A survey of chemicals and ionizing radiation for mutagenic action on *Phaseolus vulgaris* L.

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In recent years chemical substances are more often used for the induction of mutation. Among the most effective mutagenic agents, besides ionizing radiation, certain compounds should be taken into account. The most important group of mutagenically active chemicals form the alkylating agents (Héslot 1965). To this group, among others, belongs N-nitroso-N-methylurea (NMH). Alkylating agents are more specifically nucleotoxic in comparison with their general toxicity for the cells, (Ehrenberg 1960 p. 127). Inhibited growth is, among other effects, caused by these type of mutagenic agents. Such inhibition of growth may be due to interaction with the chromosome material.

Among the growth inhibiting factors there are also free radicals produced by the effects of applying ionizing radiation (Sparmann 1959, quoted from Ehrenberg 1960 p. 130—132).

It is known that certain chelate-forming compounds as, for instance, EDTA, although not inducing mutation by themselves in addition to chemical mutagenic agents may augment the effectivity of the latter this is due to the fact that substances of this kind inhibit the reattachment of chromosome fragments and hinder the process of their healing (Gustafsson 1960 p. 21, Rieger and others 1963).

Growth reduction may be caused by making the synthesis of purines more difficult, thanks to the action of certain chemicals hindering the activity of enzymes reducing the folic acid back to the tetrahydrofolic acid. It is known that folic acid together with its derivatives plays an essential role in the purine synthesis (Héslot 1960 p. 109).

In general the increase of the gamma-ray dosage causes the growth of the frequency of mutants. Proportionally to the dose the viability and fertility of plants decreases. Manner (1968) observed that in F_2 and F_3 of crosses with low-viability individuals, obtained from seed treated with high doses of gamma-rays — the frequency of chlorophyll mutants was greater than of crosses with more viable plants.

The present study was aimed at exploring the effects of gamma-rays and of chemical agents on *Phaseolus vulgaris* L., for this purpose

were observed chlorophyll changes and morphogenetic anomalies caused by applying the following mutagenic agents: gamma-rays, as well as N-nitroso-N-methylurea (NMH) alone or together with EDTA. Furthermore, it was a matter of checking how plant growth react to these chemical substances.

MATERIAL AND METHODS

A variety of dwarf kidney bean denoted by No 3 (Bańkowska 1956) was used for the experiment. In the experiment samples of dry seeds were subjected to the action of following doses of gamma rays (^{60}Co source): 3000r, 6000r, 9000r and 12000r. The treatment was performed in Radiobiological Laboratory of Plant Breeding Institute at Radziów.

Irradiated as well as control seeds were sown in pots of garden soil placed in a greenhouse. Within the limits of plants belonging to the M_1 generation — chlorophyll changes and morphogenetic anomalies were the subject for observation. In the M_2 obtained in the progeny of changed M_1 plants were examined the frequency of chlorophyll mutations and morphogenetic changes. The fertility percentage in the series of plants irradiated by the said doses was also computed.

In the experiment on the influence of chemical substances on bean solutions of mutagen N-nitroso-N-methylurea in concentrations of 0.1, 0.5, 1, 1.5 and 3 mmoles were used. As means for the augmentation of the effectivity of NMH, molar concentrations of 50 and 100 mmoles of EDTA were used. The seeds were soaked in distilled water in conical flasks and placed in a thermostat at 24°C for 10 hours. Thereafter the seeds were rinsed in water and then treated with a solution of EDTA of a fixed concentration for 5 hours. After repeated rinsing the seeds were soaked in a NMH-solution of a suitable concentration for 5 hours. Altogether the seeds were treated for 20 hours. All through the time the seeds were soaked the t° was kept at 24°C . Only distilled water was used for the control seed soaking, and it was freshly used with each change of different concentrations of the treated plants. After rinsing the seeds were used in the experiment.

A part of the seeds was used in laboratory tests and the remaining seeds in field experiments. In laboratory tests for growth response of treated plants Künzel's method was applied (Künzel 1968) originally this method was used in experiments on barley. According to this method germinating seeds were placed in rolls made of filter paper, each seed put by a plastic rod to allow a vertical growth of radicles. The whole roll was placed in a 1 lt -beaker with water. The water level was so adjusted that it always reached about 20 mm below the growth apex of the

Table 1
Combinations of chemical compounds applied in the experiments

No. of combination	Combination	Time of soaking in hours		
		10	5	5
1	Control	H ₂ O	H ₂ O	H ₂ O
2	0.1mM NMH	"	"	NMH
3	0.5mM NMH	"	"	NMH
4	1 mM NMH	"	"	NMH
5	1.5mM NMH	"	"	NMH
6	3 mM NMH	"	"	NMH
7	50mM EDTA + 0.1mM NMH	"	EDTA	NMH
8	50mM EDTA + 0.5mM NMH	"	EDTA	NMH
9	50mM EDTA + 1 mM NMH	"	EDTA	NMH
10	50mM EDTA + 1.5mM NMH	"	EDTA	NMH
11	50mM EDTA + 3 mM NMH	"	EDTA	NMH
12	100mM EDTA + 0.1mM NMH	"	EDTA	NMH
13	100mM EDTA + 0.5mM NMH	"	EDTA	NMH
14	100mM EDTA + 1 mM NMH	"	EDTA	NMH
15	100mM EDTA + 1.5mM NMH	"	EDTA	NMH
16	100mM EDTA + 3 mM NMH	"	EDTA	NMH
17	50mM EDTA	"	H ₂ O	EDTA
18	100mM EDTA	"	H ₂ O	EDTA

radicel. During the incubation period of 10 days the seeds were kept in a room thermostated at +24°C. As soon as the leaf-primordia appeared the plants were additionally illuminated (the plants were placed in a constant light of mercury lamps from 9 a. m. to 7 p. m.).

The outdoor experiment carried out in the experimental garden of the Department of Genetics at Ursynów consisted of 18 combinations in randomised blocks in two replications, with 42 seeds per plot spaced 20×40 cm. Table 1 presents the scheme of combinations of chemical compounds used in the experiment.

RESULTS

In the experiments on the influence of gamma rays nearly all the combinations in M₁ and M₂ of irradiated seeds showed chlorophyll changes and morphogenetic anomalies. The chlorophyll changes appeared in the form of light greenish-gray spots or strips against the regular green tissue of the leaves, or they are expressed in the form of yellow-hued leaf -primordia or else in a greenish-yellow hue of leaves developing mostly on the top parts of plants. The morphogenetic anomalies were characterised by leaves with 4, 5, 2 or 1 leaflets. Besides a coalescence of the leaflets curving inwards in a specific way was observed. Leaf petioles were sometimes forked and there appeared a pronounced narro-

Fig. 1 presents a leaf of M_1 plant with coalescent leaflets specifically curved in.

Fig. 2 presents the chlorophyll change in form of a light strip on the top leaflet (M_2 plant).

Fig. 3 presents leaves with assymetrically formed leaflets (M_1 plant).

Fig. 4 shows a leaf with four leaflets (M_2 plant).

Fig. 5 presents a leaf with a single leaflet (M_2 plant).

Fig. 6 shows a leaf with five leaflets (M_2 plant)

wing and assymetry in shaping of leaflets as well as deformation of leaf-primordia. Symptoms of plant dwarfing were observed, they were caused by a strong shortening of internodes.

Figure 1 shows a M_1 plant leaf with coalescent leaflets, curved in a specific way.

Fig. 2 presents a leaf with a chlorophyll change, expressed in the form of a strip of a lighter shade on the top leaflet of a M_2 plant. Fig. 3 presents leaves with assymetrically shaped leaflets of M_1 plant. Fig. 4 shows a leaf with four leaflets and fig. 5 shows a leaf with a single leaflet (of M_2 plants). Fig. 6 presents a leaf with 5 leaflets of a M_2 plant. In the M_2 generation mutational changes could be classified into 3 groups: 1) chlorophyll changes 2) morphogenetic changes and 3) a simultaneous appearance of both types of changes.

In the series of the control embracing 40 plants no mutational changes were noted. In the series treated with 3000r-dose for a total of 42 plants of M_2 no chlorophyll changes were observed. There were 15 plants with morphogenetic changes. In the series treated with a 6000r-dose, for a total number of 75 plants, 3 plants showed chlorophyll changes of which one plant had deformed leaves, and 23 plants had morphogenetic changes. In the series treated with a 9000r-dose for the

total number of 115 plants only 1 showed chlorophyll changes, 13 — morphogenetic changes and 1 plant was a dwarf. In the series treated with 12000r-dose for the total number of 81 plants there were 8 with chlorophyll changes, besides 2 of these plants had deformed leaves, and 12 plants showed morphogenetic changes. Morphogenetic changes were more frequent than chlorophyll changes. Approximately they were five times as numerous. For the total number of 353 M_2 plants 12 plants proved to be with chlorophyll changes and 66 with morphogenetic changes.

The data concerning the fertility of M_2 plants showed a decrease of fertility % in comparison with the control, proportionally to the dose of gamma-rays. Computing the % of fertility the number of normally shaped seeds in relation to the number of funiculi per pod was taken into consideration. The mean % was computed for all the pods on a plant and the mean % for all plants in each series. The mean % for the control plants amounted to 95.5%, for M_2 plant series treated with 3000r-dose — 90.73%, for series dosed with 6000r — 89.7%, for series dosed with 9000r — 82.27% and for series dosed with 12000r — 73.59%.

In M_1 all the seeds germinated and even a 12000r-dose did not produce lethal effects. Chlorophyll changes were more numerous in M_1 than in M_2 , only two plants in M_2 showed the presence of lighter spots on leaves of which one was in the form of a narrow strip of a greenish-gray hue on the topmost leaflets (Fig. 2), the other plant had a light spot inbetween the nerves on the side leaflet. Some plants with yellow hued leaf-primordia were observed.

The remaining chlorophyll mutations of M_2 comprised plants which in the early stage of growth were normally green but later produced shoots with yellow-green leaves. The chlorophyll defect could be caused by a recessive mutation of the gene determining a normal chlorophyll development. The chlorophyll and morphogenetic changes could be conditioned by chromosome aberrations, caused by the action of ionizing radiation.

The field experiment on the reaction of plants treated by NMH alone and also with addition of EDTA consisted of examining M_1 generation taking into consideration the following traits: 1) the length of the germination period, 2) the number of plants germinating on a plot 3) the height of plants 22 (1-st measurement) 33 and 41 days (second and third measurement respectively) from sowing, 4) the length of leaf-primordia 22 days from sowing, 5) the length of the largest leaflets 33 and 41 days from sowing, 6) fertility of plants (in %). The data are presented on Table 2 and on figures 7, 8 and 9.

It may be seen on the table 2 that the combinations treated with NMH alone have a marked lengthening of the germinating period pro-

Table 2

Results of the field experiment on the plants treated with chemicals

No. of combination	No. of germinated plants	% of germinated plants	Mean length of germination period in days	Mean % of plant fertility
1	63	75%	8 ± 0.14	91%
2	71	85%	8.08 ± 0.12	90.7%
3	63	75%	8.6 ± 0.25	88%
4	61	73%	8.93 ± 0.21	83.1%
5	58	69%	10.74 ± 0.26	73.5%
6	24	28%	13.41 ± 0.64	59%
7	45	54%	13.00 ± 0.32	85.66%
8	39	46%	9.04 ± 0.44	77.8%
9	33	39%	10.70 ± 0.59	80.2%
10	37	44%	10.52 ± 0.29	76.7%
11	9	11%	11.22 ± 0.62	56%
12	11	13%	10.10 ± 0.45	75.9%
13	15	18%	10.34 ± 0.54	73.7%
14	20	24%	14.40 ± 0.93	72%
15	10	12%	11.60 ± 0.66	69%
16	4	5%	14.50 ± 0.91	70%
17	19	22%	10.36 ± 0.43	81.32%
18	3	4%	14.33 ± 0.65	85%

portional to the increase of mutagen concentration. EDTA applied before NMH retarded the moment of germination only at low concentrations of NMH, at higher NMH concentrations the addition of EDTA did not have any retarding effects on germination.

With the increase of NMH concentration grew the lethal effect on M_1 plants caused by the increase of mutagen toxicity. Concentrations smaller than 1.5 mmoles did not decrease to less than 70% the number of surviving plants. However concentration = 3 mmoles caused a decrease of surviving plants to a lower level than 50% (Table 2). The experimental data were transformed after Bliss. Chi-square tests proved that NMH and EDTA have toxic effects. The differences between control and treated plants were significant. As regards the effects of joint action of EDTA and NMH in causing the increase of toxicity the experimental data have permitted to note a certain slight cooperation between these substances in producing an increase of the lethality of plants.

As concerns the effects of growth reduction measurements performed on the 22nd day after the sowing showed that together with the increase of the NMH concentration there appeared a proportional to it growth reduction of both the leaf-primordia and the stem. (Fig. 7). Fig. 7 presents the stem and leaflets growth reduction (in % of control)

in the field experiment. On the vertical scale are plotted the % of growth reduction, on the horizontal — are given concentrations of NMH in mmoles. Measurements conducted on the 33rd and 41st days after the sowing showed a still stronger reduction of stem growth at high NMH concentrations. The measurements data of these three periods

Fig. 7. Reduction of growth in % of control under the action of NMH.

permitted to state the dependence between the reduction of growth and NMH concentration. The outcome of the Duncan test established that the growth reduction of stem and leaves increased in proportion to the strength of NMH concentration showing a nearly linear dependence; NMH causes a very strong reduction of stem growth—up to 72, 68 and 50%, and for leaves — up to 65%. At a 3 mmoles NMH dose 6 plants were obtained which after forming leaf-primordia remained in this stage of growth to the end of their vegetation thus giving proof for a very strong reduction of growth at this NMH concentration. It was also observed that the same NMH concentration with an addition of EDTA (50 mmoles) also caused similar growth inhibition.

The addition of EDTA effected the increase of growth reduction only at very low concentrations of NMH, not over 0.5 mmoles (Fig. 8)

Fig. 8 presents the % of growth reduction of stem and leaflets under the joint action of EDTA and NMH in the field experiment. At higher concentrations of this mutagen = 1.5 and 3 mmoles the addition of EDTA did not effect the growth reduction of stem and leaf.

Fig. 8. Reduction of growth in % of control under the action of EDTA (50 mmoles) + NMH.

The obtained laboratory experiment data showed that simultaneously with the strengthening of NMH concentration there was an increase of the growth reduction of stem and radicle. The addition of EDTA at low NMH concentrations not reaching 1.5 mmoles caused a stronger stem reduction (Fig. 9). Figure 9 presents the stem and radicle growth reduction (in % of control) observed in the laboratory experiment. At higher NMH concentrations the addition of EDTA had no effect and did not influence the reduction of the radicle growth.

On the basis of data obtained in field experiments (Table 2) concerning the % of fertility of M_1 plants it was proved that the increase of NMH concentration had an essential influence on the decrease of plant fertility. Already at 1 mmole concentration the difference in plant

fertility between the control and the NMH treated plants was significant, EDTA alone did not decrease plant fertility neither did an addition of it to NMH cause any decrease of the % of plant fertility on any pronounced way.

Fig. 9. Reduction of growth in % of control under the action of NMH, EDTA + NMH

On the contrary to the effects observed by gamma rays treatment—in the experiments on the chemical substances influence on bean there were no chlorophyll changes obtained. In M_1 only morphogenetic changes were observed, including among others a dwarfing of plants and an appearance of leaves with supernumerary leaflets. The fact of not obtaining chlorophyll changes effected by NMH is difficult to understand. This could be caused by a not sufficient number of seeds used in the experiments, it may be that this kind of changes are less frequent in cases of chemical treatment than under the influence of ionizing radiation.

Reassuring we can state that the investigated mutagen NMH in our experiment caused in M_1 a pronounced growth reduction of stem, leaves and radicles, retarded the seed germination and augmented the lethality of plants proportionally to the strength of concentration. The lethal dose is a little over 3 mmoles.

The fact of our obtaining any chlorophyll changes in M_1 is not in agreement with data quoted by other authors who obtained numerous chlorophyll changes caused by NMH action on the leaves of *Arabidopsis* and barley (Müller, 1964; Künzel 1968).

The obtained results are only preliminary and call for further investigation.

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Wpływ promieniowania jonizującego oraz związków chemicznych na powstawanie mutacji u *Phaseolus vulgaris* L.

Streszczenie

Doświadczenia nad efektami powstającymi w wyniku stosowania promieni gamma i związków chemicznych przeprowadzono na *Phaseolus vulgaris* L. W tym celu poddano działaniu promieni gamma próbki nasion znajdujących się w stanie spoczynku. Dawki wynosiły: 3000r, 6000r, 9000r i 12000r. W wyniku otrzymano w M_1 i M_2 zmiany chlorofilowe i teratologiczne. Zmiany chlorofilowe wystąpiły w postaci niewielkich plam lub sektorów barwy jaśniejszej na tle normalnie zielonym liści. W potomstwie roślin zmutowanych zmiany chlorofilowe były mniej licznie reprezentowane. W M_1 i M_2 wystąpiły zmiany teratologiczne polegające na pojawianiu się roślin karłowatych, na powstawaniu listków nadliczbowych oraz zrastaniu ich między sobą, na pojawianiu się zdeformowanych o zwężonych blaszkach liści.

W doświadczeniach nad redukcją wzrostową roślin traktowanych związkami chemicznymi zadawano nasiona roztworami NMH w stężeniach 0,1; 0,5; 1; 1,5; 3 mM oraz roztworami EDTA w stężeniach: 50 i 100 mM. Część nasion kontrolnych i traktowanych wysiano w ogródku eksperymentalnym w Ursynowie, pozostałe nasiona poddano badaniom laboratoryjnym na reakcję wzrostową. Koncentracje NMH przekraczające 0,5 mM powodowały znaczną redukcję wzrostu i płodności roślin. Zmiany chlorofilowe nie wystąpiły. We wcześniejszym okresie rozwoju roślin zaznaczyła się wyraźna korelacja między stopniem redukcji wzrostu a zwiększeniem koncentracji NMH. Przy wysokich koncentracjach rośliny ginęły. Zarówno NMH i EDTA są toksyczne, aczkolwiek przy łącznym zadawaniu te dwie substancje nie wykazują efektu współdziałania w zwiększeniu toksyczności. Doświadczenia należy traktować jako wstępne i wymagające kontynuowania.