栽培大豆端粒相关序列的克隆及定位

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摘 要:以拟南芥的端粒重复序列(TR)为引物$(TTTAGGG)_3$,在栽培大豆中扩增并克隆了 1 个 574 bp 的 DNA 片段。序列分析表明:该片段与大豆端粒相关序列的相似度高达 92% ~99%,与白玉草 TR-TAS 的间隔区的相似度为 79%,与大麦和玉米等其它植物的 TAS 的相似度在 16% ~35% 之间;这一片段含有 14 个拷贝的拟南芥类型端粒重复单元,并且还有 30 个重复单元发生了碱基突变(缺失、替换与插入)。该端粒相关序列具有 1 个 25 bp 的保守重复单元,串联重复 13 个拷贝,且该序列的 A + T 含量高于 60%,体现了卫星 DNA 的特征。该序列被定位在大豆 3 号染色体的近末端。

关键词:栽培大豆;端粒相关序列;重复单元;克隆;定位

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Cloning and Mapping of Telomere Associated Sequence in Soybean

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Abstract: Using the Arabidopsis-type telomeric repeat $(TTTAGGG)_3$ as a primer, a 574 bp TAS was amplified and cloned from three soybean (*Glycine max*) cultivars. Sequence analysis indicated that the similarities of the TASs from different cultivars in this study and other TASs from GenBank ranged from 92% to 99%. A similarity of 79% was detected between the TAS obtained and the interval sequence of TR-TAS from *Silene latifolia*. Low similarities ranging from 16% to 35% were observed between the soybean TAS and other plants such as barley (*Hordeum vulgare*) and corn (*Zea mays*). In this TAS fragment, there were 14 copies of Arabidopsis-type telomeric repeat (TTTAGGG/CCCTAAA) and 30 copies of mutation motifs with deletion, substitution and inversion. The 25 bp conserved tandem repeat in this TAS fragment occurred in 13 copies and the content of adenine and thymine (A + T) was greater than 60%, showing the characterization of satellite DNA. This TAS fragment was mapped to the proximal end of soybean chromosome 3.

Key words: Soybean cultivar; Telomere associated sequence; Telomeric repeat motif; Clone; Mapping

端粒是真核生物线性染色体的末端结构,对于维持染色体稳定和末端复制具有重要作用。端粒 DNA 由高度相似的重复序列组成,包括简单的端粒重复序列(Telomeric repeat, TR)和端粒相关序列(Telomere associated sequence, TAS)或称亚端粒序列(Subtelomeric repeat)。TR 在同一生物的所有染色体间是一致的,在不同生物的染色体间也是高度保守的。在高等植物中,拟南芥(*Arabidopsis thaliana*)的端粒 DNA 序列最先被克隆[1]。随后,利用拟南芥类型的端粒 DNA 序列为探针,发现番茄(*Lycopersicon esculintum*)[2]、小麦(*Triticum aestivum*)[3]、玉米(*Zea mays*)[4]、水稻(*Oryza sativa*)[5]、烟草(*Nicotiana tabacum*)[6]和大豆(*Glycine max*)[7]的端粒中同样含有拟南芥类型的端粒 DNA 序列。TAS 通常与 TR 紧密相连,但二者在序列上几乎不具同源性。

TAS 主要由卫星 DNA 组成,一般只存在于生物体的部分染色体中。TAS 不但在拷贝数上具有很高的多态性,而且还具有种的特异性[8],因此可以作为遗传图谱的末端标记。Mao 等将小麦 TAS 定位到了染色体的端部[9],随后,水稻端粒相关序列 Tas 1 也定位在第 6 号染色体的端部[10]。大豆(2n = 40)染色体数目较多且短小,形态差别较小,因此大豆的细

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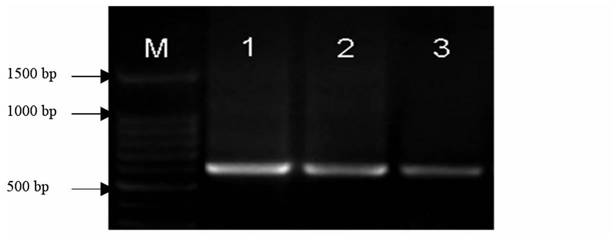

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较高的相似度,表明在大豆染色体末端存在类似区域的可能性。张德水利用荧光原位杂交技术,也将大豆端粒相关序列定位在染色体的近末端[12]。水稻端粒相关序列也被定位到了 RFLP 图谱的端部[10],这与该研究的染色体定位结果相吻合。

端粒是染色体的末端,但由于 TR 在不同生物之间、甚至同一生物的不同染色体之间的同源性较高,所以不宜作为染色体末端标记。与此不同,毗邻 TR 的 TAS 在不同的生物中多态性很高[10],而且 TAS 的克隆也相对容易。许多生物染色体的末端 TR 是不连续的,利用荧光原位杂交技术在染色体端点的近远端也可见到 TR[12]。因此,以 TR 为引物可以扩增出 TAS。TAS 可作为分子遗传图谱、细胞学图谱和物理图谱的末端标记[19]。Kato 等[20]采用多色荧光原位杂交技术,将 TAS、rDNA 和串联重复序列等 DNA 序列定位到不同的染色体上,进而区分玉米体细胞的 10 条染色体。由于大豆的细胞遗传学研究困难,大豆的遗传图谱还不能与其染色体相对应。因而,栽培大豆 TAS 的克隆,对于深入了解大豆染色体的末端信息以及整合大豆遗传图谱具有重要意义。

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