RAPD ANALYSIS OF SUNFLOWER SOMATIC HYBRID CALLI

Dragana Vasic, Institute of Field and Vegetable Crops
M. Gorkog 30, 21000 Novi Sad, Yugoslavia
Fax: +381 21 413833; e-mail: vasicd@ifvcns.ns.ac.yu

Gilbert Alibert, Laboratoire de Biotechnologie et d'Amelioration des Plantes,
INP-ENSAT/UA INRA Avenue de l'Agrobiopole, BP 107, Auzeville-Tolosane, 31326
Castanet Tolosan Cedex, France, Fax: +33 562 193518;

Andre Berville, Station d'Amelioration des Plantes, INRA-ENSAM-DAGP
2 Place Viala, 34060 Montpellier Cedex, France Fax: +33 467045415;

Dragan Skoric, Institute of Field and Vegetable Crops
M. Gorkog 30, 21000 Novi Sad, Yugoslavia Fax: +381 21 413833;

SUMMARY

Hypocotyl protoplasts of cultivated sunflower were electrofused with mesophyll protoplasts of *Helianthus maximiliani*. Fusion products were cultured according to the protocol of Trabace et al. (1995) and the calli were regenerated. In order to verify their nature, PCR analysis using ten primers unique for perennial species of genus *Helianthus* (Sossey-Alaoui et al. 1998) of regenerated calli was done. DNA extraction was done according to the protocol of Gentzbittel et al. (1994). Purified DNA was quantified on 1% agarose gel with λDNA as the reference and was adjusted to 6 ng μl^{-1} for PCR amplification. PCR amplification was performed in a 25 μl volume as described by Sossey-Alaoui et al. (1998). RAPD products were analysed by electrophoresis in 1.6% agarose gel. The presence of bands characteristic for *H. maximiliani* was detected in 75% of analysed calli.

INTRODUCTION

Somatic hybrids could be identified on the basis of morphological characteristics, by determination of number of chromosomes, as well as isoenzymatic analysis.

The development of techniques of molecular biology led to their increased utilisation for identification of somatic hybrids and determination of degree of elimination of chromosomes of donor parent in asymmetric somatic hybrids. RFLP, RAPD, Southern and dot-blot analyses are most frequently used for that purpose.

The advantage of RAPD analysis compared to other techniques is its relative simplicity that enables analysis of the great number of plants from different hybrid combinations.

In this paper, the utilisation of RAPD analysis for determination of nature of calli regenerated after asymmetric somatic hybridisation between sunflower (*Helianthus annuus* L.) inbred line CMS_1-50A and assession 1631 of *Helianthus maximiliani* is described.

MATERIAL AND METHODS

Hypocotyl protoplasts of cultivated sunflower were electrofused with mesophyll protoplasts of *Helianthus maximiliani*. Protoplasts of *Helianthus maximiliani* were irradiated with UV light prior to fusion. Fusion products were cultured in agarose droplets according to the protocol of Trabace et al. (1995).

PCR analysis of regenerated calli was done using ten primers unique for perennial species of genus *Helianthus* (Sossey-Alaoui et al. 1998). DNA was extracted from 16 calli, according to the protocol of Gentzbittel et al. (1994). Purified DNA was quantified on 1% agarose gel with λDNA as the reference and was adjusted to 6 ng μl^{-1} for PCR amplification. PCR amplification was performed in a 25 μl volume as described by Sossey-Alaoui et al. (1998).

RAPD products were analysed by electrophoresis in 1.6% agarose gel. Presence or absence of RAPD fragments characteristic for *Helianthus maximiliani* was determined.

RESULTS AND DISCUSSION

Presence of the bands characteristic for *Helianthus maximiliani* was detected in 75% of analysed calli on RAPD profiles generated with three out of ten used primers. Presence of the bands of *Helianthus maximiliani* was the most intensive on the profiles generated with the use of primer C-04 (Figure 1).

42 specific bands were observed on RAPD profile of *Helianthus maximiliani*. In hybrid calli their number ranged from one to four i.e. 90.5 to 97.4% of bands of *Helianthus maximiliani* was eliminated from the RAPD profiles of hybrid calli (Table 1). This is similar to the results obtained by Rasmussen et al. (1997) in analysis of somatic hybrids between *S. tuberosum* and *S. spegazzinii* and between *S. tuberosum* and *S. microdontum* x *S. vernei* obtained via X-ray irradiation. Forsberg et al. (1998) observed a lower degree of asymmetry (5 to 51% eliminated markers of donor parent) in somatic hybrids between *B. napus* and *A. thaliana* obtained by X and UV irradiation. The degree of elimination of markers of donor parent was higher in hybrids obtained using UV irradiation.

REFERENCES

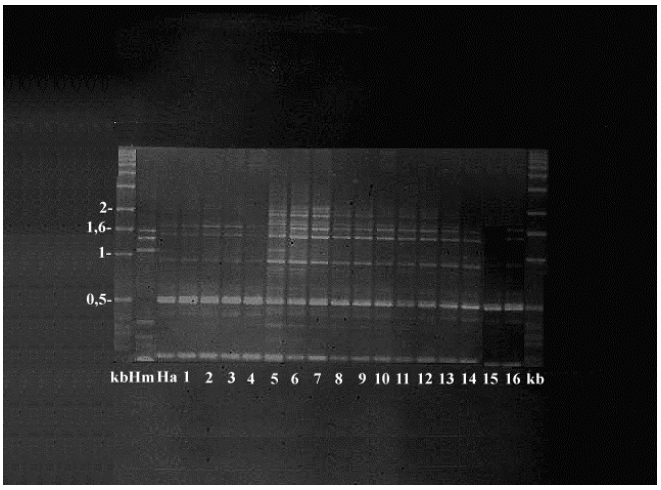

Figure 1. RAPD profile generated using primer C-04, kb – kb ladder, Hm – *H. maximiliani*, Ha – CMS_1-50A, 1-16 – calli.

Table 1. Presence of RAPD bands characteristic for donor parent in hybrid calli. Percentages are given in brackets.

Mark	**Number of bands specific for donor**	
	In donor	**In callus (%)**
5	42	4 (9,5)
6	42	4 (9,5)
7	42	3 (7,1)
8	42	2 (4,8)
9	42	2 (4,8)
10	42	2 (4,8)
11	42	2 (4,8)
12	42	1 (2,4)
13	42	2 (4,8)
14	42	1 (2,4)
15	42	2 (4,8)
16	42	3 (7,1)