DATE**:**
NAME**:**

THE TOPIC**: Direct Somatic Embryogenesis from the Flower Receptacles and Leaves of Cineraria (*Senecio* x *hybridus* Hyl.).**

MATERIAL**:** closed inflorescences and leaves of cineraria

MEDIA**:**
M-S basal salts, 0,55 mM (1 $g.l^{-1}$) myoinositol, 2,9 μM (5 $mg.l^{-1}$) thiamine-HCl, 13,5 μM (3 $mg.l^{-1}$) 2,4-D, μM 4,5 (1 $mg.l^{-1}$) BA, 88 mM (30 $g.l^{-1}$) sucrose, 0.8% (8$g.l^{-1}$) agar, pH 5,8.
Schenk and Hildebrandt 1972 macro a microelements (**SH**) supplemented see above.

Surface disinfections of plant material and explant preparation.

PROCEDURE:
1. Inflorescence (capitulum or head)
   1. Inflorescences with 2 cm of the subtending pedicle immerse in 70% ethanol and quickly pass through a flame.
   2. Flamed inflorescence soaked in 20% SAVO + 0.1% Triton X-100 for 10 min. and wash 3x in sterile distilled water.
   3. Remove the subtending pedicle; strip the involucre of phyllaries and all the florets from the receptacle.
   4. (In case of heavy infection, immerse the naked receptacle in 10% SAVO solution for 5 min and wash 3x in sterile distilled water.) Cut the receptacles into two equal halves.

2. Leaves
The first true leaves of seedlings from the sterile sowed seeds or the leaves from in *vitro* cultured cineraria plants bisect into two equal halves.

Experiment 1. The effect of basal medium on initiation of somatic embryos

MEDIUM: Petri dishes containing SH medium + 13,5 μM 2,4-D + 4,5 μM BA
Petri dishes containing MS medium + 13,5 μM 2,4-D + 4,5 μM BA

PROCEDURE:
1. The disinfected explants (see above) cut into identical halves and place one half onto the surface of the MS medium and label the Petri dish as A and the second one onto the medium SH and label the dish as B.
2. Incubate the cultures in the dark at 22 - 25°C. Observe weekly somatic embryo development and discard contaminated cultures.
3. Count the number of somatic embryos on each type of the nutrient medium and compare the results after 2 weeks of culture.

ANTICIPATED RESULTS:

In the second week of cultivation, leaf explants incubated on the SH medium (treatment A) produce crystalline callus and occasionally a somatic embryo. The explants incubated on the MS medium (treatment B) produce globular-stage yellow somatic embryos.
In the 4^{th} – 5^{th} week of culture on the SH medium leaf explants produce abundant callus and receptacle explants are curled without callus growth. The explants of treatment B are covered

with yellow globular and heart shaped somatic embryos that form callus and do not develop further.

Experiment 2. Maturation of somatic embryos and regeneration of plants

Initiation and development of somatic embryos are often determinate by basal media, supplements with vitamins and plant growth regulators (PGR), culture conditions, and a period of subculture. The composition of an induction, maintenance, maturation and a rooting media are often different. Typically, induction media contain high concentration of auxin or auxin-like compounds, whereas maturation media contain only low concentrations of PGRs or growth regulators are excluded. ABA is added to induce embryo maturation. Media for germination or rooting have reduced concentration of basal salts and sugar and low concentrations of PGRs (IBA).

MATERIAL AND MEDIA: Petri dishes containing MS medium + 0,5% activated charcoal
Petri dishes containing MS medium without PGRs
Magenta vessels containing MS medium without PGRs
Peat Pellets, Peat substrate, Plastic bags

PROCEDURE:

1. Transfer somatic embryos induced in the A variant of the first experiment to the MS medium containing activated charcoal and label as C.
2. Cultivate 3 days as before and then transfer explants to the MS medium without PGRs.
3. Paralelly to (1) transfer somatic embryos induced in the A variant of the first experiment to the fresh MS medium without PGRs. Label as D.
4. Cultivate all cultures as before for 3 weeks. Categorize somatic embryos of each treatment by developmental stage (globular, cotyledonary) and count.
5. Remove cotyledonary-stage embryos from explants and transfer them to the Magenta vessels containing MS medium without PGRs. Cultivate in 25 – 75 $\mu mol.m^{-2}.s^{-1}$ at 22°C.
6. Transfer 2 cm tall plants to moist peat pellets and cover with plastic bags in a greenhouse.
7. After 2 – 3 weeks of acclimatization transfer the plants to pots.

ANTICIPATED RESULTS:

Somatic embryos of the treatment (A) are produced directly on all types of explants. All developmental stages of SE could be observed. About 1/3 of the cotyledonary-stage embryos of treatment C produce a radicle and shoot within 10 days after transfer to MS medium without PGRs, the others (D) only form a radicle or never germinate. Positive influence of activated charcoal was observed.

Most of the plants derived from somatic embryos acclimatize easily. Flowers are smaller, nevertheless, of normal colour and shape.

LITERATURE:

Malueg K.R. *et al*. (1994): A three media transfer system for direct somatic embryogenesis from leaves of *Senecio x hybridus* Hyl. – Plant Cell Tissue, Organ Culture 36: 249 – 253.