Report: An Inducible Tobacco Sucker Control System Delivered by Intragenic Transformation

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Introduction

Shoot branching, or sucker formation, is the formation of axillary buds and subsequent growth in the axils of leaves. It is an undesirable characteristic for commercial crop production. In tobacco, sucker formation affects leaf quality, alkaloid levels and biomass, and when controlled by chemical suckerides such as flurprimidol, it results in increased production costs and chemical pollution. Therefore, effective, non-chemical sucker control that does not compromise whole plant architecture and uniformity is desirable. However, development of such a system presents a significant challenge due to our currently limited understanding of the molecular mechanism for sucker formation.

Tobacco sucker formation is induced by flowering and by topping, a production practice of removing the flower head. Topping activates a number of genes regulating axillary meristem formation. Many of these genes control biosynthesis of phytohormones, such as auxin, cytokinin (CK) and strigolactones (SLs). Auxin, from the shoot apex moves downward and down-regulates CK biosynthesis, up-regulates SL biosynthesis, and consequently, inhibits bud formation. Topping reduces auxin in the shoot apex which normally represses local CK production in the shoot. The consequent increase of CK promotes bud formation. SLs act antagonistically to CK and repress bud outgrowth (Dun et al., 2012). Some SL mutants show excess shoot branching phenotypes that can be reversed by application of GR24, a synthetic SL analogue (Gomez-Roldan et al., 2008). Some synthetic SL inhibitors also enhance shoot branching. Some SL-like chemicals are useful for controlling shoot branching (Fukui et al., 2011). It is thus possible to develop a sucker control system by genetically manipulating SL biosynthesis. However, caution must be applied in designing and developing such sucker control systems as manipulation of genes regulating phytohormones often lead to abnormal phenotypes (Sun, 2012). Therefore, a system that is turned on only after tobacco plants are topped would be ideal (or a "topping inducible" gene regulatory system).

The construction of a topping inducible system for activation/repression of target genes will require gene promoters (regions of DNA that initiate gene transcription) that are responsive to topping induction. Once topping inducible promoters are isolated, they can be used to create gene regulation constructs that are introduced into tobacco using either convention gene transformation or "intragenic" transformation in which the gene construct is comprised of native plant DNA sequences. Here we propose to isolate and characterize a number of topping inducible tobacco promoters, and to use these promoters in construction of a gene expression vector.

Objective:

To develop a sucker control system that will only turn on when tobacco is topped.

Summary of Progress

Bioinformatic analysis for identification of potential topping-inducible genes

Based on published data and our own bioinformatic analyses, we have identified sets of topping/wounding responsive genes in the model plant Arabidopsis. We identified more than 400 genes that are significantly changed after either treatment. Among them, 169 genes are up-regulated in both topping and wounding. Using Arabidopsis gene sequences as reference we searched the tobacco genome data base and identified several homologous sequences and selected twelve potential candidates for our present study. The twelve different genes belong to four different transcription factor and three structural gene families.

Expression of analyses of potential candidate genes in tobacco following topping

Leaf samples were collected from control and topped (24 h following topping) mature burley tobacco plants growing in the farm. Total RNA was extracted from the leaf samples using the RNeasy plant mini kit from Qiagen and used for cDNA synthesis using the reverse transcription system from Invitrogen. Expression of nine candidate genes in control and topped samples were verified using quantitative real-time PCR. Eight out of the nine genes selected for the present study were induced following topping (Figure 1).

Transcriptomic Analysis of Topping Induced Gene Expression

We have generated expression cDNA libraries using RNA extracted from topped tobacco leaves. Two control libraries and two topping libraries were submitted for sequencing. We are in the process of analyzing the sequencing data in order to identify genes that are differently expressed after topping and characterize the highly inducible genes for possible use in the inducible sucker control system.

Future plan

1. We will select 2-3 topping inducible genes, based on above-mentioned profiling and isolate upstream regulatory sequence (promoter sequence) of those genes.

2. We will make reporter constructs in which the expression of the *GUS* reporter gene is controlled by a newly isolated promoter. The expression construct will be cloned into a modified binary vector pCAMBIA-2300 for tobacco transformation.

3. The transgenic plants will be examined for topping-inducible *GUS* gene expression, i.e. we will determine whether the *GUS* gene only expresses upon topping. The strength and stringency of different promoters will be compared.

References

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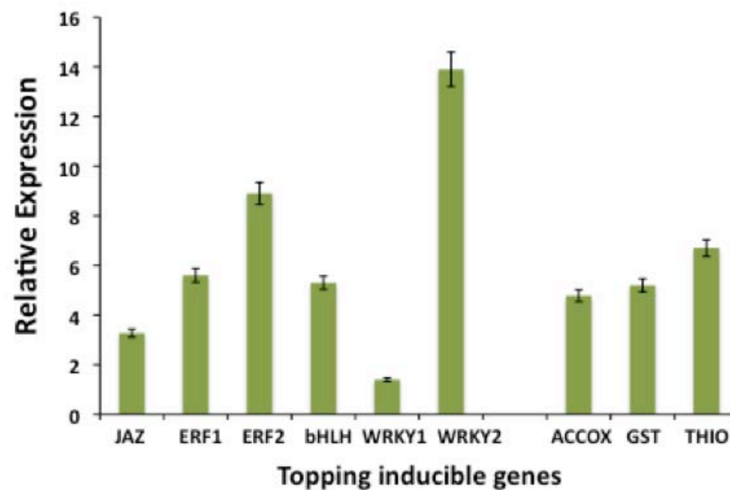

Figure 1. Expression analysis of the candidate genes in tobacco following topping. Expression levels of different genes presented here are relative to the control. JAZ: jasmonate zim domain proteins;ERF:Ethylene response factor; bHLH: basic helix-loop-helix family; ACCOX: aminocyclopropane-1-carboxylate oxidase; GST: glutathion S-transferase family; THIO: thioredoxin-2.