

Increased cuticular wax accumulation and enhanced drought tolerance in transgenic alfalfa by overexpression of a transcription factor gene

Z.-Y. Wang¹, J.-Y. Zhang¹, C. Broeckling², E. Blancaflor², M. Sledge¹ and L. Sumner²

¹Forage Improvement Division, ²Plant Biology Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, Oklahoma 73401, USA. Email: zywang@noble.org

Keywords: alfalfa, drought tolerance, transgenic plant, wax

Introduction Plant cuticular waxes play an important role in protecting aerial organs from damage caused by multiple environmental stresses such as drought, cold, UV radiation, pathogen infection and insect attack. The identification of leaf wax genes involved in stress tolerance is expected to have great potential for crop improvement. Cuticular waxes are complex mixtures of very long chain fatty acids, alkanes, primary and/or secondary alcohols, aldehydes, ketones, esters, triterpenes, sterols and flavonoids. Mutant analysis in *Arabidopsis* has contributed to the identification of the components and genes involved in wax deposition. However, no information is available on the effects of overexpression of these genes in crops of agronomic importance. Alfalfa (*Medicago sativa*) is the most important forage legume species in the world and a close relative of *Medicago truncatula*.

Materials and methods A novel AP2 domain-containing transcription factor gene, *WXP1*, was identified and cloned from the model legume *M. truncatula*. Chimeric transgene construct was made placing *WXP1* under the control of CaMV35S promoter. Large numbers of transgenic alfalfa plants were produced by *Agrobacterium*-mediated transformation. Detailed molecular and biochemical analyses were carried out for the transgenic alfalfa plants.

Results The predicted protein of *WXP1* has 371 aa; it is one of the longest peptides of all the single AP2 domain proteins in *M. truncatula*. *WXP1* is distinctly different from the most studied genes in the AP2/ERF transcription factor family, such as *AP2s*, *CBFs*, *DREBs*, *WIN1* and *GL15*. Transcript level of *WXP1* is inducible by cold, ABA and drought treatment in shoot tissues. Overexpression of *WXP1* under the control of CaMV35S promoter led to a significant increase in cuticular wax loading on leaves of transgenic alfalfa (Figure 1). The most striking phenotypic change in the *WXP1* overexpressed alfalfa plants was the more glaucous appearance in the leaves. Scanning electron microscopy revealed earlier accumulation of wax crystals on the adaxial surface of newly expanded leaves and higher densities of wax crystalline structures on both the adaxial and abaxial surfaces of mature leaves. Gas chromatography – mass spectrometry (GC–MS) analysis revealed that total leaf wax accumulation per surface area increased 29.6 – 37.7% in the transgenic lines, and the increase was mainly contributed by C30 primary alcohol. Comparison of wax accumulation in leaves and stems of the transgenics indicates that *WXP1* is mainly involved in the acyl-reduction pathway for wax biosynthesis. *WXP1* overexpression induced a number of wax-related genes. Transgenic leaves showed reduced water loss and chlorophyll leaching. Transgenic alfalfa plants with increased cuticular waxes showed enhanced drought tolerance demonstrated by delayed wilting after watering was ceased and quicker and better recovery when the dehydrated plants were re-watered.

Conclusions We identified and characterized a novel AP2 domain-containing transcription factor gene (*WXP1*) by using an overexpression approach. Our study using isogenic lines (wild type control, empty vector control and transgenic plants) clearly demonstrates the positive effects of cuticular waxes on drought tolerance. Despite the fact that both drought tolerance and wax accumulation are complicated traits that are under the control of multiple genes, our results demonstrated for the first time that overexpression of a single transcription factor gene, *WXP1*, could turn on wax production and improve plant drought tolerance in agronomically important species.

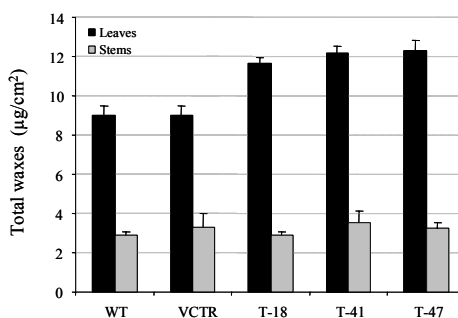


Figure 1 Cuticular wax accumulation in leaf and stem of transgenic and control alfalfa plants. WT, wild type; VCTR, empty vector control; T, transgenic lines overexpressing *WXP1*.