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# Problems and possibilities of wheat-maize somatic hybridization

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**ABSTRACT** Protoplast fusion was performed between mesophyll cells of wheat (*Triticum aestivum L.*) and albino maize (*Zea mays L.*) by polyethylene glycol (PEG) treatments. The parental protoplasts were not able to produce green plants without fusion. The hybrid calli have mixed cytoplasm and regenerated green, maize-like plants, six months after the PEG treatment. The plants were sterile although had both female and male flowers. The RAPD analysis (using total DNA, and three primer combinations) produced bands resembling the wheat profile. The cytological analysis revealed 56 chromosomes in the root tip and callus cells, but intact wheat chromosome was not observed. Genomic in situ hybridization using total wheat DNA as probe revealed the presence of wheat DNA droplets in the maize chromosomal background. The flow cytometrical analysis showed intermediate DNA concentration in hybrid nucleus. Other intermediate morphological traits of plants with hybrid origin were not found.

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**KEY WORDS**

protoplast fusion
somatic hybrid
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flow cytometry

Hybridization of somatic cells has been successfully used to combine genes a wide range of sexually incompatible species or genera. The protoplast fusion has also been applied for improvement of cultivated plant species. Genes were transferred by somatic cell fusion against bacterial, fungal, virus deseases, or even nematodes and abiotic stress tolerance, like drought and cold tolerance.

Although several hundreds of hybrids and cybrids have been produced from many different species, only few data have been published on the maize somatic hybridization (Kao and Michayluk 1974; Brar et al. 1980). One possible reason for limited successes may be resulted from difficulties in maintenance of proper maize protoplast culture systems (Prioli and Schöndall 1989; Shilito et al. 1989; Mórocz et al. 1990). At the same time protoplast fusion has a great importance in special DNA transfer and genomic projects.

Therefore here we attempted to produce maize and wheat somatic hybrids. The described molecular, cytological and flow cytometric data support a conclusion that considerable amount of nuclear DNA is present from the donor wheat genome in the chromosomes of selected hybrid plants.

## Materials and Methods

### Plant materials

The wheat parent was grown in sterile conditions from seeds of GK Öthalom variety (Cereal Research Non-Profit Co. Szeged, Hungary). Maize protoplasts were isolated from suspension culture of H1160 albino maize cell line, derived from anther culture of H229 x C2-A-18 maize hybrid (Mórocz 1991)

### Protoplast isolation

Isolation of maize protoplasts was carried out according to Mórocz et al. (1990), except that we used split incubation: 14 h at +4ºC without shaking, followed by a 2 h treatment at room temperature with gentle shaking (15 rpm).

Wheat protoplasts were isolated from leave mesophyll cells (Szarka et al. 2002)

### Protoplast fusion and culture

Isolated maize and wheat protoplasts were mixed at 2:1 ratio and suspended in UM solution (Uchimaya and Murashige 1974). A dense suspension (1.5 x $10^6$/400µl UM) of protoplasts was incubated for 20 min in a single droplet on a vibration-free place. One microliter of PEG solution was added (40 w/v% of 3500 MW Sigma PEG dissolved in sol D according to Kao and Michayluk 1974). Elution of the PEG solution started after the first fused cells appeared. The protoplasts were cultured in liquid ppN6M/89 (Mórocz et al. 1990) at room temperature in the dark. The developing calli were transferred onto hormone-free N6M regeneration medium. The differentiated well-rooted green plants were transferred into soil. Plants developing ears or tassels were self- and also cross-pollinated with other maize varieties.

### Identification of the hybrids

The chromosome number of the putative hybrid calli and plants revealed by their green color was determined by the Feulgen staining method.

The total DNA extracted with the CTAB procedure (Bousquet et al. 1990) for RAPD analysis (Szarka et al. 2002).

The albino maize chromosome plates were prepared for in situ hybridization from protoplasts (Mórocz et al. 1990), and stained with acetocarmine. In the case of wheat and the hybrid plants we used a squash preparation (Molnár-Láng et al. 2000). The GISH analysis was performed according to Reader et al. (1982) with minor modifications (Szarka et al.

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The nucleus isolation was carried out with chopping in 1 µl ice-cold LB01 buffer (Dolezol et al. 1989). After separation with 30 µm nylon filter, 40 µg/ml ethidium bromide and 50 µg/ml RNase was added. The fluorescence-intensity of the stained nuclei was measured with Becton and Dickinson Facs Calibur Flow Cytometer and analyzed with CellQuest software.

## Results and Discussion

The percentage of fused cells reached maximum of 20% in the most successful experiment, but the viable hybrid cells decreased below 2% before reaching the first division during the 1st week of culture. The hybrid cells started to divide usually on the 10th day, 5-7 days later than intact maize protoplasts. Seven embryogenic calli with green spots were selected as putative hybrids and one callus-clone yielded green plants. The recovered plants exhibited a maize morphology. The cytological analysis of the hybrid plant carried 56 chromosomes. The failure of recognition of intact wheat chromosomes emphasized the need for the additional molecular tools to uncover the origin of selected genotype. The DNA analysis was based on RAPD experiment. Three primer combinations produced bands characteristic for both parents. The DNA content measurement was shown intermediate amount of DNA in hybrid nucleus by flow cytometry. The existence of wheat DNA in the maize background was visibly shown by in situ hybridization. For these experiments we used total wheat genomic DNA labelled with fluorochrome. Considering the high number of signals distributed to several chromosomes we can predict an extensive rearrangement between the parental genomic DNAs. In interpretation of origin of genomic constitution we can rely on the results of early studies on fusion between mitotic and interphase plant protoplasts (Szabados and Dudits 1980). The cytological pictures showed formation chromatin droplets that can be incorporated into the nuclear DNA of the hybrid cells during the subsequent division cycles.

Despite of the fact that unique, unknown molecular and cellular events produced the described new genotype with maize and wheat DNA, the regenerated plants exhibit several potentials for applications in functional genomic and stress research. Further studies are in progress to search for expression of wheat specific genes or characters.

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