SELECTION FOR LOW GLUCOSINOLATE CONTENT OF RAPESEED, BRASSICA NAPUS L., USING HAPLOID EMBRYOIDS FROM MICROSPORE CULTURE.

M.C.M. Iqbal, C.Möllers

Institute of Agronomy and Plant Breeding,
Georg-August-University, von Siebold-Strasse 8,
D-3400 Göttingen, Germany.

INTRODUCTION

In vitro cultures of *Brassica napus* show varying levels of glucosinolates (GSL) as compared to the more stable contents in seeds and vegetative tissues. The total content of glucosinolates in *in vitro* cultures is very low or cannot be determined at all of *in vitro* grown materials. Hence, a screening of genotypes based on endogenous levels is of limited practical importance. However, the enzymes involved in the biosynthesis of glucosinolates are present in cell and microspore cultures. GrootWassink et al. (1987) have shown that *in vitro* cultures fed precursors in the biosynthetic pathway are able to synthesize the corresponding artificial glucosinolates. They further showed enzymes below the N-hydroxy-amino acid in the biosynthetic pathway are not specific for the side chain. Thus feeding synthetic precursors should lead to formation of artificial glucosinolates.

The precursor 2-nitrobenzaldoxime (NBA) has been described to be suitable for the synthesis of artificial GSL (GrootWassink 1987, 1988) and was found to retard growth in cell cultures. Presuming that high GSL genotypes have a higher activity of GSL synthesising enzymes compared to low GSL genotypes, the active uptake of NBA from the culture medium by microspore derived embryoids should be greater for the high GSL genotypes. This might lead to a stronger growth inhibition of these genotypes to be utilised for an *in vitro* selection for low GSL content. Such a method would enable the identification and selection of the desired genotypes at an early stage of development for selection. It would reduce the investment in time and material for further field trials and would also save costs.

In a first study the influence of the GSL precursor NBA on embryoids of a low and high GSL cultivar were investigated.

MATERIALS AND METHODS

The low GSL rape cultivar 'Duplo' and the high GSL cultivar 'Janetzski' of *B napus* were grown in the greenhouse at 20-25 °C/10 °C day/night temperatures and a 16 h photoperiod. Microspores were isolated and cultured as described by Mathias (1988). The treatments with the precursor NBA varied in concentration, duration of treatment, and size of embryoids. For continuous treatment, embryoids were cultured on a solid B5 medium (Gamborg et al. 1968) containing 5% sucrose, 1% agar and different concentrations of NBA. The cultures were maintained in an incubator

at 26 °C and a 16 h/day photoperiod. Embryoids were scored for survival (from 1:brown/dead to 10:green/vigorous) and dry matter was determined by oven-drying to constant weight.

The GSL content of the cultivars 'Duplo' and 'Janetzski' were 17.0 and 85.8 µmol/g (defatted flour).

RESULTS

Continuous treatment with the GSL precursor NBA showed a decline in growth for both cultivars (Table 1). A decrease in growth and survival of the embryoids is shown by the low GSL cv. 'Duplo' at 25 mg NBA/l, while the high GSL cv.'Janetzski' shows a decline at a lower concentration of 15 mg NBA/l.

Table 1. Effect of different concentrations of NBA on embryoids (2-3mm) from cv.'Duplo' and 'Janetzski' after 8 weeks.

Concentration	'Duplo'			'Janetzski'		
mg/l NBA	gi*	dm(g)	%	gi	dm(g)	%
0	8.78	0.737	93	8.82	0.329	83
15	8.90	0.374	73	4.82	0.112	16
20	7.60	0.303	66	4.27	0.082	25
25	7.20	0.190	66	3.55	0.061	8
30	3.16	0.064	6	3.12	0.028	8

*gi growth index from 1-10
dm(g) dry matter in gram
% % survival

The % survival gives the proportion of green embryoids capable of further growth. The remaining embryoids showed browning and necrosis at various stages of growth.

Although continuous treatment of the embryoids at concentrations of 35-45 mg NBA/l revealed differences between the two cultivars, the embryoids were injured beyond their ability to regenerate to plants, as shown by the low growth indices (Table 2). The cv. 'Janetzski' also shows a stronger decline in growth than the cv. 'Duplo'. Visually the embryoids of cv. 'Janetzski'were brown/shrivelled while those of cv. 'Duplo' were yellow.

Table 2. Effect of different concentrations of NBA on embryoids (greater than 5 mm) derived from the cv. ´Duplo´ and ´Janetzski´ after 4 weeks.

Concentration mg/l NBA	´Duplo´ gi*	dm(g)	´Janetzski´ gi	dm(g)
0	2.82	0.242	2.40	0.197
35	1.25	0.103	0.42	0.069
40	1.00	0.126	0.50	0.057
45	1.32	0.132	0.58	0.058

* gi growth index only for the leaves (1-4).

dm (g) dry matter in gram.

DISCUSSION

Aldoximes were shown to be growth inhibitory in tissue cultures by GrootWassink et al. (1987). The synthetic GSL precursor NBA exert differences in toxicity to haploid embryoids of high and low GSL cultivars in this experiment. The inhibition of growth is greater in the high GSL cv. ´Janetzski´ than in the low GSL cv. ´Duplo´. An optimal difference between the 2 cultivars is shown under continuous treatment on embryoids (2-3 mm) with 20-25 mg NBA/l. Higher concentrations, although showed differences in growth between the cultivars were toxic to both. In correlation to the high GSL content in a genotype the activity of the GSL biosynthetic enzyme system is expected to be higher. GrootWassink et al. (1990) reported the ability of tissues of Brassica spp. to metabolize NBA to 2-nitrophenylglucosinolate and other sulfated end products. Since the toxicity of NBA is also determined by the stage of the embryoid, the concentration and the duration of the treatment, further experiments are necessary to secure the possibility of selection between GSL low and high genotypes by in vitro precursor feeding.

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