Chrysanthemum indicum 'Golden Surfer' protoplast regeneration: a new horizon for somatic hybridization?

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INTRODUCTION – Commercial chrysanthemum, hybridised from *Chrysanthemum indicum* L. (Asteraceae) (2n = 6x = 54), is the second most economically important ornamental. Theoretically, somatic hybridization has a high innovative potential. However, *Chrysanthemum* protoplasts, whether fused or not, have always been very recalcitrant in regeneration experiments, yielding only calli or roots.

MATERIALS AND METHODS – Stock cultures of *Chrysanthemum indicum* 'Golden Surfer' were maintained on MS medium enriched with 20 g/l sucrose, 2 mg/l glycine, 1 mg/l kinetine and 0.01 mg/l NAA (pH 6.2). We induced organogenic callus on leaf explants on MS based medium supplemented with 3 mg/l BAP and 0.2 mg/l IAA. In vitro leaves, petioles, nodes and internodes obtained from stock culture as well as calli were used to isolate and culture protoplasts according to the culture system described by Eeckhaut & Van Huylenbroeck (2011). Microcalli were transferred to proliferation medium solidified with 4 g/l gellan gum (pH 5.8); after 3-4 weeks microcalli had grown into calli. These calli were transferred to various SIMs (shoot induction media, MS based). After 3-4 weeks on SIM, calli were transferred to shoot outgrowth medium and periodically refreshed (Fig. 1).

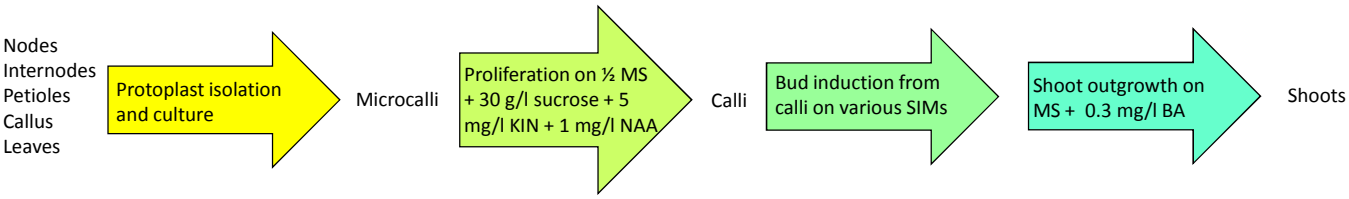

Figure 1. Schematic presentation of the steps involved in *Chrysanthemum indicum* 'Golden Surfer' protoplast regeneration.

RESULTS AND DISCUSSION – Petiole, node and internode protoplasts did not yield microcalli. In total we obtained 6781 calli derived from either mesophyll or callus protoplasts. Altogether, 5 calli (0.07%) started regenerating shoots up to 28 weeks after the initiation of the protoplast culture (Table 1, Fig. 2). All shoots regenerated from calli protoplasts in the dark. Only 2 SIMs were effective; both were supplemented with 0.02 mg/l NAA. High cytokinin concentrations (at least 5 mg/l) did not induce shoots. The plantlets were rooted and acclimatized to compare their phenotype and growth vigor to the one of regular 'Golden Surfer' in vivo plants. In following experiments, we will test the protocol on other genotypes; if protoplasts from multiple cultivars can be regenerated, it will renew prospects for somatic hybridization in this recalcitrant crop.

CONCLUSION – *Chrysanthemum indicum* 'Golden Surfer' callus protoplasts can be regenerated. The full regeneration potential of chrysanthemum callus protoplasts is yet unknown.

Table 1. Calli and shoots initiated from *Chrysanthemum indicum* 'Golden Surfer' calli derived protoplasts on different shoot inducing media and after transfer to MS + 0.3 mg/l BA.

Phytohormones (mg/l)	# calli	# shoots
0.5 IAA	164	0
0.5 IAA + 0.5 BA	192	0
0.5 IAA + 5 BA	120	0
0.5 IAA + 5 KIN	1026	0
0.5 IAA + 10 KIN	334	0
0.5 IAA + 5 ZR*	1334	0
0.02 NAA + 0.5 BA	131	1
0.02 NAA + 5 BA	92	0
0.02 NAA + 5 KIN	656	0
0.02 NAA + 1 ZR	677	4
0.02 NAA + 5 ZR	770	0
0.2 NAA + 0.5 BA	118	0

* ZR: zeatin riboside

Figure 2. *Chrysanthemum indicum* 'Golden Surfer' protoplast regeneration: calli derived shoots after culture on MS + 0.02 mg/l NAA + 1 mg/l ZR (left, middle; bars = 1 cm) and acclimatized plants from 3 independently regenerated protoplasts (right; bar = 7 cm)

Literature cited – Eeckhaut T, Van Huylenbroeck J (2011). Development of an optimal culture system for callogenesis of *Chrysanthemum indicum* protoplasts. Acta Phys Plant 33: 1547-1551.

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