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ИДЕНТИФИКАЦИЯ ФРАГМЕНТА СТИГМАСПЕЦИФИЧНОЙ ЭКСПРЕССИИ В ПРОМОТОРЕ ГЕНА ХИТИНАЗЫ КЛАССА I СОИ[1]

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Уровень экспрессии гетерологичных генов в трансгенных растениях служит важным показателем эффективности работы генов. Тонкая настройка экспрессии трансгенов ограничена небольшим репертуаром известных на данный момент эффективных промоторов. Нами клонирован и охарактеризован тканеспецифичный фрагмент промотора гена хитиназы класса I сои (*GmChi1*). Промотор *GmChi1* (GmChi1P) клонировали из сои сорта Jungery. Последовательность промотора содержит ряд предполагаемых *цис*-действующих элементов, включая тканеспецифичные мотивы и мотивы, регулируемые в условиях стресса. По результатам гистохимического анализа самая высокая активность репортерного фермента β-глюкуронидазы (GUS), находящегося под контролем GmChi1P, обнаружена в корнях трансгенного растения, *Nicotiana tabacum* cv. NC89, на стадии формирования ростка с четырьмя листьями. Интересно, что обработка салициловой кислотой эффективно подавляла высокую активность GUS в корнях трансгенного табака. Делеционным анализом GmChi1P установлено, что последовательности, расположенные между позициями −719 и −382, содержат ключевые *цис*-элементы, ответственные за экспрессию репортерного гена *uidA* (кодирующего GUS) в листьях, корнях и местах поранения *Nicotiana tabacum*. Кроме того, согласно результатам флуорометрического анализа, активность укороченных промоторов: от ChiP(−1292) до ChiP(−719) – в корнях трансгенного табака значительно подавляется абсцизовой кислотой и полностью – салициловой. Обнаружено также, что промотор ChiP(−382) экспрессируется исключительно в рыльце цветков трансгенного растения. С использованием репортерного фермента GUS не обнаружено окрашивания в других органах цветка трансгенного *Nicotiana tabacum*, включая чашелистики, лепестки, пыльники, нити и завязи, а также ни в одной из вегетативных тканей. Полученные результаты свидетельствуют о том, что фрагмент промотора ChiP(−382) может быть использован в тканеспецифичной регуляции экспрессии генов и генной инженерии растений.

Ключевые слова: *GmChi1*, соя, промотор, трансгенные растения, *Nicotiana tabacum*, стигмаспецифичный промотор

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Identification of Stigma-Specific Expression Fragment in the Promoter of the Soybean Chitinase Class I Gene

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The expression level of heterologous genes in transgenic plants serves as an important indicator of gene efficiency. The small number of currently known effective promoters, limits the possibilities in fine-tuning the expression of transgenes. We cloned and characterized a tissue-specific promoter fragment of the soybean chitinase class I gene (*GmChi1*). The *GmChi1* promoter (GmChi1P) was cloned from Jungery soybean. The promoter sequence contains a number of putative *cis*-acting elements, including tissue-specific and stress-regulated motifs. By histochemical analysis, the GmChi1P-controlled β-glucuronidase (GUS) reporter enzyme activity was shown to be highest in the roots of transgenic *Nicotiana tabacum* cv. NC89 at the four-leaf sprout formation stage. Interestingly, the high GUS activity in transgenic tobacco roots was effectively suppressed by salicylic acid (SA) treatment. Deletion analysis of GmChi1P revealed that the sequences located between positions −719 and −382 contain key *cis*-elements responsible for the reporter *uidA* gene expression (encoding GUS) in leaves, roots, and wounds of *Nicotiana tabacum*. In addition, fluorometric analysis showed that the activity of the shortened ChiP(−1292) to ChiP(−719) promoters in the roots of transgenic tobacco was significantly suppressed by abscisic acid and completely suppressed by SA. The ChiP(−382) promoter was also found to be expressed exclusively in the stigma of transgenic tobacco flowers. Using the GUS reporter enzyme, no staining was detected in other flower organs in transgenic *Nicotiana tabacum*, including sepals, petals, anthers, filaments, and ovaries, or in any vegetative tissues. The results indicate that the promoter fragment ChiP(−382) can be used in tissue-specific regulation of gene expression and plant genetic engineering.

Keywords: *GmChi1*, soybean, promoter, transgenic plant, *Nicotiana tabacum*, stigma-specific promoter