PLANTLET FORMATION FROM ROOTS OF OILSEED RAPE CULTIVARS DIFFERING IN GLUCOSINOLATE CONTENT

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ABSTRACT. Buds were induced in culture of root segments excised from oilseed rape seedlings. Their formation was precided by callus and lateral roots formation. The induction of buds depended on seedlings age from which the segments were isolated /3, 6, 9 days/, combination of growth substances /KIN + IBA or BAP + NAA/ and genotype. Cultivar differences caused by lowering the glucosinolate content were manifested in the culture of root segments by somewhat lower bud formation capacity in Start cultivars than those in Skrzeszowicki. Also the plantlets developed from buds of the Start cultivars grew less vigorously.

#### INTRODUCTION

Buds can be formed on roots of some species spontaneously, in others by adding growth substances to the medium /Peterson 1975, Lazzeri and Dunwell 1984, Espinoza and Dodds 1985, Lillo and Shahin 1986/. The present report is the continuation of our previous studies on morphogenesis of oilseed rape roots /Drozdowska and Rogozińska 1984, Rogozińska et al. 1987/ and relates to bud induction in root segments of two cultivars which differ in glucosinolate content.

# MATERIAL AND METHODS

One cm root segments /2 mm below the hypocotyl/ were isolated from aseptically grown 3, 6 and 9 days old oilse-ed rape seedlings and placed on liquid or gelled Murashige-Skoog /MS/ medium with vitamins B5 /Gamborg et al. 1968/

the liquid cultures were placed for 24h on a shaker. The gelled cultures have been grown continuously in stationary conditions. All the cultures were incubated at 25°C±2 for 6 weeks under a 12h photoperiod. The results presented are arithmetic means from 30 root segments.

#### RESULTS

After ca two weeks of root culture callus tissue appeared on the segments. Its formation was stimulated by higher concs. of growth substances, particularly by the combination of BAP + NAA /Fig. 18/.

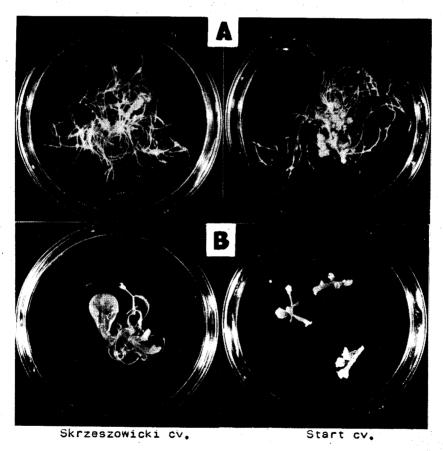


Fig. 1. Callus, lateral roots and buds formation on root segments of Skrzeszowicki and Start cultivars grown on MS medium /A/ with KIN + IBA and /B/ with BAP + NAA

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Simultaneously with callus formation laterals were developed on some of the root segments. The combination of KIN + IBA favoured more numerous than that of BAP + NAA lateral roots production /Fig. 1A, 5/. Comparing the effect of liquid and gelled media it was shown that callus and root formation was more abundant on segments grown in the liquid media.

After ca 4 weeks when callus and roots were well developed, green nodules appeared on some of the segments from which plantlets were formed. The root segments showed marked polarity and bud production occured most frequently at the end of the segment adjacent to the hypocotyl. Sud formation depended also on seedling age, liquid or gelled media and was higher in Skrzeszowicki cultivars /to 26%/than that of Start /to 18%/ /Table/.

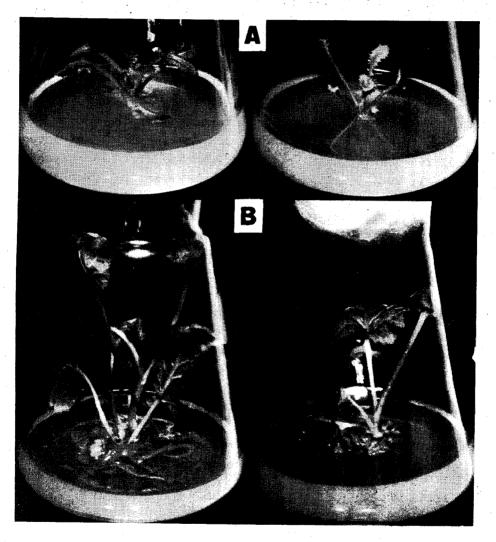
Table

Effect of seedling age and growth substances on oud formation from oilseed rape root segments of Skrzeszowicki and Start cultivars

Growth substan- ces concs. /μΜ/	Seed- lings age /days/	% of segments with plantlets			
		Skrzeszowicki cv.		Start cv.	
		liquid medium	gelled medium	liquid medium	gelled medium
2 KIN + 0.4 IBA	3	0	0	0	0
	6	6.	3	3	٥
	9	0 -	G	0	0
2 BAP + 0.4 NAA	3	0	0	0	0
	6.	12	8	6	4
	9	12	26	8	18

After 6 weeks of culture the plantlets were transferred on fresh medium with higher concs. of BAP /10 µM/ and NAA /5 µM/ where they continued their growth for 4 weeks. Next they were placed on the rooting medium with 0.1 µM IBA. After two to three weeks of growth they were transplanted into the pots with sterilised soil and placed in greenhouse

conditions. The plantlets of Skrzeszowicki cultivar were of darker green colour than that of Start. They were characterized also by faster growth in the observed culture period in comparison to the plantlets of Start cultivar /Fig. 2A; B/. Further observations are beeing continued.



Skrzeszowicki cv.

Start cv.

Fig. 2. Plantlets developed from root segments of Skrzeszowicki and Start cultivars after 4 weeks of growth on MS medium: A. /10  $\mu$ M BAP + 5  $\mu$ M NAA/ and B. further growth on rooting MS medium /0.1  $\mu$ M IBA/

## DISCUSSION

The organogenesis of roots depends both on endo- as well as on exogenous factors. Such factors as rape cultivar, seedling age, type of medium, kind and concentrations of growth substances cooperate in this process /Lazzeri and Dunwell 1984/. Greater regenerative ability of older root segments tested may be caused as suggested by Lazzeri and Dunwell /1984/ and Projetti and Chriqui /1986/ by increased pericycle activity from which both laterals and adventitious buds develop.

The comparison of organogenesis from root segments of seven Brassica cultivars revealed interspecific and intercultivar variation in regenerative capacity /Lazzeri and Dunwell 1984/. Similarly, the tested Start and Skrzeszowicki cultivars differed in the rate of caulogenesis and organogenesis in the initial period of culture.

Cytokinin usually stimulate bud formation in root segments though higher concs. were found to reduce this response /Lazzeri and Dunwell 1984/. Auxins are generally thought to inhibit bud formation /Peterson 1975/. As it was shown in our studies the combination of cytokinin and auxin were necessary for bud induction. Higher concentrations of cytokinin and auxin however were required than those reported by Lazzeri and Dunwell /1984/. The combination of BAP + NAA was found to be superior to that of KIN + IBA in the two cultivars tested.

Plantlets obtained by root organogenesis in Brassica napus may be useful in rape breeding to improve agronomic characteristics such as reduced glucosinolate content.

### LITERATURE

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