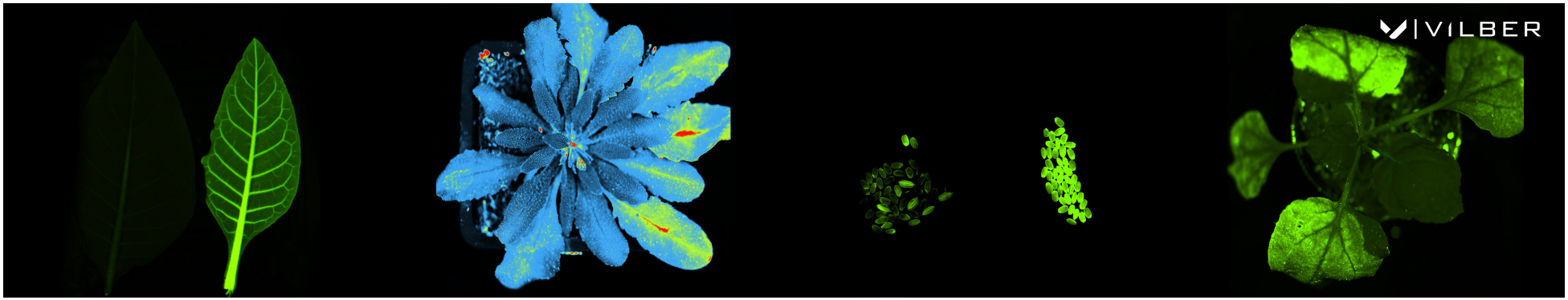

OVEREXPRESSION OF SOCYP85A1 INCREASES THE ACCUMULATION OF CASTASTERONE AND CONFERS ENHANCED BLACK SHANK TOLERANCE IN TOBACCO THROUGH MODULATION OF THE ANTIOXIDANT ENZYMES' ACTIVITIES

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INTRODUCTION

Black shank caused by Phytophthora nicotianae is one of the most devastating diseases in tobacco (Nicotiana tabacum L.) production. It causes serious disease in the roots and stems of tobacco. Plants have evolved defense mechanisms to resist pathogen infections. One such mechanism involves regulation of the biosynthesis of phytohormones, such as abscisic acid (ABA), gibberellic acid (GA), jasmonic acid (JA), and salicylic acid (SA). Brassinosteroids (BRs) are one of the most important phytohormones involved in plant growth, development and in tolerance to pathogens. However, the genetic mechanisms underlying the role of endogenous BRs in regulating the response of plants to pathogens remain poorly understood. CYP85A1 is one key enzyme involved in the biosynthesis pathway of BRs. It catalyzes the conversion of intermediate metabolites to castasterone (CS), which is the precursor of brassinolide (BL), the end-product of BRs biosynthesis pathway. It was observed that overexpression of SoCYP85A1, a spinach cytochrome P450, enhanced drought tolerance in transgenic tobacco by increasing the CS. This enhanced tolerance was demonstrated to be through the upregulation of activities of defense enzymes at the transcriptional level. In addition, CS was identified as a bioactive component in tobacco, which was induced by the P. nicotianae infection. The alteration of CS content resulted in interference of phytohormone homeostasis. This study provides new information for the disease control network and a new strategy for disease resistance breeding.

OBJECTIVE

To explore the function CS in plants subjected to pathogen invasion, this study analyzed tobacco seedlings overexpressing SoCYP85A1 infected with P. nicotianae.

MATERIAL & METHODS

Plant Materials and Growth Conditions

The genotype of tobacco that was genetically engineered was N. tabacum cv. Xanthi-nc. T3 seeds from the transgenic lines overexpressing SoCYP85A1 driven by the CAMV35S promoter were produced and confirmed in a previous study (Duan et al., 2017), in which the SoCYP85A1 gene has stable inheritance and expression. T3 seeds were grown and developed to T4 transgenic soybean plants for further analysis. The wild type (WT) and transgenic lines were grown in growth chambers under a photoperiod of 16 h light/8 h dark and maintained at 30_C and 60% relative humidity for 1 month.

GFP Analysis of Transgenic Plants

For the molecular identification of the overexpression of SoCYP85A1 in transgenic tobacco lines, a green fluorescent protein (GFP) analysis was carried out and the fluorescence signal was detected with the NEWTON 7.0 smart imaging system (Vilber Lourmat, France).

RESULTS

Figure 1. Molecular Confirmation of Transgenic Tobacco Plants Overexpressing SoCYP85A1 by GFP analysis of transgenic lines. The SoCYP85A1 gene was inserted in pB7WG2D,1-GFP to create the overexpressing vector. T4 generation of transgenic tobacco plants was confirmed by PCR analysis (see publication) and fluorescence detection of GFP protein. The 1,392-bp fragment of the SoCYP85A1 gene was amplified in 8 transgenic lines and imaged with the NEWTON 7.0 smart imaging system (Vilber Lourmat, France), thus confirming the overexpression of SoCYP85A1 in 8 lines of Transgenic Tobacco Plants.

CONCLUSION

This study provided evidence that an increase in the content of endogenous BRs could enhance the resistance of tobacco plants overexpressing SoCYP85A1 to P. nicotianae by enhancing the activities of defense enzymes. Results suggested a potential approach for enhancing black shank tolerance of tobacco plants through the regulation of genes involved in the biosynthetic pathway of BRs. SoCYP85A1 could be used as a potential candidate gene for improving resistance to black shank disease in tobacco and other economic crops. This study enlightens the usefulness of the NEWTON 7.0 smart imaging system (Vilber Lourmat, France) as a measuring tool in the molecular confirmation of transgenic tobacco plants overexpressing SoCYP85A1.

Figure 1.