

Growth response of coleoptile sections of several Polish varieties of wheat to some growth-regulators

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The investigation of the content of natural growth substances in plant extracts separated by chromatographic method requires a biological test allowing of a simultaneous determination of growth-stimulating and growth-inhibiting substances. This could be satisfactorily achieved by the coleoptile section straight growth test of some varieties of oat and wheat.

The usefulness of Polish oat varieties as test material was investigated in detail by Kentzer and Rowicka (1963). Regarding research on the content of growth inhibiting substances, preferable proved to be the wheat test owing to a greater amount of endogenous growth of coleoptile sections in wheat than that in oat.

In addition to several varieties of wheat already known and used by various authors (Luckwill 1952, Bentley, Housley 1954, Barlow, Hancock and Lacey 1957, Nečesany 1958, Phillips, Wareing 1958) a number of Polish varieties of wheat have been recently investigated for usefulness in assaying plant extracts containing natural growth substances, especially inhibitors. Instead of plant extracts, solutions of the different concentrations of some synthetic growth-regulators were tested. The results are presented below.

METHODS

The general procedure of coleoptile section straight growth test was adopted with a few modifications of the method described by Bentley, Housley (1954). Seeds were soaked and germinated, and the seedlings grew in complete darkness at 26 C until they reached a convenient size (72 to 80 hours including 2-hour soaking). Sections 10 mm long were cut from selected coleoptiles of a definite length using two-bladed cutter which removes 3 mm long apical part and the base. The leaf was left inside the coleoptile cylinder. The time of coleoptile cutting was generally 1 hour and the sections were washed with double-distilled water for another 1 hour before incubating. After washing, the sections were incubated for 20 hrs at 26 C in petri dishes 60 mm diameter containing a strip of Whatman No 3 chromatographic paper submerged in 4 ml of 2 per cent sucrose phosphate-citrate buffer pH 5.4 mixture (controls) or

in the same mixture containing additionally different concentrations of synthetic growth-regulators. During incubation the sections were continuously and gently shaken in the horizontal plane. All necessary operations were performed under safe green light. The coleoptile section lengths after incubation were determined with the help of photographic projector, and the significance of differences was calculated according to the Snedecor's variance method.

Indole-3-acetic acid (IAA) of Lab. B.D.H. Reagent prod. was applied. Coumarin, 2,3,5-Triiodobenzoic acid (TIBA), and Transcinnamic acid (t-CA) were the product of L. Light & Co. Ltd. The latter substance was kindly presented by the producers, for which the authors are greatly indebted. Seeds of 13 varieties of Polish wheat were obtained from Boguchwała Plant Cult. Sta., seeds of the "Atle" variety were kindly supplied by Dr. N. G. Smith and Dr. P. M. Robinson from the Dept. of Botany, Univ. College of Wales, Aberystwyth. The authors wish to express their many thanks to them for their courtesy.

RESULTS AND DISCUSSION

The investigation started with determining the amount of endogenous growth of coleoptile sections and the sensitivity of 13 Polish varieties of wheat to three concentrations of the promoter (IAA). To eliminate the effect of age of coleoptiles on the response, sections were cut only from seedlings within length range 16—19 mm. Controls I and II and IAA treatments contained 10 sections per dish and control III — 20 sections. The results of growth of the sections after incubation are summarized in Table 1.

Taking into account the amount of growth of controls, the sensitivity to IAA and, to some extent, also the variability among control sections, the following five varieties were selected for further investigation:

"Opolska" (1) and "R-1018" (2) showing the greatest amount of growth of controls and sensitive enough to IAA ("R-76" was omitted due to little response to promoter), "R-1030" (3) with moderate growth of controls but more sensitive to promoter than "5-lut", "Ostka popularna" (4) and "50/2" (5) showing little growth of controls but most sensitive to IAA.

Among the chosen varieties, sensitivity to a wider range of IAA concentrations and the response to different concentrations of coumarin and 2,3,5-triiodobenzoic acid were tested. 10 coleoptile sections per dish were used for each concentration. The results of this experiment are presented in Table 2. As previously, "Ostka popularna" and "50/2" showed the least amount of growth of controls and the highest sensitivity to the promoter. Especially "Ostka popularna" could be recommended for bio-assaying the plant extracts which contain small amount of growth-stimulating substances and reveal a strong inhibitory activity.

Table 1
Growth of water controls and the response to IAA of 13 varieties of Polish wheat

Variety	Controls mm					Average growth of controls I and II, mm	Response to IAA (per cent growth of controls)				
	I	II	III	Least significant difference at			Concentration of IAA mg/l			Least significant difference at	
				P=0.01	P=0.05						
				0.01	0.1		1.0	P=0.01	P=0.05		
Opolska	20.58	20.15	20.61	1.25	0.93	20.35	96.96	103.35	126.18	10.72	8.02
R-76	20.08	20.35	19.83	1.14	0.85	20.21	100.06	100.93	122.45	7.31	5.47
R-1018	19.73	19.20	19.65	1.25	0.93	19.46	95.07	106.63	121.14	8.97	6.71
5-lut	19.20	19.15	18.26	1.54	1.15	19.18	98.70	103.78	133.87	10.46	7.83
R-1030	18.88	18.30	19.39	1.59	1.19	18.59	100.47	111.63	125.49	8.74	6.54
K ₁ /61	18.25	19.00	18.98	0.98	0.73	18.62	102.28	108.72	117.04	6.72	5.02
R-1039	18.25	18.32	18.51	1.28	0.96	18.29	97.70	107.18	115.52	11.15	8.34
R-28	18.22	18.35	18.49	1.20	0.89	18.29	99.38	105.67	127.68	13.52	10.12
Lut-1	18.30	17.72	18.34	1.35	1.01	18.01	96.32	108.53	119.92	10.69	8.00
Ostka popularna	17.40	18.20	17.25	1.68	1.25	17.80	101.82	115.59	137.64	8.80	6.58
3 zb 5	16.80	18.30	17.85	1.66	1.24	17.55	98.29	105.55	126.78	12.47	9.33
50/2	16.85	17.05	17.48	1.61	1.20	16.95	104.13	105.75	140.71	11.19	8.38
Ostka chłopicka	16.88	16.60	17.01	1.34	1.00	16.74	99.78	107.24	117.10	10.92	8.17

Both varieties, however, are of less value if a simultaneous detailed determination of different concentrations of inhibitors in chromatographed plant extracts is needed, due to their little endogenous growth. The little growth of controls makes it impossible to obtain sufficiently significant differences in the response to various concentrations of the inhibitor. For this reason the variety "Opolska" showing the greatest amount of endogenous growth and most sensitive to the promoter (among the three remaining varieties) seems to be particularly valuable. This was also confirmed by the analysis of growth variability of sections in the solutions containing various concentrations of coumarin. This analysis revealed the lowest variability of the "Opolska" variety (Table 3). Consequently, the variety "Opolska" was further investigated for comparison of the response of its coleoptile sections with the response of "Atle" variety which is often used with good results by different authors for this kind of bio-assays (Phillips, Wareing 1958, 1959, Wodzicki (in press), and others). The experiment involved investigation of the response to various concentrations of IAA and to three inhibitors. Two petri dishes each containing 10 coleoptile sections were used for each concentration. The averages of two dishes are brought up in Table 4. It will be seen that the response of two varieties does not differ essentially. Growth of control sections of "Opolska" variety

Table 2

The response of five Polish varieties of wheat to coumarin, 2, 3, 5-triiodobenzoic acid and indole-3-acetic acid. Average length of coleoptile sections in millimeters or in percentages of growth of controls

Treatment	Wheat variety									
	Ostka popularna		R — 1018		R — 1030		50/2		Opolska	
	mm	%	mm	%	mm	%	mm	%	mm	%
Controls										
I	15.65		19.83		17.97		17.10		20.48	
II	15.88		19.40		18.70		17.30		19.80	
mean	15.76	100.00	19.61	100.00	18.34	100.00	17.20	100.00	20.14	100.00
Coumarin mg/l										
10	16.07	101.98	17.90	91.27	17.45	95.16	15.43	89.68	18.32	90.99
50	13.02	82.63	15.95	81.32	15.42	84.12	12.53	72.82	15.15	75.23
100	12.40	78.67	13.70	69.85	13.20	71.98	11.45	66.57	12.40	61.58
250	12.30	78.19	12.70	64.75	12.12	66.12	11.15	64.82	12.00	59.59
TIBA mg/l										
0.01	15.63	99.13	19.13	97.51	18.85	102.79	16.48	95.78	19.70	97.83
0.1	15.30	97.06	18.82	95.98	19.05	103.88	16.82	97.82	20.30	100.81
1	16.62	105.47	19.13	97.51	19.07	104.02	15.90	92.44	19.37	96.21
10	15.35	97.38	18.85	96.11	17.75	96.80	15.42	89.68	19.10	94.85
100	14.13	89.61	15.28	77.88	15.38	83.84	14.10	81.98	15.65	77.71
IAA mg/l										
0.01	17.13	108.64	18.38	93.69	18.05	98.43	16.75	97.38	20.52	101.92
0.05	17.80	112.93	20.30	103.50	19.05	103.88	18.17	105.67	20.17	100.19
0.1	19.52	123.87	20.38	103.89	18.95	103.34	17.35	100.87	21.32	105.89
1	20.77	131.80	22.67	115.61	22.05	120.24	22.37	130.09	25.75	127.87
10	25.45	161.46	28.10	143.27	25.20	137.42	23.95	139.24	27.80	138.05
100	25.92	164.47	26.95	137.41	25.80	140.69	25.57	148.69	28.88	143.39

being even somewhat greater than that of "Atle". This, probably, made the differences in the response to various concentrations of growth-retarding substances of "Opolska" variety more pronounced. This is evident when the response of two varieties to 150 and 200 of TIBA or to 75 and 100 mg/l of coumarin are compared. The differences in the growth of sections of "Opolska" variety between the above mentioned concentrations of the two inhibitory substances are significant but not in the case of the "Atle" variety. The response of the two varieties to the promoter was essentially similar, from the point of view of statistically significant (at 1 per cent level of risk) differences between the controls and between the successive concentrations investigated. These properties allow of the assumption that with regard to the usefulness for bio-assays of chromatographed extracts of different tissues or plant material containing various amounts of inhibitors, the "Opolska" variety is slightly better than the "Atle".

Table 3

Analysis of the statistical significance among differences in the growth response of coleoptile sections to four concentrations of coumarin and water (in the control series) of five varieties of wheat.

Variety	Coumarin mg/l				
	10	50	100	250	
Ostka popularna	—0.31	2.74	3.36	3.46	0***
		3.05	3.67	3.77	10
	LSD* = 0.96		0.62	0.72	50
	LSD** = 1.28			0.10	100
50/2	1.77	4.67	5.75	6.05	0
		2.90	3.98	4.28	10
	LSD* = 0.98		1.08	1.38	50
	LSD** = 1.31			0.30	100
R-1030	0.89	2.92	5.14	6.22	0
		2.03	4.25	5.33	10
	LSD* = 1.11		2.22	3.30	50
	LSD** = 1.48			1.08	100
R-1018	1.71	3.66	5.91	6.91	0
		1.95	4.20	5.20	10
	LSD* = 0.86		2.25	3.25	50
	LSD** = 1.15			1.00	100
Opolska	1.82	4.99	7.74	8.14	0
		3.17	5.92	6.32	10
	LSD* = 0.62		2.75	3.15	50
	LSD** = 0.82			0.40	100

* Least significant difference at 5 per cent level of risk.

** Least significant difference at 1 per cent level of risk.

*** Water control.

Very important for the results of bio-assays is a uniform behaviour of the sections. To some extent, it may be achieved by cutting coleoptile sections from seedlings similar by growth, as the age of coleoptile affects the response of the section to growth-regulators (Bentley 1950, Nitsch 1956, and others). Uniformity of behaviour of coleoptile sections may be influenced also by the difference in the cutting period, that is — the lapse of time between the cutting of the first and the last coleoptiles used in the same test (Barlow, Hancock and Lacey 1957). Especially, if the large single tests involving eluates of R_f strips from several plant extracts are required for a better comparison of the content of growth-substances, the time of cutting may spread over a few hours.

Thus, a further experiment was made to test the response of coleoptile sections of the "Opolska" variety cut from differently grown (differing by their developmental age) seedlings and cut at different times before

Table 4

Response of „Opolska” and „Atle” varieties of wheat to various concentrations of IAA and three inhibitors

Treatment	Wheat variety				LSD at 5 per cent level of risk	LSD at 1 per cent level of risk
	„Opolska”		„Atle”			
	Average length of 20 coleoptile sections (two test dishes) in millimeters or per- centages of growth of controls					
	mm	%	mm	%		
Water control	20.70	100.00	19.71	100.00	0.70	0.94
IAA mg/l						
0.01	20.67	99.88	20.20	102.47	1.09	1.43
0.05	22.11	106.82	21.77	110.46		
0.1	22.42	108.33	21.91	111.16		
1	24.47	118.24	29.76	150.98		
10	29.46	142.33	31.65	160.56		
100	28.55	137.92	28.35	144.96		
TIBA mg/l						
1	21.16	102.23	18.40	93.34	0.70	0.92
10	19.55	94.44	18.96	96.19		
50	17.79	85.93	18.12	91.95		
100	16.02	77.41	15.87	80.53		
150	15.35	74.15	15.30	77.62		
200	14.55	70.29	14.99	76.03		
400	13.45	64.97	13.36	67.79		
Coumarin mg/l						
1	20.89	100.90	19.21	97.46	0.56	0.74
10	19.20	92.75	18.79	95.31		
25	17.79	85.93	17.06	86.56		
50	16.10	77.78	15.81	80.22		
75	14.97	72.34	14.32	72.67		
100	14.15	68.36	14.10	71.53		
200	12.77	61.71	12.84	65.12		
300	12.35	59.66	12.72	64.55		
<i>t</i> -CA mg/l						
1	20.77	100.36	19.69	99.87	0.80	1.05
10	20.47	98.91	19.72	100.06		
50	19.76	95.47	18.60	94.36		
100	19.45	93.96	18.91	95.94		
300	14.24	68.78	14.64	74.25		

the incubation. Sections were cut separately from four groups of seedlings, namely: 16—17 mm, 18—19 mm, 20—21 mm and 22—23 mm long. These four groups of seedlings were cut during each of the three time intervals before incubation: A) the cutting operation was completed within one hour and the sections were immediately washed during another hour, B) sections were cut during one hour than left on moist filter paper in a petri dish for another hour before washing, and C) sec-

tions were left in the petri dish during two successive hours following one hour of cutting and then washed during another one hour. Thus, group *A* consisted of sections which were cut 1—2 hours before incubation, *B* — sections cut 2—3 hours and *C* — cut 3—4 hours before the start of incubation.

Only a small range of lengths (ages) of seedlings was tested as the investigation did not aim at a systematic study of the influence of age of coleoptile on the growth of section, but only at determination whether a range of lengths of seedlings sufficiently convenient for easy work may be safely applied taking a chosen Polish variety of wheat. Similarly, only three time intervals (differing by one and two hours) of cutting the coleoptiles were chosen for investigation in view of the fact that during 2 hours two persons can prepare about 1000 coleoptile sections (or 1400—1500 during three hours) which generally is a number large enough for a single test. Results of this experiment are presented in Table 5. The data were subjected to an analysis of variance. This showed that the response of water-controls and of those subjected to the lowest concentration of coumarin were not significantly influenced by the time of cutting of coleoptiles and the age of seedlings within the chosen range. The differences in growth of the sections cut at three different times before incubation were insignificant even at 10 per cent level of risk. The time of cutting was found to affect the uniformity of the results of bio-assay of the higher concentration of inhibitor and of the lower concentration of promoter. The analysis of growth of sections showed that this was caused not by the significant differences in their response to growth-regulating substances but mainly by the different original length of sections for each of the three time intervals at the starting moment of incubation. The three groups of sections differed from each other on average by 0.25 mm at the start of incubation (those cut within 3—4 hours before incubation being longest).

The time of cutting did not significantly affect the results if a higher concentration of the promoter was bio-assayed. However, the sections cut from the shortest (youngest) coleoptiles were less sensitive to 1.0 mg/l of IAA. The response of remaining groups of sections was not significantly different at the 1 per cent level of risk. This is clear in particular, if elongation of the sections over their original length at the start of incubation is compared. The analysis showed that the range of original length of seedlings \div 18—23 mm, cut at least 2—3 hours before incubation could be recommended as sufficiently safe intervals covering most uniform response of sections of lower and higher concentrations of inhibitor and promoter, and allowing of comparable results of bio-assay at 1 or even 5 per cent level of risk.

Taking into account all the results: the greatest amount of growth

Table 5

Elongation of coleoptile sections cut at different times before incubation from the variety „Opolska” wheat seedlings of various developmental age (millimeters)

Length of seedlings (Developmental age) mm	Time of cutting (hours before) incub.)	Water control		Coumarin mg/l						IAA mg/l					
				10		103				0.05				1.0	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B
16-17	1-2	20.52	9.02	19.49	7.90	14.72	3.22	22.17	10.67	23.20	11.70				
	2-3	20.80	9.05	20.40	8.65	15.40	3.65	21.10	9.35	22.87	11.12				
	3-4	21.27	9.27	19.77	7.77	15.45	3.45	22.10	10.10	26.30	14.30				
	Mean	20.87	9.12	19.86	8.11	15.19	3.44	21.79	10.04	24.12	12.37				
18-19	1-2	20.27	8.92	19.67	8.17	14.22	2.72	19.55	8.05	27.30	15.80				
	2-3	20.40	8.65	20.30	8.55	14.82	3.07	22.15	10.40	25.75	14.00				
	3-4	20.92	8.77	20.22	8.22	15.77	3.77	22.67	10.67	25.27	13.27				
	Mean	20.53	8.78	20.07	8.32	14.94	3.19	21.46	9.71	26.11	14.36				
20-21	1-2	20.77	8.20	20.37	8.87	15.42	3.92	21.97	10.47	26.20	14.70				
	2-3	21.12	9.37	20.37	8.62	14.65	2.90	21.07	9.32	26.85	15.10				
	3-4	20.02	9.27	20.70	8.70	15.57	3.57	22.07	10.07	26.80	14.80				

	Mean	20.70	8.95	20.48	8.73	15.22	3.47	21.71	9.96	26.62	14.87
21-23	1-2	20.47	8.97	21.05	9.55	14.50	3.00	21.65	10.15	27.20	15.70
	2-3	21.65	9.90	19.85	8.10	15.52	3.77	21.25	9.50	25.72	13.97
	3-4	22.10	10.10	21.22	9.22	16.20	4.20	22.47	10.47	23.32	11.32
	Mean	21.41	9.66	20.71	8.96	15.41	3.66	21.79	10.04	25.42	13.67
Mean	1-2	20.51	9.01	20.12	8.62	14.72	3.22	21.34	9.84	25.97	14.47
	2-3	20.99	9.24	20.23	8.48	15.10	3.35	21.39	9.64	25.30	13.55
	3-4	21.12	9.12	20.48	8.48	15.75	3.75	22.33	10.33	25.42	13.42

The significance of differences among average growth of various groups of sections differing by	Water control		Coumatin mg/l				IAA mg/l			
			10		100		0.05		1.0	
	A	B	A	B	A	B	A	B	A	B
Age of coleoptile	$F_e < F_t$ at $P = 0.05$	—	$F_e < F_t$ at $P = 0.05$	—	$F_e < F_t$ at $P = 0.1$	$F_e < F_t$ at $P = 0.1$	$F_e < F_t$ at $P = 0.05$	$F_e < F_t$ at $P = 0.1$	$\mu_{t0.01} = 1.39$ $\mu_{t0.05} = 1.05$	—
Time of cutting	$F_e < F_t$ at $P = 0.1$	—	$F_e < F_t$ at $P = 0.1$	—	$F_e < F_t$ at $P = 0.01$	$F_e < F_t$ at $P = 0.01$	$\mu_{t0.01} = 0.48$ $\mu_{t0.05} = 0.46$	$F_e < F_t$ at $P = 0.1$	$F_e < F_t$ at $P = 0.1$	—
Interaction of Age \times Time	$F_e < F_t$ at $P = 0.1$	—	$F_e < F_t$ at $P = 0.1$	—	—	$F_e < F_t$ at $P = 0.01$	—	—	$F_e < F_t$ at $P = 0.05$	—

A — total elongation after 20 hrs of incubation; B — elongation over the original length of sections; μ_t — least significant difference.

of controls, high sensitivity to IAA and inhibitors (as compared with the other investigated varieties of wheat), the lowest variability in growth of the sections and convenient properties making it possible to prepare sections within a suitable range of coleoptile age and during a long enough period of time — the variety "Opolska" may be recommended as one having good properties for large single bio-assays if a simultaneous determination of promoters and inhibitors in the chromatographed plant extracts is required.

SUMMARY AND CONCLUSIONS

Investigation of elongation growth of coleoptile sections of 13 varieties of Polish wheat in 2% sucrose-phosphate-citrate buffer or in the same medium with addition of indole-3-acetic acid, which was performed as the preliminary experiment, gave ground for selection of five varieties distinguished by their intensive growth of controls and sensitivity to the promoter. The response of these varieties to a number of concentrations of IAA, coumarin and triiodobenzoic acid were further tested. On the basis of the results, the variety "Opolska" was chosen for more detailed investigation, which involved its response to various concentrations of three inhibitors and the promoter and the effect of the developmental age of seedlings and the time of cutting of coleoptiles before incubation. As a result, variety "Opolska" was found to have convenient properties for large-scale routine tests requiring, a simultaneous determination of promoters and inhibitors in the single test of the chromatographed plant extracts. It showed no less amount of control growth and a similar, or even better, response to synthetic plant growth-regulators than did the "Atle" variety. Another variety, "Ostka popularna" appeared to be the most sensitive among the investigated varieties to the wide range of concentrations of IAA.

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Wpływ niektórych substancji wzrostowych na wzrost odcinków koleoptile polskich odmian pszenicy

Streszczenie

Przeprowadzono badania nad zastosowaniem polskich odmian pszenicy w teście wydłużeniowym odcinków koleoptile celem określenia zawartości substancji wzrostowych w ekstraktach roślinnych rozdzielonych chromatograficznie. W doświadczeniach użyto różnych koncentracji syntetycznych regulatorów wzrostu, a mianowicie kwasu β -indolilooctowego, kumaryny, kwasu 2,3,5-trójjodobenzoowego i kwasu *trans*-cynamonowego.

Na podstawie wyników wstępnego doświadczenia, spośród 13 odmian wybrano 5 wyróżniających się wrażliwością na działanie IAA oraz wykazujących odpowiedni wzrost w kontroli wodnej.

Dalsze doświadczenia wykazały, że odmiana „Opolska” najlepiej odpowiada wymaganym warunkom wzrostu w seriach kontrolnych i posiada potrzebną wrażliwość na działanie stymulatora, jak i inhibitorów. Odmiana „Opolska” nie ustępuje pod tymi względami odmianie „Atle”, często stosowanej zagranicą.

Badania nad wydłużaniem się odcinków koleoptile odmiany „Opolska” przygotowanych w różnych okresach czasu (3—4, 2—3 i 1—2 godziny) przed rozpoczęciem testu z siewek różnego wieku wyrażonego długością ich koleoptile (16—17, 18—19, 20—21, 22—23 mm) wykazały, że dzięki dużej jednolitości reagowania, czynniki te w wystarczająco szerokim zakresie nie wywierają istotnego wpływu na wynik testu. Ustalono mianowicie, że reakcja odcinków, ciętych od 1—3 godzin przed rozpoczęciem inkubacji z siewek o długości w granicach 18—23 mm, jest statystycznie taka sama, jeśli zastosować przedział ufności przy $P = 0,01$, a nawet 0,05 dla niskich i wysokich koncentracji substancji stymulujących i hamujących wzrost.

Wyniki te wskazują na możliwość stosowania pszenicy odmiany „Opolska” w teście wydłużeniowym odcinków koleoptile w celu badania zawartości substancji stymulujących i hamujących wzrost w ekstraktach z tkanek roślinnych rozdzielonych na frakcje metodą chromatograficzną.