Online Proceedings

Poster Session 1

Commons West, Easel 19

11:00 AM to 12:30 PM

Mannose Selection for Chloroplast Transformation in Maize Tissue Culture

Akiko Alison (Akiko) Carver, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Arnold Bendich, Biology
Mentor: Delene Oldenburg, Biology

Most procedures for DNA transformation of chloroplasts involve the use of circular DNA as vectors and green leaves as the target tissue. Although such procedures are successful with some plants, they are inefficient and do not work with other plants, especially monocots. In maize, plastid DNA (ptDNA) is comprised largely of linear molecules. Furthermore, ptDNA is rapidly degraded as the cells become green. In consequence, the introduction of transgenes into maize chloroplasts has been problematic. In order to create vectors for monocot transformation, the plastid chromosome is most efficiently targeted in its natural form. Previous work suggests that the plastid chromosome is found primarily in a complex, branched linear form with defined ends. We chose one of these ends, "End 1," which is near an origin of replication, as our target for transgene integration. We constructed vectors with the "End 1" gene sequences and a mannose-metabolizing transgene, PMI, as the selectable marker. Plastid transformation was performed by particle bombardment using gold microcarriers coated with either the circular or linear vector. The successful introduction of a transgene using the "End 1" DNA sequence indicates that the transgene integrates either by end joining of the linear vector to the linear ptDNA or by homologous strand invasion. Thus, using a linear vector with an end sequence produces optimized results when engineering monocot plastid transformants.

Poster Session 3

MGH 241, Easel 152

2:30 PM to 4:00 PM

Chloroplast Gene Integration

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Mentor: Delene Oldenburg, Biology

The goals of this project are to produce transgenic maize and wheat tissue expressing a gfp marker in chloroplasts and to regenerate homoplasmic plants from the transformed tissue. There are several advantages of transgenic chloroplasts, including multiple copies and high expression of the transgene without gene silencing. In addition, plastid DNA (ptDNA) is maternally inherited so that the transgene will not be present in pollen, thus providing containment for the transgene. We used circular and linear vectors that contain ptDNA sequences corresponding to the ends of the plastid chromosome and the gfp transgene. We used particle bombardment to deliver the vector to plastids of maize and wheat. Transgene expression was assessed by fluorescence microscopy and imaging of GFP in plastids. In addition, both standard PCR and quantitative PCR were performed using DNA extracted from the tissue and primers for the gfp transgene. Although we achieved successful plastid transformation for both maize and wheat, the tissues were heteroplasmic, containing both wild type and transgenic ptDNA. We found that a linear vector gave higher transformation efficiency than a circular vector. The next step is to develop procedures for generation of homoplasmic maize and wheat plants.