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Abstract. The *RGP* transgenic plants show good resistance to drought especially reflected in corn yield. However, little is known about root traits which contribute to drought resistance. Here, we characterized root anatomy in the transgenic plants based on micro-CT scanning. Quantitative analysis of root anatomical traits showed that the drought-resistant *RGP* transgenic plants had larger root and stele cross-sectional areas in the fourth and the fifth whorls of nodal roots. The metaxylem vessel number and total area of metaxylem vessels was higher or larger in the fifth and the sixth whorls of nodal roots from *RGP* transgenic plants.

Keywords: maize roots, anatomical traits, drought, micro-CT

1 Introduction

Drought is an important environmental factor which affects the plant growth and productivity of field crops [1, 2]. The threat of drought stress on crop growth and food production is exacerbated by global climate change [3]. Roots are the primary sites of water uptake in plants. Roots are also an important part of plant fitness in drought and water-limited environments, as they adjust their growth and water absorption and transport properties to drought stress [4]. In the case of adaptive responses of crop root to drought stress, the focus of attention can be divided into two areas: root architecture and root anatomy. Root system architecture (RSA) phenotyping has attracted extensive attention such as the wide application of destructive and non-destructive techniques for phenotyping. For example, RSA could be determined in non-destructive manners by magnetic resonance imaging or X-ray computed tomography scanning when plants are growing in soils. Consequently, the three-dimensional modelling of RSA is receiving more and more attention. However, the researches on the anatomy of crop roots are less prevalent.

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Root anatomical traits play important roles in root functions, such as water and nutrient absorption from soils, the resource transportation in plants, and the metabolic cost related with root growth [5,6,7]. Traits like the xylem vessel diameter could affect the axial water transport along the plant roots [8,9], and the number and size of root cell layers and cell wall packing would affect the radial water flow [6].

A RGP (Root Growth Promoting) factor gene is specially expressed in the roots of maize plants, which was cloned from Arabidopsis and introduced to maize plants. The *RGP* transgenic maize plants show the advantage of drought resistance by reason of their stronger water absorption of roots. However, the root anatomical traits contributing to drought resistance is unclear. Phenotyping of root anatomy in *RGP* transgenic maize plants will contribute to revealing drought-resistant mechanisms of the *RGP* transgenic maize, and breeding maize varieties of improved drought resistance. In this work, images of root anatomical organization in the *RGP* transgenic and control maize plants were obtained by X-ray micro-computed tomography system. Anatomical traits such as the root/stele cross-sectional area, and number/size of metaxylem vessels were analyzed assisted by ImageJ software.

2 Materials and Methods

2.1 Plant materials and growth conditions

Both the *RGP* transgenic maize line and the control line (inbred line Xu-178) were grown in a rainproof shelter located at Beijing Research Center for Information Technology in Agriculture. This experiment lasted from June to September in 2016. The plants were irrigated normally until 45 days after sowing, and then drought treatment began, that is, no further irrigation was applied. The plant roots of the two lines were sampled at the Silking stage (R1, eighty-six days after planting). About one centimeter root samples were obtained where were two centimeters from the root-shoot connections of the 1st to 6th whorl nodal roots, then fixed in the formalin-acetic acid-alcohol solution (70% ethanol:formaldehyde:acetic acid=90:5:5, *v*/*v*) immediately, and then stored at 4℃.

2.2 Image acquisition and analysis

The root sample preparation assay including sample drying and staining was performed according to the previous description [10]. These root samples were then placed into the micro-CT system for scanning (Type 1172, Bruker). The scanning voltage and current were set at 34 kV and 210 μA. The sample-source distance was 51 mm and camera-source distance was 281 mm. Then X-ray projections of root samples were obtained which were digitized as 2000×1332 pixel images. After that these projections could be further reconstructed to acquire a serious of root transverse-sectional images with the NRecon software (Version:1.6.9.4, Bruker). These 8-bit BMP reconstructed images were analyzed for the extraction of root anatomical parameters assisted by ImageJ 1.50i software (Http://rsb.info.nih.gov/ij/).

3 Results

3.1 Micro-CT image acquisition

Root samples from the 1st~6th whorl of nodal roots in the *RGP* transgenic and the control plants (CK) were scanned by micro-CT, and the reconstructed images of root cross-sections (2 cm from the base of nodal roots) with an image resolution of 3.4 μm per pixel were obtained (Fig. 1). The anatomical organization could be easily characterized from these micro-CT images (Fig. 1), because of the high contrast between anatomical structures including metaxylem vessels, cortex and epidermis (Fig. 1B).

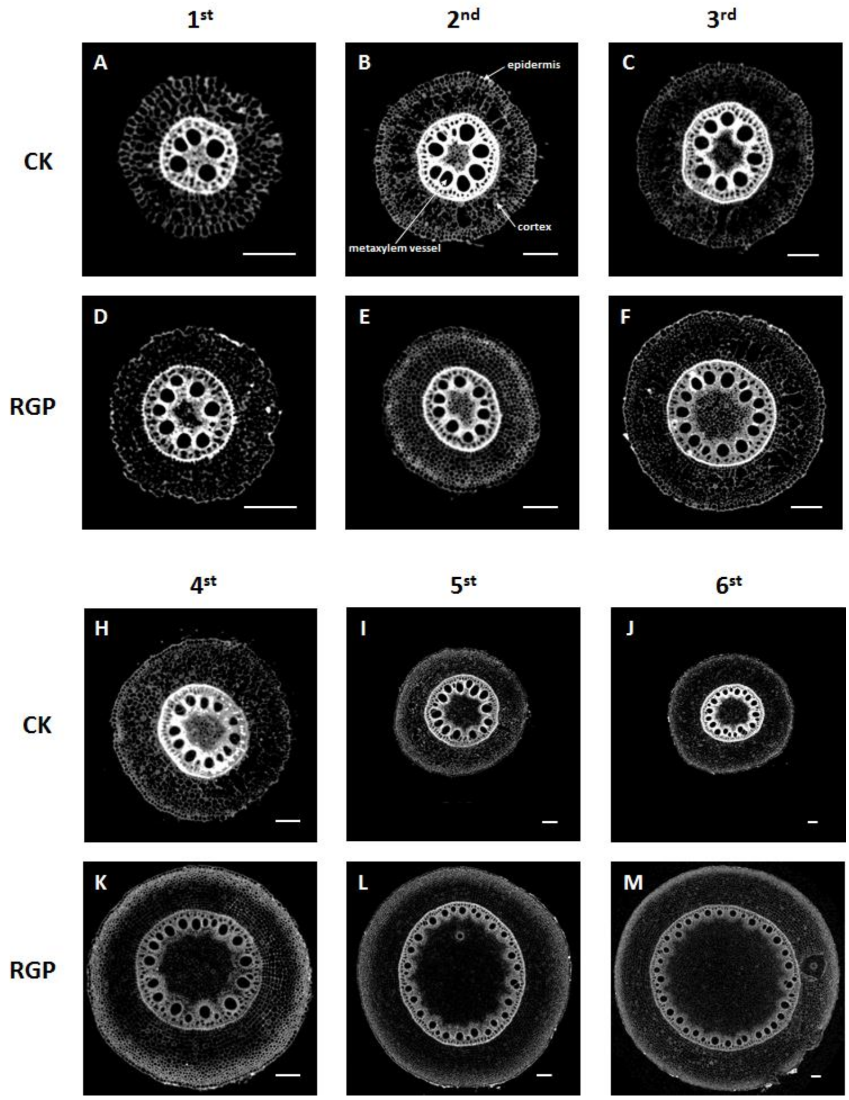

Fig. 1. Cross-section images of maize nodal roots by micro-CT scan showing anatomical organizations of the 1st~6th whorl of nodal roots from the control and *RGP* transgenic plants. The epidermis, cortex and metaxylem vessel are indicated in the picture B. Bars =0.2 mm.

3.2 Quantification of root anatomical traits

Once the micro-CT cross-sectional images were obtained, quantitative information extraction could be performed assisted by the ImageJ software. Root and stele cross-sectional area of the 1st~6th whorl of nodal roots from the *RGP* transgenic and control (CK) plants were examined. The two parameters showed no significant difference in the 1st, 2nd and 6th whorls of nodal roots from the two genotypes (Fig. 2-3). However, the *RGP* transgenic line had significantly larger root cross-sectional area in the 4th and 5th whorls of nodal roots and larger stele area from the 3rd to 5th whorl of nodal roots (Fig. 2-3).

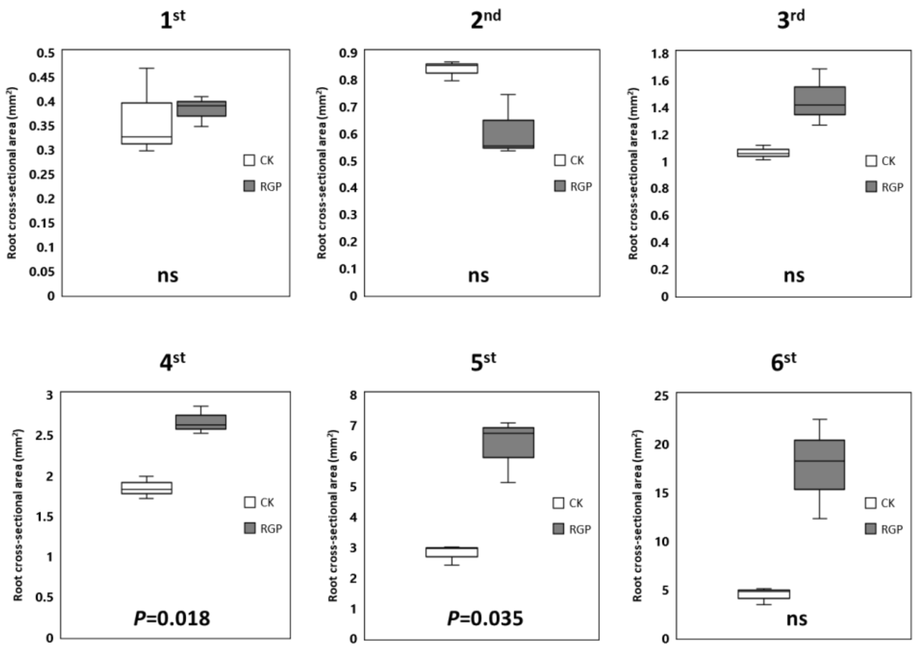

Fig. 2. Root cross-sectional area of the 1st~6th whorl of nodal roots from the *RGP* transgenic and control plants. The median value, lower and upper quartile are shown in the boxes. Error bars represent the highest and the lowest values. Student's *t*-test assay was used for *P* value calculation.

For the metaxylem vessels, both two maize lines show the increasing trend roughly in the number and the total area per root in the 1st~6th whorl of nodal roots (Fig. 4). The *RGP* transgenic plants had significantly more metaxylem vessels within the 1st~6th whorl of nodal roots compared to the control line with the exception of the 2nd whorl showing a non-significant difference (Fig. 4A). As to the total area of metaxylem vessels, the *RGP* transgenic plants also had a significant advantage in the 5th and 6th whorls of nodal roots compared to the control ones (Fig. 4B).

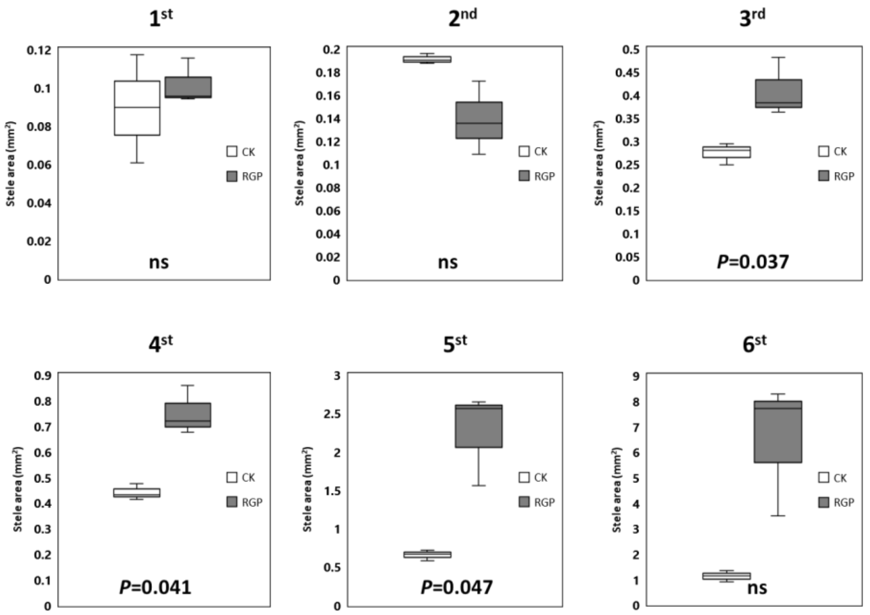

Fig. 3. Stele area of the 1st~6th whorl of nodal roots from the *RGP* transgenic line and the control line. The median value, the lower and upper quartile are shown in the boxes. Error bars represent the highest and the lowest values. Student's *t*-test assay was used for *P* value calculation.

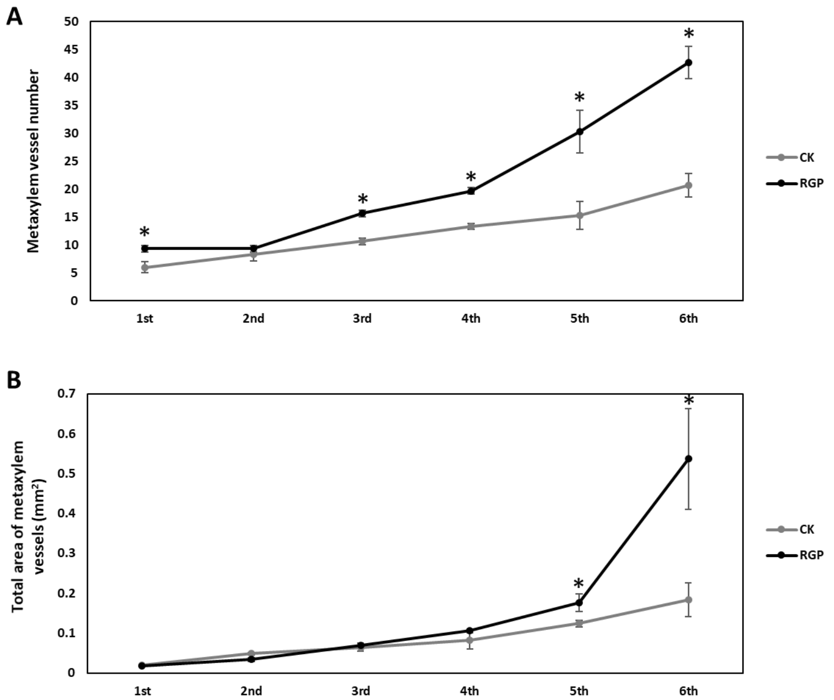

Fig. 4. Comparison of the metaxylem vessel number (A) and total area of metaxylem vessels (B) within the 1st~6th whorl of nodal roots between the *RGP* transgenic and control plants. Values are means±SD (n=3) and asterisks mean statistically significant differences between the control and *RGP* transgenic lines. Student's *t*-test assay was used for *P* value calculation, **P*<0.05.

4 Discussion and conclusions

In cereals such as maize, early work showed that roots lack secondary growth, so the metaxylem vessels play a key role in the axial water transport [6,9]. As more water is expected to reach the metaxylem vessels in the basal part of nodal roots of adult maize plants, traits including the number and the size of metaxylem vessels in the basal region could be worthy of more attention compared to the distal region [11]. Consequently, the basal nodal root segments were examined in this work.

The X-ray micro-computed tomography is a non-destructive 3-D imaging technology which enables plant tissues to be examined in their natural state without serial-slicing procedures [12]. Micro-CT has an obvious advantage in the efficiency of image acquisition. It took only about 2 hr for sample preparation (dehydration and drying), 20 min for image acquisition and reconstruction in this work. In the conventional histology, it is a time-consuming and laborious task to obtain a set of cross-sectional images. Moreover, plant tissues would be deformed or damaged during the inclusion, slicing into sections and staining processes despite cautious care using the conventional method. Recently, X-ray micro-CT has been applied to more and more plant organs, such as plant leaves [13] and crop seeds [14].

In summary, the drought-resistant *RGP* transgenic maize had obvious advantages in the root and stele cross-sectional area in the 4th and 5th whorls of nodal roots, and possessed more metaxylem vessels and larger total area of the metaxylem vessels within the 5th and 6th whorls of nodal roots in the water-limited environment. As the 4th~6th whorls of nodal roots would play significant roles at the reproductive growth stage of maize plants, these advantages could be in favor of axial water transport for further grain setting. Consequently, the distinct anatomical traits in the *RGP* transgenic maize plants could contribute to its drought resistance especially in the reproductive growth stage. In future, further researches will be carried out on the regulation mechanism of water acquisition in the *RGP* transgenic plants.

Acknowledgements. This work was supported by Beijing Natural Science Foundation (5174033), Beijing Postdoctoral Research Foundation (2016 ZZ-66), Postdoctoral Research Foundation of Beijing Academy of Agriculture and Forestry Sciences (Postdoctoral Number: 157939).

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