

Chinese cabbage (*Brassica campestris*), one of the major vegetable crops in Korea, is controlled by self-incompatibility in the reproduction process. To maintain inbred lines of the crop plant, a method in that high CO<sub>2</sub> gas is treated to the pistils to break the self-incompatibility and thereby self-pollens can successfully make germination and fertilization has been widely used in seed companies. This study shows structural alteration of papillae cell surface during the self-incompatibility breakage by CO<sub>2</sub> gas treatment that eventually allows self-pollens to germinate. Also, in order to understand what the CO<sub>2</sub> gas causes to the papillae cell at molecular level, differential gene expressions of the pistil treated with the CO<sub>2</sub> gas was investigated by DDRT-PCR and reverse northern hybridization experiments. These results suggested that the breakage of self-incompatible reaction caused by CO<sub>2</sub> gas treatment is not only done by the structural alteration of papillae cell wall but also done by gene products that are positively and/or negatively regulated by the environmental stimulation.

**D 202**

**Regeneration of Protoplast from Disintegrated Cells of the Marine Green Alga *Chaetomorpha aerea***

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When injured, the protoplasm come out from the multi-nucleate cell of a green alga *Chaetomorpha aerea* can generate numerous new cells spontaneously. Cell organelles aggregated in seawater and became surrounded with a gelatinous envelope within 20 minutes. On average, 200

protoplasts were produced from 1 ul of cytoplasm and about 15% of them survived. The total membrane surface area of individual cells was estimated by image analysis of intact cells and the protoplasts regenerated from the extruded protoplasm. About 22 % of the original cell membrane was recycled to make new protoplast membrane. Fluorescein diacetate staining showed an esterase activity inside a protoplast that increased over time. DAPI staining showed irregular distribution of nuclei, suggesting that each cell organelle was initially come out independently. Development of protoplast membrane was pH dependent and the optimum pH was 8, the pH of seawater. About 50% of regenerated cells developed a reproductive cell, aplanospore, in two weeks. The released aplanospore divided into 8 cells and each of them became a motile gamete with two flagella. This is first report on protoplast regeneration from disintegrated cell fractions of multi-cellular system.

**D 203**

**Expression of Cold-Stress-inducible cDNA Clone Isolated from Potato (*Solanum tuberosum* L.) tuber cDNA Library**

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Stress-induced proteins play a definite role in protecting plants from possible damages by drought, extreme temperatures, and flooding. Cold storage of potato tubers at 4°C was associated with the accumulation of several cold-induced transcripts. A cDNA clone encoding cold-inducible protein was isolated from a cDNA library prepared from tubers of *Solanum tuberosum* L. cv Superior.

By using a partial clone as a probe, we isolated and sequenced a corresponding full-length *ci* clone. Northern analysis of the CI7 transcript in response to low temperature, drought, exogenous abscisic acid (ABA) and high-salt treatments revealed that the transcript levels were induced by most treatments tested in tubers as well as in leaves. Whereas accumulation of CI7 transcripts during cold storage occurred within a day, CI7 transcripts in response to abiotic stresses and ABA were less expressed when compared to those of other stresses on transcript levels. The expressed pattern of CI7 was examined by reverse transcriptase-polymerase chain reaction (RT-PCR).

**D 204**

### 배추로 도입된 RAG25 유전자의 ectopic expression에 따른 표현형의 변화

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배추의 자엽절편체에 개화시기 조절유전자인 RAG25 유전자를 도입하여 이러한 유전자의 발현을 확인하기 위해 reverse transcription(RT)-PCR을 수행하였다. NPT II 유전자와 RAG25 유전자의 primer를 이용한 PCR결과 각각 0.7kb와 0.6kb에서 band를 확인하였으며 floral bud를 이용하여 in situ hybridization을 실시한 결과 자방에서의 두드러진 발현을 관찰하였다. RAG25 유전자의 발현이 확인된 형질전환체 중에서 이른 개화를 보이는 개체의 표현형을 조사하였으며 크게 mild type과 severe type의 두 유형으로 분류할 수 있었다. Mild type의 경우 wild type과 거의 유사한 외관을 가지고 있었으나 정단우성과 암술크기의 감소가 나타났다. Severe type의 경우 wild type과 다른 외관을 보이며 잎의 크기가 감소하고 정단우성의 감소가 나타났다. 이러한 severe type의 경우 floral bud가 형성되었으나 더 이상 발달되지 않았다.

**D 205**

### The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*

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The very late-flowering behavior of *Arabidopsis* winter-annual ecotypes is conferred mainly by two genes, *FRIGIDA* and *FLOWERING LOCUS C*. A MADS-domain gene, *AGAMOUS-LIKE 20 (AGL20)*, was identified as a dominant *FRI* suppressor in activation tagging mutagenesis. Overexpression of *AGL20* suppresses not only the late flowering of plants that have functional *FRI* and *FLC* alleles but also the delayed phase transitions during the vegetative stages of plant development. Interestingly, *AGL20* expression is positively regulated not only by the redundant vernalization and autonomous pathways of flowering but also by the photoperiod pathway. Our results indicate that *AGL20* is an important integrator of three pathways controlling flowering in *Arabidopsis*.

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### Effects of Auxin on the Timing of Determination for Root Formation from Internodal Explants of Cassava

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The timing for the determination in root