

Enhanced Drought Tolerance by Overexpressing of *Arabidopsis thaliana* *LOS5* Gene in Cotton (*zhongmiansuo35*)



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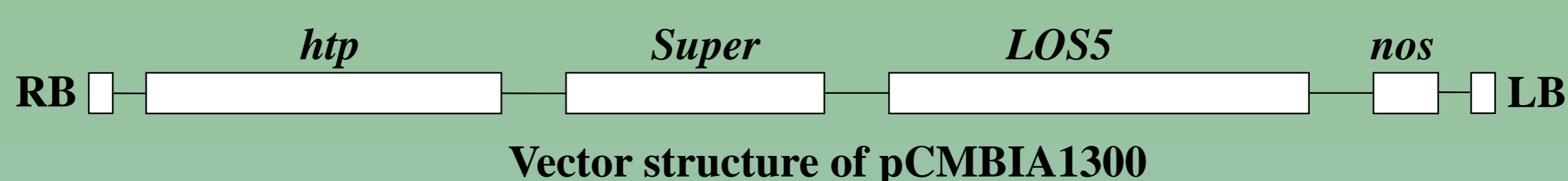
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Introduction

Drought is the most serious environmental factor limiting the productivity of agricultural crops worldwide. Drought tolerance is a typical quantitative trait, it triggers alterations in gene expression, the accumulation of metabolites such as the phytohormone abscisic acid (ABA) or osmotically active compounds, the synthesis of specific proteins and induces a range of physiological and biochemical responses in plants. Many efforts have been made to improve the crops drought tolerance and productivity under water-limiting conditions. Xiong *et al.* (2001) reported that *LOS5* locus is identical to *ABA3* which encodes a molybdenum cofactor (MoCo) sulfurase involving in regulation of ABA biosynthesis. To further understand the relationship between the functions of *LOS5* and plant drought tolerance, we over-expressed the *Arabidopsis LOS5* in cotton (*Gossypium hirsutum*) plants cv. *Zhongmiansuo35*.

Materials and methods

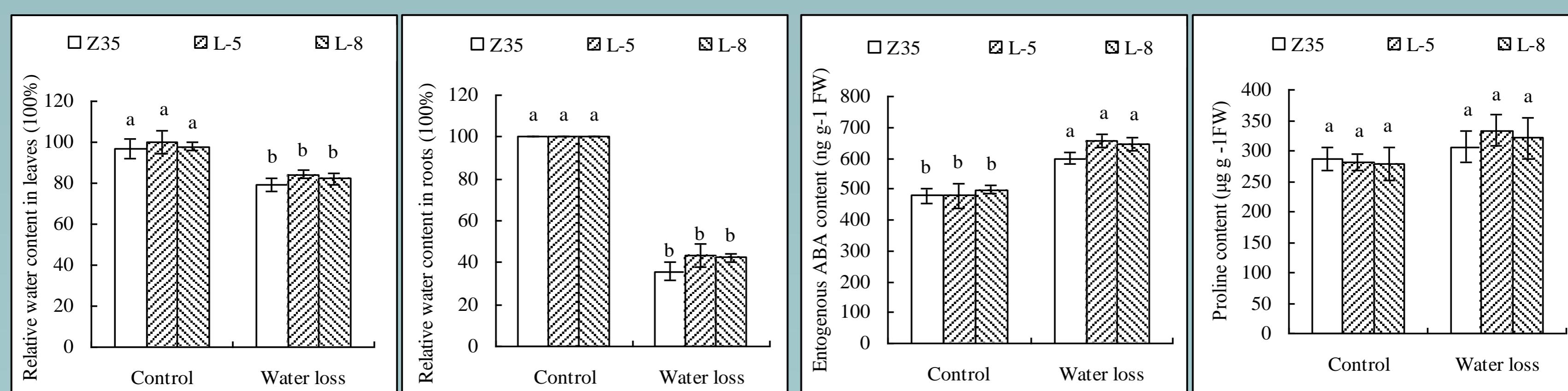
Construct and plant transformation: *LOS5* cDNA was cloned as an XbaI - KpnI fragment downstream of the super promoter in the pCMBIA 1300 binary vector containing a hygromycin resistance selectable marker. The recombinant plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105, and transformation of cotton (*Gossypium hirsutum*) plants cv. *zhongmiansuo35* (Z35) by using *Agrobacterium tumefaciens*. Two dominant lines (L-5, L-8) were selected and homozygous T3 were used for drought-tolerant analysis.



Plant growth conditions: The experiments were conducted in a growth chamber under 30/20 °C, 14/10 hours day/night, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light conditions. Seeds were germinated in sand medium and cultured hydroponically by transferring to pots (40 × 30 × 20 cm) filled with modified Hoagland's nutrient solution. A completely randomized design was used with three replications (pots).

Results

Relative water content in transgenic plants: At the five leaf stage, plants cultured hydroponically were transferred to empty pots from the nutrient solution to lose water for 5 hours. Measurement of relative water content in leaves and roots showed a tendency that transgenic plants were superior to the Z35 plants in terms of maintaining leaf water potential.



ABA and proline accumulation in transgenic plants: ABA plays significant roles in plant responses to environmental stresses, and proline accumulation can protect plants from the injury caused by abiotic stresses. Under control condition, the levels of ABA and proline in transgenic plants were similar to those in Z35 plants. Water stress enhanced the level of endogenous ABA and proline content, however, these traits in transgenic plants increased more than those in Z35 plants.

Discussion

LOS5, encodes a molybdenum cofactor (MoCo) sulfurase, is a key regulator of ABA biosynthesis, and the transcript level of *LOS5* increased significantly in response to drought treatments (Xiong *et al.*, 2001). In our study, the overexpression of *Arabidopsis thaliana LOS5* gene in cotton plants of Z35 can raise the concentration of ABA and proline under water loss conditions. By the model for stress induction of ABA biosynthesis suggested by Xiong *et al.* (2002), an initial increase in ABA from overexpressing one ABA biosynthesis gene, such as *LOS5*, could result in an increased expression of other ABA biosynthesis genes, such as *AAO3*, *LOS6/ABA1* and *NCED*, which then would lead to a coordinated increase in de novo ABA biosynthesis. And the accumulation of ABA may cause stomatal closure to avoid excessive evaporation of water and the increase in the levels of proline in response to water stress.

References

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