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Influence of hydroxyurea on the course of germination and growth of rape (*Brasica napus* L.) seedlings

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Abstract

The effects of continuous incubation in hydroxyurea (HU) solutions (0.2, 0.4, 0.8 mg/ml) on germination of rape seeds and growth of young seedling axes were studied during 132 hours from initial soaking. Germination turned out to be unaffected by the treatment. Root growth was first increasingly inhibited by the HU concentration tested, but after prolonged incubation a complete arrest of the root growth was noted at all HU concentrations. The elongation growth of hypocotyls was found to be stimulated by a HU 0.2 mg/ml concentration while it was markedly suppressed by 0.4 mg/ml, and completely arrested by 0.8 mg/ml. Inhibition of growth of the upright hypocotyl part at higher HU concentration was found to be accompanied by the unbending of the hooked under-cotyledonary part.

It is suggested that inhibition of nuclear endomitotic DNA synthesis in elongating hypocotyl cells, suppresses only partially their growth, whereas a complete inhibition of the hypocotyl growth results from arrest of the mitochondrial DNA synthesis.

INTRODUCTION

In germinating rape seeds, the growth of the embryo axis before protrusion is due to cell extension in the hypocotyl without cell division.

During protrusion, the mitotic meristematic growth of the root begins in the basal peripheral region, and spreads wavelike toward the root initials after protrusion. There are no cell divisions in the elongating seedling hypocotyl outside the provascular tissue where some few divisions occur (K u r a ś 1974). There is however DNA synthesis in the elongating epidermis and cortex cells of the rape hypocotyl before protrusion and thereafter, as shown autoradiographically (unpubl.).

Capesius and Stöhr (1974) have shown that the DNA content per nucleus in the hypocotyl cells increases continuously by endopolyploidy and, perhaps, by gene amplification in young white mustard seedlings, a species closely related to rape.

Capesius and Bopp (1974) investigated biochemically the DNA synthesis and the consequences of DNA synthesis inhibition on hypocotyl elongation in young white mustard seedlings, and have shown that continuous DNA synthesis is the indispensable prerequisite for hypocotyl cell elongation.

These results seem to suggest that continuous nuclear DNA synthesis is indispensable for hypocotyl cell elongation.

The present paper throws some new light on this problem, suggesting that it may not be the nuclear, but the mitochondrial DNA synthesis, and thereupon the multiplication of mitochondria, that is indispensable for hypocotyl cell elongation, whereas the endopolyploidy or gene amplification probably only accelerates cell elongation.

Hydroxyurea is one of the most specific DNA inhibitors (Young and Hodas, 1964; Yarbro et al., 1965; Young et al., 1967; Krakoff et al., 1968).

In the range of concentrations used here, the syntheses of RNA and protein are not affected in the plant tissues (Kihlman et al., 1966; Odmark, 1971; Habdas, 1977) and the mitotic block is reversible (Kihlman et al., 1966; Brulfert and Deysson, 1971, 1973; Habdas, 1977; Kaszyńska and Kuraś unpubl.).

MATERIAL and METHODS

Selected rape seeds (*Brassica napus* L. cf. Górczański), of equal size with black testa were sown on Petri dishes on one layer of chromatographic paper moistened with 8 ml of tap water filtered through charcoal (control) or with hydroxyurea (Serva) solution in concentrations of: 0.2, 0.4, 0.8 mg/ml. The experiment was run in darkness at 21°C (±1°C). The solutions were not changed in the course of the experiment. The per cent of germinating seeds was calculated (each time in 10 samples of 100 seeds for each combination) at 3-h intervals between 12 and 24 h after the beginning of seed swelling, and later between 24 and 108 h at 12-h intervals. As beginning of germination was assumed the breaking of the seed coat by the radicle apex. In another series of samples (5×100 seeds for each combination) the length of seedling axes (comprising root and hypocotyl) were measured. The measurements were taken with a strip of millimetre paper with the use of a 5× magnifying glass after 24, 36, 48, 60, 84, 108 and 132h. Each time 100 seedlings were measured in each combination, 20 seeds from each sample. The results are presented as arithmetic means in the tables and diagrams.

RESULTS

Influence of hydroxyurea on the course of germination

It results from tables 1 and 2 and figures 1 and 2 that hydroxyurea in the concentrations applied (0.2 — 0.8 mg/ml) has no essential influence on the course of rape seed germination. The per cent of

Table 1

Mean per cent of rape seed germination in hydroxyurea solution

Concentration of HU mg/ml	Time from beginning of swelling (h)									
	12	15	18	21	24	36	48	60	72	86
0	3.0	46.0	73.4	80.0	83.8	84.0	84.8	86.2	86.2	86.2
0.2	3.2	46.0	74.8	81.8	82.6	83.8	85.0	87.2	87.2	87.2
0.4	4.2	47.4	77.0	80.0	83.2	84.8	85.6	86.6	86.6	86.6
0.8	5.2	54.0	78.8	82.0	87.8	87.8	88.4	89.8	89.8	89.8

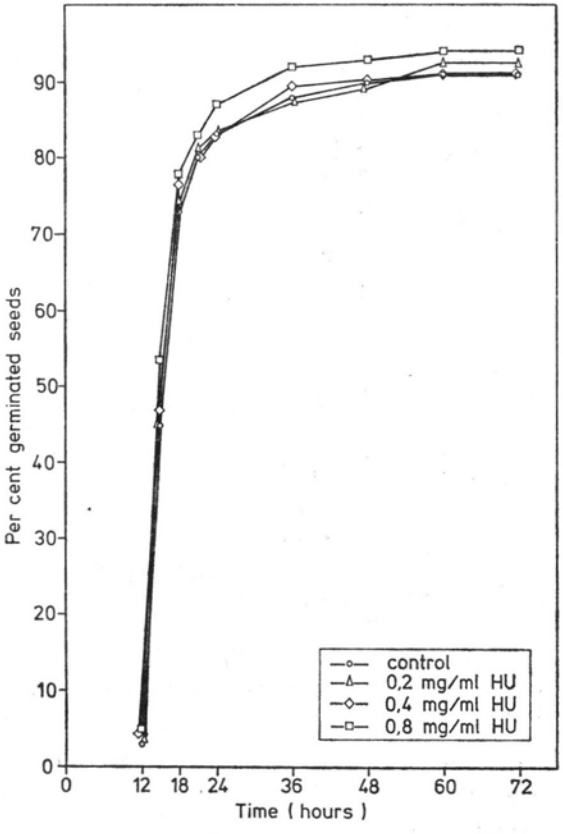

Fig. 1. Diagram of rape seeds germination during continuous incubation in hydroxyurea (%)

Table 2

Rate of rape seed germination in hydroxyurea solution

Concentration of HU mg/ml	Time from beginning of swelling (h)									
	12	15	18	21	24	36	48	60	72	86
0	3.0	43.0	27.4	6.6	3.8	0.4	0.6	1.4	1.4	1.4
0.2	3.2	42.6	29.0	5.0	3.0	1.0	1.2	2.2	2.2	2.2
0.4	4.2	43.2	29.6	2.4	3.6	1.6	0.8	1.0	1.0	1.0
0.8	5.2	48.8	24.4	3.2	5.4	0.4	0.6	1.4	1.4	1.4

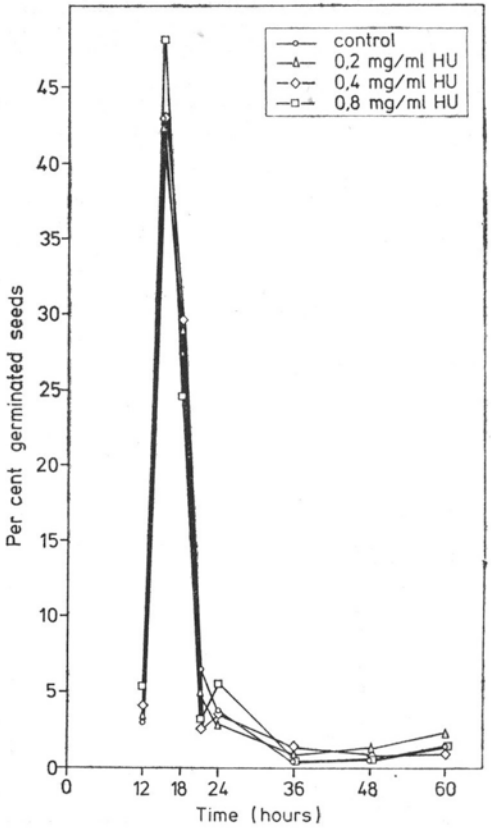

Fig. 2. Rate of rape seed germination during continuous incubation in hydroxyurea

germinated seeds after treatment hardly differs from the control result, with mean within the limits of 86 — 89 per cent. The rate of germination is also almost identical in all combinations. The germination rate curve (figure 2) shows that in all the 3 concentrations of hydroxyurea applied as well as in the control almost 50 per cent of the seeds

germinated within 3 h (between the 12th and 15th h after the beginning of soaking) and 80 per cent within 6 h (between 12 and 18 h). Thus, no inhibitory effect of hydroxyurea was noted. Most unexpected, however was its stimulating effect. This effect was slight but significant, since it increased with hydroxyurea concentration and was noted in 3 supplementary tests.

Effect of hydroxyurea on seedling growth

The growth of the protruding part of the embryo axis in all hydroxyurea concentrations was, immediately after germination the same as in the control, and 24 h after the initial soaking its length reached 0.5 to 1.0 mm. Beginning, however, with 24 h, and particulary after 36 h, a marked differences was observed between the length of the

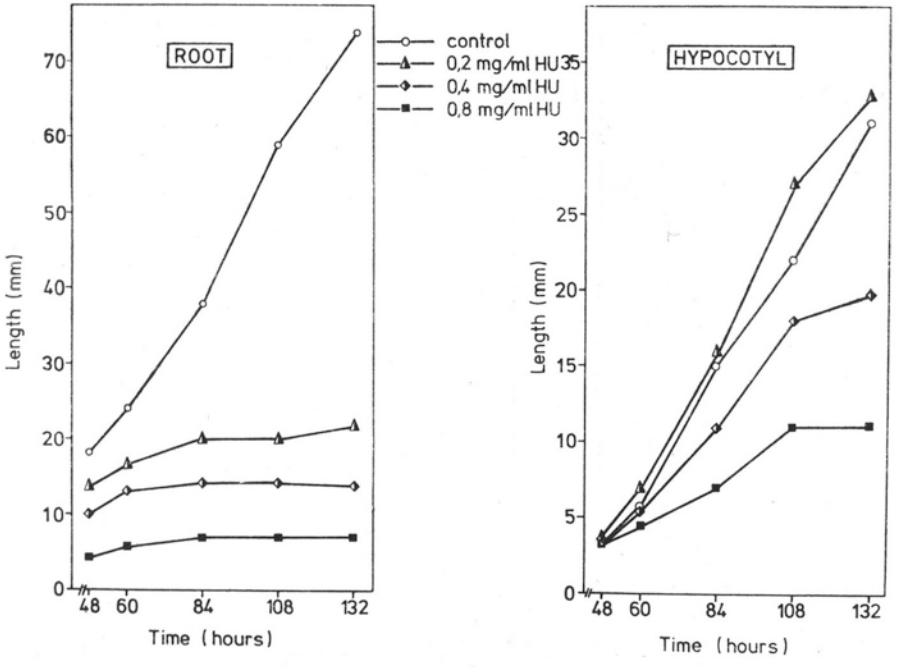

Fig. 3. Course of radicle and hypocotyl growth during continuous incubation in hydroxyurea

seedling axis in the control experiments and those with hydroxyurea. After 48 h of the experiment axes of the seedlings growing in water reached 20 — 25 mm, whereas those treated with the highest hydroxyurea concentration barely attained 8 mm. The well outlined border between the hypocotyl and the root shows that the difference in length of the seedling axis results solely from the unequal size of the roots. The hypocotyl was so far of the same length as in the control in all hydroxyurea concentrations applied.

Table 3

Mean length (mm) of rape seedlings which germinated and grew in hydroxyurea solution

Concentration of HU (mg/ml)	Part of seedling	Time from beginning of swelling (h)														
		48			60			84			108			132		
0	Hypocotyl	3.3			5.8			15.0			22.0			31.5		
	Root		18.7			24.2			38.0			59.0			73.4	
	Whole seedling			22.0			30.0			53.0			81.0			104.9
0.2	Hypocotyl	3.5			7.0			16.0			27.0			32.9		
	Root		13.8			16.7			20.0			20.0			22.1	
	Whole seedling			17.3			23.7			36.0			47.0			55.0
0.4	Hypocotyl	3.2			5.6			11.0			18.2			19.5		
	Root		10.0			13.1			14.0			14.0			13.8	
	Whole seedling			13.2			18.7			25.0			32.2			33.3
0.8	Hypocotyl	3.2			4.5			7.0			11.0			11.2		
	Root		4.4			5.9			7.0			7.0			7.2	
	Whole seedling			8.0			10.4			14.0			17.4			18.4

During further growth the difference in growth rate between the seedlings growing in water and those in 3 different hydroxyurea concentrations enhanced the difference between their axes (table 3, figure 3). After 60 h the length of the seedlings growing in the highest concentration (0.8 mg/ml) was hardly 1/3 of that of the control plants. The same as after 48 h the difference in length is mainly the result of the lower growth rate of the root (24.3% of control root length). Moreover, the mean length of the hypocotyl of seedlings grown on the highest hydroxyurea concentration at this stage reaches 77.6 per cent of that the control plants. Lower hydroxyurea concentrations either do not inhibit hypocotyl growth (0.4 mg/ml) or even have a stimulating effect (0.2 mg/ml — 120.7 per cent of control length). These differences in hypocotyl and root growth continue for the subsequent 24 h, that is between the 60 th and 84 th hour from the beginning of soaking (Figure 3, table 3). Thereafter root growth is completely inhibited in all hydroxyurea concentrations.

At this time a distinct inhibitory effect of higher hydroxyurea concentrations on hypocotyl growth is noticeable, its length attaining after 84 and 108 h at 0.4 mg/ml concentration 73.3 and 82.7, and at 0.8 mg/ml — 46.6 and 50.0 per cent, respectively, whereas at 0.2 mg/ml concentration hydroxyurea continued to stimulate growth giving 106.6 and 122.7 per cent of the control, length respectively.

In the course of the further 24 h, hypocotyl growth was still stimulated by the lowest hydroxyurea concentration, depressed by 0.4 mg/ml and completely inhibited by the highest concentration.

In control seedlings and those growing on the lowest hydroxyurea concentration the subcotyledonary part of the hypocotyl is bent forming a hook under the cotyledon during the entire experiment.

Seedlings treated with the highest concentration and the majority of those growing on 0.4 mg/ml hydroxyurea had, after 108 h, a completely erect hypocotyl. Straightening of the hypocotyl hook begins in seedlings growing on 0.8 mg/ml earlier than in those 0.4 mg/ml. Premature straightening of the bent part of the hypocotyl in plants treated with higher concentrations is, thus correlated with the increased growth inhibition of the erect part of the hypocotyl.

DISCUSSION

Rape seed germination, i.e. protrusion, is due mainly to the elongation growth of the hypocotyl (K u r a ś, 1974) in which no cell division was noted.

Immediately before germination there starts in the root basal zone a premitotic growth wave advancing in acropetal direction. It is followed, after germination, by a wave of mitoses. In the fully activated apical root meristem the rate of growth depends on cell production, as found in our earlier investigations (Kuraś, 1974). Confirmation of this conclusion on a different object may be found in the data of Barlow (1969). In the basal part of the root meristem cells stop dividing and elongate. The continuous growth of young seedling axis, comprising the root and the hypocotyl, is therefore, the function of elongation growth of the hypocotyl cells, as well as the root cells in its basal part, and of the meristematic growth of the root meristem. Thus, the different mode of reaction of root and hypocotyl to DNA synthesis inhibition by hydroxyurea becomes understandable. The organ reacting earliest and most intensively to the action of hydroxyurea is the root, the growth of which ultimately depends on mitotic activity. Inhibition of the latter by way of DNA synthesis inhibition leads of course to a gradual inhibition of root growth.

The rape hypocotyl growth is the function of cell elongation without division. It is no doubt dependent on RNA and protein synthesis which are not arrested by hydroxyurea (Young an Hodas, 1964; Schwartz et al., 1965; Young and Karnofsky, 1966; Pollak and Rosenkranz, 1967; Habdas, 1977).

The elongation growth rate of hypocotyl is probably also dependent on the endomitotic DNA synthesis observed by us in rape (unpublished autoradiographic investigations) and in white mustard by Capesius and Stöhr (1974).

Capesius and Bopp (1974) found that during 24-h incubation of young white mustard seedlings, in very high hydroxyurea concetrations (4 mg/ml, i.e. 5.2×10^{-2}M), DNA synthesis in the hypocotyl is greatly depressed and the fresh mass reduced to 50 per cent of the control, similary as under the influence of high FUdR concentrations. DNA synthesis and elongation growth inhibition in the hypocotyl of white mustard by FUdR is abolished by simultaneous application of thymidyne or thymidylate. This indicates the indispensability of continuous DNA synthesis for elongation growth of the hypocotyl, but it is not a proof of the indispensability of endomitotic reduplication of nuclear genes.

The elongation growth inhibition in the hypocotyl by high concentrations of DNA synthesis inhibitors may, namely, be not only the consequence of endomitosis or nuclear gene amplification inhibition, but may also be due to cytoplasmic DNA synthesis inhibition.

It was found in the present investigation that a low hydroxyurea concentration (0.2 mg/ml) depressing markedly DNA synthesis in plants (Kihlman et al., 1966) and gradually inhibiting root growth in rape,

not only does not inhibit, but even stimulates hypocotyl growth during prolonged incubation from the beginning of seed soaking.

Unpublished results of experiments with application of hydroxyurea for mitoses synchronization in onion roots demonstrated, that this concentration is optimal, producing maximal mitoses synchronization after almost complete inhibition of mitotic activity during 24 h of incubation. On the other hand, higher concentrations, particulary 0.8 mg/ml have a toxic effect on onion roots.

It would seem, therefore, that nuclear DNA synthesis is not indispensable for elongation growth of the hypocotyl in etiolated rape seedlings.

Complete inhibition of hypocotyl elongation growth by higher hydroxyurea concentrations may be due to inhibition of mitochondrial DNA synthesis, stopping the multiplication of mitochondria, and leading thereby to a marked decrease of the total mitochondrial activity in the cell.

The unexpected stimulation of hypocotyl growth by the lowest hydroxyurea concentration applied is probably a sign of correlative increase of the growth rate after root growth inhibition.

Premature straightening of the hypocotyl hook in seedlings grown on higher hydroxyurea concentration is more difficult to explain. In this part of the white mustard hypocotyl Capesius and Stöhr (1974) did not find any endomitotic DNA increase but only a slight increase in the amount of DNA, related probably to the transition of part of the nuclei from 2C to 4C.

The here presented observation suggest that the straightening of the bent part of the hypocotyl is the consequence of elongation growth of the cells on the concave side of the hook. The hook straightens when it rises above the filter paper imbibed with the inhibitor. It is therefore, possible that the inhibitor concentration in the cells of the hook is lower than the critical value, and then accelerated growth of this part of the hypocotyl might also be correlated with the inhibition of growth of the lower part of the hypocotyl and the root.

For elucidation of the role of DNA synthesis in elongation growth of the rape hypocotyl cells further anatomical and autoradiographic investigations will be necessary concerning the distribution of growth and endomitotic DNA synthesis along the hypocotyl during growth of etiolated seedlings and parallel anatomical and biochemical studies on the inhibition of hypocotyl growth and nuclear and mitochondrial DNA synthesis after the application of suitable inhibitors.

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Wpływ hydroksymocznika na przebieg kiełkowania i wzrost siewki rzepaku (Brassica napus L.)

Streszczenie

Zbadano wpływ ciągłej inkubacji w 0,2; 0,4 i 0,8 mg/ml hydroksymocznika (HM) na kiełkowanie nasion oraz na wzrost korzenia i hypokotyla etiolowanych siewek rzepaku.

Stwierdzono, że przebieg kiełkowania we wszystkich stężeniach HM jest taki sam jak w kontroli.

Wzrost korzenia jest coraz to silniej hamowany przez wzrastające stężenia HM i ostatecznie całkowicie zatrzymany we wszystkich stężeniach.

Elongacyjny wzrost hypokotyla jest stymulowany przez 0,2 mg/ml HM, hamowany przez 0,4 mg/ml i ostatecznie całkowicie zatrzymany przez 0,8 mg/ml.

Wzmagające się zahamowanie wzrostu wyprostowanej części hypokotyla w wyższych stężeniach HM jest skorelowane z przyspieszonym wyprostowaniem hakowato zgiętej części podliścieniowej.

Wysunięto hipotezę, że zahamowanie endomitotycznej syntezy jądrowego DNA osłabia jedynie elongacyjny wzrost komórek a całkowite jego zatrzymanie jest konsekwencją zahamowania syntezy mitochondrialnego DNA.