Transformation of microspore – derived embroys to study the myrosinase – glucosinolate system in *Brassica napus* L.

Troczynska J[1]., Drozdowska L[1]., Cegielska-Taras T[2].,
[1]Department of Plant Physiology, University of Technology and Agriculture, Bernardynska 6, 85-029 Bydgoszcz, Poland; e-mail: drozd@atr.bydgoszcz.pl
[2]Plant Breeding and Acclimatization Institute, Department of Oil Crops, Strzeszynska 36, 60-479 Poznan, Poland; e-mail: tceg@nico.ihar.poznan.pl

ABSTRACT

The myrosinase-glucosinolates system consists of one of the defense mechanism components in *Brassicaceae*. To study the function of this system, the transformation of oilseed rape (*Brasica napus* L.) via *Agrobacterium tumefaciens* was performed with the myrosinase genes TGG1 and TGG2 from *Arabidopsis thaliana*. Genomic clones of the myrosinase genes, TGG1 and TGG2, were placed downstream of an enhanced 35S promoter to generate plant expression cassettes. The myrosinase expression cassettes were subcloned separately into a binary vector containing the selectable marker gene NPTII. Microspore–derived embryos of homozygous winter oilseed rape lines (low glucosinolates line DH-O120 and high glucosinolates lines DH-GR64) were used for transformation. The shoots developed from the embryos after transformation that remained green on selectable medium were considered to be transgenic and were transferred to a root induction medium and then planted in the soil for further development. Transformation efficiency will be determined based on the presence of NPTII gene in all R_0 androgenic plants. The effect of myrosinase overexpression on the plant phenotype in R_1 transgenic lines will be discussed.

Key words: myrosinase, glucosinolates, transformation, microspore-derived embryo, *Brassica napus*

INTRODUCTION

The myrosinase–glucosinolate system have several functions in the *Brassicaceae.* In this system, hydrolysis of glucosinolates by myrosinase enzymes produces compounds with biological activities. Some of these products are involved in defense against insects, bacteria and fungi and potentially in sulphur and nitrogen metabolism as well as growth regulation (Rask et al. 2000, Bones and Rossiter 1996). Different myrosinase genes have shown tissue specific expression (Lenman et al. 1993). Over-expression of myrosinase genes in transgenic plants may be used to study the function of those genes. A fraction of lines generated from transformation with those genes may show cosupression of transgene and native myrosinase genes that will cause overall decrease in mysosinase activity. The changes in myrosinase activity may help elucidate the biological processes in which the myrosinase-glucosinolate system is involved.

This report describes preliminary results of transformation of microspore-derived embryos of winter oilseed rape *(Brasica napus*) with myrosinase genes TGG1B and TGG2B from *Arabidopsis thaliana*. Two varieties of winter oilseed rape with low or high glucosinolate content were transformed via *Agrobacterium tumefaciens*.

MATERIALS AND METHODS

Genomic clones of the myrosinase genes from *Arabidopsis thaliana*, TGG1B and TGG2B, were placed downstream of an enhanced 35S promoter to generate plant expression cassettes. The myrosinase expression cassettes were subcloned separately into a binary vector containing the selectable marker gene NPTII. Microspore-derived embryos from two winter oilseed rape homozygous lines that differ in glucosinolate content were produced according to procedure described by Cegielska-Taras et al. (2002). The double low line, DH-O120, has glucosinolate level about 5 μmol g^{-1} seed. The line DH-GR64 contains high level of glucosinolates in seed (115 μmol g^{-1} seed).

Cold treated microspore-derived embryos were used for transformation with *Agrobacterium tumefaciens*, containing the appropriate the binary plasmid pTGG1B or pTGG2B. After 3-4 days of co-culture with *Agrobacterium* embryos were transferred to the shoot induction medium (SIM) containing B5 salts and vitamins, 2% sucrose, 0.1mg/l GA_3, 500 mg/l carbenicillin, 20 mg/l kanamycin (Gamborg et al. 1968). The explants were transferred to a fresh SIM medium every two weeks. Shoots that remained green on this medium were considered to be transgenic and were placed in the root induction medium (RIM) that containing MS salts and vitamins, 1% sucrose, 10mg/l IBA, 500 mg/l carbenicillin, 20 mg/l kanamycin (Murashige, Skoog 1962). Microspore-derived embroys untreated with *Agrobacterium* and cultured on media without antibiotics were used as the control.

Ploidy level was assayed in transgenic plants regenerated from microspore-derived embroys by flow cytometry according to Śliwińska et al. (1999).

RESULTS AND DISCUSSION

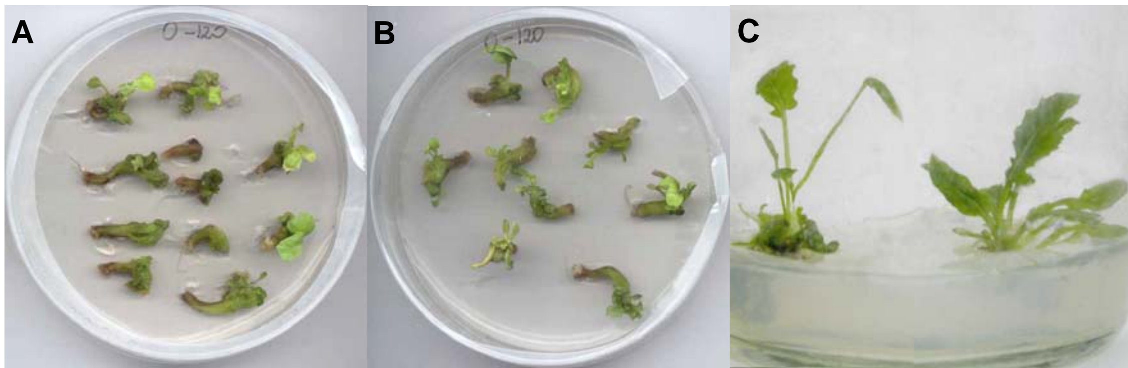
Cold treated microspore-derived embryos have a high regeneration potential; therefore they are suitable for efficient transformation (Cegielska-Taras et al. 2002). After about 7 days of culture, shoot regeneration was observed from the green embryos (Fig.1 A, B). In the subsequent passage explants were placed on the SIM medium with kanamycin where shoot elongation occurred on the green explants. The differences in number shoot formation between investigated lines of rapeseed were noticed (Tab.1). The embryos treated *Agrobacterium* were exhibited high regeneration ability. After 5-6 weeks of growth, the regenerated shoots were transferred onto the rooting medium (Fig.1C). The putative transgenic shoots developed slower (1-2 weeks) than the controls. After 2 weeks on the rooting medium, the shoots developed their first roots. The rooted plants were transferred to the soil and the ploidy level was examined. The majority of plants were haploids but there also were ascertained diploids. All of the haploid plants were grown in greenhouse, and chromosome doubling was performed using the method of colchicine treatment of secondary auxiliary shoots. The detection of NPTII protein in the transformants by ELISA will be used to distinguish true transformants from wild type shoots escaping selection.

The transformation of microspore-derived embryos via *A. tumefaciens* is a good method to obtain of genetically modified rapessed. The most commonly used transformation procedure of rapeseed uses hypocotyl explants isolated from 5-8 day old seedlings (Bade and Damm, 1995). However, the transgenic plants need to be grown for two generations in order to select homozygous plants. The transformation of haploid microspore-derived embroys, can shorten the time needed to generate homozygous plants by one generation. The transfer of genes to haploid materials led to haploid transgenic plants production from which the homozygotic diploid transformants can be produced after chromosome doubling. The usefulness of microspore-derived embryos for transformation via *Agrobacterium tumefaciens* was confirmed by using a vector containing green fluorescent protein (GFP) marker gene (unpublished data).

Table 1. Transformation and regeneration efficiencies of oilseed rape microspore-derived embryos.

DH line	Number of explants			Shoot forming embryos(%)			Number of plants produced	
	Untreated (control)	Cocultured with Agrobacterium		Control (media without antibiotics)	Transformed (media with kanamycin)			
		pTGG1B[1]	pTGG2B[1]		pTGG1B[1]	pTGG2B[1]	pTGG1B[1]	pTGG2B[1]
O120	10	108	112	95	85.2	52.7	3	3
GR64	10	18	46	100	55.6	50.0	4	10

[1] TGG1B, TGG2B – myrosinase genes TGG1 and TGG2 from *Arabidopsis thaliana*



Rys.1. Development of shoots on microspore-derived embryos

A. Non-transformed embryos after 7-10 days of culture on MS medium.
B. Shoots regenerated on kanamycin containing MS medium after 7-10 days.
C. Putative transgenic plantlets on rooting medium (MS medium with IBA and kanamycin).

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