ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 56, nr 2: 315-324
1987

Factors affecting morphogenetic potential in oilseed rape roots of the Skrzeszowicki and Start cultivars

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(Received: September 18, 1986. Accepted: October 23, 1986)

Abstract

The effect of the origin of root segments, seedling age, growth substances and gelled or liquid media were tested in respect to the morphogenetic potential of rape root segments of Skrzeszowicki (high glucosinolate content) and Start (low glucosinolate content) cultivars. Callus and roots were formed on all root segments after an approximately 2 week growth period; buds were formed after ca. 4 weeks only on segments adjacent to the hypocotyl. Higher concentrations of auxin and cytokinins were required for bud induction. Cultivar differences in the morphogenetic responses of the root segments were found. They were manifested by the more abundant callus formation (BAP+NAA) and more numerous lateral roots and buds (KIN+IBA) on segments from the Skrzeszowicki cultivar than from the Start cultivar.

Key words: Brassica napus Skrzeszowicki and Start cultivars, root culture, regeneration

INTRODUCTION

In vitro root cultures have been initiated from many plants. Continuous cultures were obtained from approximately thirty species (Butcher 1980). Many of these species exhibit regenerative potentials such as lateral roots formation, callus and, sometimes, buds. The spontaneous buds formation on roots has been obtained in several cases, mainly in species which form them in vivo. In other species, buds can sometimes be induced by adding growth substances to the media (Peterson 1975, Chaturvedi and Sinha

1979). In detailed studies on the regeneration of buds from root segments of several *Brassica* cultivars, Lazzeri and Dunwell (1984a, b) determined the effect of endo- and exogenous factors, obtaining regeneration of plants belonging to *Brassica oleracea* and *B. napus* cultivars.

This study is a continuation of previous ones (Drozdowska and Rogozińska 1984a, b) and deals with the comparison of the morphogenetic potentials of oilseed rape roots of two cultivars differing in glucosinolate content.

MATERIALS AND METHODS

Winter oilseed rape (*Brassica napus* var. *oleifera* cv. Skrzeszowicki — a high-glucosinolate variety and cv. Start — a low-glucosinolate variety) seeds were surface sterilized with mercuric chloride and rinsed three times with sterile water. Next, they were placed on Murashige and Skoog (MS) mineral

Table 1

Effect of seedling age, medium type and growth substances on bud formation from oilseed rape root segments S_1 cv. Skrzeszowicki and Start

Concentration of growth regulators (μM)	Seedling age (days)	% Segments with shoot buds			
		cv. Skrzeszowicki		cv. Start	
		gelled medium	liquid medium	gelled medium	liquid medium
1 KIN+0.2 IBA	4	6.7	0	0	6.7
	6	6.7	0	0	6.7
	8	0	0	0	0
	10	6.7	6.7	0	6.7
2 KIN+0.4 IBA	4	0	6.7	0	0
	6	0	0	0	0
	8	0	0	0	0
	10	0	20.0	0	6.7
1 BAP+0.2 NAA	4	0	0	0	6.7
	6	6.7	0	0	0
	8	0	0	0	0
	10	0	6.7	0	0
2 BAP+0.4 NAA	4	0	6.7	6.7	0
	6	0	6.7	6.7	0
	8	0	0	0	0
	10	0	13.3	0	0

The effect of seedling age (LSD = 0.25), medium type (LSD = 0.12), cultivar (LSD = 0.25) and growth substances (LSD = 0.32) was significant.

medium, diluted two-fold and gelled with agar. Roots were isolated from 4, 6, 8 and 10 day-old seedlings. One centimeter long segments were taken from the roots and labeled S_1 for the segment adjacent to the hypocotyl, S_2 — for the middle segment and S_3 — for the apical segment. Segment S_1 was taken 2 mm below the hypocotyl, S_3 from the apical end after removing 2 mm of the apex and S_2 from the middle part of the roots between S_1 and S_3.

The segments were placed in Erlenmayer flasks containing 20 cm^3 of MS medium and vitamins as in the B5 medium (Gamborg et al. 1968). Each flask contained three, S_1, S_2 or S_3, segments. The medium was either gelled with agar (0.8%) or liquid, and the following combinations of growth substances were added to it: kinetin (KIN) with indolebutyric acid (IBA) or 6-benzylaminopurine (BAP) with 1-naphthaleneacetic acid (NAA) (Table 1). After inoculation, the liquid cultures of root segments were placed for 1 week on a laboratory shaker (type 358 S, 60 cycles per min), then left for 7 weeks under stationary conditions (25°C ± 2, 16 h photoperiod, light intensity ca. 1500 lx). After 8 weeks of growth, the presence of callus, the number of lateral roots and buds were determined. The plants developed from buds were transferred to agar MS medium containing 5 μM BAP + 10 μM NAA.

The presented results are the mean values calculated on the basis of at least 15 root segments. They were subjected to variance analysis and the LSD was calculated at 95% probability.

RESULTS

CALLUS FORMATION ON ROOT SEGMENTS

During the cultivation of oilseed rape roots callus appeared. It was formed after about 2 weeks mainly on the surface where the root segments had been cut and where the lateral roots had been cut off. At the end of the culture it covered the entire surface of the segment. The intensity with which the callus was formed depended on endogenous factors such as the origin of the root segment and rape variety and on exogenous factor, type of medium (Fig. 1). The age of the seedlings (4, 6, 8 and 10 days-old) from which the segments had been isolated was not found to be significant in the formation of callus. Its formation, however, was found to depend on the variety and occurred more numerously on segments from the Skrzeszowicki cultivar than on those from the Start cultivar. In the Start cultivar, however, callus formation was more intense. In this cultivar, callus was light green in color; in the Skrzeszowicki cultivar, it was brown.

The analysis of the effect of liquid or gelled medium on the formation

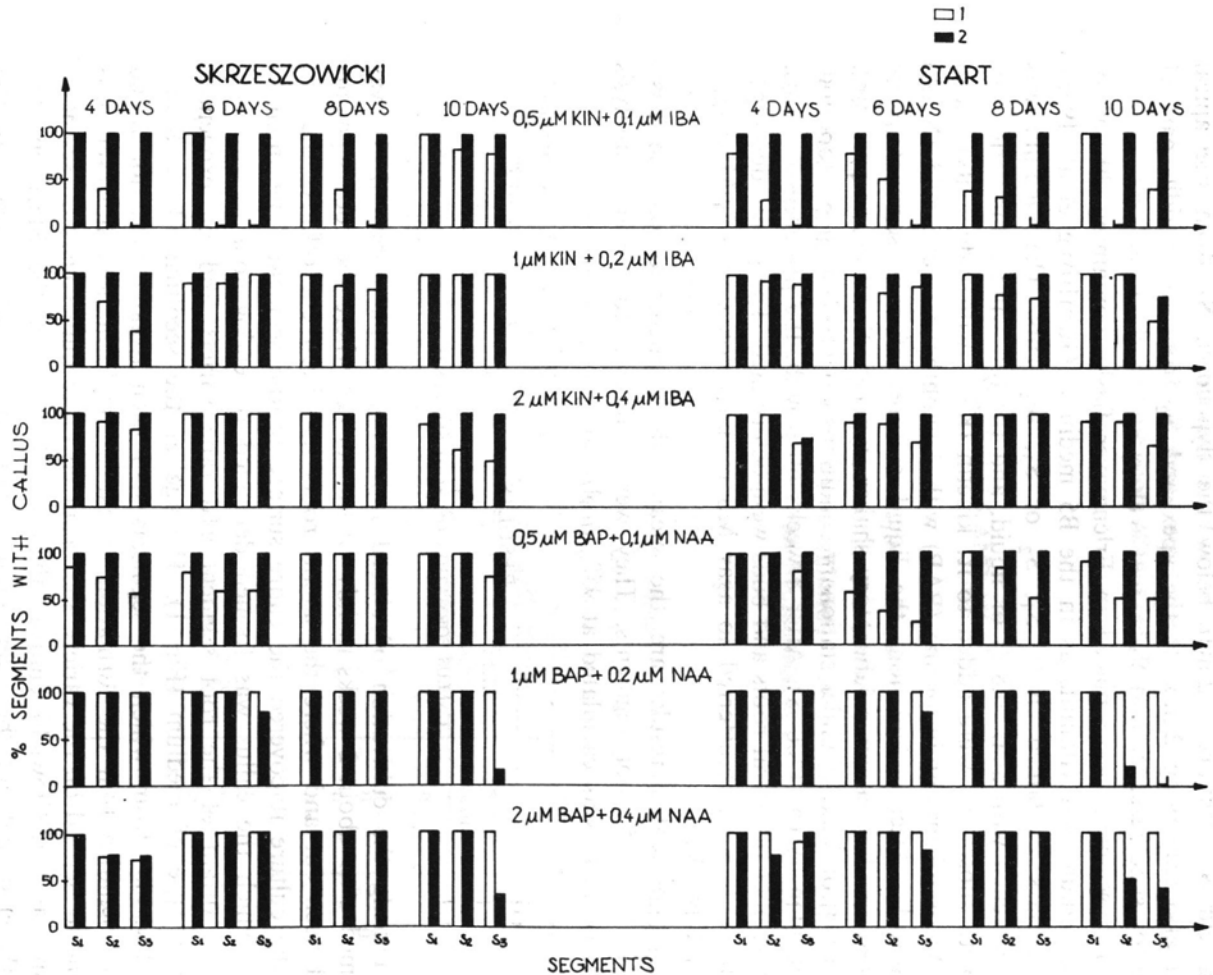

Fig. 1. Callus formation on oilseed rape root segments (cv. Skrzeszowicki and Start) depending on seedling age, segment origin, medium type and growth substances. (The effect of segment origin (LSD = 10.57), medium type (LSD = 7.16) and growth substances (LSD = 19.9) was significant)

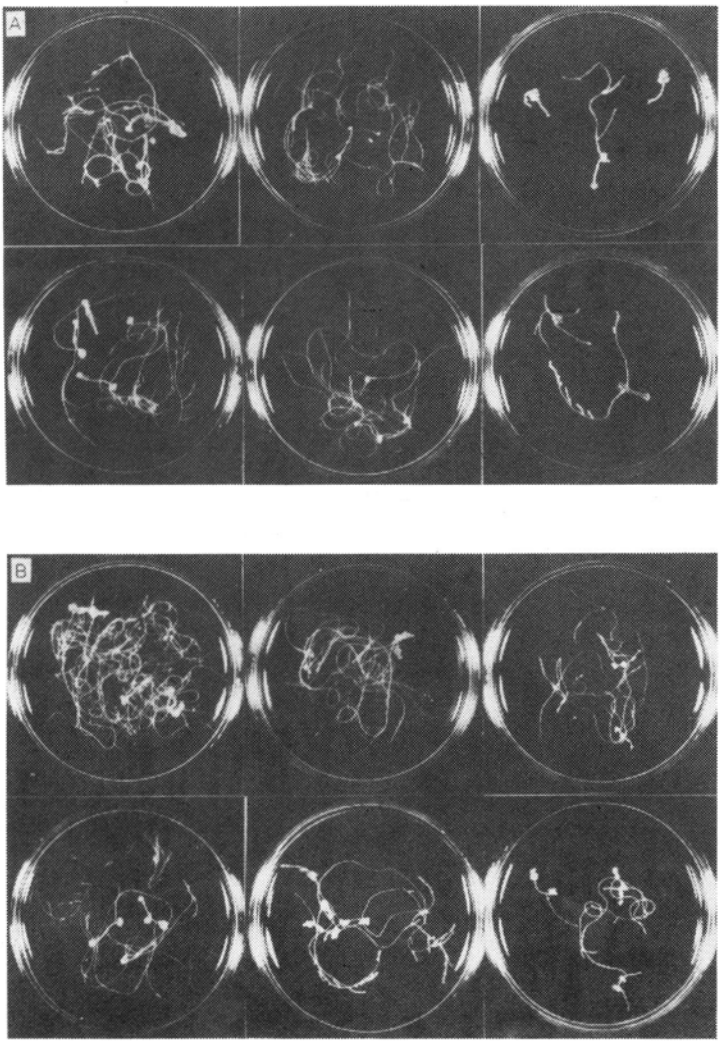

Fig. 2. Effect of KIN (0.5 μM) and IBA (0.1 μM) on lateral root and callus formation from root segments (S_1. S_2. S_3) derived from 4 day-old seedlings (A) and 10 day-old seedlings (B). (8 week culture on liquid medium)

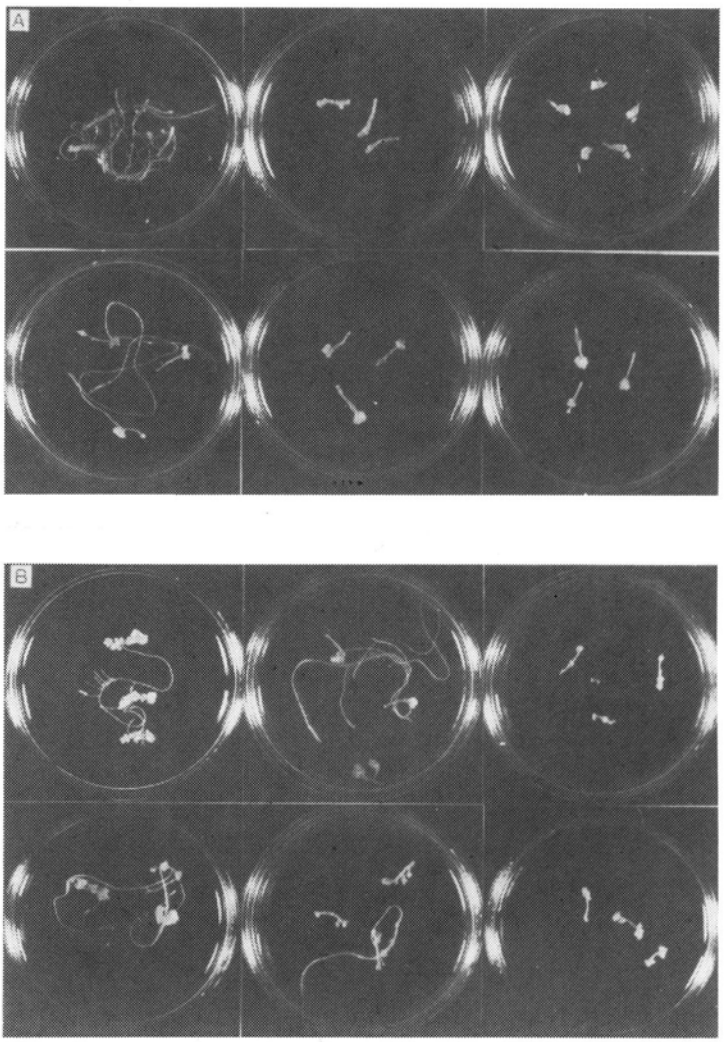

Fig. 3. Effect of BAP (0.5 μM) and NAA (0.1 μM) on lateral root and callus formation from root segments (S_1, S_2, S_3) derived from 4 day-old seedlings (A) and 10 day-old seedlings (B). (8 week culture on liquid medium)

of callus showed that it was formed more profusely and on a larger number of segments cultivated in liquid medium than on gelled medium. Growth regulators had a significant effect on callus formation. Its growth was stimulated by higher concentrations of growth regulators and the BAP+NAA combination was more effective than that of KIN+IBA. Callus was formed on the entire surface of the segments as well as on the lateral roots in the presence of all of the BAP+NAA combinations and higher KIN+IBA concentrations (Figs. 1-3).

LATERAL ROOT FORMATION

New lateral roots appeared on the root segments along with the callus during the first two weeks of culture. The lateral roots were more numerous on the S_1 segments, least numerous on S_3 (Fig. 4). In analysing the effect of the age of the seedlings, it was found that in both cultivars, the greatest number of lateral roots was formed on segments from 4 day-old seedlings. Numerous lateral roots also were formed on the segments from 10 day-old seedlings of the Skrzeszowicki cultivar. In general, the cultures of segments from this cultivar were characterized by more intensive growth (Fig. 4).

When the effects of the combinations of growth regulators on the formation of lateral roots were analysed, no greater effect of KIN+IBA was noted, while the combination BAP+NAA showed a clear inhibitory influence (Figs. 2-4). The number of lateral roots per segment and number of segments on which lateral roots were formed were greater in the agar cultures. However, on these media, the roots were shorter and less branched than on the liquid media.

BUD FORMATION

The formation of buds took place latter, after about 4 weeks of culture, and this property was possessed only by S_1 segments. Also the segments showed polarity and the buds were formed more abundantly on the part of the segment proximal to the hypocotyl. Bud formation was preceded by the appearance of green nodules.

The age of the seedling and type of medium played a part in the formation of buds (Table 1); the potential for bud formation was greater in the Skrzeszowicki cultivar (to 20%) than in that of Start (to 6.7%). Also, the developed plantlets of the Skrzeszowicki cultivar were characterized by more intensive growth in the initial weeks of culture (Fig. 5).

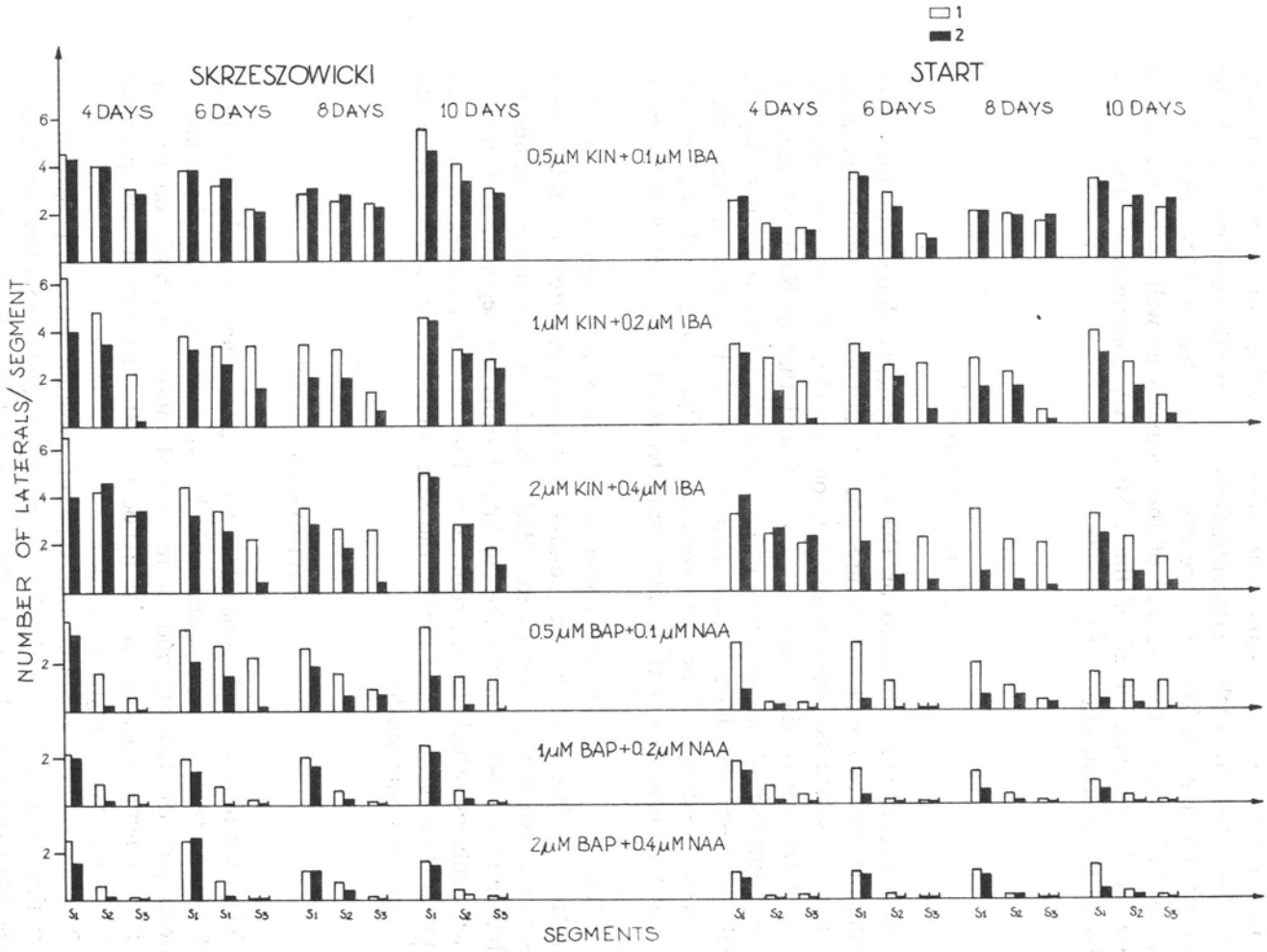

Fig. 4. Lateral root formation on oilseed rape root segments (cv. Skrzeszowicki and Start) depening on seedling age, segment origin, medium type and growth substances. (The effect of seedling age (LSD = 0.47), segment origin (LSD = 0.37), cultivar (LSD = 0.25), growth substances (LSD = 0.67) was significant)

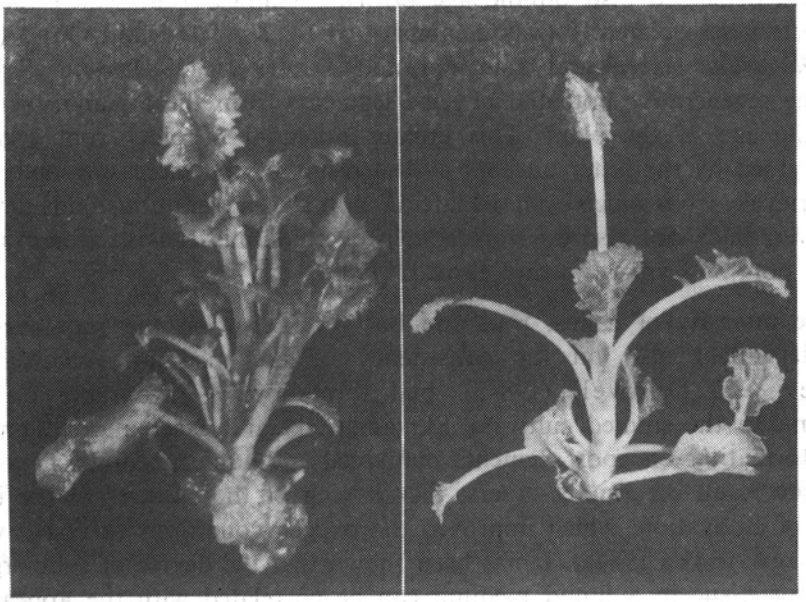

Fig. 5. Plantlets obtained from oilseed rape root segments S_1. (After 6 weeks of growth on MS medium with 5 μM BAP + 10 μM NAA)

Higher concentrations of growth regulators were necessary for the induction of buds. The greatest number of buds was formed on the medium containing 2 μM KIN + 0.4 μM IBA (20%), then on the medium with 2 μM BAP + 0.4 μM NAA (13%) (Table 1).

DISCUSSION

The morphogenetic potential of isolated roots is dependent on their genetic potential and the environment. In these studies it has been shown that all of the root segments have the ability to form callus and lateral roots, while only the segment next to the hypocotyl is able to form buds. The higher organogenetic potential of the segment next to the hypocotyl has been demonstrated in the roots of Brussels sprouts and other *Brassica* species and attributed to endogenous growth hormones (North 1953, Bonnet and Torrey 1965, Lazzeri and Dunwell 1984a). Similarly as in the study of Lazzeri and Dunwell (1984a), distinct polarity of roots in the formation of callus and buds in both rape cultivars tested has been found.

The number of lateral roots and buds in both studied oilseed rape

cultivars depended also on the age of the seedlings from which the root segments were isolated. It was demonstrated by Kefford and Caso (1972) on *Chondrilla juncea* and Lazzeri and Dunwell (1984a) on *Brassica*, that the regenerative potential of root segments from these plants increased with the age of the plant. This greater potential of older root segments is explained by the latter authors as the result of the increasing activity of the pericycle, from which both the lateral roots and adventitious buds develop. However, buds can also develop from root epidermis cells as, for example, in the potato (Espinoza and Dodds 1985).

The intensity of callus, root and but formation on the root segments was dependent also on the consistency of the medium. According to Lazzeri and Dunwell (1984a), bud formation is favourable on agar medium. In the studied cultivars, Skrzeszowicki and Start, more numerous buds formation on root segments cultivated on liquid medium may have been the result of, among others, the shaking of the cultures in the initial phase of cultivation, which improved the oxygen conditions (Drozdowska and Rogozińska 1984a). It has been shown that the degree of submergence of carrot tissue in a liquid medium can be correlated with the appearance of buds or roots (Kessel and Carr 1972). The physical state of the medium also affected the morphogenesis of *Solanum khasianum* root segments, where embryos were formed on a liquid medium, while buds on a gelled medium (Chaturvedi and Sinha 1979).

Phytohormones play a basic role in the regulation of growth and development and in the response of a plant to external conditions. Growth substances interfere in the very dynamic, endogenous hormonal balance and, in this way, influence numerous physiological processes. The studied factors interacted with growth regulators in the morphogenesis of oilseed rape roots. It has been shown that cytokinins can stimulate the formation of buds on the roots of some plant species (Kefford and Caso 1972, Bajaj and Nietsch 1975, Torrey 1976, Lazzeri and Dunwell 1984a). Auxins can also influence the formation of buds on roots and, depending on their concentration, either stimulate or inhibit this process (Peterson 1975). For the formation of buds on rape root segments, both auxins and cytokinins were required. In the oilseed rape cultivars studied by us, the necessary concentrations were somewhat higher than given by Lazzeri and Dunwell (1984a).

The potential to form buds on root segments depends on the cultivar. In the seven cultivars studied by Lazzeri and Dunwell (1984a), this ability ranged from 0-73.8%. In the studies done on two oilseed rape varieties differing in their glucosinolate content, this ability reached 6.7% in the Start cultivar and 20% in the Skrzeszowicki cultivar.

The differences between the two oilseed rape cultivars also manifested themselves in the somewhat reduced vigor of the les-glucosinolate cultivar. Cultures of isolated rape roots are a convenient simple system for the study of morphogenesis.

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Czynniki wpływające na zdolności morfogenetyczne korzeni rzepaku odmian Skrzeszowicki i Start

Streszczenie

Wykazano wpływ pochodzenia segmentu korzenia, wieku siewek, rodzaju pożywki i regulatorów wzrostu na zdolności morfogenetyczne korzeni rzepaku ozimego odmian Skrzeszowicki i Start. Kalus i korzenie przybyszowe tworzyły się po około 2 tygodniach wzrostu na wszystkich segmentach korzenia, a pączki po około 4 tygodniach tylko na segmencie przyległym do hypokotyla. Do indukcji pączków niezbędne były wyższe stężenia auksyny i cytokininy. Wykazano różnice między odmianami w reakcjach morfogenetycznych segmentów korzeni, objawiające się obfitszym tworzeniem kalusa (BAP+NAA) i liczniejszym tworzeniem korzeni bocznych i pączków (KIN+IBA) na segmentach odmiany Skrzeszowicki niż Start.