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Vitreoscilla Hemoglobin (Vhb) is a Useful Molecular Tool for the Improvement of Waterlogging in Maize

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Short Communication

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ABSTRACT

VHb derived from Vitreoscilla has been widely used to improve the waterlogging tolerance in multiple plants. In this study, *VHb* gene was transformed into maize by bombardment. Under waterlogging treatment, transgenic *VHb* maize displayed higher shoot height, longer primary root length, more lateral root number, heavier shoot and root dry weight than their WT. With the present of exogenous *VHb* gene, maize exhibited enhanced tolerance to waterlogging stress.

INTRODUCTION

Waterlogging is increasing one of major constrains of maize production and productivity, and results in serious yield loss [1]. In South and Southeast Asia, approximate 15% of total maize grown in this region is often affected by waterlogging or flooding, resulting in 20%-30% yield loss annually [1]. Transgenic technique has been widely applied to improve submergence or waterlogging tolerance in plants [2,3]. The *VHb* gene is an ideal molecular tool to improve submergence or waterlogging tolerance in plants. In previous study, with the present of exogenous *VHb* gene, the Arabidopsis, cabbage, and petunias, exhibits enhanced submergence or waterlogging tolerance [4-6]. In our research, *VHb* was transferred into maize. Transgenic *VHb* maize is more tolerance to waterlogging stress than their WT.

Transgenic VHb Maize Display Enhanced Waterlogging Tolerance

As an oxygen carrier, the present of VHb protein often enhances host growth under hypoxic condition. In this study, the full coding sequence (CDS) of *VHb* gene has been isolated from Vitreoscilla by PCR amplification, and was constructed into pCAMBIA3301 binary vector under the control of CaMV35S promoter. *VHb* gene was transformed into maize calli derived from immature Hi-II zygotic embryos by bombardment. In subsequently, the putative transgenic *VHb* plantlets were obtained. The PCR and southern blot assay has performed, and results shown that *VHb* gene has been integrated into maize genome. Exogenous *VHb* gene also exhibits high mRNA level in maize. Later, *VHb* gene was transferred into several genetic backgrounds, such as Zheng58 and CML50, using marker-aided backcrossing and self-pollination. Three homozygous Zheng58 (VHb) and five homozygous CML50 (VHb) lines were obtained [7].

Transgenic *VHb* maize and their WT with three visible leaves were subjected to waterlogging treatment in a pool at the greenhouse. All of experiments were performed three biological replicates. These traits, including seedling height (cm), primary root length (cm), the number of lateral roots, the shoot dry weight (g), and the root dry weight (g) were measure to analyze the waterlogging tolerance in maize. Under waterlogging treatment, transgenic Zheng58 (VHb) and CML50 (VHb) displayed higher seedling height and longer primary roots than their WT. A large number of lateral roots were grew out from transgenic Zheng58 (VHb) and CML50 (VHb), which was significantly greater than their WT. The shoot dry weights both in waterlogged Zheng58 (VHb) and CML50 (VHb) were heavier than their WT. However, the shoot dry weight is significantly different between waterlogged Zheng58 (VHb) and Zheng58. The root dry weights of waterlogged CML50 and Zheng58 were significantly lighter than untreated CML50 and Zheng58, while not in CML50 (VHb) and Zheng58 (VHb), which indicates that the root development is improved under waterlogging stress with overexpression of *VHb*. Higher enzyme activity and expression level of *ADH1* are showed in CML50 (VHb) and Zheng58 (VHb) compared with their controls under waterlogging stress [7]. Therefore, transgenic *VHb* maize display enhanced tolerance to waterlogging stress.

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DISCUSSION

In previous research, *VHb* gene has been transferred into several plants, excluding maize. As an important crop, maize is highly susceptible to waterlogging at the early seedling (V2) stage, resulting in serious yield loss [8]. In this study, *VHb* gene was transformed into maize by bombardment, and also transferred into Zheng58 and CML50 genetic background by maker-aided crossing. Under waterlogging treatment, several traits, such as seedling height, the length of primary roots, number of lateral roots, the shoot dry weight, and root dry weight, have been significantly improved with the overexpression of *VHb* both in Zheng58 and CML50 genetic background [7]. Transgenic Zheng58 (VHb) and CML50 (VHb) exhibit enhanced tolerance to waterlogging stress at seedling stage. Our results confirm that *VHb* gene is an ideal molecular tool to improve waterlogging tolerance in maize.

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