

## SL202

### 연사 2

#### Identification of the Regulatory Sequences Involved in Embryo-specific Expression of the Soybean $\gamma$ -Thionin-like Protein Gene

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To isolate new genes that are expressed specifically during soybean seed development, we carried out random sequencing of seed-specific cDNA clones. This led to identification of a cDNA clone, SE60, encoding a low molecular weight sulfur-rich protein in soybean seeds. Using the SE60 cDNA as a probe, we isolated a genomic clone containing the SE60 gene. The mature SE60 protein of 47 amino acids was highly homologous to sorghum  $\alpha$ -amylase inhibitors and wheat  $\gamma$ -purothionins, having 8 cysteine residues at the identical regions in these proteins. Northern hybridization indicated that the SE60 gene was expressed in a seed-specific and developmentally regulated manner. Furthermore, the RT-PCR analysis revealed that the SE60 gene was regulated differently from the glycinin(Gy2) and extensin (SbHRGP3) genes during seed development and germination. To identify the regulatory sequences for the SE60 gene expression, chimeric genes were constructed with the SE60 promoter and the GUS gene and transformed into tobacco cells. Their expression was analyzed with developing seeds of transgenic tobacco plants. GUS activity was observed specifically in the developing embryos at the globular, heart and torpedo stages and was increased gradually as seed development proceeded. GUS activity was observed mainly at the cotyledons in the light-grown seedlings, while it was mainly at the hypocotyl in the dark-grown seedlings. Histochemical and fluorometric assays for GUS activity with seeds of transgenic tobacco plants suggested that the -495 to -200 sequence may contain regulatory sequences for the embryo-specific expression of the SE60 gene. The region contained four embryo factor binding sites and a G-box motif that were identified to be involved in the seed-specific expression of other plant genes.