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The morphological characteristics of regenerative plants of hairy root cultures in maize and its effect on water use efficiency

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Abstract

The aim was to study the relationship between maize roots and water use efficiency and analyze the change of the morphological and physiological characteristics of regenerative plants of hairy root cultures in maize under water stress. The results reveal that the regenerative type maize with a large root system may be able to efficiently conserve water in case of drought stress, whereas the physiological studies from the measurement of leaf osmotic and water potential show the regenerative lines posses an enhanced drought tolerance. Thus, the high root lines of the regenerative plants can keep strong hydraulic root signal and water use efficiency under water stress.

Key words: Morphological characteristics, water use efficiency, root lines, maize.

Introduction

Drought is the most common environmental stress experienced by crop and strongly impairs production. Increasing crop resistance to drought stress would be the most economic approach to improve agricultural productivity and to reduce agricultural use of fresh water resources.

Comparison of various studies revealed that drought is thought to lead to large morphological or physiological changes [3]. Water deficit, including reduced expansion of aerial organs, maintenance of root growth, decrease of transpiration and photosynthesis, solute accumulation and osmotic stress, lower root to shoot ratios, and, in parallel, the transcriptional regulation of a large number of genes [1, 8-10, 15]. Most studies on organ size control investigate growth of aerial organs, whereas roots are only rarely considered. However, the development of plant root systems is an important agronomic trait. Plant roots perform many essential functions, including water and nutrient uptake, storage of reserves, synthesis of specific compounds, anchorage to the soil, and the establishment of biotic interactions in the photosphere [4]. Optimizing root system architecture can overcome yield limitations in crop plants caused by water shortages [13]. As a result, understanding the mechanisms of drought tolerance and breeding for drought-resistant crop plants has been the major goal of plant biologists and crop breeders. The slow pace in revealing drought tolerance mechanisms has hampered both traditional breeding efforts and use of modern genetics approaches in the improvement of drought tolerance of crop plants [14].

To develop novel methods to study a correlation between root system architecture and resistance to water stress, we began to search for new phenotypes that are conferred by drought stress ten years ago. In this study, we firstly reported the production of transformed roots using various strains of *A. rhizogenes* and regeneration of transformed plants from such transform [16]. The regenerative plants of hairy root cultures appeared special morphological characteristics, which included developed roots, high branching of adventitious roots, developed lateral roots and rapid growth. Considering the complexity of water use efficiency (drought-resistance), comparing to H99, the morphological, physiological and developmental characteristics of drought-resistance in regenerative plants of maize (*Zea mays* L.) were systemically studied. The effects of high drought resistance on the morphological, developmental and physiological characteristics under water stress were shown in strong root lines of regenerative plants from hairy root cultures in maize.

Materials and Methods

Plant materials: The regenerative plants from hairy root cultures in maize H99 were used for this study. The main steps were as follows: In terms of plant regeneration from hairy roots, different ratios of hormone exhibited varying degrees of response. Hairy roots induced by strains ATCC15834 and A4 in media (ZT 1.6 mg/L and NAA 0.2-0.4 mg/L) developed into plantlets directly [16]. Some independent transgenic lines were selected.

Growth conditions of plants: Plants were grown in a greenhouse under 16-h light/8-h dark cycles at 21°C (light period) and 18°C (dark period). Isohumic soil was used as standard soil containing 60.18 $g\cdot kg^{-1}$ organic matter, 2.00 $g\cdot kg^{-1}$ total nitrogen, 1.60 $g\cdot kg^{-1}$ total phosphorus, 12.85 $g\cdot kg^{-1}$ total potassium, 160.1 $mg\cdot kg^{-1}$ alkali hydrolyzable nitrogen, 90.21 $mg\cdot kg^{-1}$ available phosphorus and 550.23 $mg\cdot kg^{-1}$ available potassium.

For experiments with normal nutrient supply, seeds were germinated on Petri dish with half-strength Murashige and Skoog (MS) medium. After 2 weeks, seedlings per genotype were planted together in potted plastic buckets (0.45 m diameter x 0.55 m height).

Test for drought tolerance: The test for drought tolerance was processed at seedling stage (4~7 leaves). The substrate was watered to saturation, and the plants were subsequently grown for 22 d without an additional water supply. Watering was then resumed and, after a recovery phase of 7 d, comparing to the change of the morphological and physiological characteristics between regenerative lines and control.

Measurement of leaf osmotic potential and water potential: The water potential in leaves was performed in three independent experiments according to Scholander *et al.* [12] using a pressure chamber (PMS Instrument CO, Corvalis, Oregon, USA). The readings were taken at midday using healthy leaves, fully expanded and uniform in terms of age. Leaf osmotic potential was measured on the same plants as for leaf water potential. Statistical analyses were carried out with statistical software SAS 8.2.

Results

Larger root system in regenerative plants from hairy root cultures: We first analyzed reciprocal grafts between wild-type and regenerative plants showing all the aspects in roots. Thirty-five independent lines showed a moderate to strong increase in the size of the root system in more details. Fig. 1A shows that regenerative plants possess a larger root system than wild-type plants. Number of roots in regenerative plants from hairy root cultures increased by 194.6% in comparison to the wild type (Fig. 1B). This is similar to plants with larger root system, which showed 198% increase in the fresh biomass of the root system (Fig. 1C).

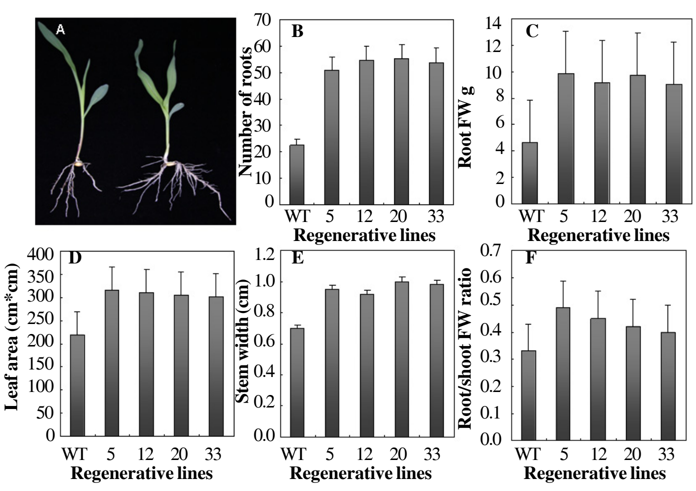

Figure 1. Root-specific regenerative lines in maize resuls in enlarged root system, leaves and normal shoots. (A) Comparison of the root systems of wild-type (left) and regenerative (right) plants grown in hydroponic culture for 30 d. (B) Total number of roots formed at 30 d. (C) Root biomass of plants grown in hydroponic culture for 30 d. FW, fresh weight. (D) Total area of leaves formed at 30 d.(E) Stem width of plants grown in hydroponic culture for 30 d. (F) Root-to-shoot biomass ratio in root-specific regenerative plants grown in hydroponic culture for 30 d compared with the wild type.

Root-specific regenerative lines in maize results in enlarged leaves and normal shoots: The regenerative lines have been described as larger root system. In contrast with the strong changes in root development, the development of regenerative leaves and shoots were comparable to the wild type. In regenerative plants, the size of the leaves and stems were analyzed in the test. In regenerative lines, the development of leaf and stem was quick, despite a normal development in the shoot. The leaf area of single plant and the stem width of the root system were 86% and 38% higher, respectively, than that of wild-type plant (Fig. 1D-E). Consequently, the root-to-shoot biomass ratios were by up to 31% in regenerative lines (Fig. 1F).

The regenerative- type plants can efficiently conserve water under water stress: To address whether the root-specific regenerative maize plants can be an adaptive response to drought tolerance, we decided to investigate the water content in regenerative lines and wild-type plants under water stress. It was found that the wild-type roots lost water faster than the regenerative plants. During the course of 22 d, the wild-type roots on average lost 46% more water than the regenerative type (Fig. 2A). The wild-type stems and leaves lost 61% and 88% more water than that of the regenerative-type plants (Fig. 2B-C), respectively. The fact suggested that the regenerative-type plants may be able to efficiently conserve water in case of drought stress.

Regenerative-type plants with an enhanced root system have higher drought tolerance: To test whether an enlarged root system can improve drought tolerance, the survival rates of regenerative-type and wild-type plants were scored after a period of complete water deprivation for 22 d, approximately 75% of the regenerative-type plants survived, whereas on average, only 12% of the wild-type plants survived. The higher survival rate of regenerative-type plants showed that the regenerative plants with larger root system competed more successfully than wild plants for limited water resources and thus increased drought tolerance.

Osmotic potential and water potential of regenerative-type plants are not almost altered under drought stress: Although roots are the major place where plants first encounter drought stress, it is likely that leaves may be able to sense and respond to the drought stress. Because many drought responses are regulated by osmotic potential and water potential, it is likely that osmotic potential and water potential may mediate drought tolerance in regenerative-type plants. We analyzed the two potentials in regenerative-type leaves and in wild-type leaves under water stress. Increased osmotic potential of the wild-type plants was observed in our studies, whereas reduced water potential in the plants was found under drought stress. Osmotic potential and water potential of regenerative-type plants are less altered in the same case of drought stress (Tables 1 and 2). Thus, it may be easy to use these data to conduct plant physiological studies of drought stress response.

Discussion

The architecture of the root system determine the plant's ability to access water and nutrients, factors that limit growth and yield [5]. Drought-resistant rice (*Oryza sativa*) varieties have a deeper and more highly branched root system than drought-sensitive varieties[11]. In this study, we investigated the regenerative maize

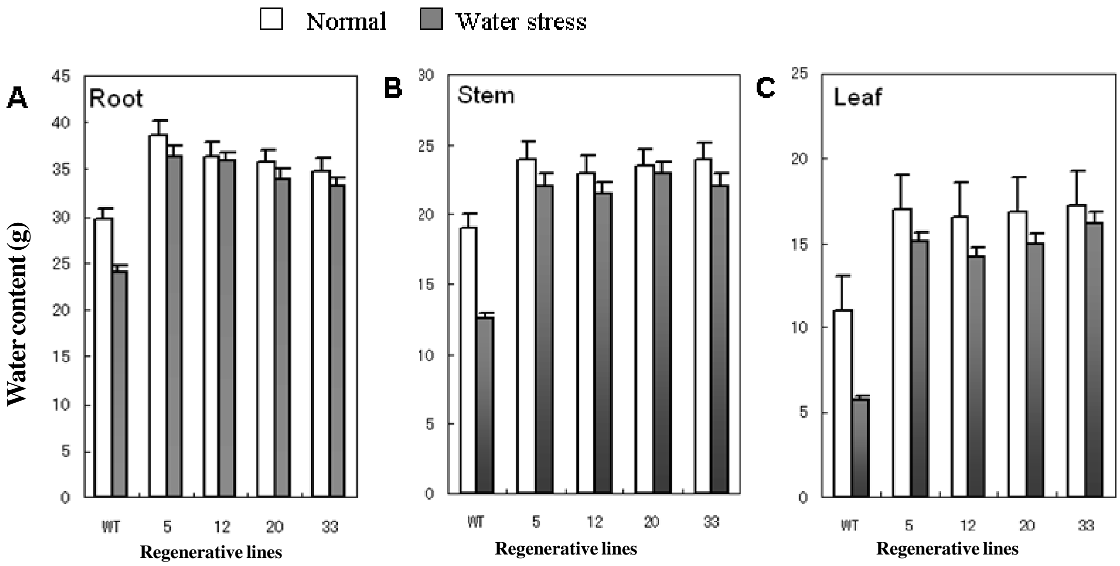

Figure 2. Water content in root-specific regenerative maize plants and in wild-type plants under water stress: A, water content in root; B, water content in stem; C, water content in leaf.

Table 1. Osmotic potential of regenerative-type leaves and wild-type leaves under water stress.

Treatment	Wild-Type	Regenerative-Type Line			
		5	12	20	33
Normal	-1.30b	-1.18a	-1.28b	-1.24a	-1.20a
Water stress	-5.80c	-1.24a	-1.34b	-1.33b	-1.23a

Table 2. Water potential of regenerative-type leaves and wild-type leaves under water stress.

Treatment	Wild-Type	Regenerative-type Line			
		5	12	20	33
Normal	-1.16ab	-0.99a	-1.10a	-1.18ab	-1.00a
Water stress	-4.65c	-1.16ab	-1.20ab	-1.27b	-1.14ab

with a large root system can use water efficiency. Elongation of the stem width, root branching and root biomass were increased in the regenerative maize. Thus, under drought stress conditions, the plants would be increased the uptake of water which is usually more available in deeper soil layers. Wild-type roots lost water faster than the regenerative plants, this is similar to other organs (stems and leaves) of the regenerative type. This study showed that the organs of the regenerative-type plants can efficiently conserve water and have higher drought tolerance under water stress.

In some studies, the inhibition of lateral root elongation was a reliable response to the drought stress [6, 14]. Our study strongly suggested that the lateral root growth in the regenerative-type plants was not inhibited, whereas an altered lateral root growth of the wild-type maize in response to drought stress was different. These genetic data strongly suggest that normal lateral root growth is an adaptive response to drought stress.

The physiological studies from the measurement of leaf osmotic potential and water potential showed the regenerative lines posses an enhanced drought tolerance. Our study from the root-specific regenerative maize suggested that the root architecture would be easy to uncover novel drought tolerance mechanisms by analyzing the morphological and physiological characteristics for guard cell response to drought stress.

In addition, the development of the root system is determined by intrinsic genetic factors and modulated by numerous environments. Root architecture is governed by many genes [2, 8, 13]. Our next work will demonstrate which genes are positive regulators of root growth in the regenerative-type maize and that its physiological function is supraoptimal for root growth under standard conditions. It establishes an approach based on the genetic engineering of the novel root system architecture and might be useful for generating crop plants optimized to grow under condition of limiting water.

Conclusions

Because of global climate change, it is predicted that drought periods will be more frequent in the future. Roots are the very place where plants may be able to sense and respond to the drought stress. To study the responses of maize roots to water stress, we investigated and analyzed the changes of the morphological and physiological characteristics of regenerative plants of hairy root cultures in maize under water stress. An advantage of the regenerative plants with a larger root system could enhance root elongation and branching increased the absorptive surface of the root. It has been shown that a larger root line of regenerative plants from hairy root cultures in maize could keep strong hydraulic root signal and water use efficiency under water stress.

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