F207

벼(Oryza sativa L.)를 형질전환하기 위한 binary vector의 제조

박 희동, 서 진교*, 심 웅섭
고려대학교 이과대학 생물학과

*Agrobacterium tumefaciens*을 이용해 Japonica type의 벼(*Oryza sativa* L.)품종을 형질전환하기 위해 새로운 binary vector(pBI-ActR1, pBI-ActF1, pBSH-ActR1)을 제작하였다. 이들 vector들은 벼에서 GUS reporter 유전자의 발현을 극대화하기 위해 벼의 actin 유전자가 클로닝되어 있는 pAct1-F로 부터 promoter 지역을 취해 GUS 구조유전자에 붙였다. pBI-ActR1과 pBI-ActF1은 pBI121의 replication origin을 가지며 pBSH-ActR1은 본 연구실에서 분리한 *A. tumefaciens* KU12의 plasmid중 하나인 pTi12의 replication origin을 가진다. 이들 vector들 중 pBI-ActR1과 pBI-ActF1을 이용하여 벼를 형질전환하기 위해 발아시킨지 3-4일 되는 유식물체 조각을 *A. tumefaciens*와 함께 배양하였으며, 형질전환 여부는 GUS 염색법으로 증명하였다. 형질전환 부위로 보이는 진한 녹색의 부위가 분열조직인 묘의 끝, 묘의 하단의 분열조직, 뿌리끝에서 나타났다.

F208

Possible Origin of Tetraploidization in Protoplast Cultures of Petunia (*Petunia hybrida*)

오만호*, 이현세, 김상구
서울대학교 자연과학대학 생물학과

To understand the origin of cytogenetic variation *in vitro* protoplast cultures of petunia, nuclear behaviours during the cultures of the mesophyll protoplasts isolated from diploid and tetraploid petunia were investigated by staining the nuclei with 4,6-diamidino-2-phenylindole (DAPI). Freshly isolated protoplasts were cultured on modified Murashige and Skoog's medium supplemented with sixteen combinations of auxin (2,4-D, NAA, IAA) and BAP. During the cultures of diploid mesophyll protoplasts, varying degrees of multinucleates prior to initiation of the first cell division were observed. At the optimum combination of plant hormones for initial cell division, formations of multinucleates induce the highest frequencies up to 53.7%. Although the majority of the protoplasts subsequently entered into morphologically normal cell division, they showed the multinucleates by staining with DAPI. In contrast with those of diploid plants, frequencies of multinucleate in protoplast cultures of tetraploid petunia were only 0.1-2.3%, and the majority of protoplasts in cultures showed normal mitotic division by staining with DAPI. The results indicate that formation of multinucleates during initiation of the first cell divisions in diploid mesophyll protoplast culture was primarily influenced by exogeneous hormones, and those multinucleates may be possible origin of tetraploidization in diploid mesophyll protoplast cultures.