Acta Bot. Neerl. 39(2), June 1990, p. 207

Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FERTILIZATION RESEARCH IN PLANTS ON 8 SEPTEMBER 1989

The selection for oligomycin-resistant Petunia

E. Flipse. Department of Plant Breeding, Agricultural University Wageningen, Lawickse Allee 166, 6709 BD Wageningen, The Netherlands.

Study of organelle segregation and recombination in fusion products requires the use of selectable, cytoplasmatically encoded mutations. Recently, Avive Galum (*Mol. Gen. Genet.* 1988, **215:** 128–133) was able to isolate a mitochondrially encoded oligomycin-resistant mutant of *Nicotiana sylvestris*. The antibiotic compound oligomycin binds to the membrane of the mitochondrial ATPase and inhibits the proton translocation. Genetic and molecular analyses of the oligomycin-resistant mutants in *Saccharomyces cerevisiae* showed that they carry nucleotide changes in the mitochondrial genes coding subunit 6 or 9 of the mitochondrial ATPase.

A procedure for the isolation of oligomycin-resistant micro-calli in Petunia has been developed. Protoplasts of different Petunia species were isolated in a medium with CPW-salts (Frearson, E.M. *et al. Dev. Biol.* 1973, **33:** 130–137) 0·3 M sucrose, 100 mg/l 2-(*N*-morpholino)ethane sulphonic acid (MES), 1% cellulase and 0·2% macerozyme. The protoplasts were washed in the same medium without enzymes and plated in culture medium TM2GZ with NAA (1 mg/l) and zeatin (1 mg/l). After 2 weeks of culturing, the dividing protoplasts were mixed with an equal amount of 0·6% Seaplaque agarose in TM2GZ. Oligomycin (0–1·5 mg/l) was added to the medium at different times during culture. The best results were found after 2 weeks of culturing. The addition of oligomycin significantly decreased the amount of calli growing. Having assessed the parameters for selection several experiments were performed. From each experiment independent calli were picked and recultured on medium with 1·5 mg/l oligomycin. Generally, calli isolated from plates with a low concentration of oligomycin did not grow as fast as those isolated from plates with a higher concentration of oligomycin. A number of Petunia species gave regeneration. The plants need to be checked for oligomycin resistance. As selection for oligomycin resistance at plant level seems impossible, protoplasts must be isolated from the oligomycin-resistant plants to determine whether they are still oligomycin resistant.

Enzymatic isolation of living embryo sacs from ovules of Petunia

H.S. Kwee and J.L. Van Went. Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

A 20–25% yield of isolated and living embryo sacs of *Petunia hybrida* L. was obtained using an enzymatic maceration procedure. This result was achieved using a maceration mixture containing 3% driselase (Sigma), 0·1% MES (2-(*N*-morpholino)ethane sulphonic acid) buffer pH 5·5, and 8% mannitol. Before use, the maceration solution had to be centrifuged to remove the insoluble fraction of the driselase. For each maceration the ovules of three flowers (±450 ovules) were incubated in 1 ml enzyme solution for 2 h at 30°C in a shaking machine (150 r.p.m.). Subsequently, the solution was centrifuged and the enzyme solution replaced by BK medium pH 6·5, supplemented with 12% mannitol (stabilizing solution). Liberated embryo sacs could be collected with a micropipette, using a dissecting microscope, and transferred to a fresh stabilizing solution. The isolated embryo sacs were intact and viable. When just-opened flowers were used the isolated embryo sac consisted of two synerigids, the egg cell, the central cell, and three antipodals. The original shape and organization of the embryo sac and individual cells was well maintained. When stored at room temperature the isolated embryo sacs remained viable for 8 h. Storing at 4°C resulted in prolongation of viability for up to 80 h. Prolonged incubation or reincubation of the embryo sacs resulted in the liberation of their consisting cells as individual, living protoplasts.