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植物和微生物遗传学

Waxy基因的RNA沉默使转基因小麦种子中直链淀粉含量下降

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摘要 通过RNAi策略转化小麦，以降低小麦种子中直链淀粉的含量。小麦中直链淀粉合成的关键酶是颗粒结合型淀粉合成酶(Granule-bound starch synthase I ，GBSSI)，通过RT-PCR方法从小麦种子中分离出Waxy基因。Southern杂交分析表明，在基因组中存在3个Waxy基因。Northern杂交分析显示出在授粉后的小麦种子中检测到Waxy mRNA。利用RNA沉默策略，将Waxy编码区683 bp的正向和反向片段以及150 bp 内含子，连接于表达载体pCAMBIA3300中玉米ubi1启动子下游。以扬麦10号授粉后15d的幼胚为外植体，利用农杆菌介导的方法进行转化。通过PCR、RT-PCR和叶片离体褪绿实验鉴定出4株转基因植株。小麦胚乳I2-KI染色和直链淀粉含量测定表明这4株转基因植株直链淀粉含量明显下降。研究结果表明Waxy基因的RNA沉默使转基因小麦种子直链淀粉的含量下降。

关键词 小麦；Waxy；RNA干涉；农杆菌介导的转化；直链淀粉

分类号

RNA Silencing of Waxy Gene Results in Low Levels of Amylose in the Seeds of Transgenic Wheat (Triticum aestivum L.)

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Abstract

In this study, the level of amylose was reduced in wheat seeds by RNAi strategy. Since the synthesis of amylose is catalyzed by the granule-bound starch synthase I (GBSSI), the Waxy gene of wheat was isolated from wheat seeds by using RT-PCR. Southern analysis confirmed that there were three Waxy genes in wheat genomes. Northern hybridization showed that Waxy mRNA accumulated in seeds following pollination. By RNAi strategy, the 683 bp sense and antisense fragments in reverse orientation separated by a 150 bp intron were cloned into pCAMBIA 3 300 just downstream of the maize ubi1 promoter. By Agrobacterium-mediated wheat transformation method, four transgenic plants (Cultivar Yangmai 10) were identified by PCR, RT-PCR and leaf painting assay. The levels of amylose in the endosperm were significantly reduced in transgenic seeds by iodine staining and analysis of amylose content. The results indicated that RNA silencing of Waxy gene resulted in low levels of amylose in the seeds of transgenic wheat.

Key words wheat Waxy RNA interference Agrobacterium-mediated transformation amylose

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