Research Progresses of Genetics and Molecular biology of Potassium uptake by Plant

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CONTENTS

- Genotypic differences of absorption and utilization of potassium in plant
- Genetic basis of plant potassium efficiency
- Molecular mechanism of potassium absorption and transportation
- Approach to potassium efficiency enhancement by genetic manipulation
- Points for future study
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Potassium uptake differs in different plant species

1979 Martin reported that the potassium uptake varies 1-14 times among different crops.

1983 Petterson and Jensen found the highest absorption rate range 3-6 \( \mu \text{mol/g.FW.h} \) with sunflower, cucumber, birch and pinaster, while for barley and wheat the rate is 10-15 \( \mu \text{mol/g.FW.h} \), 2-5 times higher than that of the former.

1994 Hu reported the Alternathera philoxeroides and Phytolaoca esculenta van Hout have strong ability to take up and accumulate potassium than other plants with solution culture, so named as potassium super-accumulation plants, so does the recently found Amaranthus.

1997 Yan et al reported that under soil conditions Alternathera philoxeroides and Phytolaoca esculenta van Hout can also absorb and accumulate potassium more than Sesbania, tobacco, wheat and broadbean.
Typical K-Hyperaccumulators:

Phytolaoca esculenta Van Hout (1)
Alternathera philoxeroides(2)
Amaranthus spp.(3)
Comparison of potassium content between K-accumulator and nonaccumulator

(Yan et al 1997 Pedosphere 7: 165-170)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Fluvo-aquaic soil</th>
<th>Yellow Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matter (g)</td>
<td>K content (gkg⁻¹)</td>
</tr>
<tr>
<td>Alternathera philoxeroides</td>
<td>8.05</td>
<td>72.4</td>
</tr>
<tr>
<td>Phytolaoca esculenta Van Hout</td>
<td>4.00</td>
<td>109.8</td>
</tr>
<tr>
<td>Feather cockscomb</td>
<td>2.91</td>
<td>90.1</td>
</tr>
<tr>
<td>Sesbania</td>
<td>2.50</td>
<td>48.2</td>
</tr>
<tr>
<td>Wheat</td>
<td>7.92</td>
<td>41.9</td>
</tr>
<tr>
<td>Broadbean</td>
<td>51.4</td>
<td>23.0</td>
</tr>
<tr>
<td>Tobacco</td>
<td>10.33</td>
<td>52.3</td>
</tr>
</tbody>
</table>
1968 With 66 bean breeds, Shea found that the difference of beans’ dry weight achieves 3 times under low potassium.

Later on, large amount of reports indicated the same phenomenon by other scientists with different plants (tomato by Markmur 1978; rice by Li 1985; wheat by Woodend 1987; tobacco by Yan et al. 1997, rice by Wang & Yang 1999; rape by Shi 2004; wheat by Liu 2006

Nowadays, potassium content difference in various kinds of species and genotypes was widely reported among wheat, maize, rice, barley, bean, tomato, ramee and tobacco.
K accumulation varieties among different wheat plants (Liu Xinhong 2006)
Distribution of available K in rhizosphere of five rape plants

(Shi et al 2004, Plant and Soil)

Depletion rate 31-48% in order:
890206>Xinongchangjiao>Luzhoujinhuang>Indian mustard=K-100
The physiology mechanism to potassium-efficiency difference

- **Absorption difference**
  - Root morphology,
  - absorption system,
  - root exudates

- **Utilization difference**

- **Transportation difference**
Characters of root architecture

- Plants of high tolerable to low potassium have characteristics of more root quantity, root number, root CEC and root surface (Steffens 1983; Liu 1987).

- Plants with higher potassium uptake have characteristics of stronger root system, less root radius, bigger root absorption surface and so on, especial high correlation between root fresh weight and total potassium absorption (Zhou 2001; Mengel 1985).

- High correlation between root hair volume and root potassium absorption rate (Classen & Barber 1980).
The Km of absorption of potassium super-accumulation plant-Alligator Alternanthera is lower than that of almost all common crops, and the Imax is higher than that (Peng 1986, Xie et al 1987, Wang & Shi unpublished).

The Km and Cmin of high potassium absorption plant-water spinach are all far below potassium concentration in common soil. (Yuan 1994)
Comparison of K uptake curve of Alligator Alternanthera vs tobacco

(Wang & Shi unpublished)
The potassium absorption rate is remarkably correlative to $H^+$ secretion in root (Glass 1981; Lin 1992).

The research on Amaranthus showed that potassium deficiency treatment enhances the secretion of photosynthetic product into soil, particularly oxalic acid (Tu 1999).
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Genetic basis of plant potassium efficiency

- As early as in 1960’s, Epstein reported that potassium efficiency is inheritable character.

- Early experiment (Shea 1967) showed that bean potassium efficiency was controlled by a single gene, the hybrid of low- and high-efficiency genotypes have no maternity effect, the controlling gene for potassium efficiency is pure recessive (KeKe).

- However, recent experiments lead the opposite conclusion: 1985 Li’s experiment on rice showed that potassium efficiency is controlled by polygene and is of quantitative genetic trait; the same conclusion with tomato by Figdore (1989), who show that potassium utilization efficiency is controlled by polygene and genetic ability is low and additive, dominant, additive × additive, epistatic effect and so on.
## Plant potassium efficiency QTLs mapping

<table>
<thead>
<tr>
<th>plants</th>
<th>chromosomes</th>
<th>QTLs No.</th>
<th>Relative trait</th>
<th>Reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>12 in total</td>
<td>175</td>
<td>Height, seedling and root dry weight, K accumulation, K absorption and use efficiency</td>
<td>1998 Wu</td>
</tr>
<tr>
<td>Rice</td>
<td>6&lt;sup&gt;th&lt;/sup&gt;, 9&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2</td>
<td>potassium absorption</td>
<td>2000 Flowers</td>
</tr>
<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;, 3&lt;sup&gt;rd&lt;/sup&gt;, 4&lt;sup&gt;th&lt;/sup&gt;, 5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4</td>
<td>fresh weight</td>
<td>2001 Harada</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>dry weight</td>
<td></td>
</tr>
<tr>
<td>Aristae</td>
<td>5&lt;sup&gt;th&lt;/sup&gt;, 15&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2</td>
<td>potassium concentration</td>
<td>2003 Atienza</td>
</tr>
<tr>
<td>Rice</td>
<td>7&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1</td>
<td>root K accumulation</td>
<td>2004 Lin</td>
</tr>
</tbody>
</table>
Localization of QTLs for K in the linkage map of Miscanthus sinensis (S. G. Atienza 2003)
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Absorption system of potassium in plant root

- **High-affinity absorption system** works at $\mu$M K level (1-200 $\mu$mol/ L).

- **Low-affinity absorption system** functions at mM K level (1-10mmol/ L).
Potassium is taken up and transported into plant tissues through high-affinity and low-affinity system, the details as follows:

<table>
<thead>
<tr>
<th>Expression Organ</th>
<th>Affinity Style</th>
<th>Low-Affinity</th>
<th>High-Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Absorption</td>
<td>K⁺ channel</td>
<td>Low-Affinity transporter(LCT1)</td>
<td>TRK/HKT transporter family</td>
</tr>
<tr>
<td></td>
<td>K⁺ channel</td>
<td>High-Affinity K⁺ channel</td>
<td>K⁺/H⁺ anti-transporter</td>
</tr>
<tr>
<td>Transportation</td>
<td>K⁺ channel</td>
<td>K⁺ channel</td>
<td>TRK/HKT transporter family</td>
</tr>
<tr>
<td>Guard Cell</td>
<td>K⁺ channel</td>
<td>K⁺ channel</td>
<td>High-Affinity K⁺ channel</td>
</tr>
<tr>
<td>Other organ</td>
<td>K⁺ channel</td>
<td>K⁺ channel</td>
<td>K⁺/H⁺ anti-transporter</td>
</tr>
</tbody>
</table>
K+ channels:

K+ channels are plasma-spanning protein, with characteristics of passive transportation, voltage dependence, ion selectivity, high efficiency transportation, passive absorption and so on. The saturation concentration is 0.3 mol/L, the velocity could reach 10^8 ion per second.

Subunit Structure of K+ Channel in Plant
**K⁺ channels:**

Shaker-K⁺ channel is the most widely found and studied K⁺ channels. Since 1992, 51 Shaker-K⁺ channel genes have been found in 16 different plant species. On the base of function, structure and cDNA sequence homology, the K⁺ channels could be divided into five group: I(AKT1), II(KAT1), III(AKT2), IV(AtKC1) and V(SKOR).
Sequence homology of maize K+ channel gene ZmKT1 to Arabidopsis KAT1 and KAT2 (Su et al 2001)

S4

ZmKT1 163  TLNMLRLWRLHRVSSLFARLEKDIRFN  189
KAT1 166  ILSMLRLWRLRRVSSLFARLEKDIRFN  192
KAT2 177  VLSMLRLWRLRRVSSLFARLEKDIRFN  203

P

ZmKT1 246  TALYWSITTLTTTGYGDLHAENPR  269
KAT1 249  TALYWSITTLTTTGYGDFHAENPR  272
KAT2 260  TALYWSITTLTTTGYGDLHAENPR  283

ZmKT1 vs KAT1/2: highly homology in the domain S4 and pore region, whole gene showed 50% identity at amino acid level.

the consensus voltage-sensitive domain (S4) and pore forming domain(P)
**ZmKT1** is highly selective and low-affinity to $K^+$

(Su et al 2001)
## Summary of isolated genes for potassium uptake and transport in plant

<table>
<thead>
<tr>
<th>Plant</th>
<th>KUP/HAK/KT family</th>
<th>TRK/HKT family</th>
<th>K⁺/H⁺antiporter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arabidopsis thaliana</td>
<td>rice</td>
<td>cotton</td>
</tr>
<tr>
<td>Identified</td>
<td>13</td>
<td>17</td>
<td>1</td>
</tr>
</tbody>
</table>
Genes for potassium uptake and transport function site

- **Stem**:
  - SPIK
  - KAT1, KAT2, KPT1, KST1, GORK

- **Leaves**:
  - SKOR, KZM1, AKT2/3, VFK1, OsAKT1, PKT2, SPICK2, VFK1, SPORK1

- **Root**:
  - HvHAK1, HAK1, HAK5, AtKUP1, AtKUP3, 4, 5, CaHAK1, TaHKT1, HvHKT1, AtHKT1, OsHKT1, 2; KEA1, 2, 3, 4, 5, 6
  - AKT1, AKT5, DKT1, LKT1, MKT1, OsAKT1, SKT1, TaAKT1, ZMK1, AtKC1, KDC1
Regulation of the genes for potassium uptake and transport in plant

- Nutrients supply level in growth medium
- Interaction with functional proteins \textit{in vivo}
### Regulation of the genes for potassium uptake and transport in roots by external K⁺ supply level

<table>
<thead>
<tr>
<th>Gene</th>
<th>Plant</th>
<th>Expression localization</th>
<th>function</th>
<th>Constitutive/induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HvHAK1</td>
<td>barley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAK1</td>
<td>rice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAK5, AtKUP1, AtKUP3, 4, 5</td>
<td>Arabidopsis</td>
<td></td>
<td></td>
<td>Induced by external K⁺ deficient stress</td>
</tr>
<tr>
<td>CaHAK1</td>
<td>pepper</td>
<td>roots</td>
<td>K⁺ influx</td>
<td>Induced by low NH₄⁺(&lt;1mM) and K⁺ deficiency stress</td>
</tr>
<tr>
<td>TaHKT1</td>
<td>wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HvHKT1</td>
<td>barley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtHKT1</td>
<td>Arabidopsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OsHKT1、2</td>
<td>rice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KEA1-KEA6</td>
<td>Arabidopsis</td>
<td></td>
<td></td>
<td>unknown</td>
</tr>
</tbody>
</table>
Overexpression of 14-3-3 protein in tobacco cell could increase $K^+$ conductance rate. 14-3-3 protein could activate KAT1 channel which affect directly KAT1 expression on plasma and then activate KAT1 channel.

G-protein could enhance $K^+$ flux of xylem organ and restrain inward-rectifying $K^+$ channels of guard cells which could be modulated by $Ca^{2+}$. 

Interaction with functional proteins \textit{in vivo}
Recently, Wu group demonstrate that CIPK23 directly phosphorylates the K+ transporter AKT1 and further find that CIPK23 is activated by the binding of two calcineurin B-like proteins, CBL1 and CBL9. They propose a model in which the CBL1/9–CIPK23 pathway ensures activation of AKT1 and enhanced K+ uptake under low-K+ conditions.
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## Approach tests to potassium efficiency enhancement by genetic manipulation

<table>
<thead>
<tr>
<th>gene</th>
<th>methods</th>
<th>Target plants</th>
<th>Phenotypes of transgenic plants</th>
<th>Reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAT1 AKT1</td>
<td>Particle bombardment</td>
<td>Arabidopsis</td>
<td>dry weight and K accumulation are remarkably higher than that of control. The transgenic tobacco has higher K absorption rate, with 18～22% more accumulation in leaves</td>
<td>2002 Shi</td>
</tr>
<tr>
<td>KAT1</td>
<td>Pollen Tube Pathway</td>
<td>cotton</td>
<td></td>
<td>2001</td>
</tr>
<tr>
<td>KAT1 AKT1</td>
<td>biolistic gun method</td>
<td>Rice, tobacco</td>
<td>K absorption rate and accumulation is significant higher</td>
<td>2003 Hu</td>
</tr>
<tr>
<td>Alligator Alternant hera DNA</td>
<td>Pollen Tube Pathway</td>
<td>rice</td>
<td>Tobacco leaves accumulate 45 % more K</td>
<td>2005 Guo</td>
</tr>
</tbody>
</table>
Identification of transgenic cotton by PCR analysis
\(\lambda/\text{HindIII marker; 240CK; 240-1; 240-4;240-60}\)
Better growth of transgenic cotton than WT
Decrease of efflux of $K^+(86\text{Rb})$ in transgenic cotton

![Graph showing the decrease of efflux of $K^+(86\text{Rb})$.]

- CK
- C1
- C2

- $y = 55460 \ln(x) - 89725$
  - $R^2 = 0.9728$
- $y = 52507 \ln(x) - 108636$
  - $R^2 = 0.9511$
- $y = 42285 \ln(x) - 88424$
  - $R^2 = 0.974$
Particle bombardment method

Regeneration of transgenic rice
### K$^+$ uptake and average absorption rate in rice seedlings

<table>
<thead>
<tr>
<th>Lines</th>
<th>K$^+$ uptake</th>
<th>Average absorption rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/plant.12h</td>
<td>µmol/plant.h</td>
</tr>
<tr>
<td>Z—8（WT）</td>
<td>36.7±0.80</td>
<td>3.33±0.07</td>
</tr>
<tr>
<td>T—22</td>
<td>55.9±0.47</td>
<td>5.09±0.04</td>
</tr>
<tr>
<td>T—30</td>
<td>46.1±0.96</td>
<td>4.18±0.09</td>
</tr>
<tr>
<td>Z—13（WT）</td>
<td>29.2±3.84</td>
<td>2.65±0.35</td>
</tr>
<tr>
<td>T—21</td>
<td>43.3±3.36</td>
<td>3.95±0.31</td>
</tr>
<tr>
<td>T—23</td>
<td>52.2±2.89</td>
<td>4.75±0.26</td>
</tr>
</tbody>
</table>
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The research of interfacial interaction between plant root and soil, especially the utilization mechanism of soil non-exchangeable potassium still needs.

Genetic basis to potassium efficiency is very important, further studies are necessary.

The structure genes involved in plant absorption and transportation system for potassium, particularly potassium channels have been well studied, but the regulation genes is still few indentified.

What’s the signal transduction pathway under low potassium stress?

The molecular technology for improving crop potassium utilization efficiency should be further studied and emphasized.
Thank You!