大豆种皮特异性启动子的克隆及功能分析

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摘 要:采用PCR技术从大豆品种吉农17基因组DNA中分离得到大豆种皮特异性启动子片段SCSP,长度约为1268 bp。序列分析表明SCSP与报道序列同源性达97%以上。将其与GUS基因融合,构建植物表达载体pSCSP-GUS后,转化到EHA105根癌农杆菌中,通过根癌农杆菌(*Agrobacterium tumefaciens*)介导法转化烟草(*Nicotiana tabacum*)NC89。转基因植株的GUS活性检测表明,仅能在种皮检测到GUS活性,而在茎、叶和花等其它组织中都未检测到GUS活性,证实SCSP片段具有种皮特异性表达功能。

关键词:大豆;种皮特异性启动子;功能分析; GUS染色

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Cloning and Identification of the Seedcoat Specific Promoter from Soybean

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Abstract: The promoter region of Seedcoat-specific gene was isolated from the genomic DNA of soybean Jilin 17 by PCR method, and obtained the promoter fragment SCSP, the length of the clone sequence is 1268bp. Sequence analysis indicated that this fragment had 97% homology compared with the reported promoters. The cloned promoter was fused to the GUS reporter gene to construct plant expression vector pSCSP-GUS, which was transferred into tobacco (*Nicotiana tabacum*) NC89 by *Agrobacterium tumefaciens*-mediated method and several transformed plants were obtained. GUS activity assays of different soybean organs indicated that expression of GUS was active only in seedcoat, which suggests the SCSP gene is seedcoat-specific promoter.

Key words: Soybean; Seedcoat specific promoter; Functional Identification; GUS staining

启动子是DNA分子与RNA聚合酶特异结合的部位,是最主要的一种转录调节因子,对于外源基因的表达非常重要。在基因表达的调控中,转录的起始是个关键。某个基因是否表达决定于特定的启动子的起始过程。在采用分子生物学手段对农作物进行遗传改良的过程中,常希望插入的外源基因能够限定在特定的组织中表达,从而使植物获得有益的性状而不影响其它特性,这就需要通过特异性启动子对靶基因的表达进行调控[1]。启动子有组成型和组织特异型,前者在所有组织中都启动基因表达,后者仅在特定的组织中和一定的发育时期启动基因表达。在组成型启动子驱动下表达的异源蛋白,可在植物细胞内大量积累。外源基因在植物内持续、高效的表达,必然大量消耗植株体内的基础物质,而基础物质的额外消耗又必然影响和抑制植物体内的其它代谢活动,不但造成浪费,往往还会引起植物的形态发生改变,影响植物的生长发育。而且重复使用同一种启动子驱动2个或2个以上的外源基因可能引起基因沉默或共抑制现象[2-3]。为了使外源基因在植物体内有效发挥作用,又减少对受体植物的不利影响,人们开始越来越多的研究组织器官特异性启动子[4-6]。种皮特异性启动子就是这类启动子,它可调控种皮表达系统改变种皮颜色,也可以提高所需要元素的表达量,还可以使外源基因在种皮表达起到保护作用等。该试验克隆了种皮特异表达启动子,将其与带有GUS报告基因的表达载体重组,利用农杆菌介导法转化烟草,并用PCR和GUS组织化学染色鉴定,并期望将其转入大豆中,使外源基因在种皮中特异表达。

1 材料与方法

1.1 供试材料

1.1.1 菌种和质粒 菌种为*E. coli* DH5α、植物表

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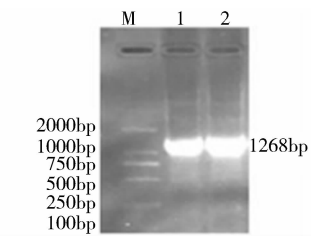

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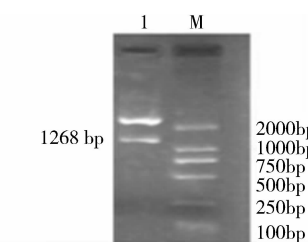

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 181 gtgtttatat agatgtgact tttgttgagg aactcatgcc aatggtactg attgtgaaac
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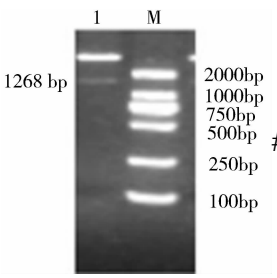

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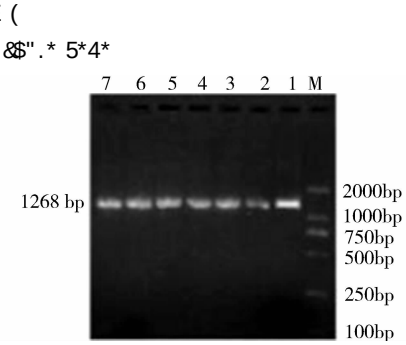

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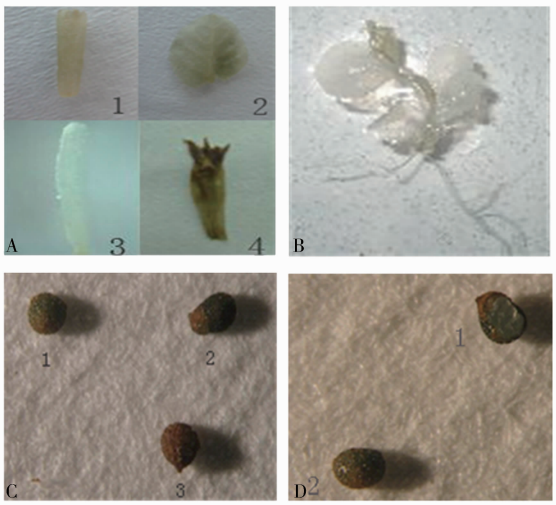

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(O) # P2W7 8 ! < A8? AGKUQ4 (2U@K 2WF2? HKCK7CFK3GW2U - IU2T@PCKUA 6? CL@7GM2U? @A27 (5) 4 . 6F3KAF - FALG8 KGK@BFB ! &" OO ! & $" O=4

(") # P2UGFB 8 +! `UE5 , ! P2W8/@ . Q4 - GA? HBK @7L IK7KU@8 ? KCB2L W2UCL@7GKUAYI IK7K AYC2 HB@7C(5) 4 (FAK7FK! &" O9 ! ! ! =$&! " : &! $&4

(&%) #5KWKUG27 8 - 4 - GGGAYI FBA? KUAF IK7KGAY HB@7CS YBK RS(IK7K: W6A27 GECBK? (5) 4 ^3@7CM23KF63@U + A2I2I E 8 KH-2UCU &" O=! 9 " >#$ $O=:>%94

(&&) #2 Ï é ! = ° X ! \ Î 5 4& ' < Â ¸ • n [' Â t ÆÇ Ã Í Î ' “ (5) 4 < d , ^ (H &)) ä ! ! %% ! $=" &! # $&%9:&&O4" ` 6 * ^! ; B26 P Y! < @7I ^ < 4 / 327A7I @7L ALK7GAWF@A27 2WCBK GKKL GHKFAWF HU2? 2CKUW2? G2ETK@7 (5) 4 526U7@82W 2UCBZKGG - q ` S: 7AKUGAE ! %%! $=" &! # $&%9:&&O4#

(&) #. AEA6FBAY ! (BA7GBAP ! (6j 6i AX 4 8 @HAL @7L CL@7GK7C@PCAVA@A27 2WCL@7GFUAHA27 2WCBK, 8 ` $ IK7K TE Z267: LA7I AY C2T@FF2 3K@KG$ ^2GA7K AY V23JK? K7C2W G< 8 X* G@7L @K2UKHUKGA27 (5) 4 526U7@8 2W+A23I AF@8 / BK? AGCUE ! %%» ! ! =" $ 99$99:99$" &4