprise the largest family of plant K^+ transporters and are thought to function as H^+–K^+ symporters (Rodriguez-Navarro et al., 1986; Maathuis and Sanders, 1992; Maathuis and Sanders, 1994). The H^+–K^+ symporters are grouped under electrochemical potential-driven transporters in the most recent Transporter Classification Database (TCDB; http://www.tcdb.org) (Saier et al., 2016). These types of transporters couple the movement of an ion downhill its electrochemical gradient (typically H^+) to the uphill movement of another ion (in this instance, K^+) against its electrochemical gradient (Blatt and Slayman, 1987; Maathuis and Sanders, 1994).

Based on their amino acid sequence, KT/KUP/HAK transporters are predicted to contain 8–15 transmembrane (TM) domains and feature rather long hydrophilic C-termini (Very and Sentenac, 2003; Gupta et al., 2008; Zhang et al., 2012; Hyun et al., 2014). Phylogenetic analysis of the KT/KUP/HAK family members from *A. thaliana* and rice shows that the proteins are arranged in four distinct clusters or subfamilies (Gupta et al., 2008). Sequence homology between the members belonging to different clusters is quite low (37–52%), while within the clusters it ranges between 53 and 91% (Grabov et al., unpublished findings). The length of N-termini in front of the first TM is probably the most distinctive feature of each cluster (Senn et al., 2001).

In terms of function there is a significant amount of data supporting the fact that Cluster I KT/KUP/HAK members act as high affinity transporters and are thus likely to be important for uptake under low environmental K^+ conditions, e.g. HvHAK1 from barley (Santa-Maria et al., 1997), AtHAK5 from *A. thaliana* (Gierth, 2005), OsHAK1 from rice (Banuelos, 2002) and LeHAK5 from tomato (Wang, 2002). A recent study has shown that tobacco BY2 cells expressing the salt insensitive Cluster I transporter OsHAK5 exhibit increased salt tolerance as they preferentially transport K^+ even when $[Na^+]_o$ is high (Horie et al., 2011). These experiments indicate that Cluster I transporters may be important for regulation of K^+/Na^+ homeostasis through highly specific and efficient uptake of K^+.

Cluster II transporters including OsHAK7 and OsHAK10 (Banuelos, 2002) are suggested to have roles in low affinity K^+ uptake. There is also data indicating that Cluster II transporters have other functions including roles in auxin transport (Vicente-Agullo et al., 2004) and in plant responses to salinity (Su, 2002; Maathuis, 2006). Cluster III and Cluster IV transporters have been found only in limited numbers of plant species and are far less studied than the other transporters, although there is evidence for their involvement in responses to salinity (Maathuis, 2006).

The oil palm (*Elaeis guineensis* Jacq.) is a perennial plant belonging to the family Arecaceae originating from West Africa where it grows in the wild. It was introduced to Malaysia in the early 1870s as an ornamental plant and has been extensively developed as a major agricultural crop (Corley and Tinker, 2015). Palm oil is a rich nutritional source of vitamins and carotenoids and displays high antioxidant activity (Edem, 2002). By 2006, palm oil had overtaken soybean oil as the largest source of edible oil (Timms, 2007). As part of the diet, palm oil has been shown to have protective effects against cardiovascular disease (Szucs et al., 2011; Wergeland et al., 2011) as well as being an effective means of treating Vitamin A deficiency (Rice and Burns, 2010). It also has significant potential as a source of biodiesel (Manik and Halog, 2013).

Despite its obvious economic importance, molecular aspects of potassium nutrition in the oil palm have been relatively under-studied compared to other important crops such as rice, potato and tomato. This research was undertaken to identify molecular determinants of the potassium transport mechanisms in *E. guineensis*. Here we describe the cloning and preliminary characterization of K^+ transporters from the oil palm. The lack of an available genome sequence for oil palm when we started this research in 2011 was at least partially circumvented by the use of the genome sequence from the related date palm (Al-Dous et al., 2011). Bourgis et al. (2011) reported that oil palm and date palm nucleotide sequences are highly conserved (92% identity) (Bourgis et al., 2011). The full gene sequences of three oil palm KUP transporters *EgKUP3*, *EgKUP8* and *EgKUP11* were obtained, by a combination of homology screening, use of short ESTs of the *EgKUPs* and RACE techniques. Expression analysis of these three transporters in root and leaf tissues under excess and depleted K^+ conditions revealed that *EgKUP8* is upregulated under conditions of depleted environmental K^+. Meanwhile, functional complementation studies showed that all three of the EgKUPs were able to support growth of a bacterial strain deficient in all the endogenous K^+ uptake systems. These studies provide the first insights into the molecular mechanisms of K^+ uptake in the oil palm.

2. Materials and methods

2.1. Plant growth and total RNA extraction

All *E. guineensis* var. *tenera* plant materials were obtained from the Malaysian Palm Oil Board, Malaysia. The plantlets were grown in liquid media (as detailed in Table 1). The plantlets were maintained in sterile test tubes (Supplementary Fig. 1) with 12 h light (2000 lx) and 12 h dark cycles and 60% humidity for the whole 3-month period prior to further processing. Roots and leaves of the three-month old rooted plantlets of approximately 100 mg were disrupted using a tissue lyser. RNA was immediately extracted from the disrupted tissues using the RNeasy extraction kit (Qiagen) according to the manufacturer's protocol. Any possible DNA contamination was removed by a DNA digestion step using DNase 1 (Qiagen). The total RNA obtained was measured for purity and concentration using a nanodrop spectrophotometer (Thermo Scientific). The quality of the RNA was then checked using formaldehyde agarose gel electrophoresis. The RNA was stored at −80 °C until further use.

2.2. cDNA synthesis

Approximately 1 μg of total RNA extracted as detailed in the previous section was used for cDNA synthesis by reverse transcription using

Table 1
Composition of plant growth media used in this study.

Media component	Treatment		
	Depleted K^+	Control	Excess K^+
1.[a] (mM)			
NH_4NO_3	20	20	20
KNO_3	0.2	10	20
$MgSO_4 \cdot 7H_2O$	1.5	1.5	1.5
KH_2PO_4	–	1.25	1.25
$NH_4H_2PO_4$	1.25	–	–
$Ca(NO_3)_2$	4	4	4
NaCl	2	2	2
2.[a] (mg/l)			
H_3BO_3	6.2	6.2	6.2
$MnSO_4 \cdot H_2O$	16.9	16.9	16.9
KI	0.83	0.83	0.83
$ZnSO_4 \cdot 7H_2O$	8.6	8.6	8.6
$CuSO_4 \cdot 5H_2O$	0.025	0.025	0.025
$CoCl_2 \cdot 6H_2O$	0.025	0.025	0.025
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25
3.[a] (mg/l)			
Thiamine HCl	1	1	1
Pyridoxine HCl	1	1	1
Nicotinic acid	1	1	1

[a] 1. = Macronutrients; 2. = Micronutrients; 3. = Vitamins.