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AN APPARENTLY PROTECTIVE EFFECT OF ADDITIONAL GENOMES ON PLANT DEVELOPMENT AFTER SEED IRRADIATION IN *PISUM ARVENSE* LAMP.[1]

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Summary. Two forms of *Pisum arvense* Lamp., diploid and autotetraploid, were used in the studies. Air-dry seeds were treated with gamma-rays from a cobalt source (^{60}Co) using the doses of 10, 20, 30 and 40 krad.

Seed irradiation impaired the dynamics of sprouts and decreased the percentage of emerged plants. Undesirable changes increased with the application of higher doses of the treatment. The impairment of sprouting dynamics and the decrease of the percentage of emerged plants were significantly lower in autotetraploid form. A relative survival of plants after seed irradiation decreased in both di- and autotetraploids. However, it was found that the response of the forms $2x$ and $4x$ to the same doses of treatment was significantly differentiated. A relative survival of diploid plants in all the cases was higher than that of autotetraploids.

The protective influence of additional genomes of an autotetraploid form of pea, observed during plant sprouting of gamma-1 generation, was levelled at the stage of generative reproduction.

Seed irradiation is one of frequently used methods of induced mutations in plants. A number of contradictory opinions (Jaranowski 1970) have appeared in a rich literature concerning the use of ionizing radiations as a mutagenic factors. Studies o plant radiosensitivity is the first significant stage of investigations dealing with the use of radiation in plant breeding (Gielo 1970). An estimate of radiosensitivity of original induced autotetraploids of *Pisum arvense* Lamp. obtained at the Chair of Genetics and Plant Breeding of the Academy of Agriculture in Poznań was one of the elements of their characteristics.

MATERIAL AND METHODS

Radiosensitivity of an autotetraploid form in comparison with that of initial diploid plants was estimated using the response of gamma-1 plants to seed irradiation at 10, 20, 30 and 40 krad doses. Air-dried seeds were treated with gamma-rays from cobalt source (^{60}Co) in the Isotope Laboratory of the Academy of Agricultur

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in Poznań. In the present studies we employed autotetraploid pea forms constituting C_6 and C_7 generations and a diploid genetic line No. 2/9 being the initial material for polyploidization.

Expecting a decline in the percentage of emerged plants with increasing doses of treatment, different numbers of seeds were sown on the plots to obtain uniform conditions of growth for plants of gamma-1 generation. Thus, in each of three replications, the number of seeds per plot depending on the size of the applied treatment dose was as follows: control (0 krad) and 10 krad — 50 seeds, 20 krad — 65, 30 krad — 80 and 40 krad — 100. Totally, the number of sown seeds of each, diploid and autotetraploid pea, was 1035. The experiment was laid out on the field of the Agricultural Experimental Station at Swadzim in split-plot design.

The effect of radiation of plant material was analysed by:

1. Observing the dynamics of sprouts. Observations were made each 3 days through the period of sprouting. They consisted in the establishment of the number of emerged plants on individual plots;
2. Calculating the per cent of emerged plants (at the seedling stage);
3. Estimating a relative plant survival on the basis of counting the percentage of fruiting plants in relation to the number of emerged plants.

Statistical calculations concerning the dynamics of sprouts were performed using the analysis of curve-linear regression. The obtained curves are presented in diagrams with determination coefficient in per cent for each of them. The calculations were made by the program ABS-37 (Malec, Mejza 1975).

A statistical analysis of the per cent of emerged plants and of a relative survival of plants was performed on the data transformed into Bliss' angular degrees. A multivariate analysis of variance was performed (Caliński, Kaczmarek 1973). It permitted to verify 3 groups of hypotheses concerning the significance of contrasts: between ploidy levels, between all applied doses of treatment with no reference to the ploidy level of the plant material and between all combinations of treatment doses and ploidy levels. Calculations were made using the program ABS-45 (Caliński et al. 1976).

RESULTS AND DISCUSSION

The development of plants in the control and gamma-1 generation was markedly differentiated. Statistically significant differences were found with regard to each of the analyzed characters in the response of the objects to various treatment doses and also in comparisons of an autotetraploid form of pea with the initial diploid material.

DYNAMICS OF SPROUTS

Seed irradiation caused a decrease in the dynamics of sprouts on the both levels of ploidy. The performed analysis of variance for curve-linear regression and the F test function made it possible to determine the significance of individual regressive effects (Fig. 1, 2).

A decrease in sprout dynamics in autotetraploid after all applied doses of treatment was smaller than that in diploids. At the same observation dates sprouts of diploid plants were weaker sometimes by over 50%. A protective action of additional genomes in the autotetraploid form became evident after the application of high doses of the treatment (Fig. 1, 2).

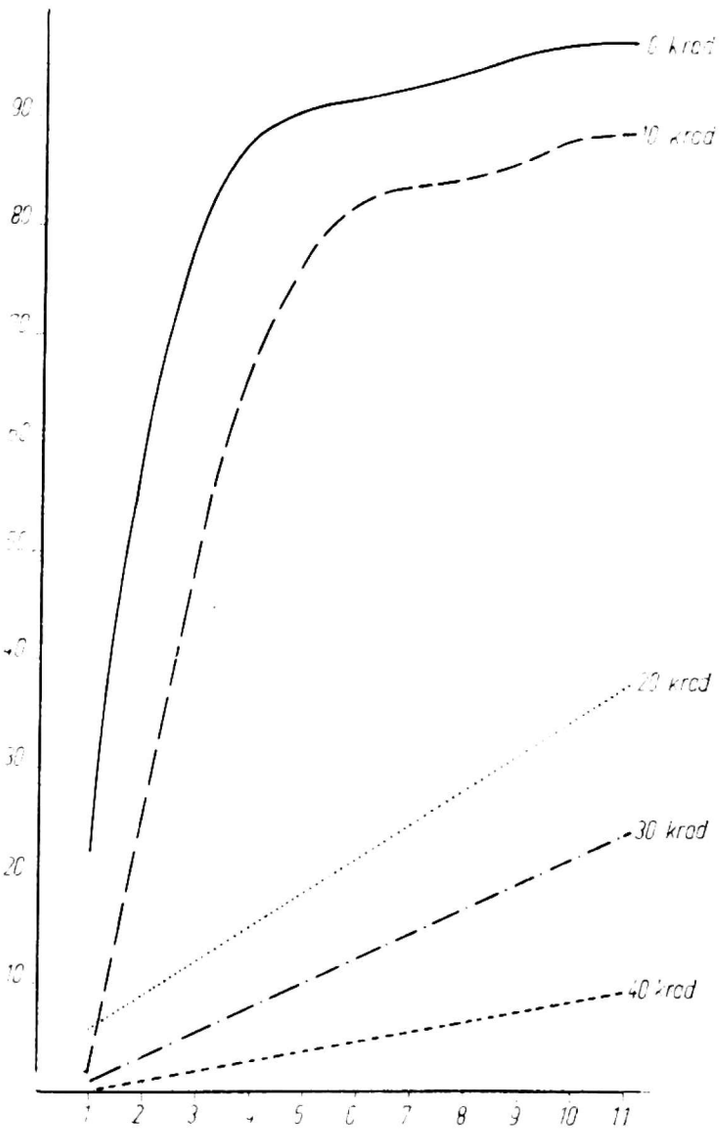

Fig. 1. Dynamics of sprouts after seed treatment of diploid pea with gamma-rays
– vertical axis: sprouts (percentage of emerged plants)
– horizontal axis: date of observations (at 3-day intervals)
Determination coefficients for regression curves:
0 krad $R^2=99.52\%$ 10 krad $R^2=98.70\%$
20 krad $R^2=85.87\%$ 30 krad $R^2=91.99\%$
40 krad $R^2=94.84\%$

Similar results concerning the dynamics of sprouts after seed irradiation were observed also by other authors in several plant species. Delays and changes in the course of sprouting were observed with increasing doses of treatment in barley (Pala 1965), field bean (Sjödin 1962), diploid fodder pea (Vasileva et al. 1969).

PERCENTAGE OF EMERGED PLANTS

Seed irradiation decreased the percentage of emerged plants in both diploid and autotetraploid forms of pea. Calculations performed by the method of simultaneous testing showed that irrespective of the ploidy level the percentage of emerged plants after irradiation of seeds with smaller doses was usually higher. Only differences between the highest treatment doses were not statistically significant. Testing differences concerning combinations of ploidy levels and treatment doses it

was found that after the application of 20, 30 and 40 krad doses the autotetraploid form reached a higher percentage of sprouts than diploid plants, whereas after seed irradiation with a 10 krad dose and in the control combination the percentage of emerged plants on the both ploidy levels was similar (Table 1).

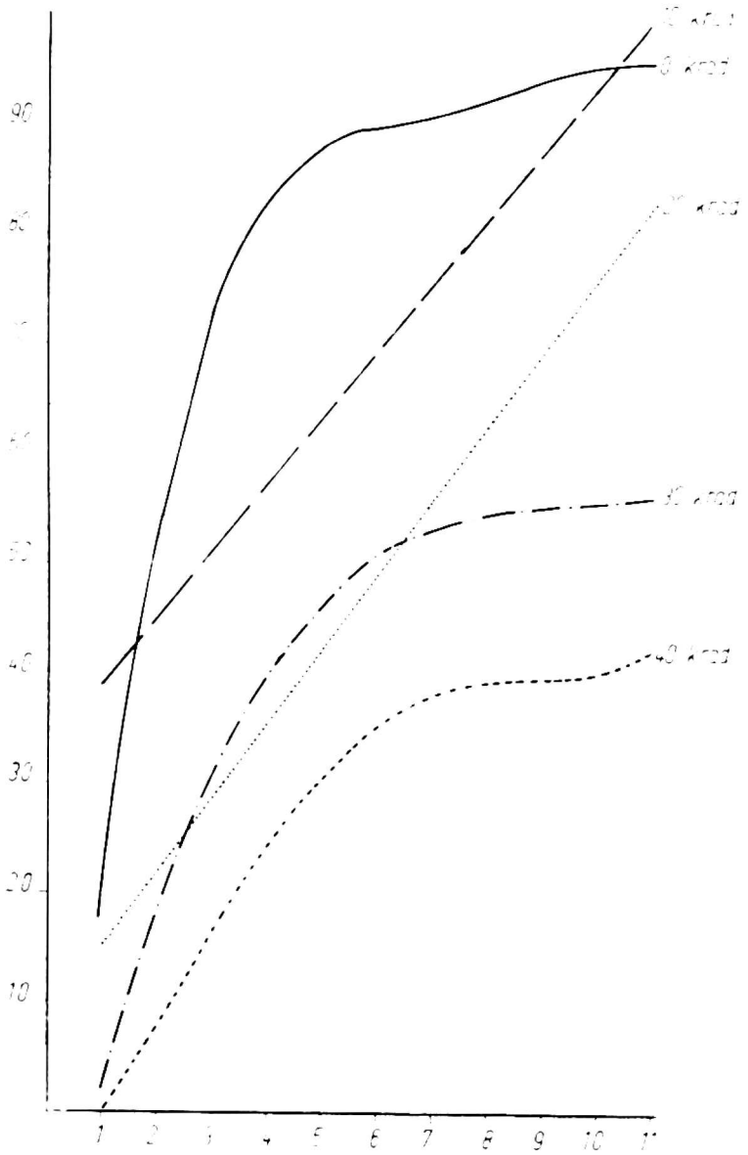

Fig. 2. Dynamics of sprouts after seed treatment of autotetraploid pea with gamma-rays

– vertical axis: sprouts (percentage of emerged plants)
– horizontal axis: date of observations (at 3-day intervals)

0 krad $R^2 = 99.66\%$ 10 krad $R^2 = 57.44\%$
20 krad $R^2 = 79.77\%$ 30 krad $R^2 = 98.64\%$
40 krad $R^2 = 97.84\%$

The obtained results of the studies did not indicate stimulation of sprouts and seedling development caused by low doses of ionizing radiation, which was observed by other authors (Fink 1965, Jaranowski 1970, Muszyński 1970, Pakendorf 1970). It may be that the lowest doses applied in the present studies were, from this point of view, too high for the both levels of ploidy.

It was found that in the diploid form of pea certain doses were critical causing an abrupt fall in the percentage of emerged plants (Jaranowski 1970). During our studies diploids were also noted to have such a phenomenon. Autotetraploids even at a 40 krad treatment dose gave about 40% of sprouts under field conditions. This fact may be related to a protective action of additional genomes of autotetraploid system. This result is analogical to observations made in the case of di-, tetra- and hexaploid wheats and in the case of di- and hexaploid clover (Palenzon 1961, Natarajan et al. 1958). Nilan (1956) also emphasized that seeds of polyploid species are more resistant to the effect of ionizing radiation.

RELATIVE PLANT SURVIVAL

The response of both di- and autotetraploid forms of pea to seed irradiation was a decrease of the relative survival of plants in gamma-1 generation. As a result of testing hypotheses taking into consideration all possible comparisons of doses (omitting ploidy levels) it was found that there is a tendency to a larger decrease of plant survival with the application of larger doses of treatment. However, differences between the largest doses were statistically insignificant. Comparing, however, the response of the forms $2x$ and $4x$ to the same treatment doses it was found to be significantly differentiated. The relative survival of diploid plants was larger than that of autotetraploid forms in all the cases (Table 1).

Table 1. Percentage of emerged plants and their relative survival in gamma-1 generation after seed irradiation of diploid ($2x$) and autotetraploid ($4x$) pea

Treatment dose (krad)	Percentage of emerged plants			Relative plant survival		
	$2x$	$4x$	mean	$2x$	$4x$	mean
0	96.0	94.7	95.3	92.5	78.9	85.7
10	87.3	86.0	86.7	90.1	64.0	77.1
20	31.8	68.2	50.0	65.5	17.9	41.7
30	20.0	56.6	38.3	65.6	12.2	38.9
40	10.3	41.7	26.0	70.6	2.5	36.5
Total means	49.1	69.4	59.3	76.9	35.1	56.0

It should be noticed that the response of many plant species to radiation is similar regarding its direction. The size of changes caused by radiation is, however, species-specific in various species and even specific in the same species. Jaranowski (1970, 1976), studying seven various genotypes of fodder pea, found that at a 50 kR dose the percentage of emerged plants widely ranged between 0 to 42.3% and that the relative survival of plants was from 0 to 78.5%. Blixt (1968) also paid his attention to genotypic, separateness, conditioning sensitivity to gamma radiation.

Changes observed after seed irradiation of di- and autotetraploid forms of pea in the dynamics and percentage of sprouts and in the relative survival of gamma-1 plants present differentiation in autotetraploid radiosensitivity in comparison with the initial diploid material. It seems that protective effect of multifold genomes concerns only somatic damages. Disturbances at the stage of generative reproduction caused a violent limitation of the reproduction ability in the autotetraploid form. The obtained results permit to infer that the absolute plant survival at the both ploidy levels declined after irradiation of seed to a similar extent. These forms differed only by the developmental stage, in which a decreased plant viability being a result of radiation became evident most clearly. The critical moment in diploids occurred earlier (a large reduction in the percentage of emerged plants) and in autotetraploids — at a later stage of ontogenesis (generative reproduction).

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POZORNIE OCHRONNY WPŁYW DODATKOWYCH GENOMÓW NA ROZWÓJ ROŚLIN *PISUM ARVENSE* LAMP. PO NAPROMIENIENIU NASION

Streszczenie

W badaniach wykorzystano dwie formy *Pisum arvense* Lamp.: diploidalną i autotetraploidalną. Powietrznie suche nasiona potraktowano promieniami gamma ze źródła kobaltowego (^{60}Co) stosując dawki: 10, 20, 30 i 40 krad.

Napromienienie nasion wpłynęło na osłabienie dynamiki wschodów i obniżenie procentu wzeszłych roślin. Niekorzystne zmiany nasilały się w miarę stosowania wyższych dawek traktowania. Osłabienie dynamiki wschodów i obniżenie procentu wzeszłych roślin było istotnie mniejsze u formy autotetraploidalnej. Względna przeżywalność roślin pochodzących z napromienionych nasion pogorszyła się zarówno u diploidów jak i autotetraploidów. Porównując

reakcję tych form na takie same dawki traktowania stwierdzono między nimi istotne zróżnicowanie. Względna przeżywalność roślin diploidalnych była we wszystkich przypadkach lepsza niż autotetraploidalnych.

Ochronny wpływ dodatkowych genomów autotetraploidalnej formy grochu, obserwowany w okresie wschodów roślin pokolenia gamma-1, został zniwelowany w fazie reprodukcji generatywnej.

КАЖУЩЕЕСЯ ЗАЩИТНОЕ ВЛИЯНИЕ ДОПОЛНИТЕЛЬНЫХ ГЕНОМОВ НА РАЗВИТИЕ РАСТЕНИЙ ПОСЛЕ ОБЛУЧЕНИЯ СЕМЯН У *PISUM ARVENSE* LAMP.

Резюме

В исследованиях использовались две формы *Pisum arvense* Lamp. диплоидная и полиплоидная. Полиплоидные растения были индуцированными аутотетраплоидами гороха. Диплоидная форма была представлена генетической линией № 2/9, которая использовалась в качестве исходного материала для полиплоидизации. Сухие семена были подвергнуты действию гамма-лучей из кобальтового источника (^{60}Co) в дозах 10, 20, 30 и 40 krad. Полевой эксперимент был проведен в трех повторениях по схеме split-plot. В поколении гамма-1 анализировались динамика и процент всходов, а также относительная выживаемость растений.

Облучение семян ухудшило динамику всходов и уменьшило процент всхожести растений на обоих уровнях плоидности. Неблагоприятные изменения углублялись по мере применения высших доз излучения. Как ухудшение динамики всходов, так и снижение процента проросших растений у тетраплоидной формы было значительно меньше, чем у диплоидной.

Относительная выживаемость растений после облучения семян снизилась как у ди-, так и у тетраплоидов. Однако, при сравнении реакции форм $2x$ и $4x$ на те же самые дозы излучения были обнаружены существенные различия. Относительная выживаемость диплоидных растений во всех случаях была высшая, чем у тетраплоидных растений.

Защитное влияние дополнительных геномов аутотетраплоидной формы гороха, выраженное в высшем проценте всходов растений поколения гамма-1, по сравнению с диплоидами, было выравнено в фазе генеративной репродукции. В результате, абсолютная выживаемость растений на обоих уровнях плоидности снизилась после облучения семян в одинаковой степени.