

结球甘蓝叶片卷曲相关7个同源异型盒基因的克隆与表达分析

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Molecular Cloning and Expression Analysis of Seven Homeobox Genes

from *Brassica oleracea*

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摘要 以结球甘蓝 (*Brassica oleracea* L.) ‘519’ 品系为试材, 通过结球期茎尖和叶片以及莲座期茎尖和叶片的转录组对比分析, 筛选出4个显著差异表达HB类基因, 分别为BoHAT2、BoHB12、BoHB7和BoHB27。进一步对上述4个基因以及调控拟南芥 (*Arabidopsis thaliana*) 叶片卷曲但在结球甘蓝转录组中未检测到表达差异的基因BoPHB、BoPHV和BoREV进行了同源克隆。序列分析表明上述7个基因都含有同源异型域 (homeodomain, HD), 具有同源异型盒 (homeobox, HB) 蛋白家族的典型结构特征。BlastP (蛋白序列与蛋白库做比对) 表明它们与拟南芥的同源蛋白相似性高, 来自结球甘蓝的BoHAT2、BoHB12、BoHB7、BoHB27、BoPHB、BoPHV、BoREV与拟南芥中的HAT2、ATHB12、ATHB7、ATHB27、PHB、PHV、REV蛋白同源, 同源性分别为86%、80%、79%、60%、94%、95%和96%。BlastP比对和进化树分析结果表明, BoHAT2属于HD-Zip II类, BoHB12和BoHB7属于HD-Zip I类, BoHB27属于ZF-HD, BoPHB、BoPHV和BoREV属于HD-Zip III类。BoHAT2、BoHB12、BoHB7、BoHB27、BoPHB、BoPHV、BoREV在结球甘蓝莲座期到结球期的茎尖和叶片中均有表达, BoHB12和BoHB7在结球期叶片中表达量显著增高, 分别是莲座期叶片的38.1倍和6.2倍, 其余基因表达量差异不明显, 表明BoHB12和BoHB7可能是结球甘蓝球叶自然卷曲过程中的主效调控基因。

关键词: 结球甘蓝 同源异型盒蛋白 基因克隆 表达分析

Abstract: Four significantly differentially expressed HB (homeobox) analogs genes, BoHAT2, BoHB12, BoHB7, BoHB27 were identified through transcriptome comparison of stem tip and leaf at rosette stage and heading stage of cabbage (*Brassica oleracea* L. ‘519’). BoHAT2, BoHB12, BoHB7, BoHB27 and else three HB analogs genes BoPHB, BoPHV, BoREV, which were no transcription difference at rosette stage and heading stage of cabbage but had been proved involving in the process of leaf curl in *Arabidopsis thaliana* were isolated respectively using homologous cloning method. Amino acid sequence analysis showed that seven genes share a homeodomain of homeobox family. BlastP analysis indicated all of proteins are highly homologous with HB protein family from *Arabidopsis thaliana*. The similarity of amino acid sequence of BoHAT2, BoHB12, BoHB7, BoHB27, BoPHB, BoPHV, BoREV genes are 86%, 80%, 79%, 60%, 94%, 95%, 96% compared with HAT2, ATHB12, ATHB-7, ATHB27, PHB, PHV, REV from *Arabidopsis thaliana*, respectively. Further BlastP and phylogenetic tree analysis indicated that BoHAT2 belongs to HD-Zip II protein, BoHB12 and BoHB7 belong to HD-Zip I protein, BoHB27 belongs to ZF-HD protein, BoPHB, BoPHV and BoREV belong to HD-Zip III protein. The real-time PCR analysis indicated the BoHAT2, BoHB12, BoHB7, BoHB27, BoPHB, BoPHV and BoREV genes express in stem tip and leaf from rosette stage to heading stage. The BoHB12 and BoHB7 genes expressed higher in the leaf of heading stage than the leaf of rosette stage, there were 38.1 times more BoHB12 transcription at heading stage than rosette stage and 6.2 times more BoHB7 transcription at heading stage than rosette stage were detected. No obvious expression difference was observed for else five genes between heading stage and rosette stage. These results demonstrate the BoHB12 and BoHB7 genes may be the major genes that involved in the process of cabbage leaf curl.

Keywords: [Brassica oleracea](#), [homeobox](#), [gene cloning](#), [expression analysis](#)

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