

研究论文

牡山羊草 *Aegilops juvenalis* Puroindoline b 基因的克隆

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摘要 Puroindoline a (Pina)和puroindoline b (Pinb)是控制小麦籽粒硬度的主效基因。根据已报道的小麦Pinb基因的保守序列,设计合成了一对特异性引物,对六倍体牡山羊草*Aegilops juvenalis* (UUMMDD)的基因组DNA和胚乳cDNA进行Pinb基因扩增、克隆和序列测定,发现了两个新型Pinb等位基因Pinb-allele-1和Pinb-allele-2.该基因全长360 bp,编码119个氨基酸残基.它编码的蛋白和麦类作物Puroindoline B(PinB)的成熟蛋白有非常高的同源性,具有麦类作物PinB蛋白所特有的WPTKWWK的色氨酸结构域和10个半胱氨酸所形成的5个二硫键结构.与软粒小麦cv.Capitole的Pinb-D1a相比较,其核苷酸同源性为93.1%、93.3%,氨基酸同源性为90.8%、92.4%.Pinb-allele-1和Pinb-allele-2分别含有11和9个氨基酸变异位点.RT-PCR证实了Pinb-allele-2基因在籽粒胚乳中的表达.Southern Blot分析结果表明,牡山羊草中含有两个拷贝的Pinb基因,其中包含着与小麦差异较大的籽粒硬度控制基因。

关键词 [牡山羊草](#) [Puroindoline b](#) [籽粒硬度](#) [克隆](#)

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Gene Cloning of Puroindoline b in *Aegilops juvenalis*

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Abstract Puroindoline a (Pina) and puroindoline b (Pinb) are major genes controlling grain hardness in wheat. According to the conserved regions of Pinb gene reported, one pair of specific primers ForB2 and RevB1 were designed and used to amplify Pinb from young leaves genomic DNA and developing endosperm RNA of hexaploid *Aegilops juvenalis* (UUMMDD). Through gene cloning and sequencing, two new alleles of Pinb, Pinb-allele-1 and Pinb-allele-2, were characterized. Both of them were 360 bp in size, encoding 119 amino acid residues which corresponded to the structure of mature Puroindoline B (Pin B) protein. They contained the tryptophan motif of WPTKWWK and five disulphide bonds formed by ten cysteines, which were specific to Pinb gene in cereal crop. There existed large differences of sequences of Pinb between *Ae. juvenalis* and *Triticum aestivum*. Compared with Pinb-D1a from *T. aestivum* cv. Capitole, the Pinb genes in *Ae. juvenalis* shared 93.1% and 93.3% homologies in nucleotide sequence and 90.8% and 92.4% homologies in amino acid sequence respectively. There were 11 amino acid mutations in Pinb-allele-1 and 9 in Pinb-allele-2. Southern blot analysis showed that there were two copies of the gene in *Ae. juvenalis*. Pinb-allele-2 was verified to express in grain endosperm by RT-PCR, but Pinb-allele-1 not. The results showed that *Aegilops* contain grain hardness-controlling genes quite different from those of *T. aestivum*.

Key words [Aegilops juvenalis](#) [Puroindoline b](#) [Grain hardness](#) [Cloning](#)

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