ORIGINAL ARTICLE

PHYSIOLOGICAL EFFECTS OF THE SYNTHETIC GROWTH REGULATOR THIDIAZUROL (DROP) ON GAMMA-IRRADIATED STRESS IN PEAS PLANTS *(PISSUM SATIVUM L.)*

ФИЗИОЛОГИЧНИ ЕФЕКТИ НА СИНТЕТИЧНИЯ РАСТЕЖЕН РЕГУЛАТОР ТИДИАЗУРОЛ (ДРОП) СРЕЩУ ГАМА-РАДИАЦИОНЕН СТРЕС ПРИ ГРАХА

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РЕЗЮМЕ

Проведени бяха лабораторни опити с млади растения грах, получени от гама-облъчени и необлъчени семена. Беше проучена възможността, синтетичния растежен регулатор Дроп да се използва като протектор срещу гама-радиационен стрес. Семената бяха облъчени с 80 и 100 Gy Co^{60} и поставени за покълнване в разтвор на Дроп в концентрация 5.10^{-4} M. Растенията бяха анализирани на тридесетия ден от тяхното развитие. Бяха определени биометрични параметри, листен газообмен и съдържание на пластидни пигменти. Беше отчетено, че под влияние на облъчването се индуцира стрес, който се изразява в депресия на растежа, свежата и сухата маса на растенията.

Уастановено беше също, че синтетичния растежен регулатор Дроп проявява модифициращ ефект срещу гама-облъчването на семена от грах, който се изразява в активиране на листния газообмен и синтеза на пластидни пигменти в тридесетдневните грахови растения.

КЛЮЧОВИ ДУМИ: гамарадиационен стрес, синтетичен растежен регулатор, растеж, листен газообмен, фотосинтетични пигменти

ABSTRACT

Young plants, obtained from the control and gamma-irradiated seeds, were studied in laboratory experiments. The possible protective effect of the synthetic growth regulator Drop, applied after the stress, was studied.
The seeds were irradiated with 80 and 100 Gy Co^{60} and germinated in 5.10^{-4} M water solution of Drop. 30-day-old plants , obtained from the control, gamma-irradiated and treated seeds, were studied .
Biometric parameters, leaf gas-exchange and plastid pigments contents were measured. Under stress conditions, the growth rates of the γ-ray-treated seeds indicated negative effect in plants, recorded by the decrease of the height, fresh and dry masses, in comparison with the control.
It was established that Drop had a modifying effect on gamma-irradiation, which effect was expressed in the growth and in the leaf gas-exchange of the 30-day-old plants.

KEY WORDS: gamma-irradiation stress, synthetic growth regulator, growth, leaf gas-exchange, photosynthetic pigments

Manuscript received: 20 October, 2002.
Accepted for publication: 25 November, 2002.

JOURNAL Central European Agriculture
ISSN 1332-9049

DETAILED ABSTRACT

The irradiation of seeds with high doses of gamma-rays disturbs a number of physiological processes - the synthesis of nucleic acids , protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity. In the case of moderate stress, the adaptability capacity of the plants is preserved and the observed changes are reversible. There are only few studies on the effect of the synthetic plant growth regulators in plants that have already been subjected to gamma-irradiation stress. The synthetic growth regulator Drop (N-Phenil-N-1,2,3-Thidiaziarol-5-UREA) has cytokinin activity. It is more active than 6-Benzil-amino-purine and in contrast to the purine cytokinins, it has no toxic effect. The phytoprotective effect of Drop on the gamma-irradiation has not been studied yet.

Three independent series of laboratory experiments with thirty-day-old peas plants, cultivar Ran 1, were carried out in a climatic box. The plants were grown as a substrate culture in a Knop nutrient solution, enriched with microelements. The experimental design involved the following treatments:1-control; 2 - irradiated seeds (80 Gy Co^{60}); 3 - irradiated seeds (80 Gy) + Drop (5.10^{-4} M) before sowing; 4 - irradiated seeds (100 Gy); 5- irradiated seeds (100 Gy)+ Drop before sowing. Growth parameters, leaf-gas-exchange and photosynthetic pigment content were measured.

As a result of the study of the parameters characterizing the protective effect of the synthetic regulator Drop in pea plants subjected to gamma-irradiation stress, the following conclusions can be drawn:

The synthetic growth regulator Drop - a compound of Phinilcarbamide nature - manifests a clear and strong cytokinin effect in young peas plants, and neutralizes partly the phytotoxic effect (growth, fresh and dry biomass, leaf area) of the gamma-ray-stressed seeds, and this is expressed better at the lower dosage.

The decreased rate of the net photosynthetic assimilation in plants, subjected to gamma-irradiation was due to the photosynthetic inhibition. The depressed photosynthetic rate results from stomatal and mesophyll limitation. Drop increases the CO_2-fixation rate in the treated peas plants. The protective effect of the Drop was established in terms of all parameters characterizing the leaf-gas exchange and the photosynthetic pigments in the stressed plants.

INTRODUCTION

It is a well-known fact, that in case of gamma-irradiation stress significant changes occur in the plant physiological and biochemical processes. The irradiation of seeds with high doses of gamma-rays disturbs the synthesis of nucleic acids [12], protein [22; 15], hormone balance [16], leaf gas-exchange [18], water exchange and enzyme activity [19]. The morphological, structural, and the functional changes depend on the strength and the duration of the gamma-irradiation stress. In the case of moderate stress, the adaptability capacity of the plants is preserved and the observed changes are reversible.

Aladjadjiyan [1, 2] also investigated the influence of some physical factors on the biological habits of peas.

The plant growth regulators (auksins, giberlines, cytokinins , etc.) and their synthetic analogues stimulate the plants physiological and biochemical processes. This provides an opportunity for them to be used as antidotes to different types of stress [3, 7, 8, 14].

There are only few studies on the effect of the synthetic plant growth regulators in plants that have already been subjected to gamma-irradiation stress [13, 18, 19].

The synthetic growth regulator Drop (N-Phenil-N-1,2,3-Thidiaziarol-5-UREA) has cytokinin activity. It was put forward by 'Shering' (Germany) as a cotton defoliant. Drop is more active than 6-Benzil-amino-purine [6], and in contrast to the purine cytokinins, it has no toxic effect [7, 8] . Drop stimulates the photosynthetic rate [7], the synthesis of beta-cyanine and retards the ageing of tissues [8]. The phytoprotective effect of Drop on the gamma-irradiation has not been studied yet.

MATERIAL AND METHODS

Plant and growth conditions: Three independent series of laboratory experiments with thirty-day-old pea plants, cultivar Ran 1, were carried out in a climatic box at a light intensity of 200 μmol (PAR) m^{-2} s^{-1} , 14 h photoperiod, day/night temperature of 22±2 °C/18±2 °C, and relative air humidity of 70 %. Plants were grown as a substrate culture in a Knop nutrient solution, enriched with microelements. The experimental design involved the following treatments:1-control; 2 - irradiated seeds (80 Gy Co^{60}); 3 - irradiated seeds (80 Gy) + Drop(5.10^{-4} M) before sowing; 4 - irradiated seeds (100 Gy); 5- irradiated seeds (100 Gy)+ Drop before sowing.

Growth parameters: Growth parameters were determined in the 30–day-old plants. Plant material was rinsed in deionized water and blotted. Dry masses of the separate organs were measured by drying the shoot and root at 75 °C, in order to give a constant mass. The leaf area was measured with a digital area meter *NEO-2* (*Technical University*, Sofia, Bulgaria). Growth analysis was made according to Beadle [5].

Leaf gas-exchange: The net photosynthetic rate, transpiration rate and stomatal conductance of the youngest fully developed intact leaves were measured with a portable infrared gas analyzer LCA-4 (Analytical Development Company Ltd,. Hddesdon, England) with a PLCB-4 chamber. Measurements were made under irradiance of 800 μ mol (PAR) m-2 s-1, at temperature of 26±2 °C, and external CO2 concentration of 400 μ mol mol-1 and a relative air humidity of 70 %.

Photosynthetic pigments: Total chlorophyll and carotenoids in the pea plants were extracted in 80 % acetone. The pigments were determined spectrophotometrically after centrifugation of the extract at 3000 g for 15 minutes [20] and calculated according to the Lichtenthaler and Wellburn [11] formulae.

Three independent experiments, each with 5 repetitions per treatment, were conducted. The results showed similar tendencies. Data from one representative experiment are given in this work. The significance of the differences between control and each treatment was analyzed by Student’s *t*-criterion.

RESULTS AND DISCUSSION

The growth analysis allows preliminary evaluation of the degree of damage induced by stress effects in the main physiological processes of the stressed plants.

Our data (Figure 1A) showed the presence of changes in the growth parameters of pea plants, subjected to gamma irradiation stress. A significant inhibition of the linear growth of gamma-stressed plants was established. Plant height decreased by 27 % (80 Gy) and 57 % (100 Gy), and the longest root length – by 30 and 59 % respectively, as compared to the control. The synthetic growth regulator Drop modified partly the gamma ray effect on those parameters, and this was better expressed at the lower doze (80 Gy) of irradiation – 11-19 % below of the control.

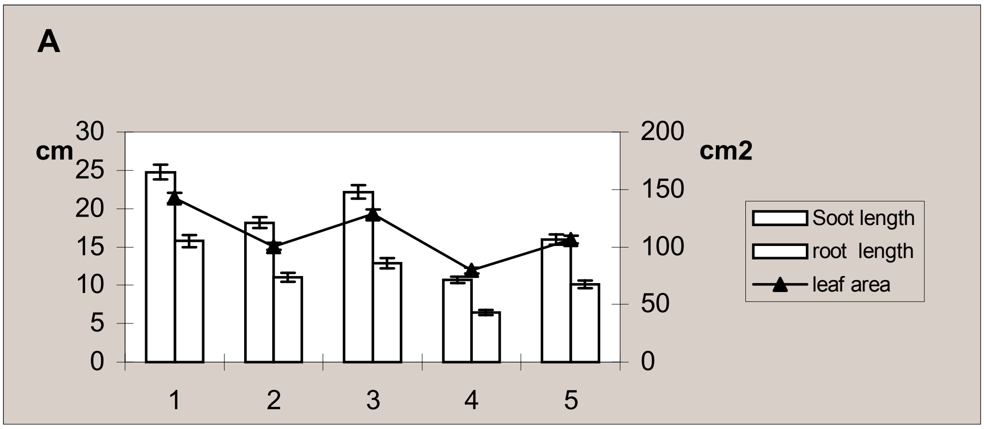
The changes in the fresh and dry biomass follow the same tendency (Figure 1 B).

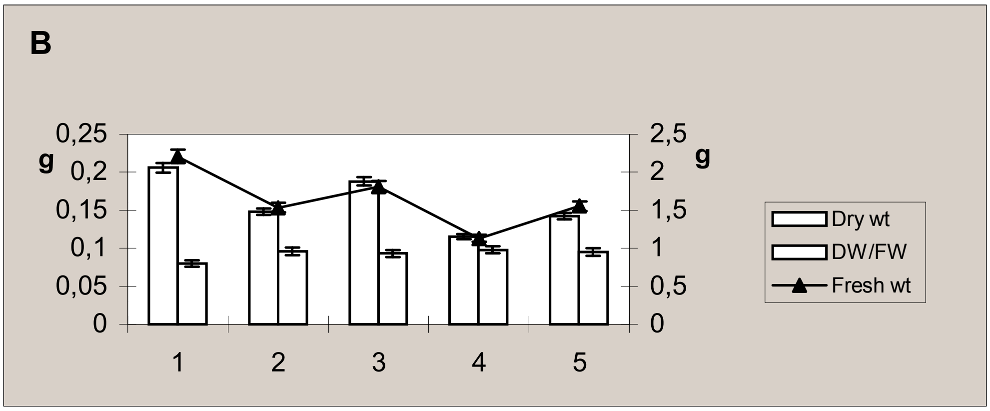
The increased correlation of dry and fresh mass (DW/FW) is a criterion indicating the presence of stress in plants [4] and in those, grown from irradiated pea seeds; it increases by 20 and 23 %. In the seeds treated with Drop, the correlation between DW and FW reaches the control level (10-15 % below of the control).

There occurs a reduction in the photosynthetic leaf area. This reduction is more considerable in the case of irradiation of the seeds with 100 Gy - 46 % below the control. Drop, when applied simultaneously with the stress factors, affected this parameter 22 % weaker than that of the control – non-irradiated.

The differences between the variants were significant and mathematically proven, ($p<0.1$ – 0.01).

Figure 1: Changes in the growth parameters (A) and the fresh and dry biomasses (B) of peas plants.





1 - control; 2 - irradiated seeds 80 Gy; 3 - irradiated seeds 80 Gy + Drop; 4 - irradiated seeds 100 Gy; 5- irradiated seeds 100 Gy + Drop.

The relative growth rate (the most integral parameter reflecting the state of growth process) in the plants obtained from the irradiated seeds, decreased by 36 and 46 % against the control (Table 1). The changes in the NAR parameter, characterizing the net assimilation rate, decreased, too (21 and 32 %). The RGR inhibition was mainly due to the suppression of NAR, and to a much lesser extent, to that of LAR. LAR decreased by 17 and 22 %, and SLA – 15 – 18 %. Drop reduced the effect of the gamma-stress on the parameters of the growth and they remained at about 10 % smaller than the control.

Table 1: Influence of gamma-rays and Drop on growth parameters in young peas plants.

	0 Gy	80 Gy	80 Gy+Drop	100 Gy	100 Gy+Drop
RGR	0.051±0.002	0.033±0.002 (64)***	0.046±0.001 (90)***	0.028±0.002 (54)***	0.040±0.002 (78)***
LAR	0.870±0.011	0.724±0.020 (83)***	0.811±0.018 (93)**	0.688±0.015 (78)	0.792±0.010 (91)***
NAR	0.587±0.019	0.464±0.11 (79)	0.573±.006 (98)	0.485±0.007 (68)	0.515±0.005 (88)
SLA	2.189±0.12	1.853±0.18 (85)	2.591±0.15 (140)***	1.932±0.15 (82)*	1.980±0.12 (90)
RWR	0.447±0.01	0.342±0.01 (77)***	0.399±0.02 (88)**	0.352±0.01 (79)***	0.406±0.02 (90)*
SWR	0.326±0.04	0.269±0.02 (82)	0.226±0.01 (67)	0.196±0.02 (58)**	0.233±0.02 (70)
LWR	0.398±0.02	0.350±0.11 (87)	0.376±0.03 (94)	0.315±0.02 (76)	0.360±0.01 (90)*

RGR-relative growth rate [mg g-1 day-1]; LAR-leaf area ratio [cm2 g-1]; NAR- net assimilation rate [mg cm-2 day-1]; SLA-specific leaf area [cm2 g-1]; LWR-leaf weight ratio[g g-1]; SWR-stem weight ratio [g g-1]; RWR- root weight ratio[g g-1]; and percentage of control. Values are means of 3 separate experiments±S.E. (n=15). *p<0.1 **p<0.01 ***p<0.001

A considerable inhibition is observed in the correlation between separate plant organs biomass (leaves, stems, roots) and total plant biomass (LWR, SWR, RWR). These values change to the positive in the variants which after the seeds irradiation were treated with Drop– concentration 5.10^{-4} M.

The negative effect of the gamma-rays on the growth–limiting factors was mainly related to changes in the photosynthesis, the dark respiration and the relative ratio of non-photosynthesizing organs, and to a much lesser extent, to those in the water exchange. The non-photosynthesizing organs in young plants are the roots. Therefore, the main growth-limiting factors in the gamma-ray-stressed plants were related to the photosynthesis and the dark respiration.

Table 2: Effect of gamma-rays and synthetic growth regulator Drop on the leaf gas-exchange in pea plants.

	0 Gy	80 Gy	80Gy+Drop	100 Gy	100 Gy+Drop
A	6.42±0.55	4.35±0.31 (67)**	5.98±0.26 (93)	2.45±0.28 (38)**	3.99±0.11 (62)***
E	2.58±0.21	2.15±0.12 (83)	2.44±0.15 (94)	1.25±0.12 (48)**	1.99±0.18 (77)***
A/E	2.48±0.15	2.08±0.08 (81)*	2.45±0.21 (92)**	1.96±0.11 (79)**	2.08±0.15 (82)
G_S	0.068±0.011	0.052±0.012 (76)**	0.060±0.002 (88)**	0.041±0.014 (60)**	0.049±0.002 (72)

A- photosynthesis rate [µmol CO2 m-2 s-1], E- transpiration intensity [mmol H2O m-2s-1], A/E-photosynthetic water use efficiency [µmol mmol-1] and gs -stomatal conductance[mol m-2s-1]. Values are means of 3 separate experiments±S.E. (n=5). *p<0.1; **p<0.01; ***p<0.001.

Photosynthesis is a unique physiological process, determining, to a great extent, the plant productivity. The data in Table 2 show the changes in the leaf-gas-exchange rate in the pea plants, grown from irradiation-stressed seeds (80 and 100 Gy) and after their treatment with Drop. In the plants, obtained from stressed seeds, a 23 and 52 % decrease in the process intensity was observed, as compared to the control. One of the reasons for that decrease was the reduced transpiration intensity (by 17 and 52 %) and the stomatal conductance (by 24 and 40 %). The photosynthetic water use efficiency, expressed as the

A/E ratio was changed with 19 –22 % in the negative aspect. After treatment with Drop, the negative effect of gamma-irradiation was significantly reduced, but at a doze of irradiation of 100 Gy, the values remained well below the control level, which is an indicator that the plants overcome the stronger stress with much more difficulty.

The decreased photosynthetic rate under stress conditions could be due both to stomatal and mesophyll limitations. The mesophyll factors could be of a different nature, such as disturbances in the pigment apparatus, light and biochemical reactions from the Calvin cycle.

Table 3: Effect of gamma-rays and synthetic growth regulator Drop on the content of total pigments (mg/g fr. w.) in leaves in young pea plants.

Indexes	0 Gy	80 Gy	80Gy+Drop	100 Gy	100 Gy+Drop
Clorophyll a	1.345	0.933 (61)	1.184 (88)	0.754 (56)	0.983 (73)
Clorophyll b	0.667	0.527 (80)	0.557 (83)	0.394 (50)	0.492 (73)
Total Caroten.	0.722	0.587 (81)	0.697 (96)	0.183 (66)	0.588 (81)
Cl. a+b	2.012	1.570 (78)	1.757 (87)	1.188 (59)	1.414 (73)
Cl a:b	2.010	1.470 (87)	1.124 (85)	1.800 (90)	1.875 (94)
Chs a+b/c	2.783	2.067 (96)	2.621 (95)	2.041 (88)	2.500 (90)

As a result of the gamma-irradiation effect, the chlorophyll content (Table 3) in the leaves of pea plants showed significant changes – chlorophyll *a* decreased by 39 and 44 % against the control. The changes in chlorophyll *b* and total carotenoids were between 20 and 59 % in negative aspects. A similar tendency was occurred in the correlation between the pigments. This can be one of the reasons for the reduced photosynthesis rate. The protective effect of the synthetic growth regulators Drop was established in all parameters, characterising the growth and leaf-gas exchange.

The treatment of the gamma-irradiated seeds with Drop leads to a stimulation of the metabolic processes, as a result of which, the studied indexes get close to those of the control plants.

It is believed [21] that the positive effect of Drop on the photosynthesis, the photosynthetic enzymes activity, and the chlorophyll content, and some other photosynthetic parameters is due to its cytokinin character.

Kurasaki [10] elaborates on the possibility that the cytokinins of carbamide type influence the biosynthesis and the metabolism of the cytokinins of adenine type.

The possibility to counteract the negative effect of the gamma-irradiation stress by means of treatment of the seeds with Drop can be connected with the two factors exerting influence on the content of the endogen phytochormones. The gamma-rays disturb the hormonal balance in the irradiated cells by decreasing the amount of the Indolil acetic acid stimulators and the kinetins and increasing that of Abscisic acid and ethylene. The exogen application of the synthetic growth regulator of citokinin type Drop can partially compensate the endogen deficiency of the phytochormone stimulators [9].

In this respect, Drop can be used as an antidote against the gamma-irradiation stress in irradiated seeds, instead of the purine cytokinins, which have mutagenic effect and toxicity.

CONCLUSIONS

As a result of the study of the parameters characterizing the protective effect of the synthetic regulator Drop in pea plants subjected to gamma-irradiation stress, the following conclusions can be drawn:

The synthetic growth regulator Drop - a compound of Phinilcarbamide nature - manifests a clear and strong cytokinin effect in young pea plants, and neutralizes partly the phytotoxic effect (growth, fresh and dry biomass, leaf area) of the gamma-ray-stressed seeds and this is expressed better at the lower dosage.

The decreased rate of the net photosynthetic assimilation in plants, subjected to gamma-irradiation was due to the photosynthetic inhibition. The depressed photosynthetic rate results from stomatal and mesophyll limitation. Drop increases the CO_2-fixation rate in the treated pea plants. The protective effect of the Drop was established in terms of all parameters characterizing the leaf-gas exchange and the photosynthetic pigments in stressed plants.

ACKNOWLEDGMENTS

The plant growth regulator Drop was provided by the Institute of Plant Physiology-Sofia, Bulgaria- for which we express our gratitude.

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