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Using rootstock to increase watermelon fruit yield and quality at low potassium supply: A comprehensive analysis from agronomic, physiological and transcriptional perspective

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ABSTRACT

Grafting is a widely used technique in watermelon production. How grafting affects watermelon fruit yield and quality at low potassium supply and the mechanism remains unclear. This study addresses the question from agronomic, physiological and transcriptional perspective. Watermelon plants [*Citrullus lanatus* (Thunb.) Matsum. and Nakai, cv. Zaojia 8424], either self-grafted or grafted onto the rootstock 'Yongshi' (*Citrullus lanatus* sp.), 'Jingxinzhen No.1' (*Lagenaria siceraria* Standl.), and 'Qingyanzhen No.1' (*Cucurbita maxima* × *C. moschata)*. Plants were subjected to 6.0 mM K (normal K) and 0.1 mM K (low K). Compared with plants treated with 6.0 mM K, those supplied with 0.1 mM K produced less fruit yield as indicated by the decrease in single fruit weight of all plants; however, a smaller decrease was observed in plants grafted onto 'Yongshi' (10%), 'Jingxinzhen No.1' (15%) and 'Qingyanzhen No.1' (19%) than the self- grafted watermelon (38%). The K^+ concentration of stem, leaf, fruit peel and flesh were obviously higher in the rootstock- grafted plants than in the self- grafted ones under 0.1 mM K. In addition, rootstock- grafted plant had significantly higher leaf zeatin riboside and chlorophyll content. Fruit quality, including contents of total soluble solid, sucrose, vitamin C, lycopene, β-carotene, were significantly decreased in the self- grafted plants under 0.1 mM K. However, 0.1 mM K treatment did not result in an obvious decrease in most of the measured fruit quality parameters in the rootstock- grafted plants. Fruit transcriptome analysis showed that there were 670, 27, 16 and 15 differential expressed genes (DGEs) responded to 0.1 mM K in the self- grafted, 'Yongshi', 'Jingxinzhen No.1' and 'Qingyanzhen No.1'- grafted plants, respectively, indicating that rootstock grafting decrease the sensitivity of watermelon fruit to 0.1 mM K at the transcriptome level, GO and KEGG analysis showed that most of the DGEs were enriched in cellular metabolic process and metabolic pathways. Low K (0.1 mM K) significantly increased the gene expression of potassium channel (*Cla020934*), but decreased the gene expression of phytoene synthase (*Cla009122*- responsible for lycopene synthesis) in the fruit flesh of self-grafted watermelon. Taken together, the above results suggested that compared with self- grafted plants, watermelon grafted onto rootstock can enhance plant fruit yield, quality and decrease the sensitivity of fruit flesh transcriptome to low K. The mechanism of improved performance under low potassium is discussed.

1. Introduction

As one of the essential macronutrients, potassium (K) plays important roles in many fundamental physiological processes in plant cells, including osmoregulation, enzyme activation, photosynthetic regulation, transport of assimilation products, and ion homeostasis (Wang and Wu, 2015). Sufficient K supply is required for optimal plant growth and development, and thus is important for crop yield as well as product quality (Zörb et al., 2014). Average soil K reserves are generally large, but most of it is not plant- available. Consequently, potassium deficiency is a widespread problem in some soils. The conventional approach to solve this problem was to apply soluble K fertilizer. The

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demand for K fertilizer is expected to increase significantly, particularly in developing regions of the world (Zörb et al., 2014). 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 plant are able to absorb higher amount of K from soil (uptake efficiency) and/or 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 (Dessougi et al., 2002; Hao et al., 2013; Fan et al., 2013; Wang et al., 2015).

Watermelon (*Citrullus lanatus*) is an economically important horticultural crop in terms of production and consumption (Guo et al., 2013). Watermelon cultivation often suffers K deficiency, which results in a negative effect on the plant growth and development (Perkins-Veazie et al., 2013; Huang et al., 2013). One way to reduce losses in plant performances caused by K deficiency would be to graft K deficient cultivars onto rootstocks with high K uptake and/or utilization 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; Gregory et al., 2013; Albacete et al., 2015). In recent years, it is reported that grafting can increase plant tolerance to abiotic stresses, such as low temperature (Ntatsi et al., 2014), high temperature (Li et al., 2014), salinity (Albacete et al., 2009; Edelstein et al., 2011; Niu et al., 2017) and drought (Cantero-Navarro et al., 2016; Sánchez-Rodríguez et al., 2016). In addition, grafting can also improve plant nitrogen uptake and utilization efficiency (Colla et al., 2010, 2011), and magnesium uptake (Huang et al., 2016). Rootstock can increase watermelon tolerance to low K at seedling stage (Huang et al., 2013). The increased performances of grafted plants under abiotic stresses are often associated with the maintenance of ionic (Edelstein et al., 2011; Niu et al., 2017) and hormonal homeostasis, such as the cytokinin under salt stress, which is important to regulate leaf senescence (Albacete et al., 2009). However, the effects and mechanism of rootstock on the fruit yield and quality of watermelon under low K were still unclear.

Adequate K nutrition has been associated with increased fruit size, content of total soluble solid and vitamin C, improved fruit color and shelf life of many horticultural crops (Lester et al., 2010). The development of high throughput technologies for large-scale analyses of transcriptomes represents powerful tools for unravelling the molecular mechanisms of plant to abiotic stresses (Urano et al., 2010). The response of transcriptome to low K has been intensively studied in the root and leaf (Ma et al., 2012; Fan et al., 2014; Zeng et al., 2014; Lu et al., 2015; Zhang et al., 2017); however, only fewer studies investigated the fruit gene expression to low K at the genome-wide level (Shen et al., 2017).

In this study, our hypothesis is that watermelon fruit yield and quality can be improved under low K by grafting onto rootstocks, and ionic and hormonal homeostasis may contribute to this improvement. In addition, the results of a transcriptomic approach, undertaken with the aim of elucidating molecular mechanisms and identifying candidate genes involved in the responses of watermelon fruit to low K are reported here.

2. Materials and methods

2.1. Plant material and treatment

The experiment was conducted in a plastic film greenhouse (length × width × height = 50 m × 6 m × 2.7 m) 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). In this study, watermelon cultivar 'Zaojia 8424' [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] was grafted onto three rootstocks: 'Yongshi' (*Citrullus lanatus* subsp. *lanatus*) (Z/Y), 'Jingxinzhen No.1' (*Lagenaria siceraria* Standl.) (Z/J), and 'Qingyanzhen No.1' (*Cucurbita maxima* × *C. moschata)* (Z/Q), using the procedure of 'insertion grafting' described by Lee (1994), whereas self- grafted 'Zaojia 8424' plants (Z/Z) were used as control. 'Zaojia8424' was selected as a representative watermelon hybrid commercially cultivated in China.

When the third true leaf of plants emerged, they were transplanted into plastic pots, each containing 10 L of substrate (peat: vermiculite: perlite = 7:3:1, v/v). Before transplanting, the exchangeable K^+ concentration of the substrate was 270 mg/kg dry weight. Each pot contained one watermelon seedling. The pots were arranged at a 150 cm row spacing, spaced 50 cm apart. The plants were treated with 6.0 mM K (normal K) and 0.1 mM K (low K) at day 5 after transplanting. The 6.0 mM K treatment nutrient solution was composed of 4 mM $Ca(NO_3)_2$, 3 mM K_2SO_4, 1 mM $NH_4H_2PO_4$, 2 mM $MgSO_4$, 89.2 μM Na_2Fe-EDTA, 46.3 μM H_3BO_3, 9.5 μM $MnSO_4$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.1 μM $(NH_4)_2MoO_4$. Only 0.05 mM K_2SO_4 was kept in the nutrient solution for 0.1 mM K treatment, while other component of nutrients was the same as in the 6.0 mM K treatment, except for the SO_4^{2-}. The EC and pH for 6.0 mM K treatment were 1.9 dS m^{-1} and 6.5, respectively, while the respective value for 0.1 mM K treatment were 1.5 dS m^{-1} and 6.4. All treatments were replicated three times and arranged in a randomized complete block design. Each replicate consisted of 24 plants. Plants were trained vertically, only the main stem was kept up with rope during growth. The plants were pollinated by hand. The fruits with the same pollination date were marked in each treatment. One fruit approximately at the same node was allowed to develop on each plant. During the culture under the plastic greenhouse, the day temperature was between 15 °C and 36 °C (mean temperature 28.5 °C), night temperature never lower than 14 °C, and day relative humidity was 35%–95% (mean relative humidity 65%).

The nutrient solution was pumped from tanks through a drip-irrigation system, with two emitters per plant at emitter flow rate of 2 L h^{-1}. The amount of irrigation solution applied for each plant was 0 L to 3 L per day, depending on plant growth stage and environmental conditions.

2.2. Single fruit weight measurement

The plants and ripe fruits were harvested at day 30 after pollination (60 days after low K treatment), and nine fruits per treatment were used. Each replicate had 3 fruits. The whole plant were cut into roots (rootstock), stem, leaf, fruit (peel and flesh), and then roots, stem, leaf, peel (1/8 fruit), flesh (1/8 fruit) were placed in an oven at 105 °C for 15 min, followed by 70 °C for 72 h. The dried materials were used for measuring K^+ concentration. Single fruit weight (fresh weight) is the mean of three randomly chosen fruits per replicate. After determination of single fruit weight, all flesh tissues (after peel and seed removal) were chopped into small pieces, and were homogenized and used for the measurements of fruit quality (total soluble solid, sucrose, glucose, fructose, vitamin C, lycopene, and β-carotene) and transcriptome.

2.3. K^+ concentration measurement

The dried powder of roots, stem, leaf, fruit peel, and flesh were digested in a mixture of H_2SO_4-H_2O_2 (volume ratio 5:1). K concentration was measured according to the method described by Yedidia et al. (2001), using the inductively coupled plasma optical emission spectrometry (ICP-AES, IRIS Advantage, Thermo Jarrell Ash/Baird, Massachusetts, USA).

2.4. Measurement of leaf zeatine riboside (ZR) and relative chlorophyll content

The second fully expanded leaf at day 55 after low potassium