F208 Characterization and Purification of *rbc*L promoter R2 region binding factors in *Zea mays*.

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It has been reported that chloroplast DNA binding factors bind to *rbc*L promoter R2(-33 to -229 from ATG) and R3(-230 to -418 from ATG) region in *Zea mays*(Lee and Sim, 1996). In this study, we identified *rbc*L R2 region specific binding proteins. Electrophoretic mobility shift assay(EMSA) showed that two factors, R2 region binding factor-1(RBF-1) and R2 region binding factor-2(RBF-2), from chloroplast membrane high-salt extract bind specifically to R2 region. The two proteins were partially purified by precipitation of different concentrated ammonium sulfate and DEAE-Cellulose anion-exchange chromatography. RBF1 is included in 0-34% ammonium sulfate precipitation fraction and RBF2 is in 34-53% Ammonium sulfate precipitation fraction. These are further purified into several fractions on DEAE-Cellulose column, of which 200mM and 400mM KCl elution fraction included RBF-1 and RBF-2, respectively. Their binding activity was confirmed by EMSA.
As a result, RBF-1 and RBF-2 are purified about 140-fold and 66-fold from chloroplast high-salt extract, respectively. And they have a specific binding activity to R2 region. These factors may play an important role in the regulation of transcription of *rbc*L.

F209 Isolation and Molecular Characterization of Proteasome α -Subunit Gene in *Petunia Hybrida*

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A cDNA clones was isolated from a cDNA library prepared from cultures of petunia petal protoplasts. DNA sequence analysis and database search revealed that the transcript encodes a protein which is highly homologous to eukaryotic proteasome α-subunit. It contains an open reading frame corresponding to a protein of 249 amino acids with a predicted molecular mass of 27,000 daltons. The deduced amino acid sequences of the clone were 89%, 57% and 56% identical with spinach, yeast, and human, respectively. When the genomic DNA extracted from petunia was digested with EcoRI, HindIII and XbaI which have no recognition sites in the cloned cDNA, only single band was hybridized in each digest. These results are consistent with the assumption of the existence of a single copy gene. Transcript of the gene was aboudant in the root and the petal but rare in fully expended leaf and sepal, no detectable in stamen and pistil. It strongly expressed in the earliest stage of flower development. These results indicate that the gene is differentially regulated in various tissues and developmental stages.