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Statistical Analysis of Arabidopsis T-DNA-flanking Sequences

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Agrobacterium-mediated T-DNA transformation of plant genome has been instrumental in tagging and subsequent isolation of genes important in a plant life cycle. Underlying hypothesis of using T-DNA in plant functional genomics is that the T-DNA insertion occurs randomly in plant genomes. To test this hypothesis, we analyzed the T-DNA flanking sequences isolated by and available in the Salk Institute Genomic Analysis Laboratory (SIGnAl) database. Examining the data of 29,084 Arabidopsis genes containing insertion information of approximately 120,000 T-DNA insert lines and functional annotations of the genes revealed that 70% of the 29,084 genes have at least one T-DNA insert, whereas 8760 genes are still left without an insert. This is quite surprising, because statistically the 120,000 lines should cover more than 95% of the genes with an insertion. This result led us to further analyze if the T-DNA insertion events truly take place randomly in the genome. Arabidopsis genome consists of 56.8 Mb (48.4%) of genic region and 60.5 Mb (51.6%) of intergenic region. Mapping the T-DNA insertion lines to the genome showed that 55% of the 120,000 T-DNA lines landed on the genic region, and 45% was assigned to intergenic regions. Nonparametric correlation tests indeed revealed that T-DNA insertion events occurred preferentially in the genic regions. Interestingly, the gene At2g25610 annotated as a putative vacuolar ATP synthase proteolipid subunit possess as many as 622 T-DNA inserts, again, suggesting a preferred T-DNA insertion region in the genome.

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벼 글루탐산 탈탄산효소 유전자의 클로닝 및 GABA 생산 능력이 증진된 벼 계통 개발 전략

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GABA(γ -aminobutyric acid) 고품유 쌀 생산 전략의 일환으로 글루탐산을 GABA로 전환하는 벼 (*Oryza sativa* L.) 글루탐산 탈탄산효소(glutamate decarboxylase, GAD) 유전자 (OsGAD, OsGAD1, OsGAD2)를 벼 뿌리 cDNA libraries와 현미에서 추출한 RNA로부터 분리하였다. 뉴클레오티드 서열분석 결과 OsGAD 클론은 505개 아미노산을 코딩하는 하나의 open reading frame (ORF)을 포함하는 총 1,712 bp로 구성 되어 있었다. OsGAD ORF로부터 얻어진 505개 아미노산 서열은 OsGAD1과는 67.7%, OsGAD2와는 61.9% 서열 동질성을 보였다. 이들 유전자를 대장균용 발현 벡터인 pVUCH와 pKK388 플라스미드에 클로닝하여 발현시킨 후 효소활성을 측정하여 본 결과 모두 글루탐산 탈탄산효소 활성을 보였고, 칼슘과 칼모듈린에 의하여 그 활성화 정도가 달라짐을 알 수 있었다. 또한 벼 형질전환체를 생산하기 위하여 rice endosperm-specific promoter를 클로닝하였고, 이 promoter와 OsGAD, OsGAD1, OsGAD2 유전자가 각각 삽입된 재조합 벡터를 제작 하고 있다. 이 재조합 발현 벡터를 이용하여 GABA 생산 능력이 증진된 벼 계통을 개발할 계획이다. (This work was supported by Korea Research Foundation Grant, KRF-2002-042-F00005).

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AtBAT1 (Binding Ability to Telomeric DNA), a Novel Telomeric-repeat Binding Protein of Arabidopsis, Binds to Both Human and Plant Telomeric Repeats

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Telomeres are nucleoprotein complexes at the physical ends of linear eukaryotic chromosomes for a protective cap. There are several genes in *Arabidopsis thaliana* known to encode plant telomere binding proteins. We have recently identified AtBAT1 containing a single Myb domain similar to those in AtTBP1 and hTRF1. Despite its sequence similarity, its Myb domain is located at its N terminus and Histone H1/H5-like domain is located at the center of the AtBAT1, which shows similar protein architecture to that of hTRF1. Northern blot analysis indicated that AtBAT1 gene is expressed ubiquitously in *A. thaliana* and transiently expressed the GFP-fused AtBAT1 was found mainly in the nucleus of onion epidermal cells. To elucidate DNA binding property of AtBAT1, we have expressed the Myb domain of AtBAT1 in *E. coli*. Expressed GST-fused Myb domain was capable of binding specifically to the double-stranded plant telomeric DNA ([TTTAGGG]_n). However, it showed much higher binding affinity to human telomeric DNA ([TTAGGG]_n). These data suggest that AtBAT1 encodes a putative plant telomere binding protein(TBP) which constitutes telomere structure. Furthermore, as it also binds to human telomeric repeats with higher affinity, it is a novel protein with distinct properties distinguished from those of known AtTBPs. Possible role of AtBAT1 in *Arabidopsis* will be discussed. [Supported by grant from KRF (2002-015-DP0415)]

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Identification and Characterization of Antiviral Peptide in Various Potato Cultivars

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PLRV (Potato Leafroll Virus), PVX (Potato Virus X), PVY (Potato Virus Y) are major viral pathogen in potato (*Solanum tuberosum*) diseases. We infected PVX and PVY onto wounded potato leaves because the physical barrier of the cell wall had been breached by injury, thereby facilitating entry of the virus. Virus infected plants using sap-inoculation(virus amplex with 0.1M phosphate buffer) method showed various symptoms including mosaic, yellowing, necrosis, mottle, vein clearing, vein bending or changelessness etc. We have used Enzyme-Linked Immunosorbent Assay and PCR for virus detection and screening for virus-resistant potato cultivars from 13 potato species including Golden, Early, Winter, Purple, Alchip. We selected new virus-resistant potatoes in which we assume that there are antiviral peptides or antibacterial peptides among expressing changelessness symptom potatoes. To minimize the damage of potato viruses, CA(1-8)-MA(1-12), PMAP-23, SMAP-29, HP (2-20) and analogous peptides can be applied to sensitive to virus infected potato cultivars. Those peptides can be overproduced by transformation to the plants to get virus-resistant potatoes.