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Differentiation of shoot elements from the rachis of *Secale cereale* L.

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Abstract

In vitro culture of young *Secale cereale* spikes is described with special attention given to changes in development of the rachis. 7 mm explants were cultivated on a modified Murashige and Skoog (1962) medium (MS) supplemented with 2,4-D (dichlorophenoxyacetic acid), NAA (α-naphthaleneacetic acid), IAA (β-indoleacetic acid), 2,4,5-T (trichlorophenoxyacetic acid), and ZEA (zeatin), KIN (kinetin), BAP (6-benzylaminopurine), IPA (izopentenyladenine or 2 iP) in numerous combinations and concentrations. Rachises differentiated branches with node construction in the presence of synthetic auxin. Rhizogenesis of explants was stimulated by 2,4-D and 2,4,5-T. 2,4,5-T + IPA appeared to be the best combination for callus regeneration. Many meristematic centres were found previously and combinations of NAA + KIN stimulated direct, whereas 2,4-D + ZEA indirect, differentiation of shoot elements. Development was observed from plantlets to flowering plants.

INTRODUCTION

Investigations on morphogenetic abilities of cultivated plant cells and tissues of the *Graminae* family were hitherto carried out on explants taken at various developmental stages of plants.

Roberts and Street (1955) initiated studies on the morphogenetic abilities of rye. Carew and Schwartin (1958) cultured 15-20 day-old rye plant embryos, Sheridan (1973, 1974) 2 mm shoot fragments of 2-4 week-old rye seedlings from which he obtained callus tissue incapable of differentiating shoot elements.

Until now first stages of androgenesis have been observed in anther culture of rye (Stolarz, 1974, 1976; Orlikowska, 1977), however haploid plants were sporadically obtained (Malepszy, 1975;

Thomas, Wenzel, 1975; Wenzel, Thomas, 1974; Wenzel et al., 1975). Root and shoot differentiation from callus tissue has been observed in cultures isolated from fragment of mature plant embryos, and from immature diploid and tetraploid rye plant embryos (Rybczyński, 1978a, 1978b, 1979).

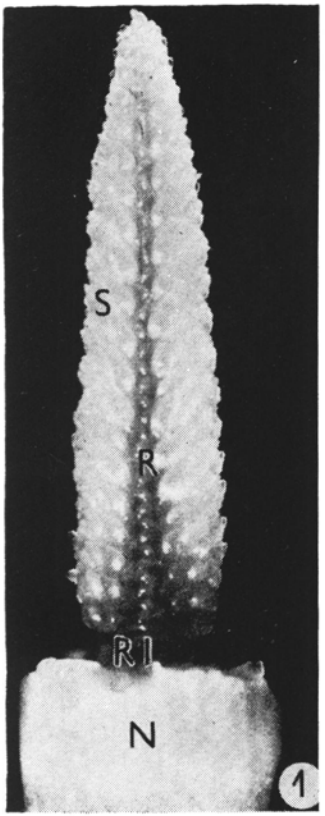
It has been noted from available literature that until now in *in vitro* cultures of rye and other members of the *Graminae* family, the process of differentiating shoot elements was preceded by the formation of callus tissue showing morphogenetic abilities.

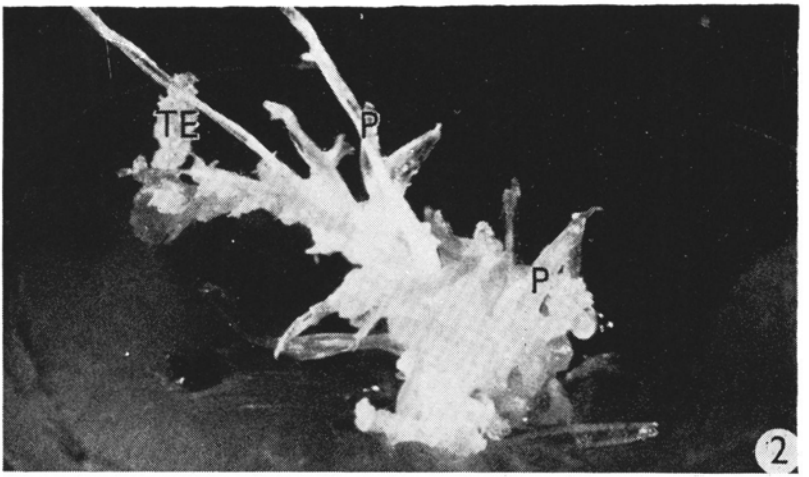
The purpose of the present study was to describe the morphogenetic processes accompanying the earlier presented problem (Rybczyński 1980) of direct differentiation of shoot elements from the rachis of rye under *in vitro* conditions.

MATERIAL AND METHODS

Young spikes of common diploid rye *Secale cereale* L. cv. 'Strzekęcińskie' were taken for these investigations. Shoots were cut out from several week-old rye plants tillering in green houses. The first three leaves were removed from the shoots, then the material was sterilized for 1 min. in 70% ethanol and for 10 min. in 5% calcium hypochlorite, and washed five times in sterile distilled water. After total defoliation of the shoots explants 7 mm in length were isolated by means of an ocular scalpel under a stereoscopic microscope. Fig. 1 shows the place of cutting explants from shoots.

The basal Murashige and Skoog (1962) medium was supplemented with vitamins, growth substances, yeast extract and sucrose. 1000.0 mg/l yeast extract and 0.25 mg/l calcium panthothenate were used for supplementing all of the nutrient media. In addition, nutrient media containing 0.5 mg/l GA_3, nicotinic acid, glycine-HCl, pyridoxine-HCl, thiamine-HCl, 100.0 mg/l myo-inositol and 5% sucrose were used for cultures of whole explants. Explant segments, on the other hand, were cultured in a nutrient media supplemented with 1.0 mg/l thiamine, 100 mg/l myo-inositol, and 1.5 and 3.0% sucrose. Auxines and cytokinins were used in the following combinations: IAA + BAP, 2,4-D + ZEA, NAA + KIN; 2,4,5-T + IPA. In the case of explant segment cultures also NAA + KIN was added to the nutrient medium, but without GA_3. A total of 20 combinations of nutrient media were applied (Table 1). Thirty explants were set out in each culture medium combination. All of the organic supplements were sterilized by Sartorius Membrane Filter 0.45 mm. All of the media were solidified with Difco Bacto agar at pH 5.6 or 6.0. Culturing was effected in Erlenmeyer flasks and





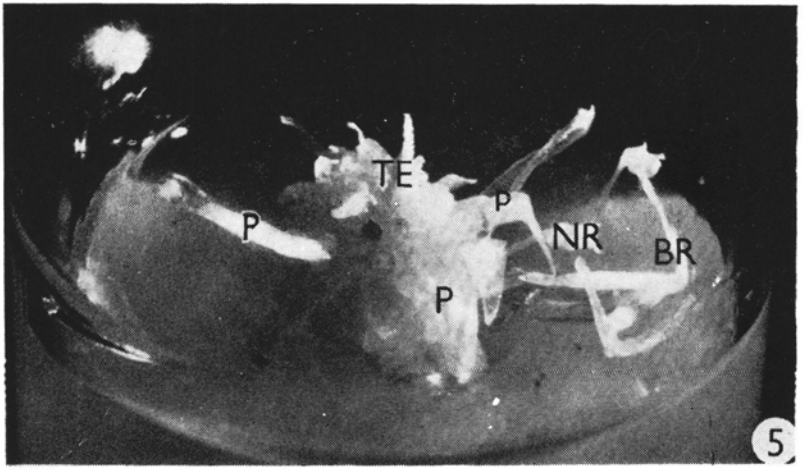
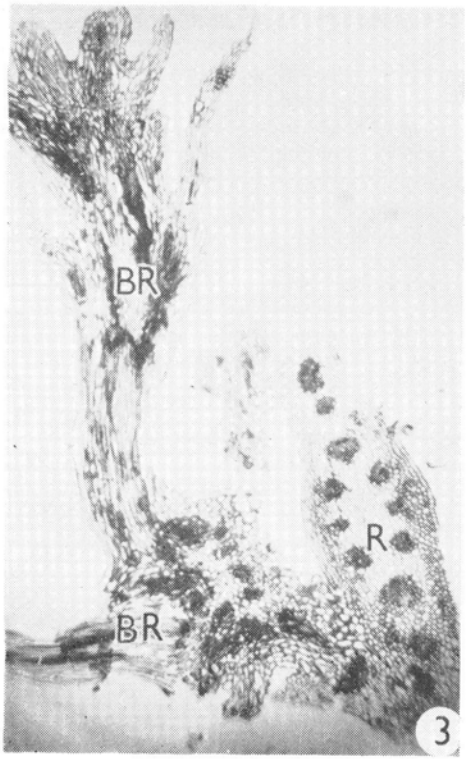


Fig. 1. Defoliated upper node of the rye shoot and young spike (7 mm) as the explant. (20 ×)
N — upper node of the shoot, R — rachis, RI — region of explant isolation, S — spiklet.

Fig. 2. Control culture. Elongation of some paleas of the explant after 28 days of culture. (6 ×)
P — palea, TE — top of the explant.

Fig. 5. Differentiation of repeated rachis branches with glumes on the MS medium added with 0.5 mg/l 2,4-D + 0.5 mg/l ZEA after 28 days of culture. (5 ×)
BR — branch of the rachis, NR — node of the rachis branches with glumes, P — palea, TE — top of the explant.

PLATE II



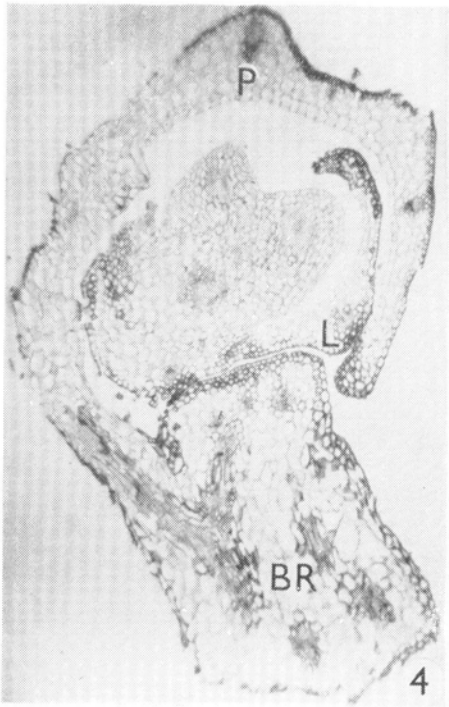
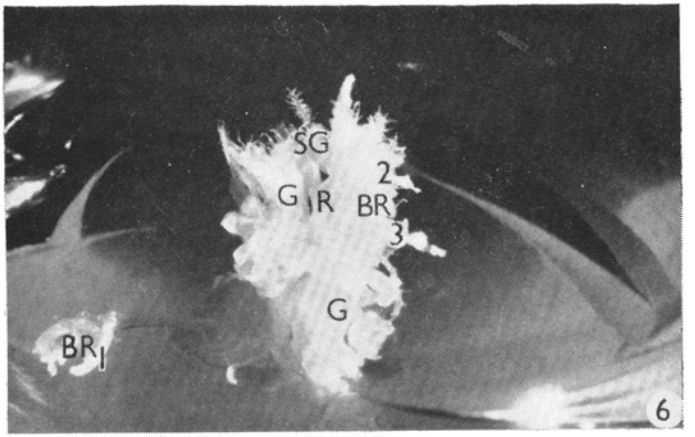
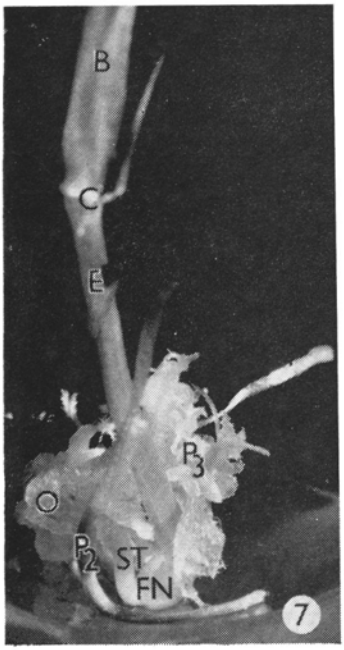


Fig. 3. Cross section of the rachis and longitudinal section of rachis branches. (160 ×)
BR — branches of the rachis, R — rachis.

Fig. 4. Section of the node of rachis branch with palea and lemma. (160 ×)
BR — branch of the rachis, L — lemma, P — palea.





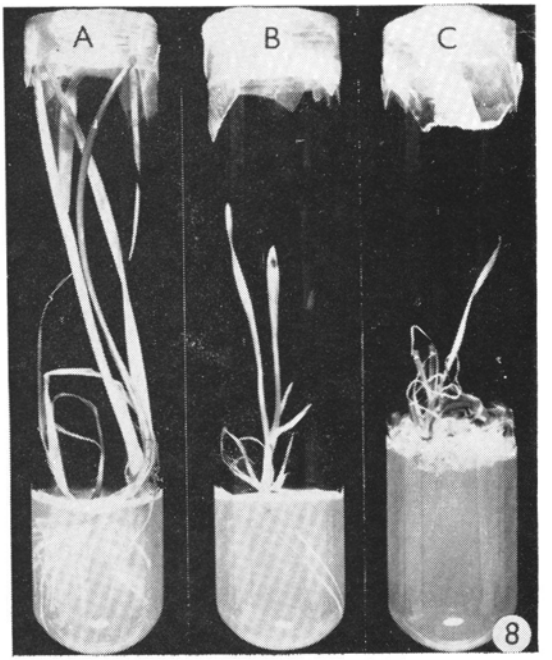


Fig. 6. Differentiation of succeeding rachis branches. Inhibition of glume development and intensive growth of stigma on MS medium with added 0.5 mg/l 2,4,5-T + 1.0 mg/l IPA after 28 days of culture. (6 ×)

BR_{1-3} — succeeding rachis branches, G — glumes, R — rachis, SG — stigma showing intensive growth.

Fig. 7. Green shoot differentiated directly from the rachis after 49 days of culture on MS medium with added 1.0 mg/l NAA + 2.0 mg/l KIN. (4 ×)

B — leaf blade initiating shoot elements, C — conection from the sheath to the blade, E — sheath of the leaf, FN — first node, O — callus-like overgrowth of the explant, P_2, P_3 — plantlets in different developmental stages, ST — structure initiating differentiation of the succeeding leaves of the first plantlet.

Fig. 8. Differentiated development of 15 week plantlets cultured on MS medium with added 1.0 g/l NAA + 2.0 mg/l KIN (A), 2.0 mg/l NAA + 0.3 mg/l KIN (B), and 1.0 mg/l 2,4-D + 1.0 mg/l ZEA (C).

in test tubes 24 mm in diameter and 120 mm in length, in darkness, at temperature of 24°C ± 1°C. Cultures were kept in darkness for six weeks and later transferred to light, the photoperiod being maintained for 16 hours.

Table 1

Growth substance combinations used *in vitro* rye rachis culture (mg/l)

Culture of whole explant							Culture of explant segments		
Control	without growth substances								
Culture	GA_3 0.5								
IAA+BAP	1.0			2.0					
	2.0	|	4.0	0.5	|	1.0			
2,4,5-T+IPA	0.5			1.0					
	0.5	|	1.0	2.0	|	4.0			
2,4-D+ZEA	0.5			1.0					
	0.5	|	1.0	0.5	|	1.0 ○			
NAA+KIN	1.0			2.0		●	0.2	2.0	●
	2.0	|	4.0	0.5	|	1.0	1.0	0.3	

○ — regeneration of plantlets.

● — direct regeneration of plantlets.

A Linsmaier and Skoog (1965) nutrient medium without growth substance of 1.0 sucrose and 0.7% agar was used for rooting.

The material designated for cytological examination was prepared as given in previous studies (Rybczyński, 1978b, 1979).

RESULTS

Only sporadic elongation of the lemma was observed in the control cultures in the presence of GA_3 only (Fig. 2).

The rachis reacted most strongly to the combination with growth substances.

During a two-week culture in darkness on nutrient media with an addition of 2,4-D+ZEA and 2,4,5-T+IPA a general growth of explants could be observed. Explants in these cultures differentiated branches of the rachis (Fig. 3) on which transparent glume-like structures were formed at unequal spacings (Fig. 4). In this way branches of the rachis formed nodes and internodes (Fig. 5). At the same time several branches of the rachis were observed with variable numbers of nodes and internodes (Fig. 6).

After four weeks of culture also branches of the rachis were noted in the presence of NAA. Spacings between newly regenerated nodes on branches of the rachis were greater on the 2,4-D nutrient medium than on the NAA one.

In the presence of 2,4,5-T the rachis lost its nodular structure and sporadically formed lateral branches. Apart from lateral branches rhizogenesis of numerous roots was also noted (Table 2). Regeneration of roots took place both at the base of explants, as well as their tips. In long term cultures the mentioned processes were accompanied by the formation of callus tissue. Rhizogenesis of this callus was observed.

Table 2

Growth substances combinations and rye rachis reaction

Rachis reaction	Growth substances	Time of the culture (days)
Callus formation	2,4,5-T+IPA	38
Direct rhizogenesis	2,4,5-T+IPA+	38
	2,4-D+ZEA	38
	NAA+KIN	28
Rachis branches differentiation	2,4-D+ZEA+	14
	2,4,5-T+IPA	14
	NAA+KIN	28
Green plantlets differentiation	NAA+KIN+	28
	2,4-D+ZEA	81

+ — combination of growth substances which had the most intensive influence on the rachis reaction.

Both after 28 days of culturing 7 mm explants in the presence of 1.0 mg/l NAA + 2.0 mg/l KIN and after six weeks of culturing explant segments on a nutrient medium containing 2.0 mg/l NAA + 0.3 mg/l KIN rachises differentiated green shoot elements (Fig. 7). After another two weeks the new centres began forming further leaves. Formation of successive leaves in the newly regenerated plantlets took place very rapidly. In these combinations explants did not form any callus tissue, and rooting was observed only after 49 days of culturing (Figs. 8a, b).

Abundant formation of shoot elements on a nutrient medium containing 2,4-D and ZEA did not take place until the 12th week of culture. This process was preceded by differentiation of albinotic leaves and intensive rhizogenesis of the rachis (Fig. 8c).

Regenerated plantlets formed a root system, and after being transplanted into soil developed, blossomed, and formed caryopses (Fig. 9). Table 2 summarizes the results obtained.

DISCUSSION

The investigations carried out till now on rachis were connected with practice and agricultural genetics (Surma et al., 1974; Mathanets, 1968).

Attainment of callus tissue, roots and lateral branches, and even direct regeneration of plants from rachises under *in vitro* conditions, point to new possibilities in investigations on this plant organ.

Direct rhizogenesis of the rachis was induced by 2,4-D as was the case in cultures of immature rye embryo scutellum (Rybczyński, 1978b).

Differentiation of lateral branches of the rachis was induced by synthetic auxins only. In principle IAA and sporadically NAA stimulated elongation of the lemma. Differentiation of rachis branches did not take place identically. In the presence of 2,4-D rachis branches separated into internode-like structures joined by the formation of glume-like structures. Photograph 4 showing a cross section through a node indicates similarity in the arrangement of glumes to that in the flowers of rye; it was not possible, however, to collect sufficiently convincing proof that generative elements of flowers are formed, might be suggested by the above structures.

Dudits et al. (1975), Nemet and Dudits (1977) presented the way callus tissue is obtained from 1.5 cm segments of the rachis in their studies on callus cultures and wheat protoplasts. Both the primary callus and passaged tissue showed rhizogenous and caulogenous capabilities. 2,4-D, IAA and ZEA used together as a supplement to nutrient medium "T" gave optimal stimulation to the formation of shoots. O'Hara and Street (1978) obtained callus tissue originating from various plant organs, including rachis segments on a nutrient medium containing 1.0 mg/l 2,4-D. Rachis segments regenerated callus tissue on a nutrient medium containing a high concentration of 2,4-D. The callus formed was smaller than that from node parts. Callus obtained from all of the explants formed roots when transferred to a nutrient medium lacking 2,4-D. Formation of shoot buds was also noted on its surface.

In our experiments direct regeneration of new plants took place only in the presence of NAA + KIN, irrespective of other supplements present in the nutrient medium. In the case of plants obtained on nutrient media containing 2,4-D + ZEA differentiation of shoot elements was preceded by abundant rhizogenesis. Due to the simultaneous formation of callus tissue and shoot elements, it was difficult to define the source of plant regeneration. Differentiation of green plant parts was likewise preceded by regeneration of albinotic shoot elements and took place during the 12th week of culture.

Similar processes were observed on a scutellum culture of immature rye embryo, where induction of callus tissue and differentiation of green plantlets took place in the presence of 2,4-D and ZEA (Rybczyński, 1979). These results are in contrast to a number of publications emphasizing that callus tissue cultured on nutrient media with 2,4-D are not capable of regenerating shoots (Sheridan, 1973, 1974; Shimada et al., 1969; Trion et al. 1968). This capability, however, can be obtained after transferring to a medium lacking 2,4-D or containing IAA or NAA (Cheng, Smith, 1975).

Regeneration of plants from callus tissue has been frequently noted in *in vitro* cultures of monocotyledonous plants, preceded by formation of albinotic leaf-like structures (Tamura, 1968; Rybczyński, 1979). The process of differentiation of shoot elements in our cultures took place directly from the rachis, and the differentiating leaves already possessed a typical structure for *Graminae* plants during the first stages of development. Hence, it appeares probable that regenerated leaves did not originate from glume primordia which were sporadically able to elongate.

The process of shoot differentiation in cultures of monocotyledonous plants continues to present numerous difficulties. Hence, the above study constitutes an effort to indicate another organ of rye which can constitute a source of shoot differentiation in *in vitro* cultures of monocotyledonous plant organs.

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Różnicowanie elementów pędu z osadki kłosowej żyta (Secale cereale L.)

Streszczenie

W wielotygodniowej hodowli 7 mm kłosów *Secale cereale* na zmodyfikowanej pożywce Murashige i Skoog (1962) obserwowano zmiany w rozwoju osadki kłosowej. W obecności syntetycznych auksyn osadki kłosowe różnicowały odgałęzienia o węzłowej budowie. Rizogeneza eksplantatów była stymulowana przez 2,4-D i 2,4,5-T. Osadka kłosowa w obecności 2,4,5-T + IPA regenerowała tkankę kalusową. W obecności NAA + KIN w osadce kłosowej powstawał szereg centrów różnicowania związków pędu, które następnie rozwijały się w pełni wykształcone rośliny.