Journal of Environmental Protection and Ecology 2, No 2, 293–298 (2001)

Environmental radioactivity

MODIFYING EFFECT OF DIPHENYLUREA ON GAMMA-IRRADIATED SEEDS OF BEANS (*Phaseolus vulgaris* L.)

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Abstract. The importance of the problem of the radioactive contamination of agricultural products is determined mainly by the necessity to find ways of restricting and reducing the effects of this phenomenon. The practical use of synthetic growth regulators can be a successful approach to it. The aim of the research was to study the modifying effect of diphenylurea on young bean plants (*Phaseolus vulgaris* L.) grown from pre-sowingly irradiated seeds.The results show that the synthetic growth regulator diphenylurea modifies the growth processes, the leaf gas-exchange and some enzyme systems in young bean plants, disturbed as a result of the gamma-irradiation of the seeds.

Keywords: young bean plants (*Phaseolus vulgaris* L.), gamma-irradiation of the seeds, diphenylurea, leaf gas-exchange.

AIMS AND BACKGROUND

The anthropogenetic contamination of the environment is a major ecological problem. In recent years the sources of radioactive contamination have been treated with increased public and scientific interest.

It was established that gamma-irradiation with high doses causes stress in the plants. This causes a series of negative effects on the growth and development of the crops, and affects almost all physiological processes. It was found that the irradiation of seeds with high doses of gamma-rays disturbs the synthesis of nucleic acids[1], protein[2,3], hormone balance[4], leaf gas-exchange and enzyme activity[5].

Of no less interest to the science is the search for ways of restricting and reducing the effects of this phenomenon. A successful approach to it is the use of growth regulators as radioprotectors and modifiers of the ray stress in the plants[6,7]. The resistance of the cereal crops to unfavourable environmental factors is increased when they are treated with cytokinins of phenylcarbamide type in the early phases of the vegetation. The protective effect of diphenylurea on the gamma-irradiation in beans has not been studied yet. We have presumed that if it does have positive effect on the gamma-irradiated bean seeds, it can be used to modify and improve the growth processes inhibited by the irradiation.

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The main objective of the research was to study the effect of the synthetic growth regulator of cytokinin type N-N'-diphenylurea, as a modifier of the ray stress in young bean plants (*Phaseolus vulgaris* L.), grown from pre-sowingly irradiated seeds.

EXPERIMENTAL

Dry bean seeds, cultivar Plovdiv 10, were irradiated with two doses of gamma-rays Co^{60}, close to those causing moderate and strong radiation stress – 150 and 200 Gy. The irradiated and the control seeds were superficially treated with a 1% (w/v) solution of calcium hypochloride – $Ca(OCl)_2$ in 10% (w/v) ethanol. Some of the irradiated and non-irradiated seeds were treated (soaked) with a solution of N-N'-diphenylurea with a concentration of 10 mg/l for 16 h. The seeds germinated in thermostat at a temperature of 22-24°C.

The plants were grown as substrate crops (Pertit) in 50% nutrient solution of KNOP, and micro elements from A to Z (Hogland), in a climatic box under the following conditions: light intensity 200 $\mu mol\ m^{-2}s^{-1}$ (PAR), photo period 14 h (light), 10 h (darkness), temperature of 22±2°C/18±2°C day/night and relative humidity of the air 60-70%. On the 30th day of their development, an analysis of the growth[8], the leaf gas-exchange by means of a Photosynthetic Measuring System LCA-4 (UK), and the content of plastid pigments[9], was made.

RESULTS AND DISCUSSION

The growth analysis allows us to assess the degree of the caused by the stress impact disturbances in the main physiological processes – photosynthesis, water exchange, respiratory exchange, etc.

The results in Table 1 show that the applied doses of irradiation of 150 and 200 Gy inhibit the growth parameters of the young bean plants. The relative growth rate (RGR) is reduced with 13 and 36%, respectively. The modifying effect of diphenylurea is more strongly expressed at lower doses of irradiation. The changes in the neto assimilation rate (NAR) index, indicating the rate of the netophotoassimilation in plants, follow almost closely the tendency of RGR, regarding both the radiation stress and the recovering effect of diphenylurea. The decrease of the correlation leaf area/dry tissue (LAR) and the specific leaf area (SLA) is more considerable at 200 Gy. There is a tendency towards an increase of these indices in treated seeds as a result of the cytokikin effect of the substance. The lowered results for relative weight of roots (RWR) and the lowered values of SLA and LAR give us a reason to presume the presence of disturbances in the process of water exchange in plants, and those of NAR - disturbances in the processes of photosynthesis and respiration. What can also be of interest is to study the effect of the substance diphenylurea on these processes.

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Table 1. Influence of gamma-rays on the basic indices of growth in young bean plants, cultivar Plovdiv 10

Indices	Variants					
	0 Gy	0+DU	150 Gy	150+DU	200 Gy	200+DU
RGR (mg/g/day)	68±2.2	73±2.6	58±4.1*	64±3.2	46±2.6***	52±1.5*
%	100	107	87	94	64	76
LAR (cm^2/g)	0.376±0.012	0.379±0.028	0.372±0.015	0.382±0.001	0.356±0.035	0.366±0.026
%	100	100	96	101	94	97
NAR($g/cm^2/day$)	0.180±0.06	0.217±0.05***	0.155±0.05**	0.167±0.08	0.117±0.08***	0.154±0.02**
%	100	120	86	92	65	85
SLA ($cm^2/g/day$)	0.748±0.01	0.682±0.09***	0.552±0.07	0.731±0.01**	0.346±0.02***	0.549±0.02***
%	100	91	72	97	46	73
PWR (g/g)	0.227±0.008	0.220±0.030	0.190±0.038	0.204±0.006	0.151±0.040	0.202±0.060
%	100	97	85	89	66	88
LA (cm^2)	146.21±11.2	168.15±3.15	136.15±2.80*	150.20±2.60*	71.32±1.10***	98.30±4.15*
%	100	115	93	102	48	78

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2. Transpiration (E – mmol H_2O $m^{-2}s^{-1}$) and stomato conductivity (g_s – mol $m^{-2}s^{-1}$)

Indices	Variants					
	0 Gy	0+DU	150 Gy	150+DU	200 Gy	200+DU
E	4.10±0.16	4.15±0.25	3.60±0.15***	3.80±0.11**	2.95±0.19*	3.48±0.015
%	100	101	89	91	74	84
g_s	0.12±0.02	0.15±0.5	0.10±0.05	0.10±0.02	0.07±0.01	0.09±0.02
%	100	125	83	88	58	77

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2 shows the results of measuring of transpiration and the stomata conductivity in control and test plants. The results show that and at the two different doses of irradiation the two values, being measured, decrease, and at the higher dose there is a more considerable inhibition – 26% (E) and 42% (g_s) in comparison to the control, respectively. The stronger inhibition probably is a result of the suppressed growth of the root system, which we have observed in plants grown from irradiated seeds, and hence the disturbed water supply and the closure of the stomata cells. We have registered a more strongly expressed modifying effect of diphenylurea with regard to the higher dose of irradiation – 200 Gy, and this holds true for both indices – (E) 10% and (g_s) 20% higher than the irradiated ones. As a growth regulator of cytokinin type the diphenylurea has a stimulating effect on the root system, which restores the disturbed water supply of the cells, and hence optimizses the transpiration.

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The changes in the photosynthesis rate and the plastid pigments in the control and test plants are presented in Table 3.

Table 3. Photosynthesis rate (A – μmol CO_2 $m^{-2}s^{-1}$) and content of pigments (mg/g fr.w.) in young bean plants, the cultivar Plovdiv 10

Indices	Variants					
	0 Gy	0+DU	150 Gy	150+DU	200 Gy	200+DU
A	6.53±0.18	6.65±0.28***	506±0.32***	5.66±0.38***	4.41±0.35***	5.15±0.52***
%	100	103	81	88	67	78
Chloroph. "a"	0.942±0.05	0.969±0.04	0.954±0.03	0.950±0.03	0.930±0.06	0.940±0.05
%	100	103	102	101	99	100
Chloroph. "b"	0.320±0.01	0.322±0.06	0.332±0.01	0.335±0.02	0.305±0.01	0.310±0.02
%	100	105	102	101	95	98
Carotinoids	0.318±0.01	0.328±0.02	0.336±0.02	0.330±0.01	0.320±0.01	0.320±0.04
%	100	103	102	100	100	100
Chl. "a" + "b"	1.263	1.291	1.283	1.285	1.235	1.250
%	100	102	101	102	98	99
Chl. "a"/"b"	2.943	3.028	2.873	2.923	3.046	3.032
Chls/carot.	3.971	3.935	3.935	3.898	3.859	3.906

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The results show that there is a tendency towards a decrease of the rate of this process in both of the irradiated variants (plants), and at the higher irradiation dose it is 33%. One of the reasons for this may be the decreased transpiration intensity – 26% (Table 2), and on the other hand, this may be caused by the changes in the concentration and the correlation between the plastid pigments, which participate in the light reactions of the photosynthesis. In plants, grown from treated seeds, there is a clearly expressed tendency towards a more intensive photosynthesis " and at 150 Gy the recovering effect of the growth regulator is 7%, and at 200 Gy – 11% higher than in the irradiated seeds.

The data on the changes in the plastid apparatus of the young bean plants subjected to moderate and strong radiation stress do not differ considerably from the control. The insignificant deviations in the concentration of and the correlation between the pigments are within the norm and are not the cause of the observed tendency towards a decrease of the photosynthesis rate in the irradiated plants. This is probably a result of the decreased LAR, and hence of SLA.

In the plants grown from seeds treated with this substance there is a tendency, yet weaker, towards an increase of the chlorophyll content. It is a well-known fact that the cytokinins, to which type is diphenylurea, not only prevent the chlorophyll destruction, but also cause a new synthesis.

The data on the modifying effect of the synthetic growth regulator diphenylurea on the respiration intensity in young bean plants, the cultivar Plovdiv 10, are shown

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in Table 4. It is evident that the applied doses of irradiation increase the intensity of the leaf respiration during darkness with 12 and 23%. This is probably due to the energy deficiency caused by the inhibited photosynthesis.

Table 4. Respiration (R_D – mg CO_2) and peroxidase activity (PA – in UE/g.fr.w.min^{-1})

Indices	Variants					
	0 Gy	0+DU	150 Gy	150+DU	200 Gy	200+DU
R_D	0.619±0.052	0.460±0.035*	0.690±0.015	0.530±0.080*	0.773±0.033*	0.682±0.056
%	100	74	112	111	123	113
PA	834±24	820±48	975±25**	880±30	1240±21***	980±61***
%	100	99	116	106	150	118

$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

The respiration intensity in plants of the variant 200 Gy+diphenylurea is reduced by 10% as a result of the modifying effect of the substance.

The functional activity of the peroxidase is connected with the extinguishing of the harmful oxygen radicals which number is increased in the case of stress impacts, particularly in the case of gamma-irradiation. The results show increased activity of the enzyme by 16% at 150 Gy and by 50% at 200 Gy and confirm the data of Li Guoquan[10].

As a result of the protective action of the growth regulator the amount of the harmful oxygen radicals is reduced, and the peroxidase activity remains only with 6 and 18% above the control.

CONCLUSIONS

The synthetic growth regulator of cytokinin type N.N′-diphenylurea has a positive effect on the basic parameters of the growth analysis – RGR, LAR, NAR and SLA, and on the photosynthesis rate. It increases the photosynthetic potential of the plants grown from treated seeds, which most probably is a result of the stimulating effect of the substance on the root system and of the optimization of water supply and water potential. Diphenylurea reduces the irradiation effect by normalizing the energy balance, as a result of which the respiration and the peroxidase activity are reduced.

The synthetic growth regulator of cytokinin type diphenylurea has a positive effect on the recovery of the physiological condition of young bean plants, the cultivar Plovdiv 10, which proves its quality of being a radio modifier of the ray stress, a fact which confirms our hypothesis. Under its influence, a metabolism occurs in the young plants, which secures the recovery functions during the vegetation period, and as a result of this the protective activity in the plants is increased and the physiological processes and the disturbed enzyme systems are activated.

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Received 24 November 2000
Revised 5 February 2001

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