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# Grafting onto different rootstocks as a means to improve watermelon tolerance to low potassium stress

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ABSTRACT

To test the possibility that using appropriate rootstock could improve the tolerance of watermelon to low potassium (K) stress, a greenhouse experiment was conducted to determine plant growth and some physiological parameters of watermelon plants [*Citrullus lanatus* (Thunb.) Matsum. and Nakai, cv. Zaochunhongyu], either ungrafted or grafted onto the rootstock 'Hongdun' (*C. lanatus* sp.), 'Jingxinzhen No.4' (*Cucurbita moschata* Duch.) and 'Nabizhen' (*Lagenaria siceraria* Standl.). Plants were subjected to two levels of K (6.0 and 0.6 mM, supplied as $K_2SO_4$) for 20 days in hydroponic systems. Compared with plants treated with 6.0 mM K, those supplied with 0.6 mM K produced less biomass as indicated by the significantly decreased shoot and root dry weight in both ungrafted and grafted plants. However, a less decrease in shoot dry weight was observed in plants grafted onto 'Hongdun' (5%) and 'Jingxinzhen No.4' (3%) than in the ungrafted plants (23%). The xylem sap volume, total amount of K in the xylem sap, shoot K accumulation, K uptake efficiency, and leaf transpiration rate were significantly higher in the plants grafted onto 'Hongdun' and 'Jingxinzhen No.4' than in the ungrafted plants under 0.6 mM K conditions. However, the K utilization efficiency of shoot was quite similar among all the plants. In addition, the reduction in the shoot dry weight induced by low potassium stress (0.6 mM K) was similar in the plants grafted onto 'Nabizhen' (18%) and ungrafted plants (23%). Taken together, the above results supported the hypothesis that watermelon grafted onto adequate rootstocks ['Hongdun' (*C. lanatus* sp.) and 'Jinxinzhen No.4' (*C. moschata* Duch.)] can enhance plant tolerance to low potassium stress. The enhanced tolerance is mainly attributed to higher K uptake efficiency, rather than K utilization efficiency.



## 1. Introduction

Potassium (K) is an essential nutrient for plant growth and development (Schachtman and Liu, 1999), which plays vital roles in plant cells including osmoregulation, photosynthesis, enzyme activation, formation of carbohydrates, nucleic acids, and proteins (Fageria et al., 2001). It is the most abundant inorganic cation in plants, and can comprise as much as 8% of plant dry weight (Grabov, 2007). Potassium availability for plants is highly variable, due to complex soil dynamics, which is strongly influenced by root–soil interactions (Ashley et al., 2006). Consequently, potassium deficiency is a widespread problem in some soils. The conventional approach to solve this problem was to apply K fertilizer. However, the demand for K fertilizer exceeded the productive capacity (Liu, 2009). Therefore, the improvement of K efficiency in crops is an attractive way to reduce the cost of agricultural production (Rengel and Damon, 2008).

K efficiency consists of K uptake and K utilization efficiency, which is described as the yield potential that can be achieved under K deficiency (Damon and Rengel, 2007; Damon et al., 2007). The K efficient genotypes are able to absorb higher amount of K from soil (uptake efficiency) and produce more dry matter per unit of K taken up (utilization efficiency) (Rengel and Damon, 2008). Plant species and cultivars of a given species differ in their K uptake and utilization efficiency (Trehan and Claassen, 1998; Dessougi et al., 2002; Trehan and Sharma, 2002; Zhang et al., 2007; Hafsi et al., 2011; Wu et al., 2011).

Watermelon is an important crop that often suffers K deficiency, which results in a decrease in the plant growth, content of fruit sugar, vitamin C, and rind thickness etc. (Perkins-Veazie et al., 2003; Wang et al., 2010). One way to reduce losses in the plant performances caused by K deficiency in plants would be to graft K deficient cultivars onto rootstocks with high K uptake and use efficiency (Savvas et al., 2010). In the past, grafting was used widely in vegetable crops to limit the effects of soil-borne pathogens (Lee, 1994). In the recent years, it is reported that grafting can be used as an effective way to increase plant tolerance to abiotic stresses, such as low temperature (Zhou et al., 2007; Venema et al., 2008), copper toxicity (Rouphael et al., 2008), salinity (Huang et al., 2010),

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and alkalinity (Colla et al., 2010a). In addition, it is suggested that grafting can improve plant nutrient uptake and nitrogen utilization efficiency (Ruiz et al., 1997; Colla et al., 2010b). Nevertheless, no published data is available concerning the effects of grafting on the watermelon tolerance to low potassium stress (Savvas et al., 2010).

In our previous study, we found that fruit of watermelon plants grafted onto 'Hongdun' (*Citrullus lanatus* sp.) had higher K concentration, fruit yield and quality as compared with ungrafted plants (cv. Zaochunhongyu) under normal conditions, whereas the value in the plants grafted onto 'Nabizhen' (*Lagenaria siceraria* Standl.) was similar with ungrafted plants (Li et al., 2009). Therefore, our hypothesis is that grafting watermelon onto adequate rootstock can raise plant tolerance to low potassium stress by enhancing the uptake and utilization efficiency of potassium.

## 2. Materials and methods

### 2.1. Plant material and treatments

The experiment was conducted from September to October in 2009 in the greenhouse at the National Center of Vegetable Improvement in Huazhong Agricultural University, central China (latitude 30°27′N, longitude 114°20′E and altitude 22 m above sea level). 'Zaochunhongyu' [*C. lanatus* (Thunb.) Matsum. and Nakai., Mikado Kyowa Seed Co., Ltd, Japan] was grafted onto three rootstocks: 'Hongdun' (*C. lanatus* sp., Chendu Jiachen seedling Institute, China), 'Nabizhen' (*L. siceraria* Standl., Mitsuo Seed Co., Ltd, China), and 'Jingxinzhen No.4' (*Cucurbita moschata* Duch., Beijing Vegetable Research Center, China), using the procedure of 'insertion grafting' described by Lee (1994), whereas ungrafted 'Zaochunhongyu' plant was used as control. When the third true leaf emerged, the grafted plants were transplanted into 20 L plastic container (55 cm × 33 cm × 16 cm). There were 12 plants in each container. The full strength Shizuoka University nutrient solution formula was used as the base solution (Miyajima, 1994). Two potassium levels (6 mM, 0.6 mM) were used, using $K_2SO_4$ as the substance source. In the nutrition solution, the concentration of $K_2SO_4$ for 6 mM and 0.6 mM K treatment is 522 mg L$^{-1}$ and 52.2 mg L$^{-1}$, respectively, while other macronutrients and micronutrients were the same in solutions with two potassium levels: $Ca(NO_3)_2{\cdot}4H_2O$, 944 mg L$^{-1}$; $NH_4H_2PO_4$, 114 mg L$^{-1}$; $MgSO_4{\cdot}7H_2O$, 492 mg L$^{-1}$; $Na_2$Fe–EDTA, 35 μg L$^{-1}$; $H_3BO_3$, 2.86 μg L$^{-1}$; $MnSO_4{\cdot}H_2O$, 1.61 μg L$^{-1}$; $ZnSO_4{\cdot}7H_2O$, 0.22 μg L$^{-1}$; $CuSO_4{\cdot}5H_2O$, 0.08 μg L$^{-1}$; $(NH_4)_6$ $Mo_7O_{24}{\cdot}4H_2O$, 0.02 μg L$^{-1}$.

In the experiment, there are 8 treatments, with 4 graft combinations [Zaochunhongyu (Z), Z/Hongdun, Z/Jingxinzhen No.4, Z/Nabizhen] and 2 potassium levels. The 8 treatments were replicated 4 times with 12 plants in each replicate and were arranged in a randomized complete block design. The nutrient solutions were renewed at an interval of 5 days to avoid excessive depletion of any particular ions. Nutrient solutions were aerated using a pump every other hour during the day and were not aerated during the night. During the culture, plants were grown under natural light conditions at an average photosynthetic photon-flux density of 300 μmol m$^{-2}$ s$^{-1}$, and the day temperature of greenhouse was between 16 and 32 °C and day relative humidity between 50 and 95%. Plant samples were taken 20 days after potassium treatment.

### 2.2. Determination of plant growth

Four plants per treatment were harvested 20 days after potassium treatment, plants were dissected into shoot and root, for ungrafted plants, the part above the cotyledon node was regarded as "shoot" and the part bellow as "root", for grafted plants, the part above the graft union was regarded as "shoot" and the part bellow as "root". The shoot and root were placed into a forced air oven at 105 °C for 15 min, and then at 70 °C for 72 h to determine the shoot, root, and whole plant (shoot + root) dry weights (DW).

### 2.3. Collecting and measuring of xylem sap volume

Xylem sap was collected for continuous 4 h (08.00–12.00 h a.m.) at 18 days after K treatment from 4 plants per treatment according to Dong et al. (2008). The plants were cut off 1.5 cm above the graft union. The cut stem was connected to low-volume plastic tubing which ran into a glass tube. The volumes of the sap samples were then determined, and samples were frozen for determining K concentration. The total amount of K in the sap was calculated (sap volume × sap K concentration).

### 2.4. Determination of K concentration

In this process, $K^+$ extraction and determination were performed as described by Xu et al. (2006). $K^+$ concentration was analyzed with atomic absorption spectrophotometer (Varian spectra AA 220, Varian, Palo Alto, CA, USA).

### 2.5. Determination of leaf transpiration rate

Leaf transpiration rate was measured using a gas exchange system (CIRAS-2, PP-systems, Hitchin, UK). The assimilatory chamber was controlled to maintain the leaf temperature at 25 °C, $CO_2$ concentration at 360 μmol mol$^{-1}$, and photosynthetic photon-flux density at 800 μmol m$^{-2}$ s$^{-1}$. Four replicate plants per treatment were measured between 09:00 a.m. and 12:00 a.m. on sunny day (19 days after K treatment).

### 2.6. Calculation of K uptake and utilization efficiency

K uptake efficiency was estimated as the total $K^+$ uptake accumulated in the plant, K accumulation (mg) = K concentration (mg g$^{-1}$) × plant dry weight (g) (White et al., 2010). Potassium utilization efficiency (KUE) was calculated from the ratio of the changes in plant biomass (dry weight) to the amount of potassium accumulated in plant, which is equal to the reciprocal of plant K concentration ($1/[K]_{plant}$) (Rengel and Damon, 2008).

### 2.7. Statistical analysis

The data are presented as means of four replicates. A two-factorial analysis of variance (ANOVA) was performed to study the effects of graft combination, potassium treatment and their interactions on the plants. Duncan's multiple range test was performed at $P = 0.05$ on the significance of graft combination measured. Statistical analyses were performed using SAS statistical software (version 8.0, SAS Institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. Plant growth

The dry weights of shoot, root and whole plant were significantly affected by graft combination and K treatment (Table 1). When the means were calculated over the two potassium levels (6 mM and 0.6 mM) for each graft combination, the shoot, root and whole dry weight of plants grafted onto 'Jingxinzhen No.4' and 'Hongdun' were significantly higher than the plants grafted onto 'Nabizhen' and ungrafted plants (Table 1). Compared with