Interspecific somatic hybridization between *Gentiana cruciata* L. and *G. tibetica* King with application of electrofusion

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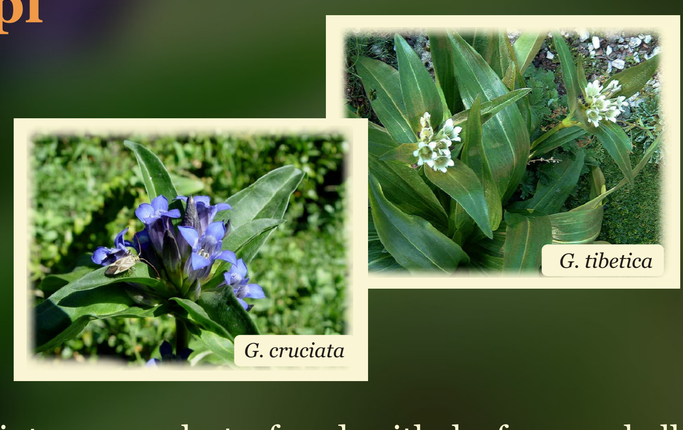
INTRODUCTION

For today no somatic hybrids have been obtained within *Gentianaceae* family. Some experiments were only carried out to optimalize conditions for electrofusion of *Eustoma grandiflorum* protoplasts with those of *G. lutea* and *G. scabra* (Takahata et al. 1995). The aim of our work was to produce interspecific somatic hybrids between *G. cruciata* and *G. tibetica* by electrofusion of protoplasts.



MATERIAL AND METHODS

Protoplasts from embryogenic cell suspension of *G. cruciata* were electrofused with leaf mesophyll protoplasts of *G. tibetica* **(Fig. 1)**. For fusion treatment two DC pulses of 1170 V/cm (30 µs) followed an AC pulse of 1 MHz and 60 V/cm (2 s). Obtained heterokaryons were cultured with unfused protoplasts on agarose modified MS medium supplemented with 0.5 mg/l 2,4-D and 1 mg/l KIN or 2 mg/l NAA and 0.1 mg/l TDZ. For microcalli proliferation agar-solidified MS media with the same combinations of plant growth regulators were applied. Somatic embryogenesis was induced after placing of calli on different plant regeneration media **(Tab. 1)**. Obtained plantlets were transferred to ½ MS medium for further development.

Flow cytometry analysis. Determination of the nuclear DNA content of parental and 16 regenerated plants as well as estimation of ploidy level of 20 calli were carried out. *Petunia hybrida* cv PxPc6 (2C = 2.85 pg DNA) served as an internal standard.

AFLP. Leaves of both parental species, cell suspension of *G. cruciata* and calli and leaves of regenerants obtained after fusion were used to extract total DNA. AFLP analysis were performed according to Vos et al. (1995). DNA was digested with *Eco*RI and *Mse*I. 3 primer combinations were used for selective PCR . Products of amplification were analysed on polyacrylamide gel and visualised by autoradiography.

Tab. 1. Composition of media used for plant regeneration

Medium Symbol	Compounds
PRM1	MS + 0.1 mg/l NAA + 5.0 mg/l TDZ
PRM2	MS + 0.1 mg/l NAA + 5.0 mg/l BAP
PRM3	MS + 1.0 mg/l KIN + 0.5 mg/l GA_3 + 80 mg/l SA

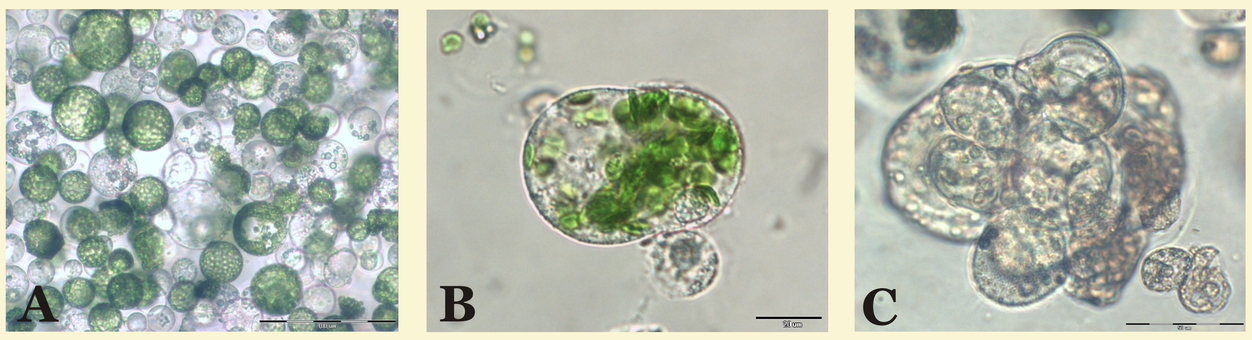


Fig. 1. Protoplast fusion and culture: mixture of cell-suspension protoplasts of *G. cruciata* with leaf mesophyll protoplasts of *G. tibetica* **(A)**, a single heterokaryon **(B)**, heterokaryon-derived cell cluster after 2 weeks of culture **(C)**

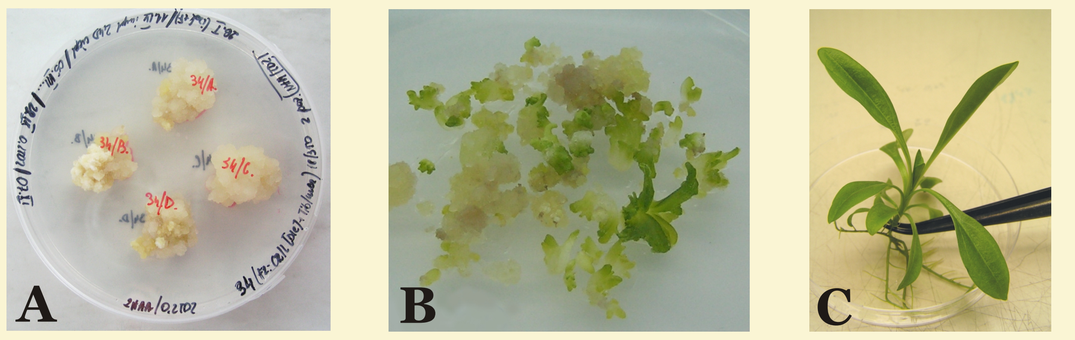


Fig. 2. Callus culture and plant regeneration: callus lines obtained after protoplast fusion **(A)**, somatic embryogenesis on callus tissue **(B)**, regenerated somatic hybrid plant **(C)**

RESULTS

134 callus lines were obtained from 10 independent fusion experiments **(Fig. 2A)**. Among them only one showed embryogenic potential **(Fig. 2B)** on PRM3 medium giving rise to plant regeneration. Finally 24 plants were obtained **(Fig. 2C)**. No morphological disturbances among them were observed, all regenerants easily developed root systems. Flow cytometry analysis revealed that all of them had a 12.41 ± 0.15 pg DNA, which is a little less than the sum of the parental DNA contents (7.89 ± 0.06 pg DNA for *G. cruciata* and 6.81 ± 0.06 pg for *G. tibetica*) **(Fig. 3A)**. Most of tested calli were mixoploids **(Fig. 3B)**. AFLP analysis showed that all regenerants and 5 calli tissues possesed specific bands from both parents which confirmed their hybrid nature **(Fig. 4)**.

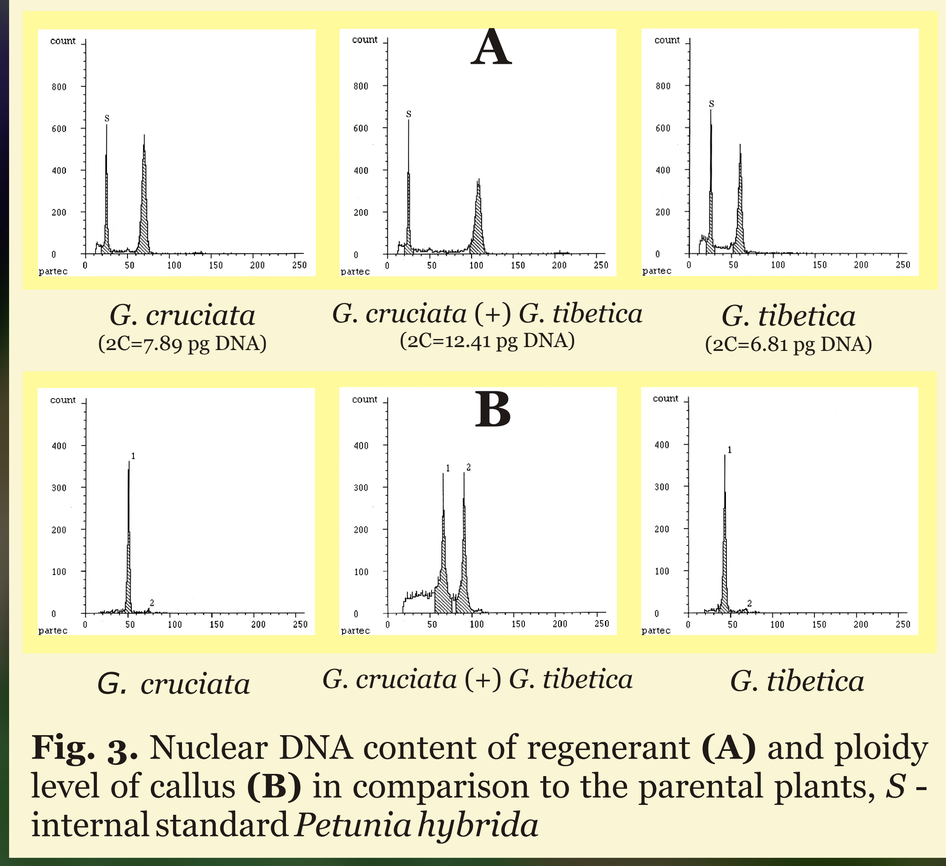


Fig. 3. Nuclear DNA content of regenerant **(A)** and ploidy level of callus **(B)** in comparison to the parental plants, *S* - internal standard *Petunia hybrida*

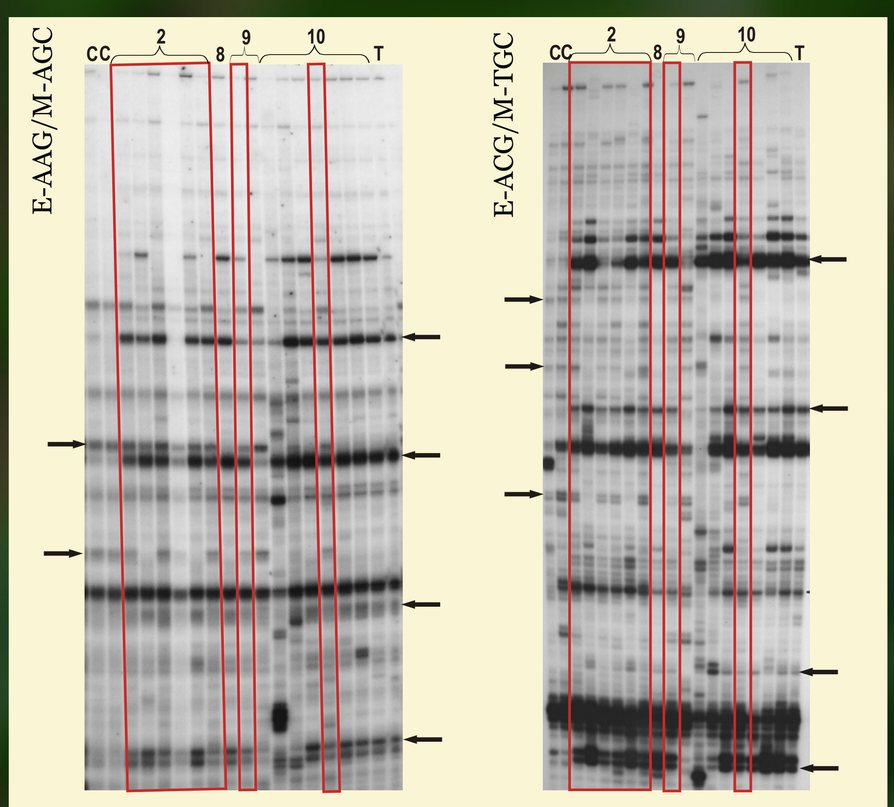


Fig. 4. AFLP profiles of calii and plants obtained after fusion and their parental genotypes: *C* - *G. cruciata*, *T* - *G. tibetica*, 2-10 - numbers of individual fusions, *black arrows* indicate polymorphic bands, in *red frames* - lines of true somatic hybrids

CONCLUSIONS

1. *G. cruciata* (+) *G. tibetica* somatic hybrid tisssues and plants were produced after protoplast fusion, their hybrid nature was confirmed by AFLP and FCA.
2. Nuclear DNA content of obtained regenerants indicates that chromosomes elimination probably occurred, which reqiures to be confirmed by chromosome counting.
3. Acclimatization and further analysis of regenerated somatic hybrids are planned.

REFERENCES

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2. Vos P., Hogers R., Bleeker M., Rijans M., Van de Lee T., Hormes M., Frijters A., Pot J., Peleman J. Kuiper M., Zabeau M., 1995. AFLP: a new techniqe for DNA fingerprinting. Nucleic Acids Res., 23: 4407-4414