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INVESTIGATION OF RADIOPROTECTIVE EFFECTS OF NOVEL N,N'-DISUBSTITUTED THIOUREAS IN IN VIVO AND IN VITRO SYSTEMS OF *PISUM SATIVUM* L.

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Introduction. There are many investigations on man, animals and plants radioprotection. However, in the atomic energy epoch, search for novel, more-effective radioprotectors has greater and up-to-date importance. Increasing attention is paid to investigations of plants in respect to modification of the biological and genetical effect of ionizing radiation [1-5]. We have observed that thiourea derivatives demonstrate some radioprotective effect. For example, some N,N'-disubstituted thioureas as ATB (o'-allylthioureidobenzoic acid), A-2-PTU (N-allyl-N'-2-pyridylthiourea), P-2-PTK (N-phenyl-N'-2-pyridylthiourea) have considerable radioprotective effect [6-8]. Later, we observed that the urea, derivative N-3-chlorophenyl N-2-pyridylurea also demonstrates clear radioprotective effect [9].

Ionizing radiation is used in plant biotechnologies in investigations with theoretical and applied character. It could be important for establishment of effective systems for somatic embryogenesis, androgenesis, asymmetric somatic hybridization and most of all in experimental mutagenesis in vitro [10].

The aim of the presented investigations was to examine the biological activity of the novel compounds – PTB (o'-phenylthioureidobenzoic acid) and ATB using in vivo and in vitro pea systems, respectively.

Materials and methods. Experimental work was performed under laboratory, greenhouse and field conditions. Calibrated seeds of pea (c.v. Gradinsky) were irradiated with gamma-rays (^{60}Co) in a dose of 80 Gy with dose rate 350 rad/min. Irradiated seeds were immediately treated with solution of PTB in three concentrations: 10^{-3}M, 10^{-4}M and 10^{-5}M. As a control were used seeds irradiated with 80 Gy and treated with distilled water. The alone effect of PTB was investigated in the same concentrations (10^{-3}M, 10^{-4}M and 10^{-5}M). As absolute control was used the variant without irradiation and chemical treatment. The influence of PTB on biological action of γ-rays after irradiation alone or after irradiation followed by treatment with the examined substances was assessed by the following criteria: frequency and spectrum of induced chromosome aberrations, percentage of plant germination, radio suppression of stem and root growth under laboratory conditions.

To study the biological effect of new compounds in vitro system for plant development from immature pea embryos (c.v. Sredetz) was used [11]. ATB was added into the culture media in concentrations from 10^{-6}M to 10^{-3}M. When mutagenic treatment was applied, immature seeds were irradiated by ^{60}Co γ-source, and immature embryos were irradiated and cultured in vitro. The effect of ATB or γ-irradiation both

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applied alone or in combination was studied on the basis of several criteria: survival, organogenic potential and shoot growth.

Results and discussion. Data on cytological analysis are presented in Table 1, showing the effect of PTB on the frequency of induced chromosome aberrations in meristem cells of pea roots. The frequency of chromosome disturbances reach 10.7% after γ-irradiation at a dose of 80 Gy. The number of aberrations decreases almost twice as a results of post irradiation PTB treatment. The best effect (5.55% and 5.22%) is achieved after PTB treatment with concentrations of 10^{-3}M and 10^{-4}M, respectively. PTB treatment alone causes chromosome aberrations from 2.4% to 2.9%, which is in the limits of the control.

Analysis of the cytological data (Table 1) reveals that the studied substance applied in the post γ-irradiation period has caused three folds decrease in the frequency of chromosome bridges, i.e. in the recombinant type of chromosome disturbances. Out of 51 chromosomal and chromatid bridges (4.74%), they decreased to 17 at 10^{-4}M, which represents 1.39%. The frequency of the observed fragments decreased from 3.81% to 0.82% (variant 3), i.e. the chromosome fragmentation decreases more than 4 times. The most pronounced radioprotective effect was at concentration of 10^{-4}M.

Table 2 presents data about PTB influence alone and after irradiation followed by treatment with this substance on the first generation (M_1). As a result of the applied post-irradiation treatment with concentrations of 10^{-4}M and 10^{-5}M, the percentage of germinated seeds has increased by 46%. Higher concentration of 10^{-3}M has inhibitory effect on the process of germination. The substance under investigation has considerable effect on the initial growth of stem and root system (Table 3). The laboratory test of 14-day-old plants shows that irradiation at a dose of 80 Gy caused significantly shorter stem (from 15.15 to 6.15 cm). In that case, the radiation depression is calculated to be 59.41%. In the case of post-irradiation treatment with 10^{-4}M PTB, the stem height is 10.57 cm, compared to 6.15 cm, and radiation depression decreases dramatically to 30.23%. Stem weight increases from 0.25 g in the case of irradiation to 0.42 g when 10^{-4}M PTB treatment is applied. The radiation depression decreases from 56.14% to 26.32%, i.e. more than two folds.

During root growth radiation depression decreases 10 folds, from 36.71% to 3.41%. Here the highest concentration of 10^{-3}M had inhibitory effect on the root development,

Table 1

Cytological analysis of radioprotective effect of o′-PTB on pea (*Pisum sativum* L.) c.v. Gradinsky

Variants	Total nmb of anaphases	Normal anaphases	Anaphases with aberration		Bridges				Fragments				Backward chromosomes	Micronucleus
					chromosomal		chromatid							
	nmb	nmb	nmb	%	nmb	%	nmb	%	nmb	%	nmb	%	nmb	nmb
Control – H_2O	1380	1354	26	1.88	13	0.94	-	-	1	0.07	3	0.22	9	-
γ 80 Gy+H_2O	1075	960	115	10.70	45	4.19	6	0.56	31	2.88	10	0.93	23	-
γ 80 Gy+o′-PTB 10^{-3}M	1171	1106	65	5.55	16	1.37	4	0.34	13	1.11	7	0.60	18	7
γ 80 Gy+o′-PTB 10^{-4}M	1207	1144	63	5.22	13	1.07	4	0.32	6	0.50	4	0.33	26	10
γ 80 Gy+o′-PTB 10^{-5}M	862	806	56	6.50	14	1.62	3	1.04	9	1.04	7	0.81	23	-
o′-PTB 10^{-3}M	1504	1461	43	2.86	16	1.06	1	0.06	1	0.07	5	0.33	14	6
o′-PTB 10^{-4}M	1304	1266	38	2.91	9	0.69	6	0.46	3	0.23	7	0.54	9	4
o′-PTB 10^{-5}M	1499	1463	36	2.40	13	0.87	-	-	3	0.20	4	0.27	13	3

nmb – number

Table 2

Effect of o′-PTB alone or after irradiation on seed germination of pea (*Pisum sativum* L.) c.v. Gradinsky

Variants	Seeds, number	Germinated plants, number	Germinated plants, %	Germination in comparison with control
Control $+H_2O$	100	44	44	100.00
γ 80 Gy $+H_2O$	100	26	26	100.00
γ 80 Gy + o′-PTB 10^{-3}M	100	23	23	88.46
γ 80 Gy + o′-PTB 10^{-4}M	100	38	38	146.15
γ 80 Gy + o′-PTB 10^{-5}M	100	38	38	146.15
o′-PTB 10^{-3}M	100	16	16	36.36
o′-PTB 10^{-4}M	100	39	39	88.63
o′-PTB 10^{-5}M	100	38	38	86.86

as well, while concentrations of 10^{-4}M and 10^{-5}M PTB had proven radioprotective effect on stem and root growth.

The effect of ATB on pea immature embryos in vitro is presented in Table 4. There is no significant effect of ATB, with the exception of the suppressive highest concentration of 10^{-3}M on the percentage of bud formation. However, stimulation effect of development at concentrations of 10^{-5}M, 5×10^{-5}M and 10^{-4}M was observed, taking as criteria the number of buds formed by an explant from 3.26 - 4.28 compared to 2.33 in the control and the bud site being 0.4 - 0.6 cm for the control and 0.6 - 0.8 cm in the cases of 5×10^{-5}M and 10^{-4}M ATB. After irradiation of immature embryos at dose of 15 Gy which is close to LD_{50} for organogenic processes, ATB treatment has radioprotective effect. The percentage of explants forming buds increases to 78.9% and 100% at 5×10^{-5}M and 10^{-4}M ATB, respectively, compared to 64.9% at 15 Gy. The mean number of buds is restored and the size of buds is normal (Table 5).

Stimulation effect on the growth of irradiated shoots into plantlets was detected too. Buds developed from irradiated explants could grow to 3.96 cm on media supplemented with ATB, while the height of the control plantlets was 3.11 cm.

This system can be applied in experimental mutagenesis in vitro, as well as to create a model system to study the mutagenic effect of environmental pollution.

At this stage of investigations, it is difficult to the explain the exact mechanism of radioprotective effect of these substances. It is known that thioureic bridge of PTB is linked with the nitrogen atoms by benzene nucleus, as one of them has carboxyl group in o′-position. The structure of ATB is analogous, as one N-atom is linked with allyl

Table 3

Effect of o′-PTB on initial growth and development of 14-day-old plants of pea (*Pisum sativum* L.) c.v. Gradinsky under greenhouse conditions

Variants	Roots				Stems			
	length, cm	depression, %	weight, g	depression, %	height, cm	depression, %	weight, g	depression, %
Control $+H_2O$	8.50	-	0.19	-	15.15	-	0.57	-
γ 80 Gy $+H_2O$	5.38	36.71	0.14	26.32	6.15	59.41	0.25	56.14
γ 80 Gy + o′-PTB 10^{-3}M	4.80	43.53	0.12	36.84	7.10	53.14	0.28	50.88
γ 80 Gy + o′-PTB10^{-4}M	7.92	6.82	0.18	5.26	10.57	30.23	0.42	26.32
γ 80 Gy + o′-PTB 10^{-5}M	8.21	3.41	0.20	5.26	8.98	40.73	0.36	36.84
o′-PTB 10^{-3}M	7.33	13.76	0.21	10.53	15.04	0.73	0.54	5.26
o′-PTB 10^{-4}M	8.00	5.88	0.18	5.26	15.40	1.65	0.51	10.53
o′-PTB 10^{-5}M	8.23	3.18	0.19	5.26	14.20	6.27	0.53	7.02

Table 4

Effect of o′-ATB on pea (Pisum sativum L.) development in vitro

Variants	Bud forming, %	Average number of buds	Size of buds, cm
Control	95	2.33	0.4 - 0.6
o′-ATB 1×10^{-6}M	100	2.62	0.4 - 0.6
o′-ATB 5×10^{-6}M	90	2.24	0.4 - 0.6
o′-ATB 1×10^{-5}M	95	3.26	0.4 - 0.8
o′-ATB 5×10^{-5}M	95	4.28	0.6 - 0.8
o′-ATB 1×10^{-4}M	100	3.28	0.6 - 1.0
o′-ATB 1×10^{-3}M	70	1.82	0.1 - 0.2

Table 5

Radioprotective effect of o′-ATB on in vitro development of pea (*Pisum sativum* L.) after irradiation of explants with γ-rays

Variants	Bud forming, %	Average number of buds	Size of buds, cm
Control	90.0	2.27 ± 0.17	0.5-0.7
γ 15 Gy	64.9	1.66 ± 0.21	0.2-0.4
o′-ATB 5×10^{-5}M	100.0	2.20 ± 0.18	0.5-0.8
o′-ATB 1×10^{-4}M	100.0	3.10 ± 0.22	0.5-1.0
γ 15 Gy + o′-ATB 5×10^{-5}M	78.9	2.00 ± 0.25	0.5-1.0
γ 15 Gy + o′-ATB 5×10^{-4}M	100.0	2.33 ± 0.25	0.5-1.0

group and the other – with a benzene nucleus in which also a carboxyl group is in o′-position.

Conclusion. The radioprotector PTB belonging to the thiourea group was created. Applied after irradiation of seeds this compound decreases significantly the frequency of chromosome aberrations and the level of induced radiation suppression at active concentrations of 10^{-4}M and 10^{-5}M. The treatment with PTB has favorable effect on the initial development of pea. The protective effect is more pronounced on the height and weight of the stem than on the root system.

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