# Root K affinity drivers and photosynthetic characteristics in response to low potassium stress in K high-efficiency vegetable soybean

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**Research Article**

**Keywords:** chlorophyll a/b ratio, potassium, potassium high-efficiency, root bleeding-sap, vegetable soybean

**Posted Date:** April 7th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-349341/v1

## Abstract

**Aims** Vegetable soybean is highly demanded on potassium (K) application. Significant variations of K absorption and utilization exist in vegetable soybean. This study aim at exploring mechanisms of K absorption and utilization of high-efficiency in vegetable soybean by studying the characteristics of root K affinity-associated drivers and photosynthesis in vegetable soybean (edamame) (*Glycine max* (L.) Merr.).

**Methods** Pot and hydroponic experiments were carried out to examine the characteristics of root K affinity-associated drivers and photosynthesis in vegetable soybean genotypes with different K efficiency. Two K high-efficiency vegetable soybean genotypes and two K low-efficiency genotypes were investigated in low K and normal K conditions.

**Results** The root of K high-efficiency genotypes had a higher $K^+$ affinity associated with higher maximum $K^+$ uptake rate (*I*max), but lower Michaelis constant for $K^+$ absorption (*K*m) and lower compensation concentration for $K^+$ uptake (*C*min). Seedlings of K high-efficiency genotypes also had higher root vigor (TTC reduction method) and greater absorbing activity (methylene blue method), especially in the low K condition. Besides, the root bleeding-sap rate per root length and K upward fluxes rate per root length of K high-efficiency genotypes in beginning seed stage were consistently higher than that of K low-efficiency genotypes. The root of K high-efficiency vegetable soybean genotypes exhibits $K^+$ high-affinity and driving advantages. Photosynthetic parameters of K high-efficiency vegetable soybean genotypes were less affected by low K stress. Low K stress decreased the net photosynthetic rate of K high-efficiency genotypes by 6.1~6.9%, while that of K low-efficiency genotypes decreased by 10.9~15.7%. The higher Chl a/b ratio with enhanced relative content of Chl a in response to low K stress might be an adapted mechanism for K high-efficiency genotypes to maintain photosynthetic capacity.

**Conclusion** Stronger root K affinity drivers associated with photosynthetic adaptability to low potassium stress are the key factors in determining the K high-efficiency of vegetable soybeans.


## Introduction

Potassium application benefits vegetable soybean yield and quality (Liu et al., 2017), while the direct absorption and utilization for available potassium by plants in cultivated soil is always essential (Singh and Reddy, 2017; Chen et al., 2020). Selecting and breeding potassium efficient varieties of vegetable soybean is an important biological means in making full use of potassium resources (Rengel and Damon, 2008). Previous studies have shown that there are great differences in potassium efficiency among different genotypes. For instance, intraspecific variation in potassium efficiency have been reported in many crops including rice (*Yang* et al., 2003), wheat (*Zhang* et al., 1999; Damon and Rengel, 2007), sweet potato (*Wang* et al. 2015), tomato (*Chen* and *Gabelman*, 1995) and soybean (*Sale* and *Campell*, 1987; Wang et al., 2012a; Liu et al., 2019a).

Differences in potassium efficiency of crops can be understood from two main aspects: the difference of potassium uptake efficiency and the difference of potassium utilization efficiency (Rengel and Damon, 2008). The utilization efficiency of potassium refers to the ability of the crop to convert unit potassium into dry matter yield (Wang et al., 2018). K high-efficiency vegetable soybean genotypes are good at redistributing K and dry matters with higher harvest index (HI) and higher K harvest index (KHI) (Liu et al., 2019a). K uptake efficiency emphasizes the capacity of root K absorption (Tsialtas et al., 2017). The higher specific K uptake rate (total K content/total root length) in K high-efficiency vegetable soybean genotypes ensures the supply of K to the whole plant. Besides, K high-efficiency genotypes also have a strong ability to regulate their root architecture to adapt to low K conditions (Liu et al., 2019b).

Except for root morphology, K uptake kinetic parameters, root bleeding-sap, and root vigor are also demonstrated as effective parameters in evaluating K uptake efficiency (Teo et al., 1992; Hao et al., 2015; Cui et al., 2016; Zhang et al., 2017). Sufficient potassium can increase plant hydraulic conductance and transpiration (Pettigrew, 2008). The vigorous root can increase the nutrient and water uptake, and thus promote the whole plant growth. The root bleeding-sap and the upward fluxes of K are higher in the cotton cultivars with high potassium efficiency (Yang, 2011). As substrates for photosynthesis, water and mineral elements absorbed by plant roots are transported upward by transpiration (Liu et al., 2016). Improved root characteristics may contribute to plant-water status, enhanced photosynthesis, biomass, and yield of soybean cultivars (Cui et al., 2016). Thus, the uptake power of the root system determines the supply of nutrients in the above ground part of the plant.

Less photosynthetic assimilates and reduced assimilate transport out of the leaves to the developing fruit greatly contribute to the negative consequences that deficiencies of potassium have on yield and quality production (Pettigrew, 2008). Genotypic variations in photosynthetic decline caused by K deficiency have been reported. For instance, compared with K-inefficient cotton cultivar, the K-efficient cultivar has a higher net photosynthetic rate (*Pn*) associated with higher biomass products (*Wang* et al., 2012).

In our previous studies, K high-efficiency vegetable soybean genotypes were demonstrated with a strong K uptake and redistribution ability (Liu et al., 2019a; 2019b). But how the K high-efficiency genotypes uptake K and what characteristics of the roots in terms of absorptive power are not clear yet. Furthermore, the basic processes of synthesis and partitioning of photosynthetic assimilates that affect K use efficiency also need further examination (Hao et al., 2016). This study compared the differences of root activity, root bleeding-sap, the upward fluxes of K, K kinetics parameters, photosynthetic parameters, and chlorophyll content between K high-efficiency and K low-efficiency genotypes. The data obtained will reveal the mechanisms underlying K absorption and utilization of high-efficiency in vegetable soybean.

# Materials And Methods

## Plant Material

Based on previous K efficiency selection (Liu et al., 2019b), two K high-efficiency vegetable soybean genotypes (Line 19 and Line 20) and two K low-efficiency genotypes (Line 7 and Line 36) were used in this study. Compared with K low-efficiency genotypes, the K high-efficiency genotypes have higher K agronomic efficiency (KAE), recovery, internal utilization-efficiency rate (KIUE) and specific K uptake rate (Liu et al., 2019a, b). Greater reductions in K concentration of vegetative organs were found in K high-efficiency genotypes than K low-efficiency genotypes in low K conditions (Liu et al., 2019a). All genotypes were released by the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China.

## Experimental Design

A pot experiment was conducted at the agronomy farm of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China (45$^{o}$732N, 126$^{o}$612E; altitude 128 m a.m.s.l.) in 2017. The soil used was a typical Mollisol (Black soil) with the following properties: soil pH 6.6, organic matter 28.9 g $kg^{-1}$, total N 2.3 g $kg^{-1}$, total P 1.3 g $kg^{-1}$, total K 18.9 g $kg^{-1}$, available N 159 mg $kg^{-1}$, available P 57.0 mg $kg^{-1}$, and available K 85 mg $kg^{-1}$ (insufficient for vegetable soybean yield and quality; Liu et al. 2017). The experiment was performed in a randomized complete design with three replications, and 5 pots were used for each replication. Three seeds per pot were sown in May 2017 with regular manual pest and weed control. The pot size was 32 cm in diameter by 27 cm tall. A uniform fertilizer application at seeding included 70 kg $ha^{-1}$ diammonium phosphate and 98 kg $ha^{-1}$ urea. Treatments consisted of two K-fertilizer rates at seeding: $K_2SO_4$ 0 kg $ha^{-1}$ (K0) and $K_2SO_4$ 120 kg $ha^{-1}$ (K120). Plants were randomly harvested at fourth-node (V4), full bloom (R2), beginning seed (R5), and full seed (R6) stages (Fehr et al. 1971) for root bleeding-sap and K upward fluxes measurement.

A hydroponic experiment was used for $K^+$ absorption kinetic parameters, root vigor, and root absorbing activity test. Seeds of the four selected genotypes were sterilized and germinated on moistened filter paper in a plant growth chamber at 60% humidity and 28$^{o}$C, under a 16 h light, 8 h dark cycle for 3–4 days. After that, the seedlings were transferred into light-proof glass boxes (volume 15 × 20 × 20 $cm^3$) with half-strength modified Hoagland nutrient solution, as described by Wang et al. (2012a). There were six replications in each box. The cotyledons of all samples were excised to eliminate any additional supply of nutrients. When the plants grew to the first trifoliolate stage (V1), they were treated with half-strength Hoagland nutrient solution with different concentrations of $K^+$ (0.5 and 3.0 mmol $L^{-1}$). The potassium source was $KNO_3$, and the difference of $NO_3^-$ was adjusted by $NH_4NO_3$ in the low-K treatment. The initial pH value of the nutrient solution was 6.0, which was adjusted by 0.1 mol $L^{-1}$ NaOH or 0.1 mol $L^{-1}$ HCl. After transplanting, the samples were continuously cultured for 9 days. The nutrient solution was changed every 3 days and kept enough $O_2$ by regular ventilation with an air pump. The pH value of the solution was adjusted to 6.0 when the nutrient solution was changed.

## Measurements

Collection of root bleeding-sap: root bleeding-sap collection was based on the method used by Cui et al (2016) with slight improvements in pot experiment. The root bleeding-sap was conducted from 9:30 a.m. to 13:30 p.m. at V4, R2, R5, and R6 stages. The plants were cut with branch scissors at cotyledons position, and the cross-section was cleaned with a small amount of absorbent cotton and then immediately connected to the root bleeding-sap collection device and the joint was sealed. The collected root bleeding-sap was temporarily stored in a refrigerator at 4 $^{o}$C for measurement. The formula was as followed: root bleeding-sap rate (g $h^{-1}$ per plant) = bleeding-sap weight/4 h.

K upward fluxes = root bleeding-sap rate × K concentration, K concentration was determined by flame spectrophotometer (Liu et al., 2017; Nguyen et al., 2017).

$K^+$ absorption kinetic parameters: After potassium deficiency and starvation for 48 hours, samples under the treatment of K3.0 at 9 d were immersed in 0.2 mmol $L^{-1}$ $CaSO_4$ solution for 3 times, dried and put into a 200 mL absorption solution. The composition of the absorption solution was 0.05 mmol $L^{-1}$ KCl + 0.2 mmol $L^{-1}$ $CaSO_4$. The absorption solution was placed in a dark conical flask, the culture temperature was (25 ± 2) $^{o}$C, and the light intensity was 210–260 mol $m^{-2}$ $s^{-1}$. Parameter calculation including the maximum $K^+$ uptake rate (*I*max), Michaelis constant for $K^+$ absorption (*K*m), compensation concentration for $K^+$ uptake (*C*min) and statistical analysis refer to the method of Hao et al. (2015).

Root vigor, as determined by enzymatic reduction assay of triphenyltetrazolium chloride (TTC) was conducted according to the modified protocol of Duncan and Widholm (2004).

Root absorption activity was determined by methylene blue adsorption (Song et al., 2005). Firstly, the root volume was tested by drainage method, and the root surface water was blotted with an absorbent paper, and then the root was put into a solution with a known concentration of methylene blue for 1.5 min. Removed and placed the root into the second beaker, repeated twice. Colorimetric determination of methylene blue was conducted in the remaining solution in the three beakers at 660 nm using the Uv-visible spectrophotometer.

Total absorbing area ($m^2$) = [(C1- C1′)×V1] ×1.1 + [(C2 - C2′)×V2]×1.1

Actively absorbing area ($m^2$) = [(C3- C3′)×V3]×1.1

Ratio of actively absorbing area to total absorbing area (%) = 100 × actively absorbing area/ total absorbing area

Ratio of total absorbing area to root volume ($m^2$ $cm^{-3}$) = Total absorbing area/Root volume

**Note**

C, the original concentration of the solution (mg $ml^{-1}$); C′, concentration of the solution after leaching (mg $ml^{-1}$); 1, 2, 3 is the beaker number; V, Volume of the solution; When 1 mg methylene blue forms a monolayer, it covers an area of 1.1 $m^2$.

Photosynthetic properties of the youngest fully expanded main-stem leaf (the 3rd leaf from apex) were determined at 10:00–12:00 a.m. at V4, R2, R4 (full pod), R5, and R6 stages with a Li-6800 (Li-COR, Lincoln, USA) at 25°C, 60% relative humidity, 500 μmol $mol^{-1}$ $CO_2$ concentration and 1200 μmol $m^{-2}$ $s^{-1}$ quantum flux.

Determination of chlorophyll (Chl) content: 0.2 g fresh leaves were cut into pieces, and put into a 50 mL centrifuge tube, with additional 20 mL of 80% ethanol solution, and then soaked in a dark place for 8 h. Absorbance was determined at 665 nm and 649 nm respectively by the Uv-visible spectrophotometer.

Chl a = $13.95A_{665}$-$6.88A_{649}$

Chl b = $2.96A_{649}$-$7.32A_{665}$

Total chlorophyll concentration = Chl a + Chl b

### Statistical Analyses

Analysis of variance, mean separation and correlation analysis were performed using SPSS 17.0 and Genstat 12.0. LSD test was used for means separation when treatments were significant.

# Results

### Comparison of $K^+$ Absorption Kinetic Parameters Between Two K Efficiency Genotypes

Table 1 shows the $K^+$ kinetics absorption parameters of the four vegetable soybean genotypes in a hydroponic experiment. The maximum $K^+$ uptake rate (*I*max) in K high-efficiency genotypes was around 58.2 ~ 65.5 μmol $g^{-1}$ $min^{-1}$ FW, which was significantly higher than that of K low-efficiency genotypes around 42.4 ~ 54.1 μmol $g^{-1}$ $min^{-1}$ FW ($P < 0.05$). The compensation concentration for $K^+$ uptake (*C*min) in K high-efficiency genotypes was 0.83 ~ 1.32 μmol $L^{-1}$, which was lower than that of K low-efficiency genotypes (3.16 ~ 3.22 μmol $L^{-1}$) ($P < 0.05$). The Michaelis constant for $K^+$ absorption (*K*m) is a parameter evaluating the affinity between root and $K^+$. Higher affinity was found in K high-efficiency vegetable soybean genotypes with a lower *K*m of 32.8 ~ 35.0 μmol $L^{-1}$ than that of 48.6 ~ 49.0 μmol $L^{-1}$ in K low-efficiency genotypes ($P < 0.05$).

Table 1

Comparison of $K^+$ absorption kinetic parameters of root systems between two potassium efficiency types under low potassium stress

| | **Line 19** | **Line 20** | **Line 7** | **Line 36** |
|---|---|---|---|---|
| *Imax*(μmol $g^{-1}$ $min^{-1}$ root $FW^{-1}$) | 65.0 a | 58.2 b | 42.4 d | 54.1 c |
| *Km*(μmol $L^{-1}$) | 35.0 b | 32.8 c | 49.0 a | 48.6 a |
| *Cmin*(μmol $L^{-1}$) | 1.32 b | 0.83 c | 3.16 a | 3.22 a |
| Note: ANOVA, LSD test, $P \leq 0.05$. | | | | |

### Root Bleeding-Sap and Upward Fluxes of $K^+$ in Pot Experiment

As shown in Fig. 1, root bleeding-sap rate per plant showed a trend of increasing first and then decreasing from fourth-node (V4) to full seed (R6) stage with the maximum at full bloom (R2) stage. The root bleeding-sap rate per plant of Line 20 was the highest among the four genotypes with 1.8 mL $h^{-1}$ $root^{-1}$ in K0 and 1.6 mL $h^{-1}$ $root^{-1}$ in K120 respectively. Compared with K120 treatment, K0 treatment increased the average of the root bleeding-sap rate per plant over the four stages by 9.9%~24.3% in K high-efficiency genotypes, but by -2.2 ~ 5.1% in K low-efficiency genotypes. On the other hand, the root bleeding-sap rate per root length in four genotypes was found higher at V4 ~ R2 stage, dropped down at R5 ~ R6 stage. Interestingly, K high-efficiency genotypes had a higher root bleeding-sap rate per root length compared with K low-efficiency genotypes at beginning seed stage ($P < 0.05$). At this time, root bleeding-sap rate per root length ranged 0.09 ~ 0.13 mL $h^{-1}$ $cm^{-1}$ in K0 and 0.10 ~ 0.15 mL $h^{-1}$ $cm^{-1}$ in K120 treatment, while in K low-efficiency genotypes kept around 0.05 mL $h^{-1}$ $cm^{-1}$ in K0 and 0.05 ~ 0.07 mL $h^{-1}$ $cm^{-1}$ in K120 treatment.

Higher K upward fluxes were found in K120 treatment over the four genotypes, which was accompanied consistently by a higher concentration of potassium in the root bleeding-sap (Fig. 2). The K flux rate per root length was highest at fourth-node stage, with plant growth K flux rate per root length decreased. At beginning seed stage, K high-efficiency genotypes had higher K flux rate per root length and K concentration of root bleeding sap compared with K low-efficiency genotypes ($P < 0.05$). The K flux rate per root length of K high-efficiency genotypes was 19.4 ~ 32.4 μg $h^{-1}$ $cm^{-1}$ in K0 and 24.1 ~ 37.2 μg $h^{-1}$ $cm^{-1}$ in K120 treatment, that of 7.6 ~ 8.7 μg $h^{-1}$ $cm^{-1}$ in K0 and 9.0 ~ 15.2 μg $h^{-1}$ $cm^{-1}$ in K120 treatment in K low-efficiency genotypes. Meanwhile, K concentration of root bleeding-sap in K high-efficiency genotypes reached 51.1 ~ 63.2 μg $mL^{-1}$ in K0, and 60.2 ~ 64.0 μg $mL^{-1}$ in K120 treatment at beginning seed stage, which was significantly higher than that of K low-efficiency genotypes with 41.1 ~ 48.0 μg $mL^{-1}$ in K0 and 46.1 ~ 52.4 μg $mL^{-1}$ in K120 treatment ($P$ □ 0.05).

Correlation analysis revealed that K upward fluxes rate per root length and K concentration of root bleeding-sap were positively correlated with K concentration per plant ($P < 0.05$) (Table 2).

Table 2
Correlation analysis of root bleeding-sap rate per root length, K upward fluxes rate per root length, K concentration of root bleeding-sap, and plant K concentration in vegetable soybean

| | K upward fluxes rate per root length | K concentration of root bleeding-sap | Plant K concentration |
|---|---|---|---|
| Root bleeding-sap rate per root length | 0.884** | 0.415* | 0.707** |
| K upward fluxes rate per root length | | 0.745** | 0.831** |
| K concentration of root bleeding-sap | | | 0.598** |

* $P < 0.05$, ** $P < 0.01$ for significance of correlations (Pearson). Plant K concentration data based on the study of Liu et al., (2019b).

### Root Vigor And Absorbing Activity

The root vigor was tested in a hydroponic experiment using the measurement of respiratory activity with triphenyltetrazolium chloride (TTC) (Fig. 3). At the seedling stage (9d after treatment), the root vigor of potassium high-efficiency genotypes was obviously induced by low potassium stress. Low K stress (K0.5) increased the root vigor of K high-efficiency genotypes by 46 ~ 85% compared with normal K treatment (K3.0) ($P < 0.05$), Although no consistent trend was observed in K low-efficiency genotypes, low K stress decreased root vigor by 21% in Line 36 ($P < 0.05$). The highest root vigor of 516 μg $g^{-1}$ FW $h^{-1}$ was found in Line 20 under low K condition.

The root absorbing activity parameters are shown in Table 3. Higher total absorbing area and actively absorbing area were found in K high-efficiency genotypes. Meanwhile, in K high-efficiency genotypes, low K stress increased the actively absorbing area by 21.4 ~ 30.6% and total absorbing area by 9.6 ~ 19.0% ($P < 0.05$). In contrast, an opposite trend was found in K low-efficiency genotypes. Under low potassium stress, the actively absorbing area decreased by 6.1 ~ 10.3% and the total absorbing area decreased by 6.6 ~ 15.7% ($P > 0.05$). Besides, K high-efficiency genotypes also had a higher ratio of total absorbing area to root volume, especially in Line 19 with 62.3 $m^2$ $cm^{-3}$ (K0.5) and 60.2 $m^2$ $cm^{-3}$ (K3.0). Low K stress increased the ratio of actively absorbing area to total absorbing area by 9.3 ~ 9.4% in K high-efficiency genotype ($P < 0.05$), but by 0.6 ~ 5.0% in K low-efficiency genotypes ($P > 0.05$).

Table 3
Root adsorption activity of vegetable soybean genotypes with different potassium efficiency

| | | **Total absorbing area** ($m^2$ $plant^{-1}$) | **Actively absorbing area** ($m^2$ $plant^{-1}$) | **Ratio of actively absorbing area to total absorbing area (%)** | **Ratio of total absorbing area to root volume** ($m^2$ $cm^{-3}$) |
|---|---|---|---|---|---|
| Line 19 | K0.5 | 0.103 a | 0.051 a | 49.5 a | 62.3 a |
| | K3.0 | 0.094 b | 0.042 bc | 45.3 bc | 60.2 a |
| Line 20 | K0.5 | 0.094 b | 0.047 ab | 49.9 a | 52.9 b |
| | K3.0 | 0.079 c | 0.036 cd | 45.6 bc | 52.5b |
| Line 7 | K0.5 | 0.070 d | 0.035 de | 50.1 a | 52.1 b |
| | K3.0 | 0.083 c | 0.039 cd | 47.7 ab | 44.2 c |
| Line 36 | K0.5 | 0.071 d | 0.031 e | 43.9 c | 40.6 cd |
| | K3.0 | 0.076 cd | 0.033 de | 43.6 c | 38.8 d |
| Note: ANOVA, LSD test, $P \leq 0.05$. | | | | | |

### Comparison Of Photosynthetic Parameters Between Two K Efficiency Genotypes

Pot experiment was conducted to determine photosynthetic parameters of two K efficiency vegetable soybean genotypes affected by K deficiency (K0) are shown in Fig. 4. K deficiency decreased photosynthetic rate (*Pn*) of the four vegetable soybean genotypes. During the whole growth stages, the *Pn* of K high-efficiency genotypes decreased by 6.1 ~ 6.9%, while that of low-efficiency genotypes decreased by 10.9 ~ 15.7%. From full bloom to full seed stage, the *Pn* increased first and then decreased. The maximum value occurred at full pod (R4) stage of the potassium low-efficiency genotype Line 36, which reached 28.8 μmol $CO_2$ $m^{-2}$ $s^{-1}$ and 33.8 μmol $CO_2$ $m^{-2}$ $s^{-1}$ under K0 and K120 treatments, respectively. Meanwhile, from full pod to full seed stage, dramatic decline of the *Pn* was found in K low-efficiency genotypes. The *Pn* of Line 36 decreased by 87.2% in K0 and 86.2% in K120 treatment ($P < 0.05$), and that of Line 7 decreased by 54.3% in K0 and 59.0% in K120 treatment. While, the decline of the *Pn* in K high-efficiency genotypes was around 33.5 ~ 51.8% in K0 and 36.7 ~ 41.1% in K120 treatment, which was not as much as that of K low-efficiency genotypes.

K deficiency also decreased the stomatal conductance (*Gs*) in all genotypes, but K high-efficiency genotypes were less affected. The decrease of *Gs* by K deficiency was 7.5 ~ 7.8% in K high-efficiency genotypes, but 9.4 ~ 12.5% in K low-efficiency genotypes. Low K treatment had the most obvious effect on *Gs* at beginning seed stage.

As opposed to *Pn* and *Gs*, K deficiency increased the intercellular $CO_2$ concentration (*Ci*), especially in K low-efficiency genotypes. The increase of *Ci* by K deficiency was 7.3 ~ 7.9% in K low-efficiency genotypes, but 2.0 ~ 6.0% in K high-efficiency genotypes.

K deficiency decreased transpiration rate (*Tr*) of all genotypes. Across the five stages, *Tr* of K high-efficiency genotypes was around 8.8 ~ 9.8 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K0 and 9.0 ~ 10.1 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K120 treatment, which was higher than that of 7.1 ~ 8.2 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K0 and 7.1 ~ 8.6 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K120 treatment in K low-efficiency genotypes. At full seed stage, higher *Tr* was found in K high-efficiency genotypes with 4.0 ~ 4.5 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K0 and 4.2 ~ 4.7 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K120 tretment, but only 1.0 ~ 1.2 mmol $H_2O$ $m^{-2}$ $s^{-1}$ and 1.4 ~ 1.6 mmol $H_2O$ $m^{-2}$ $s^{-1}$ in K low-efficiency genotypes.

## Chlorophyll

Pot experiment indicated that K deficiency significantly decreased leaf chlorophyll content of the four vegetable soybean genotypes (Table 4). The average total chlorophyll content (Chl) in the four growth stages of K high-efficiency genotypes was 2.29 ~ 2.53 mg $g^{-1}$ in K0 and 2.89 ~ 2.91 mg $g^{-1}$ in K120 treatment, which was lower than that of K low-efficiency genotypes with 2.57 ~ 3.20 mg $g^{-1}$ in K0 and 3.08 ~ 3.50 mg $g^{-1}$ in K120 treatment. However, K deficiency increased the ratio of Chl a to Chl b, especially in K high-efficiency genotypes. The ratio of Chl a to Chl b was increased by 36.2 ~ 38.1% in K high-efficiency genotypes ($P < 0.05$), but only by 6.0 ~ 9.8% in K low-efficiency genotypes.

Table 4

Changes of chlorophyll a, chlorophyll b, and total chlorophyll concentrations (mg $g^{-1}$) of vegetable soybean with different K efficiency in pot experiment

| Stage | | Line 19 | | Line 20 | | Line 7 | | Line 36 | |
|---|---|---|---|---|---|---|---|---|---|
| | | **K0** | **K120** | **K0** | **K120** | **K0** | **K120** | **K0** | **K120** |
| V4 | Chl a | 1.66 d | 1.99 ab | 1.36 f | 1.86 c | 1.83 c | 1.94 b | 1.46 e | 2.06 a |
| | Chl b | 0.26 d | 0.59 b | 0.25 d | 0.68 a | 0.48 c | 0.54 bc | 0.31 d | 0.47 c |
| | Total | 1.92 c | 2.58 a | 1.61 e | 2.54 a | 2.31 b | 2.48 a | 1.77 d | 2.53 a |
| | Chl a/b ratio | 6.36 a | 3.36 f | 5.33 b | 2.73 g | 3.78 e | 3.61 ef | 4.77 c | 4.41 d |
| R2 | Chl a | 1.90 d | 1.97 d | 2.11 c | 2.22 b | 2.30 b | 2.53 a | 2.08 c | 2.21 b |
| | Chl b | 0.95 d | 1.05 c | 1.12 b | 1.28 a | 1.03 c | 1.28 a | 1.12 b | 1.25 a |
| | Total | 2.84 e | 3.02 d | 3.23 c | 3.50 b | 3.34 bc | 3.81 a | 3.21 c | 3.46 b |
| | Chl a/b ratio | 2.00 b | 1.86 bc | 1.88 bc | 1.74 c | 2.23 a | 1.98 b | 1.86 bc | 1.76 c |
| R5 | Chl a | 2.03 c | 2.26 b | 1.68 d | 2.11 c | 2.31 b | 2.48 a | 2.18 bc | 2.56 a |
| | Chl b | 1.01 c | 1.17 b | 0.86 d | 1.15 b | 1.17 b | 1.33 a | 0.97 c | 1.20 b |
| | Total | 3.04 c | 3.44 b | 2.53 d | 3.26 bc | 3.48 b | 3.80 a | 3.15 c | 3.76 a |
| | Chl a/b ratio | 2.01 bc | 1.93 bc | 1.96 bc | 1.83 c | 1.97 bc | 1.87 c | 2.24 a | 2.13 ab |
| R6 | Chl a | 1.52 de | 1.63 d | 1.17 f | 1.47 e | 2.53 b | 2.68 a | 1.59 de | 1.80 c |
| | Chl b | 0.81 cd | 0.88 c | 0.63 de | 0.87 c | 1.13 b | 1.23 a | 0.57 e | 0.78 cd |
| | Total | 2.33 de | 2.51 cd | 1.80 f | 2.34 de | 3.67 b | 3.92 a | 2.16 e | 2.58 c |
| | Chl a/b ratio | 1.87 cd | 1.84 cd | 1.87 cd | 1.69 d | 2.23 b | 2.17 bc | 2.77 a | 2.31 b |

Note: ANOVA, LSD test, $P \leq 0.05$.

# Discussion

Potassium high-efficiency including K uptake- and use-efficiency two aspects, that is crops should have strong K uptake capacity and dry matter conversion capacity (Damon and Rengel, 2007). Consistent with this, our previous studies indicated that K high-efficiency vegetable soybean genotypes have higher K recovery rate and K agronomic efficiency rate combined with more adaptable root morphology parameters and more adequate dry matter partitioning rate (Liu et al., 2019a, b). Based on these, how the K high-efficiency vegetable soybean roots absorb K and how their photosynthetic assimilation performed needs to be further revealed.

## Characteristics of Root K Uptake Drivers in K High-Efficiency Genotypes

According to the theory conducted by Silberbush et al. (1983), the most important factor affecting K uptake would be a long fine root system. The second characteristic would be the value for maximal influx. After that, K kinetic parameters and root activities also act as important factors assessing K efficiency when plants suffering low K stress (*Silberbush* et al. 1983; Cui et al., 2016; White et al., 2018). Potassium high-efficiency vegetable soybean genotypes are more efficient in root architecture adjustment to adapt to low K conditions. Meanwhile, K high-efficiency genotypes have higher specific root K uptake rate (total K accumulation/total root length) than K low efficiency genotypes, which ensures an adequate supply of potassium (Liu et al., 2019b). Based on these, in this study, we further investigated the root bleeding-sap rate and K upward fluxes rate, which are important indicators assessing root pressure, root activity and K absorption abilities (*Doussan* et al., 2006; Wang et al., 2020b). The results indicated that K high-efficiency vegetable soybean genotypes against with low K condition by increasing root bleeding-sap rate per plant and maintaining higher root bleeding-sap rate per root length at beginning seed stage. In K high-efficiency cotton cultivars, higher root bleeding-sap and K upward fluxes could also be induced by low K stress (Yang, 2011). Suitable grafting would help watermelon seedlings accumulate more K by increasing root bleeding-sap volume and the total K in the root bleeding-sap (*Huang* et al., 2013). Due to the rate of root bleeding-sap is closely related to plant nutrient supply, water transport and even photosynthesis (*Guan* et al., 2014; *Jia* et al., 2018; He et al., 2019), increased rate of root bleeding-sap under K deficiency might be an important regulation mechanism for vegetable soybean efficient uptake K. Besides, beginning seed stage is a most important period for seed establishment, the higher root bleeding-sap rate per root length in K high-efficiency vegetable soybean genotypes accompanied by higher K upward fluxes rate per root length and higher potassium concentration of root bleeding-sap is positively correlated with plant K concentration ($P < 0.05$). This is another evidence demonstrating that the root of K high-efficiency genotypes has a stronger affinity for $K^+$.

The absorption kinetic parameters of ion uptake are useful indexes of the level of adaptation of the genotype to the nutrient condition in the soil (Crowley, 1975; *Daniel et al.*, 2020). Hao et al. (2015) also recognized that K kinetic parameters could be used to test crop low-K adaptability. The present study revealed a lower Michaelis constant ($K$m) and compensation concentration for $K^+$ uptake ($C$min), and higher maximum $K^+$ uptake rate ($I$max) from K high-efficiency genotypes, compared with K low-efficiency genotypes. Lower *K*m and *C*min indicate higher affinity between the roots and $K^+$ and stronger ability to use the low-concentration $K^+$, while higher *I*max ensures a faster K uptake rate (Glass, 1980; Teo et al., 1992). Therefore, it was reasonable to say that K high-efficiency vegetable soybean genotypes had better K adaptability than K low-efficiency genotypes.

Root activity is another important heritable trait in evaluating root absorption ability, which also influences nutrients acquisition and initial canopy cover and, thereby, crop yields (Liu et al., 2015; Cui et al., 2016; White et al., 2018). In present study, both root vigor and absorbing activity (including actively absorbing area, total absorbing area and the ratio of actively absorbing area to total absorbing area) are consistently enhanced by low K stress in K high-efficiency genotypes, while those of K low-efficiency genotypes are inhibited. Therefore, K high-efficiency genotypes are more adapted to low K stress through regulating root activities, which is beneficial for nutrients upward flux (Liu et al., 2015).

### Photosynthetic Characteristics Of K High-efficiency Vegetable Soybean Genotypes

Photosynthetic parameters affected by K levels are direct references to characterize the photosynthetic capacity of crops (Wang et al., 2020a). Many investigations have shown that crops with high K efficiency should be more efficient in photosynthesis, or that photosynthetic capacity is less affected by low potassium stress (Wang et al., 2014). For instance, under K deficiency, *Pn* of K-efficient cotton cultivar Liaomian 18 is 19.4% higher than that of K-inefficient cultivar NuCOTN99[B]. Besides, photosynthetic parameters of Liaomian 18 was less affected by low K stress (*Wang* et al., 2012). In present study, photosynthetic parameters are also less affected in K high-efficiency genotypes. We did not find absolute superiority of *Pn* in K high-efficiency genotypes, but they have a higher harvested index (HI) and K harvest index (KHI) (Liu et al., 2019a). A high harvest index is fundamental to efficient utilization of all resources taken up by the plant, and the photosynthate transport and distribution rather than photosynthesis rate are critical for low K adaptation in K high-efficiency genotypes (Rengel and Damon, 2008; Hao et al., 2016).

Consistent with photosynthetic parameters, chlorophyll (Chl) content also reflects the photosynthetic activity in leaves (Szafrańska et al., 2017; Choudhury et al., 2019). The effect of photosynthetic photon flux density on the leaf Chl a/b ratio is one of the most characteristic differences between sun and shade leaves (Abtahi et al., 2019). Typically, total Chl content per unit leaf area is lower and the Chl a/b ratio is greater in sun compared to shade soybean leaves (Anderson, 1986; *Fritschi* et al., 2007). In present study, K high-efficiency vegetable soybean genotypes exhibit greater Chl a/b ratio but lower total Chl content when suffering low K stress. The higher Chl a/b ratio with enhanced relative content of Chl a in response to low K stress might be an adapted mechanism for K high-efficiency genotypes to maintain photosynthetic capacity, because Chl a is the main pigment in leaves that absorbs light energy, which ensures light absorption as much as possible (Abtahi et al., 2019). Similar results were also revealed in the research of Wang et al. (2008). The present study recognized that the high efficiency of photosynthesis, including more adaptable photosynthetic parameters and chlorophyll proportion, was essential for K utilization efficiency in K high-efficiency vegetable soybean genotypes.

## Conclusions

This study identified some important characteristics of K high-efficiency vegetable soybean genotypes from both K uptake and utilization aspects (Fig. 5). Firstly, higher affinity of root to $K^+$ associated with root activity under low K stress is essential to promote root K absorption. The strong drivers, represented with higher root bleeding-sap rate and *Tr* induced by low K stress, ensure the upward flux of $K^+$ and other essential nutrients as much as possible. After that, the photosynthetic system of K high-efficiency vegetable soybean genotypes is less affected and reasonably regulated by low K stress to maintain photosynthates. Therefore, the ability to redistribute photosynthetic products seems more important than photosynthetic capacity in K high-efficiency genotypes. Overall, crop K high-efficiency should be holistic, and factors involved are not isolated. Stronger root K affinity drivers associated with photosynthetic adaptability to low potassium stress are the key factors in determining the K high-efficiency of vegetable soybeans.

## Declarations

### Acknowledgments

This research was partially funded by National Natural Science Foundation of China (grant No. 41977096), and Strategic Priority Research Program of Chinese Academy of Sciences (XDA24030403-3).

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# Figures

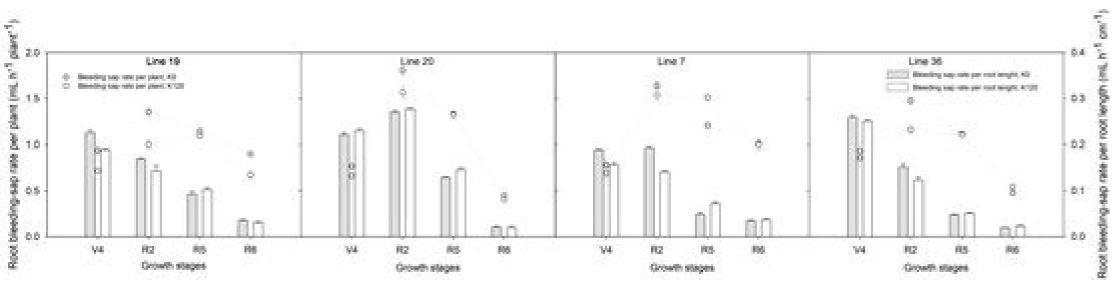
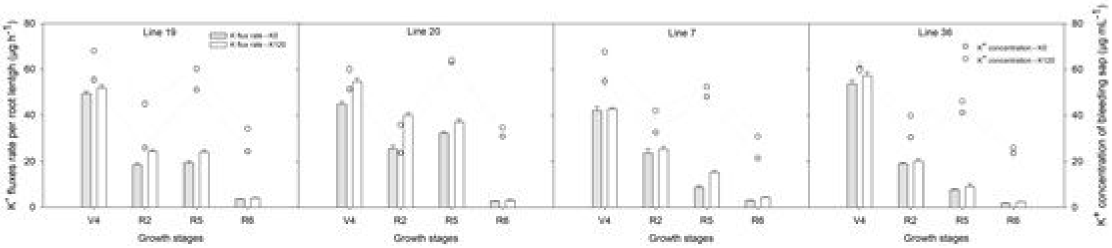


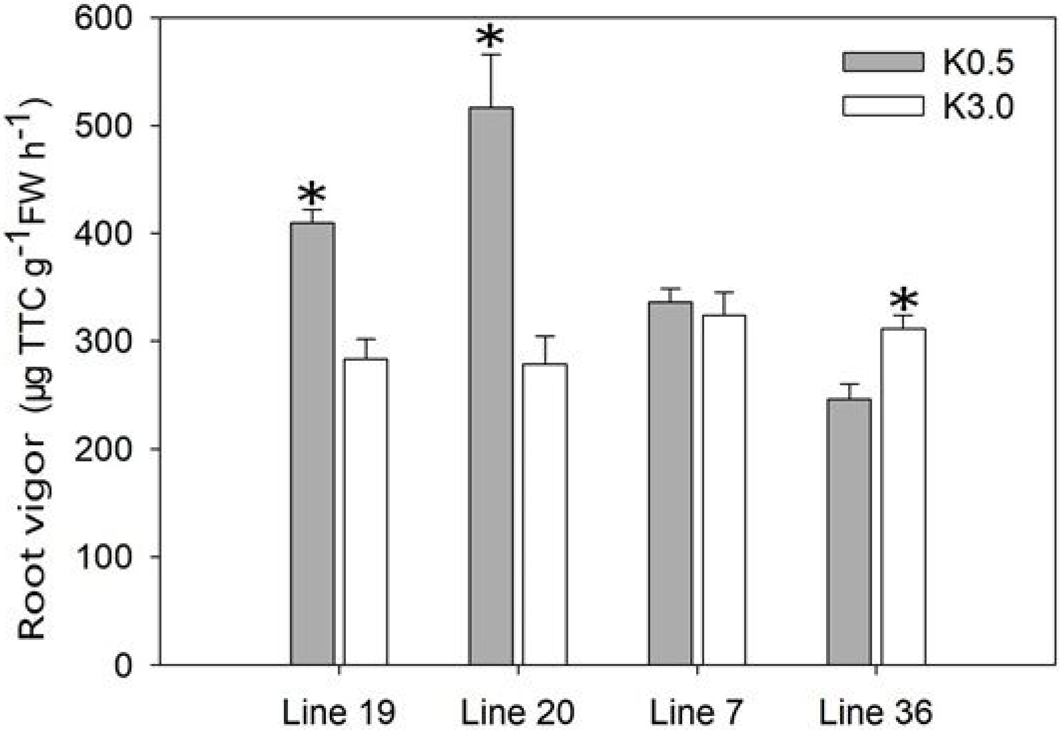

**Figure 1**

Root bleeding-sap of distinct potassium efficiency genotypes under low K application in pot experiment



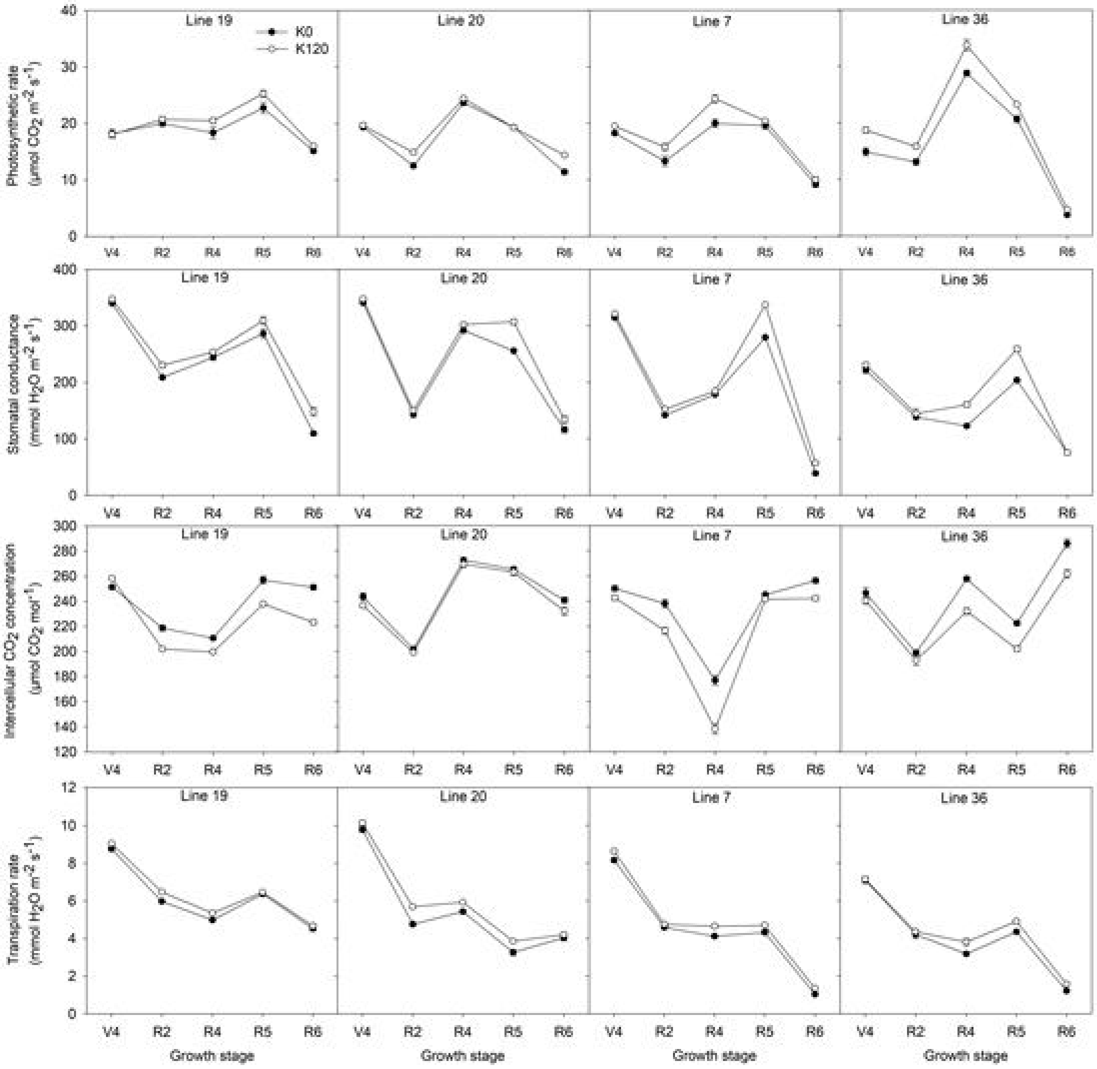

## Figure 2

Potassium upward fluxes and concentration in root bleeding sap between two potassium efficiency genotypes in pot experiment



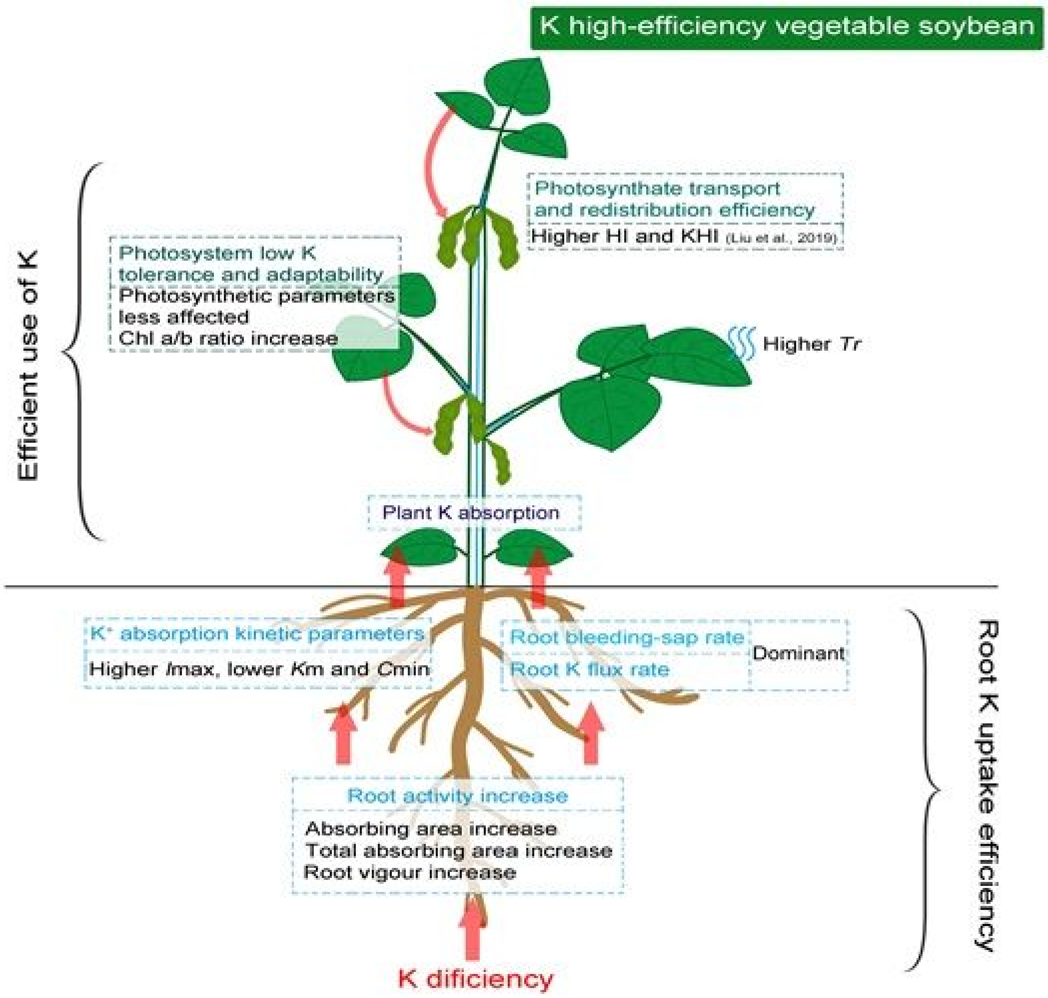

## Figure 3

Effect of low potassium treatment on root vigor of vegetable soybean with different potassium efficiency in hydroponic experiment *, P ≤ 0.05




## Figure 4

Changes of the photosynthetic parameters of vegetable soybean genotypes with different K efficiency in pot experiment




## Figure 5

Schematic diagram demonstrating efficient potassium absorption and photosynthetic assimilation in K high-efficiency vegetable soybean under low K condition