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The Effect of N-Nitroso-N-Methylurea, Buthylmethane Sulphonate and X-Rays on the Germination and Production of Chlorophyll Mutations in Einkorn Wheat

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Abstract. Grains of *Triticum monococcum* L. var. *sofianum* KÖRN. were treated with 0·1 mM, 0·2 mM and 0·3 mM solutions of N-nitroso-N-methylurea (MNH), with 0·03 M solution of buthylmethane sulphonate (BMS) and with X-rays in doses of 5 000r and 10 000r. The germination and development of individual colors of chlorophyl mutants were observed by the system developed by LAMPRECHT (1960). All the mutants induced were classified according to their color changes into three main categories-homogenous unicolor, homogenous multicolor and heterogenous multicolor. In the last type the colors of individual leaves of the same plant varied. Anthocyanin mutations "*albina*" and "*albino-transvirgata*" sometimes coincided with the chlorophyll mutations. Some chlorophyll mutations showing complicated groups of colors appeared which were beyond the scale of classification by ordinary systems. The largest proportion in the spectrum of chlorophyll mutations, induced by MNH and X-rays was occupied by mutations of the *albina* type. The broadest mutation spectrum in our experiments was induced by the application of 0·3 mM MNH. The doses of X-rays used induced relatively higher numbers of *albina*-type chlorophyll mutations than MNH and BMS. In our experiments we succeeded in inducing on medium size samples of *Triticum monococcum* L. var. *sofianum* KÖRN not only almost all types of chlorophyll mutations, induced by FUJII (1960, 1962) and MATSUMURA (1960), but in addition also a great number of other even more complicated chlorophyll mutations, which have never been previously described in *Triticum monococcum.* L.

Einkorn wheat can be considered a model species of the genus *Triticum L.*, useful in the study of radiation and chemical mutagenesis concerning macromutations as well as micromutations, owing to its high sensitivity to the action of mutagens. In addition the cultivation of this species is very easy and its genome is closely related to the genomes of tetraploid and hexaploid species.

Most of the visible mutations scored in the X_2 generation of diploid monocotyledons (70 to 90%) affect the production of chlorophyll (SMITH 1939, 1950 and others). Papers, dealing with type and heritability of chlorophyll mutations induced in *Triticum monococcum L.* dealt mostly with monofactorial

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recessive mutations, calculations of mutation frequencies and sometimes the additiveness of double recessive constitutions (FUJII 1962).

The application of chemomutagens, some of which can induce a relatively high proportion of point mutations and a lower proportion of aberrations (SHAMA and SEARS 1964), opened new possibilities of attacking this problem. NILAN and KONZAK (1961), EHRENBERG et al. (1961) and NILAN et al. (1964) induced chlorophyll mutations in barley by ethylmethane sulphonate, diethylsulphate and ethylenimine and X-rays. All the alkylating agents used in comparison with radiation induced relatively lower proportions of exter me types *albina* and higher proportions of *xantha* and *viridis*. Similar results were obtained by RAO and GOPAL (1964) in rice.

Chlorophyll synthesis is a complicated stepwise biochemical process, which is controlled by a large number of genes. The resulting phenotype is considerably influenced by external condition and the degree of influence varies in different mutants during development of the plants. In our experiments, therefore, we observed the mutants in regular intervals during their development, from germination up to full ripeness. Our experiments on einkorn wheat *Triticum monococcum L.* var. *sofianum* KÖRN. were focused on the detailed study of quantitative mutations induced. As far as we know, this technique of evaluating chlorophyll mutations induced by chemical mutagens has not been previously employed.

Material and Methods

Irradiation and Mutagen Treatment

We used *Triticum monococcum* L. var. *sofianum* KÖRN. The grains were irradiated by X rays in doses of 5 000 and 10 000 r/230 kV, 12 mA, filter = = 0·2 mm Cu, coherent filtration = 1 mm of Cu, focus distance 20 cm, intensity 485 r (min.). The other grains were rinsed in the mutagen solutions of MNH 0·1 mM, 0·2 mM and 0·3 mM and BMS 0·03 M, the amount of which reached double the layer of the grains treated. The temperature of solutions was 24° to 25° and the duration of treatment was always 24 hours. Irradiated as well as control grains were rinsed in distilled water for 24 hours. After another 24 hours of washing (in all varieties of experiments) all the grains were sown on the same day in the experimental field. M_1 grains were sown again to make the M_2 generation.

Evaluation of Results

The nature of our experiments did not require sowing of individual lines separately. The number of grains of M_2 sown in individual variants, never exceeded 1800 grains. The frequency of chlorophyll mutations is expressed in relation to the overall number of plants in M_2. The same number of grains was sown from each of 100 M_1 plants in each variant under study, which ensures that the sample used can be considered as a representative one. The germination of seeds was scored in 2 to 3 day intervals and within

Abbreviations used: MNH = N-nitroso-N-methylurea
BMS = buthylmethane sulphonate

intervals of the same length the development of chlorophyll mutations was observed. As there was a great variability in color changes of some mutants, especially in those induced by MNH and BMS the color classifier of LAMPRECHT (1960) was used.

Results

Germination

Intervariant differences, given in Fig. 1 (germination in percentage of the control), were most strongly expressed in the first days and later the relative numbers of germinating seeds approached that of controls. The lowest relative number of germinating seeds and the largest delay of germination was observed after 0·03 M BMS, where after 17 days only 50% of plants germinated. Strong depression of germination activity was observed also after treatment by 0·3 mM MNH (82% of control). After treatment by 0·2 mM MNH and 0·1 mM MNH the delay of germination and the number of germinating seeds was suppressed to a lesser extent (90—93% of control).

In the early intervals after irradiation by 5 000 r there was enhancement of germination (113%), but on the 18th day the number of germinating seeds was only 3% higher than in the controls. After irradiation by 10 000 r the number of germinating seeds approached that of controls from the beginning, but later it was fixed at 96% of control.

Chlorophyll Mutations

The classification of chlorophyll mutations was in the early days after germination complicated by the presence of considerable amounts of anthocyanine in the germinating plants which partially overshadowed the chlorophyll mutations. In addition mutations occurred which changed the genetic determination of anthocyanine synthesis.

The overall frequency of chlorophyll mutations is given in Table 1 and the percentage of relatively more frequent types of mutations is given in Table 2 The inexactitude of the most commonly used classification can be illustrated in one of the mutations, induced by 0·2 mM MNH (Fig. 3). After germination the plant was pale green with lighter vermilion leaves on its basis and apex. Later transversal pale yellow to orange spots appeared on the first two leaves the next leaves had pale yellow transversal strips with pale violet, unclearly limited spots. Even later, the green parts of leaves with transversal pale yellow strips became dark green and the violet spots remained only in a few sectors. One week later this mutant showed pale yellow transversal strips with pink-violet spots on the first two dark green leaves, the third leaf was yellow at the base and in the apical direction it had a light-green violet color, more apicaly light green with transversal yellow and green strips with violet spots on the strips. The violet spots later disappeared. The fourth leaf was yellow at its base, in the apical direction pink-violet and orange and bright green on the apex. The fifth leaf was white at the base, pink-violet in the middle and orange on the apex. In the stage of 10—11 leaves the last ones were of bright yellow color with light violet to pink sectors and as the plants grew older, its youngest leaves were

TABLE 1
FREQUENCY OF CHLOROPHYLL MUTANTS in M_2

Variants	Control	0·1 mM MNH	0·2 mM MNH	0·3 mM MNH	0·03 M BMS	5 000 r	10 000 r
Number of seedlings in M_2	1546	1438	1419	1262	378	1698	1487
Number of mutant seedlings in M_2	0	48	77	83	66	42	93
Number of mutants in percent in M_2	0	3·3	5·5	6·6	17·4	2·5	6·2

white without any violet shade. The amount of chlorophyll gradually decreased, all the plant grew white though at the time of formation of the spike, green straw appeared, but the resulting ear was sterile and eventually the plant died (Fig. 4). It is obvious that anthocyanine mutation interfered with the phenotype expression of complicated chlorophyll mutation.

In some anthocyanine mutants of the *albina* type the anthocyanine was distributed in transversal strips and in addition the plants had violet to red growing strips (Fig. 5). Irradiation did not induce this type of change. In

TABLE 2
SPECTRUM OF CHLOROPHYLL MUTATIONS in M_2 (values in per cent)

Mutants	Control	0·1 mM MNH	0·2 mM MNH	0·3 mM MNH	0·03 M BMS	5 000 r	10 000 r
albina	0	16·7	27·5	28·7	27·0	33·6	41·5
xantha	0	12·5	19·5	9·6	18·2	28·8	14·0
lutea	0	8·3	7·8	7·2	0	4·8	0
chlorina	0	4·2	5·2	4·8	45·5	4·8	6·5
chlorotica	0	0	0	1·2	1·5	0	0
flavoviridis	0	6·2	1·3	1·2	0	2·4	6·5
claroviridis	0	4·2	5·2	1·2	0	4·8	2·2
viridissimus	0	8·3	5·2	1·2	0	0	0
viridalba	0	0	1·3	2·4	0	2·4	4·4
caneovirens	0	0	2·6	1·2	0	0	4·4
albina virido apicalis	0	0	1·3	2·4	0	4·8	2·2
xantha virido apicalis	0	2·1	3·9	1·2	0	0	1·1
aureo apicalis	0	10·2	2·6	2·4	0	0	0
albino striata	0	4·2	2·6	7·2	1·5	4·8	1·1
flavovirido striata	0	6·2	1·3	9·6	0	0	0
albino-vario maculata	0	4·2	2·6	4·8	1·5	4·8	2·2
albino-xantho-vario-maculata	0	0	1·3	1·2	0	0	0
others (characteristic in text)	0	22·8	28·8	13·2	4·6	2·4	14·0

the beginning of their development *albina* mutants were either violet or completly white.

We were able to classify most of the mutants in agreement with other authors into two groups. The first group corresponds to the group of homogenously unicolor mutants, while the other group consists of mutants with homogenouslym ulticolor in one plant. The mutations *albina*, *xantha* (Fig. 6),

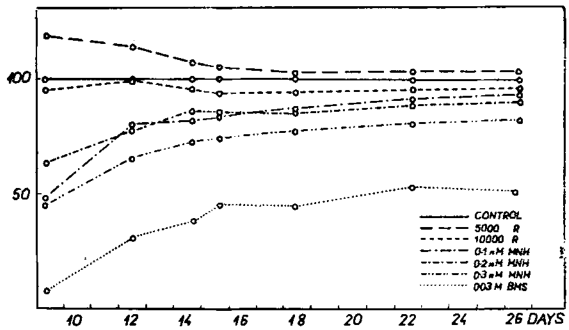

Fig. 1. The germination of M_2 grains, expressed in percentage of control.

lutea (Fig. 7), *chlorina*, *chlorotica*, *flavoviridis*, *viridialba*, *claroviridis*, *viridissimus*, *aurea*, *caneovirens* and *griseovirens* belong to the first group; the second group consists of types such as *albino striata*, *albino-vario maculata* (Fig. 8) *albino-xantho-vario maculata*, *albino-virido apicalis*, *albino transvirigata*, *xantho-fusci vario maculata*, *aureo apicalis*, *chlorotica viridissimo-luteo-albino-vario maculata*, *flavovirido striata*, *xantho-micromaculato striata*, *chlorotica viridissimo-luteo-albino-vario maculata*. Other types characterized by various colors on one plant, but not included in conventionally used categories were included in the third category of heterogenously multicolored plants (Fig. 3 and 9).

In the first two groups, anthocyanine completly disapeared 1—2 weeks after germination and in the later stages of ontogenesis it did not interfere with the observation of chlorophyll mutations. Mutations *albina* and *albina xanthescens* never survived longer than one month after germination. Mutations *xantha*,*xantha albescens* and *xantha aurescens* did not survive either as well as mutations *lutea* and *lutea xanthescens*. Mutation aurea appeared just once, after treatment by 0·3 mM MNH and very soon died. *Chlorina* mutations except for *chlorina albescens*, survived. One of the induced mutations of the *chlorotica* type died while the other one survived up to ripeness. Mutations *flavoviridis*, *claroviridis* and *viridissimus* survived while mutations *viridalba* and *caneovirens* did not. Bi- and multicolor mutations usually survived, but fertility was depressed. Mutation *albina virido apicalis* died; mutations *xantha viridio apicalis* on the other hand grew into a green color and survived till ripeness. All the mutations *albino-vario maculata*, *albino-striata*, *albino-transvirgata*, *albino-xantho-vario maculata*, *albino apicalis*, changed to *albino striata*, *flavovirido striata*, *flavovirido vario maculata*, *xantho micromaculato-striata*, *xantho costata*, *viridissimo costata*, *diffundere*, *viridissimo-*

spurio maculata, *viridissimo-vario maculata*, *flavorido-vario transvirgata* and *aureo apicalis* survived to ripeness.

On the basis of visual classification of all the mutations mentioned, except *albino-striata*, the green leaf area increased during ontogenesis. This phenomenon was expressed especially in the two mutations *xantho-fusci-maculata*, which grew into a uniform yellowish green color.

Of special interest among multicolor mutations are *chlorina luteo-aureo-vario maculata*, *chlorina viridissimo-luteo-albino-vario maculata*, *flavoviridis virido-striata*, *chlorina xantho-vario maculata* and *claroviridis albino-vario maculata*. A few remaining mutants differed from each other in the color of leaves within one plant and it was impossible to classify by the scale of LAMPRECHT (1960). After treatment by 0·3 mM MNH there was a strong increase in the relative number of uniformly multicolored mutants (*flavo virido striata* 9·6%, *albino striata* 7,2%, *albino maculata* 4·8%). After treatment by 0·03 M BMS there was a strong relative increase of the *chlorina* mutations and after treatment by 10 000 r a great proportion of mutation *flavoridis* (6·5%) was observed.

Discussion

In the experimental plants treated by 5 000 r an enhancement of germination was observed which probably can be considered as the stimulating activity of the irradiation of M_1 prolonged to the next generation. The stimulating activity of irradiation on M_1 was described by a number of authors (PIERRE 1959 et al.). The absence of stimulation in M_1 of our experiments (VAGERA, in press) can be explained by the delay in germination of strongly damaged M_1 grains, which on the average masked the stimulation activity. The most strongly damaged material was eliminated during M_1 generation and the stimulation was evident as late as in M_2; it could be explained as "hybridisation" by radiation (ANDREJEV 1963).

Investigation of the spectrum of chlorophyll mutations confirmed that in *Triticum monococcum L.* var. *sofianum* KÖRN. X-rays, MNH and BMS induced also not only all the types of chlorophyll mutations described most completly by FUJII (1960, 1962) and MATSUMURA (1960) and those induced in *Triticum monococcum* L. var. *flavescens* KÖRN, but also any other types with more complicated phenotype. Especially the treatment by MNH and BMS influenced the production of anthocyanine. Chlorophyll synthesis is controlled by a number of genes (the estimated number in barley is over 200) and the phenotype expression of chlorophyll mutations depends on a great number of additional factors, as mentioned by NYBOM (1955). For this reason we compared our mutants not only with the classification scale, but also with each other.

FUJII (1960) divided his chlorophyll mutations into two main categories those showing one color and those showing more colors. On the basis of our results, however, we think that it would be more comprehensive to divide induced mutations into three groups: Homogenously unicolored, homogenously multicolored and heterogenously multicolored, where the colors of individual leaves or groups of leaves on the same plant differ.

Mutants of the last group were induced only by the action of MNH and BMS. It seems to prove that in accordance with observation of some authors working on barley (NILAN and KONZAK 1961, EHRENBERG et al. 1961) and rice (RAO and GOPAL 1964), chemomutagens act by a "finer" than radiation and they induce not only fewer extreme chlorophyll mutations, but they also effect a much finer change of the complicated genetic system of control of chlorophyll synthesis, which causes more complicated variability of the color changes within individual plants, in some cases making possible gradual restoration of the original amount of chlorophyll.

With respect to the presence of anthocyanine in individual layers of cells it was possible to divide the chlorophyll mutations into three groups: 1) white, 2) uniformly violet, 3) with transversal violet strips. White plants constituted the smallest relative proportion and they probably arose in the consequence of simultaneous mutative disappearance and anthocyanine mutation of the *albina* type. Homogenously violet mutations were the most common category observed. They arose obviously in consequence of chlorophyll mutation alone and anthocyanine, which is normally present in the seedlings of *Triticum monococcum L.* var. *sofianum* KÖRN. was synthesized without any change. The third type was found in our experiments only after treatment by MNH and BMS and it probably corresponds to the anthocyanine mutation "*albino transvirgata*".

In almost all the mutations studied anthocyanine disappeared during 7—14 days after germination, which is the normal situation, occuring also in non-mutant seedlings of the var. *sofianum*. In some cases, however, anthocyanine did not disappear and it interfered with the expression of chlorophyll mutations, for instance, during development of the mutants, described in Results. In this case the effects of two multicolor mutations chlorophyll and anthocyanine (chloprohyll mutation was probably *xantho-albino-vario-maculato albescens* type, but anthocyanine mutation was unidentified) obviously overlapped each other. The differences in the colors of individual leaves on the same plant in this and some other mutations demonstrated the more complicated nature of mutations induced by MNH which interfere not only with the chlorophyll system, but probably also with other lipochromes.

According to recent data, the development of most surviving mutations of *maculata* type and some *striata* types can be characterised by a gradual reversion to the original chlorophyll content. In our experiments this phenomenon took place in later stages; early stages of the development of mutations *albino maculata* and *albino striata* were characterised by the change of original pale green or yellow colors to white, which was expressed to the largest degree two months after germination. As late as at the end of the vegetation period the white sectors partially disappeared. From external factors, the greatest part in increasing the chlorophyll content was played by elevated temperature at the begining of summer, as was already found before in similar chlorophyll mutations of *Triticum monococcum L.* var. *flavescens* KÖRN. by FUJII (1960).

The largest proportion in the spectrum of chlorophyll mutations induced by MNH and radiation consisted of *albina* mutations. The broadest mutation spectrum in our experiments was induced by the application of

0·3 mM MNH. The doses of X-rays used induced relatively higher numbers of *albina* chlorophyll mutations than MNH and BMS. It confirms the hypothesis of finer action of chemomutagens, which at the same time induce a smaller number of lethal chlorophyll mutations. In this connection it is of special interest that after treatment by 0·03 M BMS only a few plants survived as the dose strongly exceeded LD_{50}, but most of the mutations in M_2 (45%) were of the *chlorina* type, while only 27% of them were of the *albina* type and 18% of *xantha* type.

For further experiments it is advisable to use especially higher concentrations of MNH — 0·4 mM and 0·5 mM and 0·01—0·02 M solutions of BMS.

As far as we know, chlorophyll mutations in *Triticum monococcum L.* were never observed in detail during their entire ontogenesis and neither are such data available for some mutant species.

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J. VAGERA, Pedagogická fakulta, Palackého universita, Olomouc: **Účinek N-nitroso-N-methylmočoviny, buthylmethansulfonátu a paprsků X na vzcházení a typy chlorofylových mutací u pšenice jednozrnky v M_2.** — Biol. Plant. **11** : 408—416, 1969.

Obilky *Triticum monococcum* L. var. *sofianum* KÖRN. byly ovlivněny 0,1 mM, 0,2 mM a 0,3 mM roztokem N-nitroso-N-methylmočoviny (MNH), 0,03 M roztokem buthylmethansulfonátu (BMS) a 5 000 r i 10 000 r paprsků. X V M_2 bylo sledováno vzcházení a byl zaznamenáván individuální barevný vývoj všech indukovaných chlorofylových mutantů klasifikačním systémem podle LAMPRECHTA (1960). Indukovaní mutanti tvořili podle typů barevných změn 3 skupiny — rovnoměrně jednobarevné, rovnoměrně vícebarevné a nerovnoměrně vícebarevné, kde se odlišovaly zbarvením od sebe i jednotlivé listy téhož individua. U některých chlorofylových mutantů typů albina se objevily anthocyanové mutace typu „*albina*" a typu „*albino transvirgata*". Několik barevně složitějších chlorofylových mutantů nebylo možné podle dosavadních systémů klasifikovat. Největší procentuální zastoupení ve spektrech chlorofylových mutací indukovaných MNH a paprsky X připadalo na mutaci *albina*. Nejširší mutační spektrum ze všech variant indukovala 0,3 mM koncentrace MNH. Použité dávky paprsků X indukovaly relativně větší počet chlorofylových mutací albina než MNH a BMS. V práci se podařilo u *T. monococcum* L. var. *sofianum* KÖRN. indukovat na nepříliš velikém materiálu nejen téměř všechny typy chlorofylových mutací popsané Fujiim (FUJII 1960, 1962) a Matsumurou (MATSUMURA, 1960) u *T. monococcum* L. var. *flavescens* KÖRN., ale i řadu dalších, zejména složitějších chlorofylových mutací, u *T. monococcum* L. doposud nepublikovaných.

The plates will be found at the and of the issue.

Fig. 2. Mutation *albina,* masked by anthocyanine.

Fig. 3. Heterogenously multicolored mutation induced by 0·2 mM MNH in the stage of three leaves.

Fig. 4. The last stage of heterogenous multicolored mutation from Fig. 3.

Fig. 5. *Albina* mutation, showing simultaneously anthocyanine mutation "*albino transvirgata*."

Fig. 6. *Xantha* mutation.

Fig. 7. *Lutea* mutation.

Fig. 8. *Albino-vario maculata* mutation.

Fig. 9. Plants showing chlorophyl as well as anthocyanine mutation.