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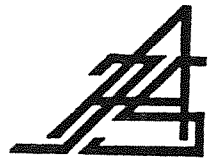

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A R A B I D O P S I S

I N F O R M A T I O N S E R V I C E

No. 6 Göttingen March, 1969

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A. Brief Notes

(continued)

It is a pleasure to acknowledge the dedicated and efficient services of Miss Friederike BUGGERT and Miss Elisabeth BISKUP in the assembly and typing of this Newsletter.

G.RÖBBELEN

A. BRIEF NOTES

Information on current genetical-taxonomical studies within the genus Arabidopsis

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The only purpose of this brief report is to inform Arabidopsis research workers of our current studies on taxonomic relations within this genus. Two different approaches to this problem seem to be promising: (I) studies on interspecific hybrids, and (II) application of methods of numerical taxonomy. These two approaches may provide independent and complementary information on the taxonomic relations under question. By means of the former method some conclusive data were already obtained by KRIBBEN (1965). Certain advantages of the latter, at least for the purposes of examination of inter-strain relations, were recently illustrated by CROVELLO (1968).

In our studies a comparison of several species of the genus Arabidopsis, and several subspecies of A. thaliana on the background of variation between morphological mutations obtained in race Enkheim is carried out. Five of the nine species found in the USSR (A. thaliana (L.) HEYNH., A. wallichii (J. D. HOOK) BUSCH, A. pumila (STEPH.) BUSCH, A. griffitiana (BOISS.) BUSCH, and A. korshinskyi BOTSCH.) and represented by twelve natural populations from the North-West and Central regions of the European part of the USSR and from the Middle Asia are included, as well as five morphological mutations of the race Enkheim. The degree of similarity of the studied taxons is estimated on the basis of twenty eight different quantitative characters specifying the shape of cotyledons, the shape and number of rosette and stem leaves, the type of leaf margine, the number of stems, lateral branches, siliques, seeds, etc.

Except for purely taxonomical problems the results of such comparison may comprise a basis for further population genetical, population geographical and microevolutionary studies.

References:
CROVELLO, T.J.: Arabid.Inf.Serv. 5, 7-9 (1968)
KRIBBEN, F.J.: "Arabidopsis Research", Rep.Int.Symp., Göttingen 1965. Pp. 26-30

Distribution and fruiting time of Arabidopsis species in Tajikistan

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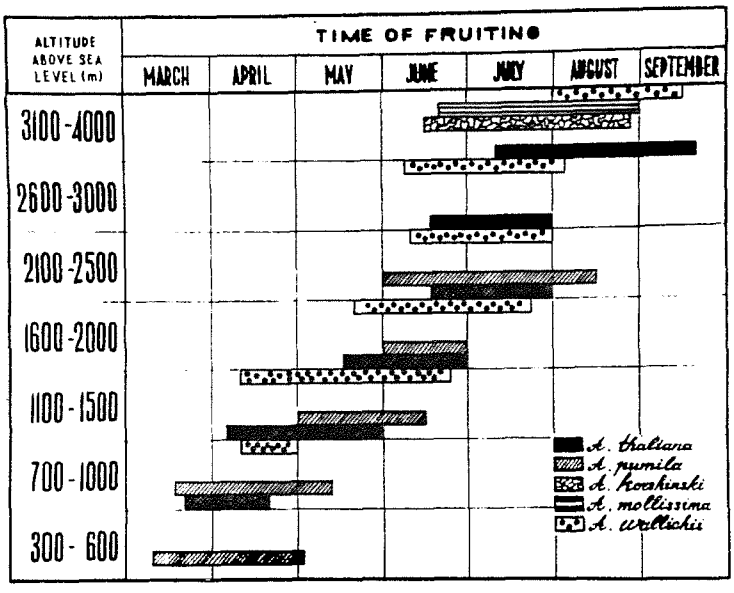

Preliminary studies revealed that species of Arabidopsis in Tajikistan are very polymorphic. Therefore it is important to collect and investigate different forms from various environment. Species of Arabidopsis are localized almost on whole territory of Tajikistan. Due to the very different climatic and ecological conditions, the period of vegetation and fruiting time throughout Tajikistan is very variable (Figure) The physiological peculiarities of different ecotypes of Arabidopsis thaliana, A. pumila, A. mollissima and A. korschinskyi are under study.

Analysis of within family phenotypic variation in a natural population of Arabidopsis

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A group of nine families of the H1-2 population were previously found to be highly variable for the phenotypic criterions of rosette size and number of days to flowering (KARBE and RÖBBELEN, 1968). Six of these families were further tested to determine whether the variation was genetically controlled. Such variation would suggest that hybridization was taking place among the "pure lines" within a race, or that genetically variable lines were being maintained in the natural population.

Seeds of single S_1 plants that appeared to be phenotypically different were planted for progeny testing. The analysis of the data showed that with a few exceptions, the means of the S_2 progeny of parent plants from the same family were not significantly different with respect to rosette diameter (Table 1). Therefore, it appears that the phenotypic differences between the individual S_1 plants were due largely to non-genetic factors. It is possible, that the some seed dormancy and following retardation of growth contributed to the phenotypic variability in the S_1 generation.

Table 1: Rosette size (cm) of selection lines from the H1-2 population

Family	Selection No.	n	Mean rosette size	$s_{\bar{x}}$	Rosette size of parent
10	0	18	4.0	.19	-
	1-1	19	3.9	.12	1.2
	1-2	18	4.3	.21	1.5
	2-1	25	4.2	.12	6.5
	2-2	19	3.8	.13	8.0
	2-3	20	3.4	.22	7.5
16	0	18	4.5	.18	-
	1-1	17	4.5	.16	9.0
	2-1	25	4.4	.18	7.0
	2-2	20	4.4	.10	8.5
	3-1	20	4.1	.17	1.0
	3-2	19	4.2	.22	0.5
	3-3	24	4.4	.17	1.5
	4-1	18	4.8	.10	0.6
	4-2	20	4.4	.12	1.5
19	0	-	-	-	-
	1-1	19	4.5	.30	9.0
	1-2	25	5.0	.18	8.0
	1-3	20	5.2	.21	7.5
	2-1	19	5.0	.30	8.0
	2-2	19	4.6	.28	7.0
	2-3	23	5.1	.25	7.5
	3-1	20	5.1	.26	0.6
	3-2	20	5.4	.10	1.0
	3-3	19	5.0	.24	0.8
	3-4	25	5.0	.17	0.5
	3-5	20	4.8	.20	1.5
27	0	20	5.4	.13	-
	1-1	19	4.8	.17	9.0
	1-2	25	4.7	.13	7.5
	1-3	20	4.7	.17	8.0
	2-1	19	4.8	.17	1.0
	2-2	19	4.7	.11	1.5
	2-3	24	4.9	.17	1.5
	2-4	20	4.2	.19	2.0
40	0	18	4.2	.22	-
	1-1	20	4.3	.12	7.5
	1-2	24	4.2	.10	8.0
	2-1	20	4.2	.12	7.5
	2-2	20	4.5	.13	7.0
	3-1	19	4.5	.16	2.0
	3-2	24	4.3	.12	1.5
42	0	23	4.0	.13	-
	1-1	20	4.1	.19	9.0
	1-2	19	4.6	.13	7.5
	1-3	20	4.2	.10	8.0
	2-1	23	4.4	.14	1.0
	2-2	20	4.3	.20	0.5
	2-3	20	4.1	.09	1.5

$lsd_{.05} = .49$

$lsd_{.01} = .75$

Reference:
KARBE, Christine, and G. RÖBBELEN: Arabid.Inf.Serv. 5, 13-15 (1968)

Effects of 8-azaguanine and p-fluorophenylalanine on the development of Arabidopsis

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The inhibitory effects of the antimetabolites 8-azaguanine (AG) and para-fluorophenylalanine (FPhe) on the development and metabolism of some plants have already been reported (BULL and FAULKNER, 1964; HEYES, 1963; MILLER and ROSS, 1966). The purpose of this study is to make an evaluation of the overall effects of these two analogues on the developmental aspects of the M_1 plants of Arabidopsis thaliana.

The different concentration gradients of these analogues were prepared in the mineral medium (JACOBS, 1964) on a weight to volume basis. The basic substrate-perlite was supplemented with these culture solutions. Plants were subjected to continuous illumination with a temperature of 24 $\pm$ 1.5°C. Light intensity and relative humidity were 9000 lux and above 70%, respectively.

Critical concentrations of these analogues which adversely affect the development were found to range from $1x10^{-5}$ to $1x10^{-4}$M for AG and $5x10^{-5}$ to $5x10^{-4}$M for FPhe. Rate of germination was practically unaffected at lower concentrations but higher concentrations partially or completely inhibited the normal process of germination. Greater concentrations of these substances also prevented the production of a normal tap root system; such concentrations of FPhe (for example, a concentration of $2.5x10^{-4}$M) induced the profuse development of hairy lateral roots. As regards the development of leaves, these concentrations of both analogues either delayed or prevented the formation of a full rosette (cotyledons and 6 leaves). The leaves were darker in colour when the AG concentration was high. Plants grown in FPhe showed considerable reduction in the size of the rosette. Time of flowering presented great variations. The visible initiation of the floral apex and the opening of the first flower bud were remarkably delayed in various concentrations of the analogues. Survival at maturity and fertility were also affected with increase in the concentration levels. The Table given below shows the important inhibitory effects of these two analogues in Arabidopsis thaliana when supplied at moderate levels.

Table 1: (1) Rate of germination 8 days after sowing(%). (2) Full rosette 16 days after sowing(%). (3) (4) Average fresh and dry weight of plants 14 days after sowing(in mg). (5) Flowering after 24 days(%). (6) Fertility(%). (7) Survival at maturity(%)

Culture conditions	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Mineral medium (Control	100	100	9.7	1.7	100	100	100
MM+AG ($2.5x10^{-5}$ M)	100	60	4.6	0.5	65	69.2	90*
MM+FPhe ($1x10^{-4}$ M)	100	40	1.4	0.2	65	57.1	95*

* Some of these plants were completely sterile

An attempt is also now under way to establish a correlation between these developmental aspects and biochemical events.

References:
BULL, A.T., and B.M. FAULKNER: Nature 203, 506-507 (1964)
HEYES, J.K.: Proc.roy.Soc.Lond., Ser. B, 158, 208-221 (1963)
JACOBS, M.: Arabid.Inf.Serv. 1, 36-37 (1964)
MILLER, J., and C. ROSS: Plant Physiol. 41, 1185-1192 (1966)

Experimental conditions for the study of the uptake of foreign DNA by Arabidopsis thaliana

L. LEDOUX and M. JACOBS

(Section of Cellular Biochemistry, Department of Radiobiology, C.E.N., Mol, and Laboratory for Genetics of Higher Plants, Free University of Brussels, Belgium)

Sterile seeds have been treated, under continuous illumination, with Escherichia coli DNA^2H^3H obtained from the C.34 strain, thymineless, adapted for growth on deuterated water and grown in about 1 μCi/μmg (2.5×10^6 dpm/μg); its bouyant density in CsCl being 1.730 g/cm^3. The DNA has been isolated from Arabidopsis thaliana (d = 1.698 g/cm^3) by a method using pronase for deproteinisation (LEDOUX, 1968).

a) T r e a t m e n t o f s e e d s . Dry sterile seeds or sterile seeds preincubated for 24 h in water were treated with the labelled DNA for 3 days. The plantlets were then washed with sterile water and further grown on mineral medium for 2 days. Fig. 1a and 1b show the result obtained. It can be seen that the

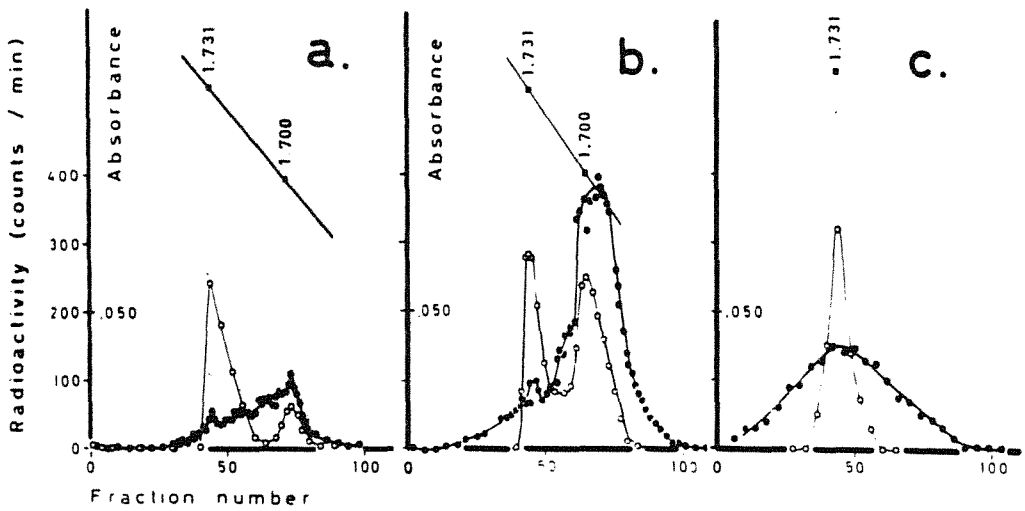

Figure 1: CsCl gradient ultracentrifugation of DNA's from

a) 60 dry seeds
b) 60 wet seeds treated with E. coli DNA^2H^3H
c) 60 seed coats corresponding to b)

●——● radioactivity o——o optical density

Density markers: M. lysodeikticus (1.731 g/cm^3) and mouse (1.700 g/cm^3) DNA's

(60 pl) $\frac{6\ d}{DNA^2H^3H}$ + 50 d (60 pl) Apex + $\frac{DNA^2H^3H}{16^{th}\ d}$ + 39 d

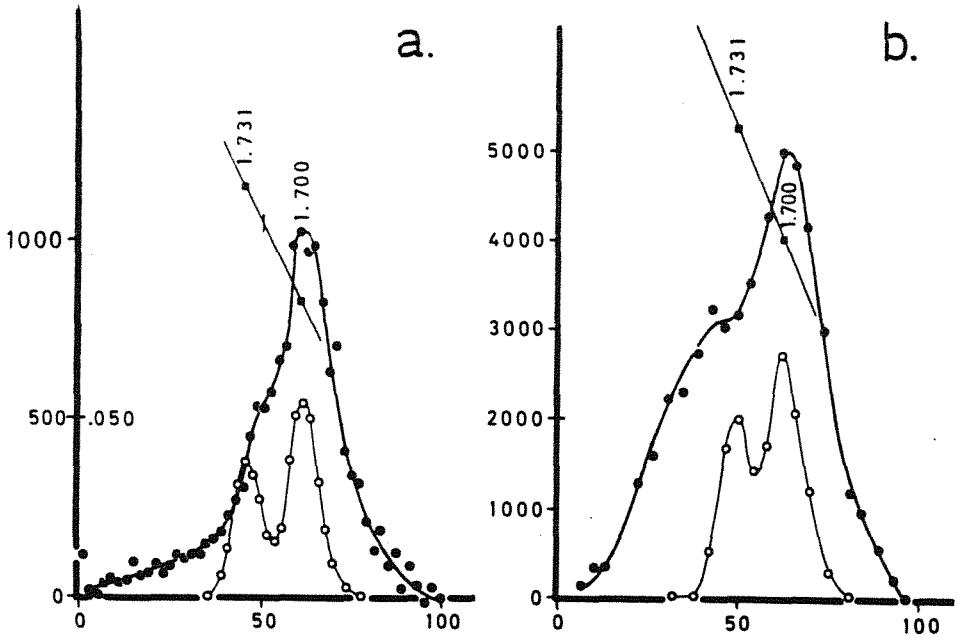

Figure 2: CsCl gradient ultracentrifugation of DNA's from adult plants (see text)

a) from 60 seeds treated with the foreign DNA
b) apex of 60 plants treated with the foreign DNA

●——● radioactivity o——o optical density

Density markers: M. lysodeikticus and mouse DNA's

uptake is much larger in the dry seeds than in the wet ones. Fig. 1c shows that the seed coats contain less radioactivity than the seedlings and that this radioactivity corresponds to depolymerised E.coli DNA^2H^3H.

b) T r e a t m e n t o f a p e x . Fig. 2 shows a comparison between the results obtained with mature plants coming from treated seeds (Fig. 2a). It can be seen that with the latter method a higher amount of radioactive molecules can be recovered, but that their quality is very similar in both cases.

Reference:
LEDOUX, L.: L'absorption des acides desoxyribonucléiques par les tissus vivants. Vaillant-Carmanne, Liége 1968

Fate of exogenous DNA during growth and development of Arabidopsis

L. LEDOUX and M. JACOBS

(Section of Cellular Biochemistry, Department of Radiobiology, C.E.N., Mol, and Laboratory for Genetics of Higher Plants, Free University of Brussels, Belgium)

The short life span of Arabidopsis makes this plant quite a convenient tool for studying the biological effects due to different exogenous DNA. Preliminary results, obtained by means of the technique of chromatography on DEAE-cellulose paper pulp, on the penetration and translocation of a highly polymerised E. coli ^{3}H-DNA have been already reported in Arabidopsis (BONOTTO et al, 1965). In this note, some data on biochemical analyses of plants treated with DNA-^{2}H^{3}H of E. coli, in optimal conditions previously defined (LEDOUX and JACOBS, this issue, page 5), are given by using ultracentrifugation in CsCl gradients.

Sterilized dry seeds of Arabidopsis thaliana, race Wil-2, were immersed in various DNA-^{2}H^{3}H of E. coli, in continuous light, at 24°C for several days (DNA density = 1.730g/cm^{3}; Fig. 1a). After treatment, seeds were washed and sown on sterile mineral

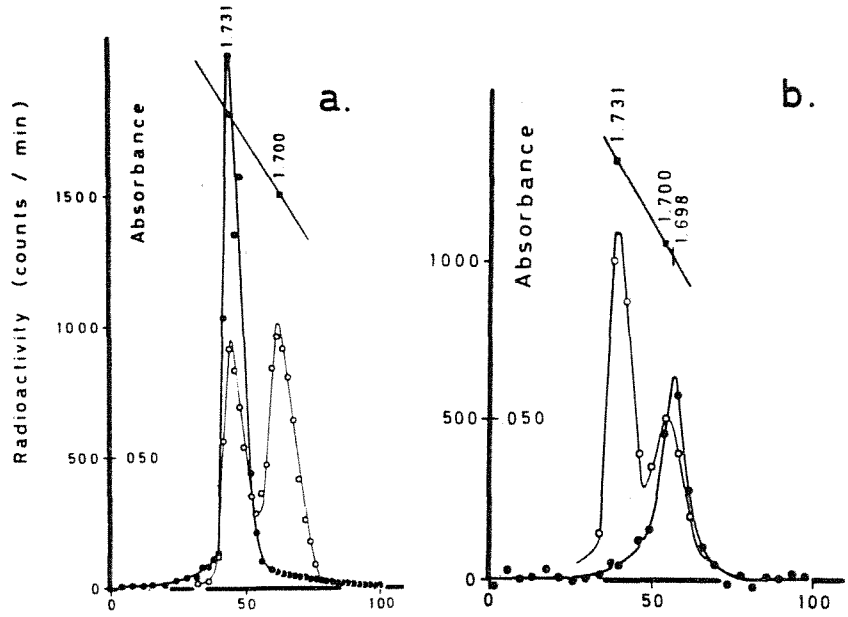

Figure 1: CsCl gradient ultracentrifugation

a) DNA ^{2}H^{3}H E. coli. b) DNA ^{3}H Arabidopsis thaliana

●——● radioactivity o——o optical density (260 mμ)

Density markers: M. lysodeiktious (1.731 g/cm^{3}) and mouse (1.700 g/cm^{3}) DNA's

medium with perlite as substrate. Control seeds were treated with ^{3}H-thymidine under similar conditions (Arabidopsis DNA density = 1,698/cm^{3}; Fig. 1b).

Plant samples were taken at different times of the life cycle. Their DNA is extracted and analysed in CsCl gradients.

The results summarized here are taken from an extensive study of the uptake and retention of the labelled E. coli DNA:

(1) At the cotyledon stage (Fig. 2a) radioactive DNA found in the seedlings exhibits heterogeneity from the point of view of density. The quantity of DNA is equal to more or less 0.01 μg per seed, each seed weighting 13 μg (fresh weight).

(2) With the expansion of leaves (Fig. 2b) radioactive molecules are distributed in two main populations having a density of 1.719 ± 0.002 and 1.698 ± 0.002 g/cm^{3}, respectively. Each population has been studied separately following the method already described for plant material (LEDOUX and HUART, 1968; LEDOUX, 1968). The effects of ultrasounds, of denaturation and of ultrasounds + denaturation suggest the formation of a structure made up of exogenous and endogenous DNA, double-stranded and bound by covalent linkages.

(3) In flowering and mature plants (Fig. 2c) the distribution of labelled molecules is strictly comparable quantitatively to the one reported in (2).

(4) In mature seeds radioactivity represents about 1% of the total amount initially measured in the treated seed. The population of these labelled molecules is either very heterogenous for density or it includes highly depolymerised DNAs (P M ± 500.000 daltons) (Fig. 2d).

(5) In M_2 seedlings selected after 6 and 12 days, the labelled DNA has a density intermediate between that of exogenous DNA and that of Arabidopsis DNA (Fig. 2e).

In the control ^{3}H-thymidine treated seeds, the analysis in neutral or alkaline CsCl gradients of polymerised or ultrasonicated DNAs obtained from plants of different ages, shows the presence of a homogenous population of labelled Arabidopsis DNA molecules.

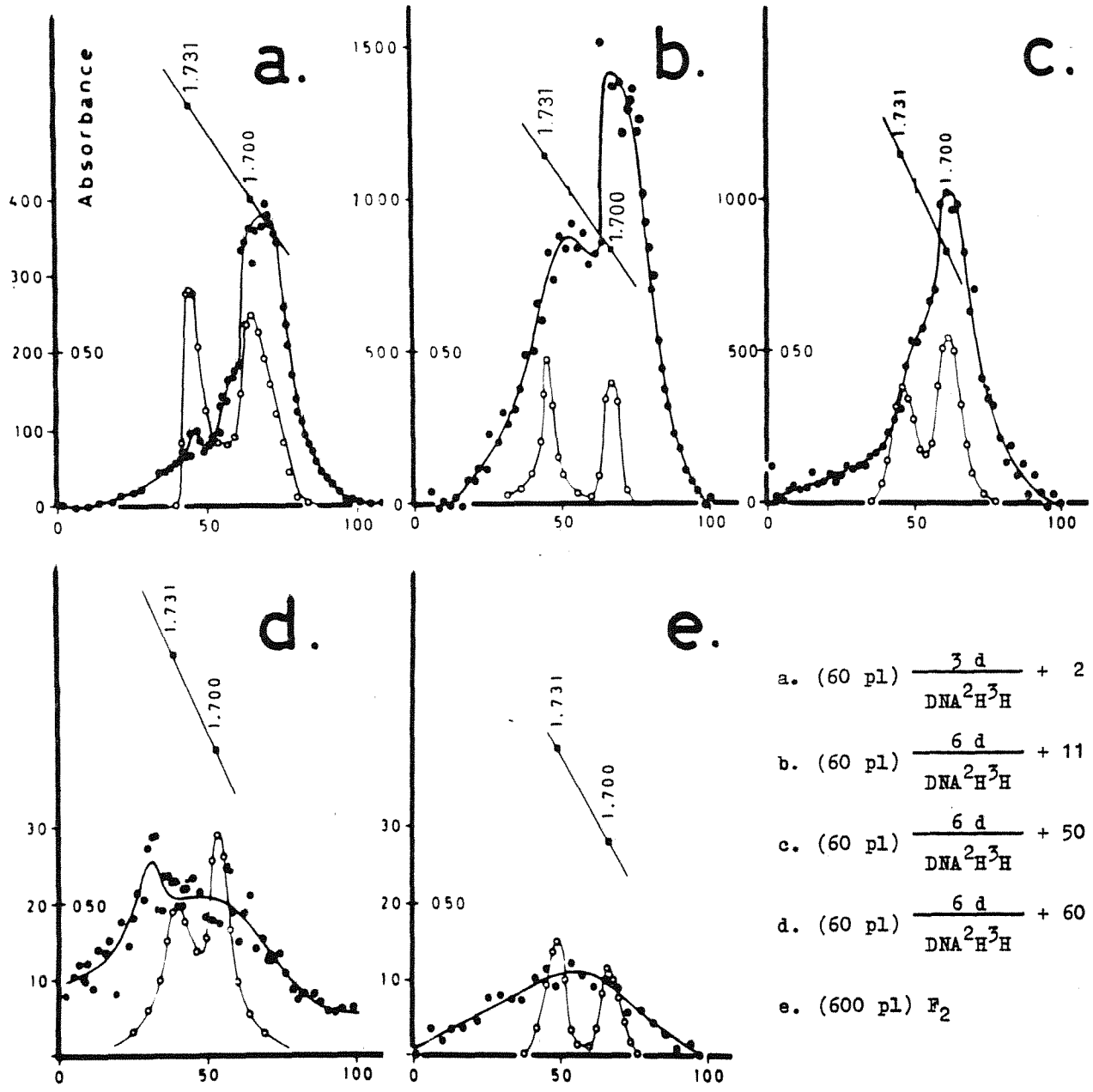

Figure 2: CsCl gradient ultracentrifugation of DNA's from plants treated with E. coli DNA^2H^3H (cf. text)

a) cotyledons
b) plants at rosette stage
c) mature plants
d) seeds
e) M_2 seedlings

●——● radioactivity o——o optical density

Density markers: M. lysodeikticus and mouse DNA's

In the seeds harvested from these control plants, no radioactivity was found though the seeds were soaked in ^3H-thymidine with a specific radioactivity twenty times higher than that of the exogenous DNA.

This study shall be completed with results obtained from the analysis of separated plant organs and from experiments on the biological effects of exogenous DNA.

References:
BONOTTO, S., M. JACOBS, and L. LEDOUX: Arabidopsis Research, Rep.Int.Symp.Göttingen, pp. 171-183, 1965
LEDOUX, L.: L'absorption des acides désoxyribonucléiques par les tissus vivants. Vaillant-Carmanne, Liège, 1968
- , and H. HUART: Nature 218, 1256-1259 (1968)

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Sedimentation constant of Arabidopsis RNA

S. BONOTTO and M. JACOBS

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We have previously described a method for the extraction of Arabidopsis RNA (BONOTTO and JACOBS, 1967) and for the separation of ribosomal RNA (r-RNA) from soluble RNA (s-RNA) (BONOTTO and JACOBS, 1968).

The RNA extracted by these methods from 1 month old Arabidopsis plants was tested for its sedimentation constant. Unlabelled Arabidopsis RNA was layered together with a small amount of labelled RNA (^{3}H-RNA) from Acetabularia mediterranea, on the top of 4.6 ml of 5-20% sucrose gradients, containing 50 µg/ml PVS, and centrifuged 5 hrs at 37.500 rev/min in the SW 40 rotor of the Martin Christ ultra-centrifuge. Two drop fractions, collected by puncturing the bottoms of the tubes, were diluted with 0.9 ml of distilled water and the absorbance at 260 mµ was measured in the Cary spectrophotometer. The radioactivity of each fraction was measured in a Packard liquid scintillation counter.

The figure shows the centrifugation pattern of unlabelled Arabidopsis RNA and of labelled Acetabularia RNA having sedimentation values of 23 S, 17 S and 8 S respectively (BONOTTO and JANOWSKI, unpublished results).

The approximate sedimentation constant of Arabidopsis RNA, calculated from these values, amounts to 25 S, 16 S and 4 S for the heavy r-RNA, the light r-RNA and the soluble RNA respectively. In another experiment, not reported here, unlabelled Arabidopsis r-RNA was centrifuged together with labelled Acetabularia RNA (^{3}H-RNA), having a sedimentation constant of 15 S. The results obtained showed clearly that the light r-RNA of Arabidopsis is heavier than 15 S Acetabularia RNA; again its calculated constant was 16 S.

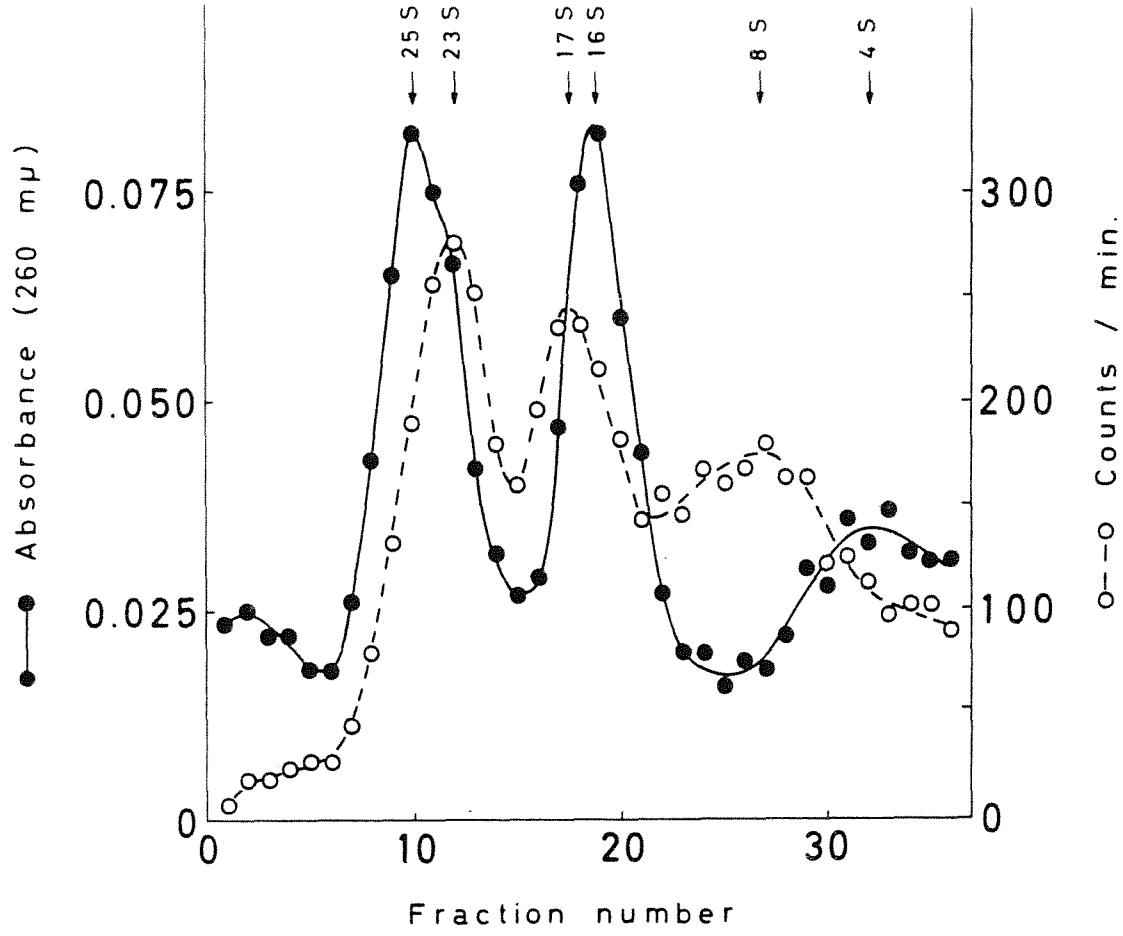

Figure: Sedimentation pattern of unlabelled r-RNA and s-RNA extracted from 1 month old Arabidopsis plants (•—•) and of labelled RNA from Acetabularia mediterranea (o—o). The unicellular algae were incubated 30 min in sea-water medium containing 10 uc/ml of Uridine-5-^{3}H. The numbers at the top of the graph represent the sedimentation constant values.

The values of the sedimentation constant reported here for total Arabidopsis r-RNA (25 S and 16 S) are in agreement with several recent reports on the RNA of higher plants (CLICK and HACKETT, 1966; SPENCER and WHITFELD, 1967; STUTZ and NOLL, 1967; HADZIYEV, MEHTA and ZALIK, 1968).

References:
BONOTTO, S., and M. JACOBS: Arabid.Inf.Serv. 4, 65-67 (1967)
- , - : Arabid.Inf.Serv. 5, 59-60 (1968)
CLICK, R.E., and D.P. HACKETT: Biochim.Biophys.Acta 129, 74-84 (1966)
SPENCER, D., and P.R. WHITFELD: Arch.Biochem.Biophys. 121, 336-345 (1967)
HADZIYEV, D., S.L. MEHTA, and S. ZALIK: Plant.Physiol. 43, 229-237 (1968)
STUTZ, E., and H. NOLL: Proc.nat.Acad.Sci. (Wash.) 57, 774-781 (1967)

We thank Mrs. Eliane BONNIJNS-Van GELDER for her technical assistance during part of this work. This work was carried out with help of the "Fonds National de la Recherche Scientifique Fondamentale".

Methylation with tRNA-transmethylase enzymes from Arabidopsis

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In No. 5 of this newsletter we demonstrated the existence of tRNA transmethylase enzymes in Arabidopsis thaliana (ABEELS, 1968); that existence is here further described using tRNA from E. coli, strain K-12 58/161, grown in minimal or in complete growth medium. As for the study of DNA transmethylase enzymes (ABEELS and DIGNEFFE, 1969) we took more tRNA sources because a methylation pattern made in vitro is dependent of the number of recognition sites for transmethylase enzymes. The technic was tested using enzymes from E. coli with tRNA from starved or not starved bacteria, because except for E. coli 58-161 starved it seems impossible to make homologous methylation (tRNA and enzymes from the same strain) (GOLD, HURWITZ and ANDERS, 1963).

A more interesting result came from the study of the chromatographic pattern of the digestion with T1 ribonuclease enzyme of tRNA after incubation with methylation.

R e s u l t s:
(1) tRNA rich in methyl groups + enzymes of E. coli = no methylation

(2) tRNA starved + enzymes of E. coli = methylation

(3) tRNA rich + enzymes from Arabidopsis thaliana = very low methylation

(4) tRNA starved + enzymes from Arabidopsis thaliana = good methylation.

Chromatography of 1n-HCl hydrolysis 1 hr, 100°C, of tRNA from (2) and (4) indicates the presence of 6-dimethylaminopurine, 6-methylaminopurine, methylguanine, methyluracil, and methylcytosine. The oligonucleotide T1 patterns of (2) and (4) were similar in localization of radioactivity but different in extension; the level of (4) was half that of (2). T1 pattern of (3) was very difficult to establish because of the very low radioactivity of the spots. I n c o n c l u s i o n : tRNA transmethylase enzymes of Arabidopsis thaliana certainly recognizes a good part of the methylation sites of E. coli, coding properties must be similar for a number of loci, but it should be very interesting to analyze the relationships between tRNA synthetase of Arabidopsis thaliana and tRNA from E. coli; such an investigation is presently made in our laboratory.

T e c h n i c a l p r o c e d u r e : The same technics were used as in No. 5 of this newsletter, except
- the sRNA from E. coli was extracted following the more precise method of RAMMLER et al. (1965),
- localization of nucleotides was made with right angle chromatography
  solvent I n-butanol-H_2O 86:14 v/v, NH_3 gas,
  solvent II isopropanol-conc. HCl-H_2O (170:41:39 by vol.),
- T1 digestion was made in phosphate buffer, pH 7.5.

References:
ABEELS, M.: Arabid.Inf.Serv. 5, 27 (1968)
- , and C. DIGNEFFE: Arabid.Inf.Serv., this issue, p. 11
GOLD, J., J. HURWITZ, and M. ANDERS: Proc.nat.Acad.Sc. U.S. 50, 164-169 (1963)
RAMMLER, D.M., T. OKABAYASHI, and A. DELK: Biochem. 4, 1994-2002 (1965).

Existence of DNA transmethylase enzymes in Arabidopsis

M. ABEELS and C. DIGNEFFE

(Laboratoire de Cytogénétique, Université Catholique, Louvain, Belgium)

In No. 5 of this newsletter we have described the existence of tRNA transmethylase enzymes in Arabidopsis thaliana (ABEELS, 1968). More informations on those enzymes were also given in this issue (ABEELS and DUBOIS, 1969), but it was of interest to investigate the existence of DNA transmethylase enzymes in Arabidopsis thaliana.

A methylation pattern for a specific strain can be made either after purification and separation of the deoxyribonucleotides from a DNA extraction, or with an in vitro reaction using a submethylated source of DNA, enzymes from the investigated strain and methyl ^{14}C groups from the sole methyl donor, S-adenosylmethionine methyl ^{14}C. A homologous methylation between DNA and enzyme from the same species seems to be impossible except for the E. coli strain K12 58/161 which synthesizes DNA in the absence of methionine (BOREK and RYAN, 1958; GOLD, HURWITZ and ANDERS, 1963 a, b).

For those reasons in vitro methylation of DNA from other species with enzymes from Arabidopsis thaliana was prefered for technical convenience but also because that system supposes the recognition of a DNA structure and therefore similarities in the genetic coding properties are tested.

Three different sources of DNA were prepared: (1) E. coli strain K12 58/161 normally grown, (2) E. coli strain K12 grown in starvation conditions with minimal medium (3) Streptomyces strain S17. Methylated deoxyribose nucleotides are well known for many species of bacteria and bacterial viruses (WYATT, 1950; DUNN and SMITH, 1955) mammalian tissues (CHARGAFF et al., 1953) and recently they had been found in algae (PAKHOMOVA, 1968). As for those organisms we found no other nucleotides than 6-MAP (6-methylaminopurine) and 5-MC (5-methylcytosine). The ratio 6-MAP/5-MC for the different sources of DNA methylated by Arabidopsis enzymes were 1.38 for (1) DNA from E. coli rich in methyl groups, 1.63 for (2) DNA from E. coli poor in methyl groups, and 1.27 for (3) DNA from Streptomyces. Methylation level was another source of difference because the radioactivity of (1) was the third of (2), and that of (3) the half of (2).

C o n c l u s i o n s :

- Methylation activity exists by Arabidopsis thaliana.
- 6-MAP and 5-MC are found.
- Extensions of the methylation level are different for the different species of DNA.
- Similarities of coding properties must exist.
- Investigations with endonuclease digestion enzymes are necessary for more informations.

E x p e r i m e n t a l p r o c e d u r e :

E. coli growth medium:

- for rich DNA :

Difco Bacto Tryptone	10 gr
NaCl	8 gr
Glucose	1 gr
Distilled water	1000 ml

- for poor DNA the medium utilized is a variant of the M9 medium with 0.003 gr of l-methionine for 1,000 ml of minimal medium,
- for Streptomyces : Nutrient Broth 8 gr, NaCl 1 gr for one liter of growth medium.

The DNA was extracted following the method of MARMUR (1961) with very little modifications. The plants 2 to 5 weeks old were frozen and homogenised. The medium for the extraction of enzymes contained : 0.1 M Tris buffer, pH 7.2, with 16 mM $MgCl_2$, 20 mM potassium-metabisulfite and 0.45 mM sucrose (ANDERSON and ROWAN, 1966). The assay mixture for the methylation consisted of 1 µM 2-mercaptoethanol, 10 µM triethanolamin buffer, pH 8.8, 10 mµM C^{14}-CH_3-S-adenosyl-methionine, 2 mg of heated ribonuclease, 100 mµM nucleotides as methyl deficient DNA and sufficient enzyme to incorporate at least 1 µM of C^{14}-CH_3 groups (HURWITZ et al., 1964) for a total volume of 0.25 ml. After incubation for 30 min at 38°C 5 µM sodium pyrophosphate, 0.05 ml of 0.5% bovine plasma albumin, and 0.2 ml of 7% $HClO_4$ were added and the insoluble material removed by centrifugation. The pellet was resuspended and washed twice again. One part from the washed pellet was dissolved with 1.5 ml of 0.2 M NH_4OH, decanted on metal planchets, dried and counted. The other part after hydrolysis with ln-HCl, 1 hr at 100°C, was submitted to right angle chromatography; solvent I n-butanol - H_2O 86:14 v/v, NH_3 gas, solvent II isopropanol-conc. HCl - H_2O (170:41:39 by vol.).

Purine bases and pyrimidine nucleotides were localized in the UV-light, a UV-photography was made and the cut out spots were counted for their radioactivity.

References:

ABEELS, M.: Arabid.Inf.Serv. 5, 27 (1968)
- , and E. DUBOIS: Arabid.Inf.Serv., this issue, p. 10
ANDERSON, J.W., and K.S. ROWAN: Biochem.J. 101, 9 (1966)
BOREK, E., and A.J. RYAN: Bacteriol. 75, 72 (1958)
CHARGAFF, E., C.F. CRAMPTON, and R. LIPSCHITZ: Nature 172, 289 (1953)
DUNN, D.B., and J.D. SMITH: Nature 175, 336 (1955)
GOLD, M., J. HURWITZ, and M. ANDERS: Proc.nat.Acad.Sci. (Wash.) 50, 164-169 (1963 a)
- , - , - ; Biochem.Biophys.Res.Comm. 11, 107 (1963b)
HURWITZ, J., M. GOLD, and M. ANDERS: J.Biol.Chem. 239, 3462-3474 (1964)
MARMUR, J.: J.mol.Biol. 3, 208-218 (1961)
PAKHOMOVA, M.V. et al.: Dokl.Akad.Nauk SSSR 182, 712-715 (1968)
WYATT, G.R.: Nature 166, 237 (1950)

Study of the DNA content of diploid and tetraploid nuclei of Arabidopsis thaliana after treatment by cysteamine and X-rays

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It is well known that some organic coumpounds increase the resistance of the organisms against the damage induced by ionizing radiations. Several workers are studying the mechanism of radioprotection at several stages of the DNA synthesis. Our purpose was to compare the action of X-irradiation on the DNA content of the resting nuclei of Arabidopsis thaliana after treatment by a radioprotector, the cysteamine (S-mercaptoethylamine). The behaviour of diploid and tetraploid strains was also compared.

We have used the roots of young seedlings of diploid and tetraploid Arabidopsis, after a five day germination of the seeds on wet filter paper. The dosis of X-rays was 20,000 R, this is the lethal dosis for a high proportion of the seedlings. The concentration of cysteamine was 0.01%; the soaking of the filter paper by that solution for 24 hours did not reduce the vitality and the growing of the seedlings. For each chromosome type, four items were compared : control plants, treatment by the radioprotector, irradiated material and treatment by cysteamine followed by irradiation. The seedlings were fixed in acetic-alcohol immediately after the treatment and stored in 70 % alcohol. The roots were stained according to the classical Feulgen technique and squashed in a drop of 50 % acetic acid. The squashes were made permanent by dehydration and mounting in Canadabalsam.

The Barr and Stroud integrating microdensitometer was used for measuring the relative quantity of DNA in the nuclei. That estimation was done by comparing the relative absorption of light in the nuclei and in the surrounding cytoplasm. One hundred nuclei were measured for each item and their distribution in term of relative absorption is reported in Fig. 1. The number of available nuclei in a root meristem

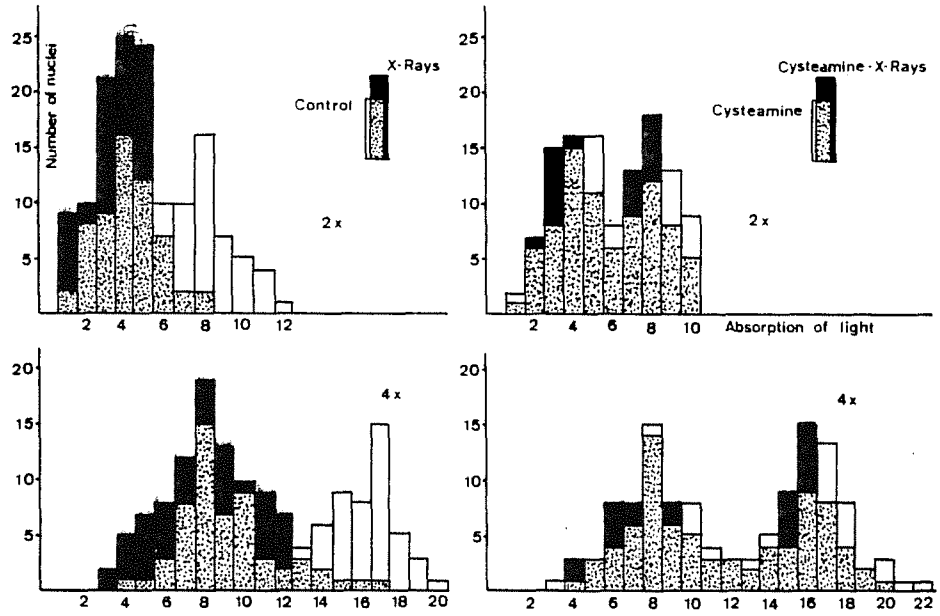

Fig. 1: Distribution of nuclei according to their DNA content (arbitrary units of light absorption) in diploid (upper diagrams) and tetraploid Arabidopsis (lower diagrams)

of Arabidopsis is relatively low and, on the other hand, the absorption of the light is very poor, because of the small size of the chromosomes in regard to the nucleus volume. The upper left diagram shows (white and dotted parts) the distribution of the nuclei in control seedlings. The discrimination between the peaks corresponding to the G_1 and G_2 stages is not very clear; these peaks were noted for 4 and 8 arbitrary units of absorption. An absorption of about 8 units was always observed for prophase and metaphase nuclei, 4 units corresponding to the DNA content in anaphase and telophase. The G_1 and G_2 nuclei were more distinct in the tetraploid seedlings (lower left diagram) and the peaks indicated a double amount of DNA (8 and 16 units respectively).

The seedlings fixed after irradiation showed a very clear accumulation of the nuclei in G_1, in tetraploid as well as in diploid plants (dark and dotted parts of the diagrams). Very few nuclei showed an absorption corresponding to the G_2 phase.

The right part of the Fig. 1 shows, for diploid and tetraploid plants, the results of measurements performed in seedlings treated by cysteamine (white) and irradiated after the treatment by the radioprotector (dark). The distribution of the nuclei was similar in the control and in the plants only treated by cysteamine. On the other hand, the pretreatment by cysteamine has concealed the effect of X-rays since both peaks are visible again; the two distributions are similar, in spite of a light shifting of the G_2 peaks to the left after irradiation.

Table 1: Mean absorption rates (arbitrary units)

	diploid	tetraploid
Control	5.98 ± 0.254	12.37 ± 0.441
Irradiated	4.00 ± 0.149	8.57 ± 0.271
Cysteamine	6.13 ± 0.249	12.43 ± 0.471
Cysteamine and X-rays	5.72 ± 0.234	11.55 ± 0.447

The mean absorption values have been compared by analysis of variance and the means are reported in Table 1. The comparisons did not show any significant differences between control and plants treated by cysteamine as well as between "cysteamine" and "cysteamine and X-rays", in diploid and tetraploid strains. On the contrary, the differences between control and irradiated seedlings were very highly significant.

It may be concluded, from Fig. 1 and Table 1, that the DNA content in irradiated plant was similar to the G_1 peak of the control. The effect of the cysteamine was very effective at the considered point of view, since a normal distribution was maintained after irradiation. The radioprotection was perhaps not perfect, since the G_2 peaks for irradiated plants were a little shifted to the left in comparison to the unirradiated ones. That shifting was responsible of a light reduction of the general means; the decrease of the means was not statistically significant, but it was observed in diploid and in tetraploid strains. Thus the irradiation seemed to block the nuclei at the G_1 stage, the disappearance of the G_2 nuclei being possible during the time of irradiation (75 min). The cysteamine gave an almost normal distribution of the nuclei in irradiated seedlings. These results may be interpreted as a true radioprotective effect of the cysteamine which would allow DNA synthesis after irradiation and the production of new G_2 nuclei. But the presence of the second peak in irradiated material may also be the consequence of a decrease of the mitotic activity induced by the cysteamine; according to that second interpretation, the G_2 nuclei would be nuclei which have not had the opportunity of completing their mitosis during the irradiation time.

Attempted callus culture of spontaneous callus-forming albino mutant

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A mutant stock derived from Professor RÖBBELEN has the Göttingen identification number V52. Phenotypically this mutant is identical with mutant im and we have considered it to be the same mutant as im.*

It was observed that our seed stock showed a marked tendency toward abnormal or callus growth. In 14% of seedlings, spontaneous callus formation was noted when the seedlings were grown on 1% glucose supplemented mineral agar under incandescent white light (2600 microwatts/cm^2/sec). Since in experiments on the control of chlorophyll formation it would be desirable to have large amounts of material, and since seeds of albino V52 are difficult to obtain, culturing of the spontaneously formed calluses was attempted. It was hoped that with the addition of growth factors the calluses could be grown very rapidly. The experiments reported by LOEWENBERG (1965), ZIEBUR (1965) and SHEN-MILLER and SHARP (1966) were used as a guideline in determining which media to try.

M a t e r i a l s a n d M e t h o d s . For callus growth 50 ml of each of the following media was placed in each of two 250 ml Erlenmeyer flasks:

(1) Basic nutrient medium for Arabidopsis: Mineral salts (M) + 1% glucose + 0.78% agar
A Basic medium + 10% coconut milk by volume
B Basic medium + vitamin solution (V) + amino acid solution (A)
C Basic medium + kinetin (0.64 mg/l) + Indole acetic acid (2 mg/l)
AB Basic medium + coconut milk + vitamins + amino acids
BC Basic medium + vitamins + amino acids + kinetin + I.A.A.
AC Basic medium + coconut milk + kinetin + I.A.A.
ABC Basic medium + coconut milk + vitamins + amino acids + kinetin + I.A.A.

In one flask of each type of medium was placed a piece of callus from a V52 plant; on the other, an excised hypocotyl from a wild-type plant. The callus tissue was obtained from two 32-day-old albino V52 plants grown aseptically on glucose-mineral agar in continuous white light; the hypocotyls were excised from approximately one-week-old seedlings of wild-type Arabidopsis grown under the same conditions. The flasks were then placed in continuous white light and observed periodically for growth.

*Recent crossing data confirm this assumption (RÖBBELEN, 1968)

The composition of the used solutions was as follows:

M = M i n e r a l s a l t s for basic medium for Arabidopsis (mg/l medium)

$MgSO_4 \cdot 7H_2O$	739	$MnSO_4 \cdot 4H_2O$	2.3
$Ca(NO_3)_2 \cdot 4H_2O$	944	$CuSO_4 \cdot 5H_2O$	0.24
$NaH_2PO_4 \cdot H_2O$	552	$ZnSO_4 \cdot 7H_2O$	0.29
KNO_3	607	H_3BO_3	1.86
$FeSO_4 \cdot 7H_2O$	12.5	Ammonium Molybdate	0.035

V = V i t a m i n s (mg/l medium)
(Note: Vitamin concentrations in B are twice as large as the following)

pyridoxine HCl	0.4	choline chloride	0.4
nicotinamide	2.0	i-inositol	16.0
folic acid	0.4	p-amino benzoic acid	0.4
calcium pantothenate	0.4	riboflavin	0.2
ascorbic acid	8.0	biotin	0.2
thiamine	0.4		

A = A m i n o A c i d s (mg/l medium)

glycine	2.44	D-L serine	1.80
p-alanine	1.72	L threonine	1.80

O b s e r v a t i o n s . Only one of the hypocotyls showed significant growth - the one cultured on medium (1). From this hypocotyl was regenerated a whole new plant, or cluster of several plants, which flowered and set seed. Hypocotyls grown on A and B grew short roots from one end, but showed no further growth. All of the hypocotyls (except (1)) eventually turned brown and shrunk somewhat in length.

All of the calluses grew quite slowly. Most of them, however, did not remain undifferentiated, but started to proliferate albino leaves. Callus tissue cultured on AB was the only to remain undifferentiated throughout the six weeks of the experiment. Only tissue grown on B produced roots as well as leaves. - Two of the "calluses" - (1) and B - started to grow quite quickly about 25 days after planting, when the agar was beginning to dry out somewhat.

Each piece of tissue was about 2mm in diameter at the start of the experiment, each hypocotyl was approximately 5mm in length when planted. Final measurements, made six weeks after planting, were as follows:

Medium	Diameter of calluses in mm	Length of hypocotyls in mm
(1)	8	Regenerated entire plant - flowered and set seed
A	4	5
B	9	3
C	3	3.5
AB	3	3
BC	3	3
AC	3	3
ABC	2	3

D i s c u s s i o n : The results of the experiment on callus growth show that the tissue on (1) - with no organic additives - and on B - with vitamins and amino acids added - grew the best. That the coconut milk - known to contain plant growth factors - and kinetin and IAA produced no acceleration in growth, but actually inhibited growth as compared to the control, would seem to indicate that these spontaneous calluses produce their own growth hormones.

Since the growth of tissue on (1) and B was accelerated after around 25 days, when a fair amount of water had evaporated from the agar, it is speculated that high osmotic pressure will speed the growth of the tissue. To test this speculation, an experiment is now in progress in which the "callus" tissue from this experiment has been subcultured onto media with higher osmotic pressures, due to either the glucose, the mineral salts, or both.

Evidently, none of the media were able to induce the wild-type hypocotyl tissue to form callus, demonstrating that this tissue is quite different from the V52 spontaneous calluses, as assumed. Yet the regeneration of an entire plant from the hypocotyl on (1) shows the remarkable regenerative capacity of this tissue.

References:

LOEWENBERG, J.R.: Arabid.Inf.Serv. 2, 34 (1965)
- , and P.J. THOMPSON: Arabid.Inf.Serv. 4, 68 (1968)
RÖBBELEN, G.: Planta 80, 237-254 (1968)
SHEN-MILLER, J., and W.R. SHARP: Bull.Torrey Bot.Club 93, 68-69 (1966)
ZIEBUR, N.K.: Arabid.Inf.Serv. 2, 34-35 (1965)

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Isolation of germination temperature-sensitive mutants in Arabidopsis thaliana
A preliminary report

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Genetical and biochemical analysis in microorganisms has been greatly facilitated by the use of conditional lethal mutations. Affected organisms die under "restrictive" conditions but survive in a "permissive" environment.

One class of conditional lethals, the temperature-sensitive mutants, have been detected also in higher organisms, e.g., in Arabidopsis (LANGRIDGE, 1965) and in corn (ROBERTSON and ANDERSON, 1961). In Drosophila melanogaster (SUZUKI et al., 1967) temperature-sensitive mutants have been created by artificial mutation and extensively analyzed. To the writer's knowledge, no similar work has been done with plants. Yet, of particular interest would be morphological mutants that would lead to a detailed study of plant development by locating various intermediate steps, made apparent by the effects of defective genes.

Seeds of the variety Estland were treated with N-methyl-N-nitro-N'-nitrosoguanidine at 10 or 100 micrograms per ml for 24 hours, rinsed in distilled water, and germinated on minimal medium (VELEMÍNSKÝ and GICHNER, 1964) in 200 mm tubes (one seed per tube).

At high concentration, see Table below, germination seemed to have been impaired. Of 78 seeds, 15 germinated. Among those, 3 lacked chlorophyll. The remaining 12 grew slowly, though they reached maturity.

Treatment	Grown at	Number of plants seeded	Germinated		% Germination
			Green	Albino	
Control	16°C	260	210	0	80.7
Control	28°C	81	71	0	87.6
Nitrosoguanidine 10 µg/ml	16°C	282	267	0	94.6
" 10 µg/ml	28°C	183	155	0	84.5
" 100 µg/ml	16°C	78	12	3	19.2

At low concentration of the mutagen, however, no injury to the embryo could be detected. The first generation of treated seeds were harvested and seeded on minimal medium in petri dishes (100 seeds in one petri dish) and placed in a growth chamber at 28°C. All the seedlings that germinated at that temperature were discarded and the petri dishes were transferred in another growth chamber at 16°C. The seedlings which germinated at that low temperature were then transferred into test tubes and placed at 28°C to continue their life cycle. Seeds of these suspected mutants were seeded at low and at high temperature. Only 8 of the 20 tested were indeed germination temperature-sensitive.

Among the treated plants, three albino were obtained but could not be rescued on complete medium and two did not shoot a flower stalk at either 16°C or 28°C.

References:

LANGRIDGE, J.: Austr.J.Biol.Sci. 18, 311-321 (1965)
ROBERTSON, D.S., and I.C. ANDERSON: J. Heredity 52, 2 (1961)
SUZUKI et al.: Proc.nat.Acad.Sci. (Wash.) 57, 907-912 (1967)

Photomorphogenesis in Arabidopsis thaliana

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During 1968 an investigation of the action spectrum for floral induction of Arabidopsis thaliana, race "Estland", was carried out in collaboration with Dr. W.H. KLEIN at the Radiation Biology Laboratory, Smithsonian Institution, Washington D.C. Initial observations were reported (BROWN and KLEIN, 1968), and details of the methods used and results will be published elsewhere.

The absolute light requirement for seedling morphogenesis was fulfilled with continuous far-red irradiation. Further photomorphogenesis to development of the primary bud cluster and elongation of the bud stem can be accomplished, without development of photosynthetic capability, when plants are cultured on mineral agar supplemented with 1% glucose under continuous far-red light. The quantum flux density required for seedling photomorphogenesis was lower than that required for meristem activation and floral induction. The peak far-red wavelength for floral induction was near 730 nm, the region from 710 to 740 was effective, but 750 nm light was completely ineffective. At 700 nm germination and vegetative growth were activated, but not floral induction. The entire waveband from 700 to 520 nm, at energies from 100 to 200 microwatts/cm^2/sec, was ineffective in floral induction, though maximum vegetative growth occurred in the red region. The blue region from 400 to 500 nm was effective in floral induction. Under conditions where irradiation with monochromatic light is continuous from the time the dry seed is sown, it was found that the total quantum dose delivered to saturation of floral induction with either blue light (485 nm), or with far-red light (730 nm), was similar for either wavelength. The dose required at 485 nm was 1.25×10^{-3} Einstein/cm^2, and at 730 nm it was 1.30×10^{-3} Einstein/cm^2. The action spectrum in the far-red region can be interpreted in terms of the hypothesis of HARTMANN (1966) as involving phytochrome by continuous excitation of a circa 1% steady-state far-red absorbing fraction of the total phytochrome pool. However in the blue region the evidence indicates that a second photoreceptor absorbs the light and interacts with phytochrome in mediating photomorphogenesis, since the presence or absence of a far-red component in a four hour white light period preceeding 20 hours of monochromatic blue light markedly influenced the quantum effectiveness of blue light. A similar interaction has been suggested to explain blue and far-red effects on photoregulation of oat mesocotyl (SANDEMEIER, 1968) though in this tissue both red and far-red had equal effects on growth. In the case of floral induction in Arabidopsis under steady state irradiation, the ineffectiveness of the red region may be ascribed to loss of photoregulation subsequent to vegetative meristem activation, because of destruction of P_{fr} and depletion of the phytochrome pool, rather than to maintenance of an inhibitory ratio of the far-red versus red absorbing form of phytochrome.

References:
BROWN, J.A.M., and W.H. KLEIN: Proc.Canad.Soc.Plant Physiol. 9, 22-23 (1968)
HARTMANN, K.M.: Photochem. and Photobiol. 5, 349-366 (1966)
SANDEMEIER, M.: Photochem. and Photobiol. 7, 391-401 (1968)

This work was carried out during tenure of a National Academy of Sciences, National Research Council Senior Smithsonian Fellowship, which is gratefully acknowledged.

Induced somatic reversions in recessive chlorina mutants

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The efficiency of various mutagenic agents on several recessive leaf colour mutants of Arabidopsis was tested. After treatment of mutant seeds, green sectors within the yellow M_1 mutant plants were scored as "reversions". The percentage of plants with reversions varied from 0% to almost 30%, depending on the specific mutant (Table 1).

Table 1: 0.2% EMS-treatment of seeds, 16 h submers

Mutant	Plants	Green sectors %
ch[1]	2410	2.4
V 81	2235	19.8
V 89	2135	4.1
V 93	2000	0.0
V 95	2360	8.2
V 157	1520	29.7
V 300	2100	0.9
V 318	1890	3.3

Equivalent data were obtained in 3 similar experiments. Thus the reversion frequency seems to be a characteristic feature of the mutant gene in question. Of course, the reversion rate also depends on the treatment conditions (Table 2). But, throughout

Table 2: Effect of EMS-dose on seeds, 16 h submers

EMS	V 89		V 157	
	Survival %	Sectors %	Survival %	Sectors %
0.1 %	94.3	3.8	97.1	11.6
0.2 %	73.2	4.1	68.4	29.7
0.3 %	16.9	7.6	21.6	51.3
0.4 %	0.7	9.4	2.3	63.1

the doses tested, the difference between mutants, like V 89 and V 157, remains the same. Even sublethal doses of EMS never yielded as high reversion frequencies in V 89 as in V 157.

In order to determine the genetic nature of the phenotypic reversions, the sectored M_1 plants were grown to maturity and the seeds harvested from each green sector separately. - The first result was (as expected), that many of the inflorescences, produced from these sectors, were completely or partially sterile. Probably chromosomal defects were involved in these instances. On the other hand, sectors with normal fertility are likely to be less affected by chromosomal rearrangements. Those revertant sectors, selected by this means for structurally normal chromosomes, were further tested.

In the M_2 generation two types of progenies were clearly distinguishable. In some instances green wild type and yellow mutant plants segregated 3:1 indicating that the green M_1 sector was heterozygous for the corresponding locus. These somatic sectors may be attributed to "backmutations" (though deletions or closely linked suppressors are not necessarily excluded). - Within the other kind of progenies, two types of rosettes, light green and darker ones, appeared besides the mutant. As for example in the mutant V 81 the wild type is fully dominant; the light green plants cannot represent the heterozygous state of the mutant gene. For further analysis these intermediate types were crossed to both, the wild type and the original mutant. Segregation ratios of 1 yellow : 2 light green : 13 green plants indicated, that the sectorial reversion in the M_1 resulted from a mutation in a locus, different from the original mutant one. In some cross-combinations with the wild type, the F_2 even showed 4 phenotypic classes:

	yellow	yellow-green	light green	green
Possible genotypic explanation:	$\frac{xa}{xa}\ \frac{+}{+}$	$\frac{xa}{xa}\ \frac{S}{+}$	$\frac{xa}{xa}\ \frac{S}{S}$	$\frac{xa}{+}\ \frac{\circ}{\bullet}$; $\frac{+}{+}\ \frac{\bullet}{\bullet}$

xa being the mutant locus and S its suppressor. - In Table 3 all these different types

Table 3: Genetic analysis of green sectors after 0.2% EMS, 16 h, on seeds

Mutant	Number of sectors			
	total	analyzed	backmutations %	suppressors %
V 81	411	28	7.1	92.9
V 95	184	17	5.9	94.1
V 157	442	41	2.0	98.0

of reversions, not segregating 3:1, are combined as "suppressors". The table shows, that the frequency of reverted green sectors, originating from suppressor mutations, is very high after EMS treatment with less than 10% of these cases being true backmutations.

Eight of the suppressors of the mutant V 81 were intercrossed and all appeared to be identical. In 12 test combinations with suppressors of the mutant V 157, 4 different ones were found; but only one of these showed linkage to the mutant locus. To date

little is known about suppressors in higher plants. By further analyzing the suppressor situation in leaf colour mutants, we hope to contribute to an understanding of gene interaction during chlorophyll synthesis or chloroplast differentiation.

All of the suppressors, which we obtained thus far, do not fully revert the mutant to the wild type if in the heterozygous state, and some suppressors do not even do so in the homozygous condition. In Table 4 from the total number of reversions analyzed

Table 4: Phenotypic estimate of the backmutation frequency by determining the percentage of complete revertants

Treatment (V 157)	Sectored plants %	Revertants tested	
		total	wild type %
EMS, 25 mM, 16 h	14.3	31	1.9
NIL*, 10 mM, 1 h	9.7	54	22.2
X-rays, 15 kR	4.1	26	19.1

* Nitrosoimidazolidon

by progeny tests, those were noted, that gave complete reversion to wild type. The figures in the last column, therefore, are most probably equivalent to the percentage of backmutations. In this experiment 3 different mutagenic treatments were compared. It is evident from the data, that EMS, which produced the highest overall number of revertant sectors, yielded only 1.9% backmutations. On the other hand, X-rays, despite of their relatively low somatic sectoring, induced a much higher number of backmutations. With other mutants, the figures may vary somewhat; but for 3 different mutants tested so far, the tendency was the same. Accordingly, whenever the action of different mutagenic treatments is to be analyzed, it appears not to be sufficient to score the number of somatic sectors only.

Linkage relationships between mutant genes for flowering time in Arabidopsis thaliana (L.) HEYNH.

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HUSSEIN (1968) identified 20 X-ray and EMS-induced single-gene flowering time mutations (see also HUSSEIN and VAN DER VEEN, 1968) by means of diallel crosses within 5 groups of mutants, based on parent of origin (line C or line 51), direction of change, and magnitude of change. Line 51 is a single-gene mutant (e_1e_1) towards late from line C. Linkage groups established from this study (HUSSEIN, 1968) are:

(A) $e_3 - e_4$ among the "large" effect late mutants from C and 51 (e_2, e_3, e_4, e_6; e_1 included).

(B) $e_7 - e_8$ among the "small" effect late mutants from line 51 (e_7, e_8; e_1 included).

(C) e_1-l_3-X_2 among the "small" effect late mutants from line C (l_1, l_2, l_3, X_1, X_2, X_3; e_1 included).

(D) $e_1 - v_6$ among the "small" effect early mutants from line 51 (v_1, v_2, v_3, v_4, v_5, v_6; e_1 included).

(E) $R_1 - R_2$ among the second-cycle early mutants (R_1, R_2; e_2 included) induced in line CA (e_2e_2).

N.B. No inferenees were made about the linear order. It is seen that (C) and (D) form one linkage group ($e_1/l_3/X_2/v_6$).

Additional crosses between mutant lines of different diallel sets were made during the summer and autumn 1968 (Dept. Genetics, Wageningen). The data (F_1's and F_2's) so far analysed, revealed that (A) and (B) belong to one linkage group ($e_3/e_4/e_7/e_8$).

As the character (flowering time) studied shows much overlapping variation, special methods were adopted for identification of the single genes (cf. HUSSEIN, 1968). Even though, only relatively close linkages could be detected. Since so much linkage was found among the 20 mutations, it is concluded that the loci involved are not evenly distributed all over the genome (2n=10), but rather occur in clusters.

References:
HUSSEIN, H.A.S., and J.H. VAN DER VEEN: Arabid.Inf.Serv. 5, 30 (1968)
HUSSEIN, H.A.S.: "Genetic analysis of mutagen-induced flowering time variation in Arabidopsis thaliana (L.) HEYNH." Ph.D. thesis; 1968, Wageningen. Also published as Meded. Landbouwhogeschool Wageningen 68-11 (1968) Reprints available.

Genetic analysis of mutagen-induced flowering time variation in Arabidopsis thaliana (L.) HEYNH.*

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Arabidopsis thaliana (L.) HEYNH. was chosen as model plant to study the genetic system of flowering time (start of flowering) and, often closely correlated to it, number of rosette leaves (vegetative production). The duration of the vegetative phase is of interest from the point of view of both natural selection (local adaption) and artificial selection (plant breeding).

Genotypic variation was induced by seed treatment with EMS (ethyl methanesulfonate) and with X-rays. Two pure lines were used as starting material, viz. the very early flowering Landsberg-'erecta' (line C) and a later flowering single gene mutant line derived from it (line 51). In total 24 flowering time mutants with good fertility and vigour, and of independent origin, were obtained through sector selection in M_1 (for fertility) and individual plant selection in M_2, followed by line selection in M_3 and further generations (for flowering time and fertility).

After grouping the 24 lines according to parent of origin, and direction and magnitude of effect, diallel crosses (F_1 and F_2) were made within groups, in order to identify the individual mutations by means of classical Mendelian methods, rather than to describe and analyse the induced quantitative variation by the statistical methods of quantitative inheritance. In nearly all cases, including the small-effect lines, single gene differences with the parent of origin, could be successfully identified with the experimental and analytical procedures used for this purpose.

The analysis of the flowering time mutants led to the following conclusions:
(1) No significant differences could be detected between EMS and X-rays with respect to magnitude and direction of effect. There is some indication that X-rays induce more (small-effect late) dominant mutations. With respect to mutant frequency at equal levels of M_2-fertility, EMS is 2 to 3 times as efficient as X-rays.
(2) The majority of the mutant lines selected differ in one single recessive gene; a few contain intermediates and a few contain dominants. All mutations identified are at different loci. These results have a number of practical implications:
(a) Mutant lines with more than one gene mutation for flowering time appear to be relatively rare, although such lines, if present, would have an increased likelihood of being selected in M_2 and later generations.
(b) As in general flowering time mutants selected in M_2 are single gene homozygotes, at least when the mutations are recessives, no further segregation will occur in M_3. This means that once individual plant selection has been applied in M_2, no within line selection for flowering time is necessary in further generations.
(c) When no large-effect mutants of the desired phenotype appear in M_2, but only small-effect mutants in the desired direction, crosses can be made already between individuals of different M_2-lines, in order to obtain recombinants with phenotypic effect of sufficient magnitude. This procedure finds its justification in the fact that the M_2-mutants selected are most often single gene recessives, and in the fact that the mutations are at different loci.
(3) Mutagenic treatment of the medium early line 51 and of the late mutant line CA (from our selections), releases variability in both directions: towards early and towards late. However, no earlier mutant lines were obtained from the very early line C. Moreover, line C did not respond to vernalization treatment, and early mutant alleles when transferred to line C did not come to expression. Therefore, it is concluded that line C represents a 'physiological limit' towards early.
(4) There was no evidence for allelism of mutations of independent origin. Several linkage groups were established (See HUSSEIN elsewhere in this issue). On the one hand, the loci for flowering time in Arabidopsis are not distributed at random over the genome, on the other hand they are not restricted to a few chromosome segments either.
(5) Except for occasional evidence for non-allelic interaction (epistasis), the effects at the individual loci are additive over loci. Crosses between recessive and intermediates gave, in connection with the relative magnitudes of the gene contrasts, interesting numerical examples for the dominance theory of heterosis.
(6) Pronounced genotype x environment interactions were frequently met: homozygotes effect x season, dominance effect x season and also genotype x vernalization x season interactions were described.

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The estimation of heritability of developmental characters with the aid of the intraclass correlation coefficient in natural populations of Arabidopsis

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In an earlier communication (DOBROVOLNÁ, 1968), an attempt was done to estimate the heritability of two developmental characters in a natural population by means of the offspring-parent regression coefficient. This procedure required to grow both mother plants and their progenies in strictly the same experimental conditions. This necessity is sometimes difficult to ensure (cf. BARTHELMESS, 1964).

Another appropriate method which lacks these difficulties and which can give valuable results by analyzing only one generation is based on the calculation of the intraclass correlation coefficient, W. In such simple situations the W values correspond directly to the required coefficients of heritability.

For this purpose, progeny lines derived from single plants of six natural populations, Bo-1, Dou, He-2, Kr-4, Pi, and Stř (cf. DOBROVOLNÁ, 1967) were studied at 25±3°C under continuous illumination of 1250 lux. Three developmental characters were scored in individual progeny plants: the number of days to appearance of the flower primordia (X), the number of rosette leaves (Y), and the number of rosette leaves per day (Y/X) or the "leaf formation rate" according to CETL (1966). In the population Stř it was possible to compare the results with those obtained by means of the offspring-parent regression (DOBROVOLNÁ, 1968).

In estimating heritability with the aid of the intraclass correlation coefficient the following formula was used (modified according to LE ROY, 1966):

$$W = \frac{V_B - V_W}{\bar{n}} \Big/ \frac{V_B - V_W}{\bar{n}} + V_W$$

where V_B and V_W are the between and within progeny variances, and $\bar{n}$ the average number of plants per progeny. Furthermore, the standard errors (s_W) and the reliability intervals were calculated.

Table: The values of intraclass correlation coefficient, its standard error and reliability interval (P=0.05) for the three developmental characters X, Y, and Y/X in different natural populations

Population	Number of progenies	X		Y		Y/X	
		W ± s_W	reliability interval	W ± s_W	reliability interval	W ± s_W	reliability interval
Bo-1	23	.60 ± .02	.48 - .74	.53 ± .02	.40 - .67	.53 ± .02	.41 - .68
Dou	30	.60 ± .02	.49 - .72	.73 ± .01	.64 - .82	.70 ± .02	.60 - .80
He-2	60	.69 ± .01	.62 - .76	.60 ± .01	.51 - .68	.34 ± .01	.26 - .42
Kr-4	37	.63 ± .01	.54 - .73	.53 ± .01	.43 - .64	.46 ± .01	.36 - .58
Pi	91	.60 ± .01	.54 - .66	.63 ± .01	.57 - .69	.45 ± .01	.39 - .52
Stř	162	.53 ± .01	.48 - .58	.51 ± .01	.46 - .56	.40 ± .01	.35 - .45

The results show that in the character X the values were from 0.53 to 0.69, in the character Y from 0.51 to 0.73, and in the character Y/X from 0.34 to 0.70, respectively. These figures appear to be well comparable with those obtained by the offspring-parent regression method in the population Stř as it can be seen from the comparison with the cited paper (DOBROVOLNÁ, 1968).

The results confirm that in the populations studied the heritability of the three developmental characters is relatively high and that the used method provides a remarkable simplification of the procedure in estimating heritability in natural populations of Arabidopsis.

References:

BARTHELMESS, I.: Z.Pflanzenzücht. 52, 273-332 (1964)
CETL, I.: Arabid.Inf.Serv. 3, 8-9 (1966)
DOBROVOLNÁ, J.: Arabid.Inf.Serv. 4, 6-7 (1967)
- : Arabid.Inf.Serv. 5, 17 (1968)
LE ROY, H.L.: Elemente der Tierzucht. Bayerischer Landwirtschaftsverlag, München-Basel-Wien, 1966.

EMS-induced dominant restorers of EMS-induced recessive male sterility in Arabidopsis

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Prior to the present experiment, VAN DER VEEN and WIRTZ (1968) carried out a small-scale model selection experiment to assess the frequency of EMS-induced genic male steriles: Having treated Landsberg-'erecta' seeds with 10 mM EMS (24°C, 24 hrs), they selected 116 M_1-plants for harvesting one f u l l y f e r t i l e fruit per plant, and screened the 116 M_2's (size 12 plants) for fruit-length resp. pollen-release. Six promising recessive male steriles were carried on to M_3 (via heterozygotes), back-crossed to the parent of origin, and reselected. A 6 x 6 diallel between heterozygotes showed the 6 mutations to be at 5 different loci. Four of the six lines were discarded for incomplete expression of m.s. and/or partical expression of m.s. in the heterozygote. Of the remaining two, line Ms_1-ms_1 fulfilled the following criteria: 1) complete expression of m.s. throughout the plant, 2) no traces of m.s. in the heterozygote, 3) complete female fertility, and 4) no phenotypic differences with the parent of origin other than those resulting from sterility. The second line (Ms_2-ms_2) is equally good, but ms_2ms_2 switches to male fertility in the later flowers. Both lines have normal meiosis in pollen mother cells and young pollen formation, but the pollen decays while the anthers fail to open.

MÜLLER (1968) reported an X-ray induced recessive male sterile in race Dijon G.

The present authors treated seeds of line Ms_1-ms_1 with 10 mM EMS (24°C, 24 hrs), and the M_1 consisted of 754 fertiles and 796 male steriles (expected 775 and 775; P = 0.30 - 0.20). On 9 of the male steriles a fully or nearly fully fertile sector was found, either in the main inflorescence or as a side branch, the remaining parts of these plants being c o m p l e t e l y m a l e s t e r i l e. N.B. Spontaneous crosspollination produces at the most an occasional seed on ms_1ms_1-plants.

Descent No.	M_2's from the 9 M_1-sectors RR + Rr	rr	q'	M_3's from Rr (pooled) R.	rr	q'
1	5 + 14	10	0.69	218	91	0.59
2	6 + 15	11	0.69	202	73	0.54
3	2 + 15	18	1.00	205	96	0.64
4	0 + 19	17	0.94	219	158	0.84
5	0 + 24	12	0.67	420	130	0.47
6	0 + 20	16	0.89	314	138	0.61
7	0 + 18	18	1.00	268	96	0.53
8	0 + 21	14	0.80	334	135	0.58
9	0 + 21	15	0.83	374	121	0.48

In all 9 cases, restored fertility was transmitted to the M_2 (see Table), i.e. the revertant M_1-sectors were not "physiologically" induced by EMS-treatment, but resulted from dominant mutations, which moreover were carried by the sporocyte tissue itself! (Though cytoplasmic restoration seems unlikely in view of the segregation patterns in M_2 and M_3, we are nevertheless carrying out the test ms_1ms_1 x pollen from fertiles).

Further points shown by the Table are:

1. The 9 M_2's (36 seedlings transplanted per fam.) had fertile : sterile segregations varying from 2:1 (No. 5) to 1:1 (No. 7). In the case of EMS-induced pyrimidineless-to-normal revertants (VAN DEN BERG and FEENSTRA, 1968), the mutant h o m o z y g o t e s were phenotypically non-revertant (leading to 1:1), which indicates interallelic complementation. In our case, however, a progeny-test of all fertile M_2-plants by means of M_3 (24 seedlings transplanted per fam.), revealed resp. 5, 6 and 2 homozygous fertiles in No's 1, 2 and 3. Moreover, the consistent decrease of revertant-deficit from M_2 to M_3 (all segregating fam. pooled per descent), leads us to expect that also in No's 4 till 9, fertile (= RR) homozygotes will be found.

2. The revertant-deficit can be explained on the basis of gametic selection. A high level of seed-set excludes selective non-fertilization of egg-cells. Here, we will test the model of certation, thus excluding selection in the female tetrads, i.e. assuming 1:1 among the eggcells. Denoting the relative frequencies of R- and r-pollen by p' and q', and the observed rel. freq. of RR, Rr and rr by f_1, f_1 and f_0, the expected rel. freq. are 1/2p', 1/2 and 1/2q'. Then q' (see Table) can be estimated as 2 f_0. (Of course, after progeny testing q' can be obtained via $f_0 + 1/2f_1 = 1/2q' + 1/4$). Significant is that the M_2 contains about 50% Rr (expected 1/2N). It should be noted that phenotypic extension (by enzymatic crossfeeding) of genotypic Rr-sectors in M_1 will also lead to an rr-excess in M_2, but at the same time to l e s s than 50% Rr. In fact, No's 5, 6, 8 and 9 rather show a small excess of Rr. Finally, it is seen that certation (q') consistently decreases from M_2 to M_3, though at varying rates. Similar trends with chlorophyll mutants have been ascribed by various authors to certation, diminished in successive generations by natural selection in the pollen.
   At the moment we are trying to find RR in all 9 descents, and make a 10 x 10 diallel (including Ms_1Ms_1) to test for allelism with the Ms_1ms_1 locus, resp. for allelism at other loci (external suppressor loci). This involves 45 large F_2's to distinguish between non-segregation and at best 15:1 segregation.

References:
MÜLLER, A.J.: Arabid.Inf.Serv. 5, 53-54 (1968)
VAN DEN BERG, B.I., and W.J.FEENSTRA: Arabid.Inf.Serv. 5, 27-28 (1968)
VAN DER VEEN, J.H., and P. WIRTZ: Euphytica 17, 371-377 (1968)

A telotrisomic in Arabidopsis thaliana

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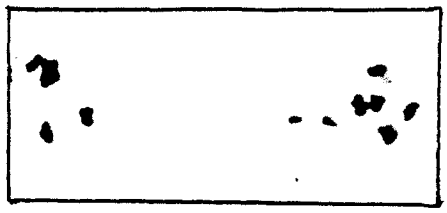

Telophase I. At each pole 5 chromosomes; near the right pole telotrisomic divided. 2300 x

Narrow-serrated (Ns) and Narrow-curved (Nc) trisomics were illustrated by STEINITZ-SEARS in 1963. They closely resemble each other in morphology, except the former has a more serrate leaf margin and a less curved petiole. However, studies showed Ns was triplex for both lu and tz, whereas Nc gave a positive result for tz but not lu. This would be expected if Nc is a secondary, tertiary or telosomic trisomic. Nc was found to be a telotrisomic by cytological examination. It is therefore established that tz and lu are on the different arms of this chromosome.

Reference:
STEINITZ-SEARS, L.M.: Genetics 48, 483-490 (1963)

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The lack of pollinating ability in the Fragilis trisomic

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Although Arabidopsis thaliana is an autogamous plant, the Fragilis trisomic does not set seeds without artifical pollination. This trisomic has two flower anomalies, either or both of which may be responsible for the failure of pollination: (1) Heterostyly, the stigma protruding beyond the anthers. (2) Protogyny, the female gametophyte maturing a few days earlier than the male.

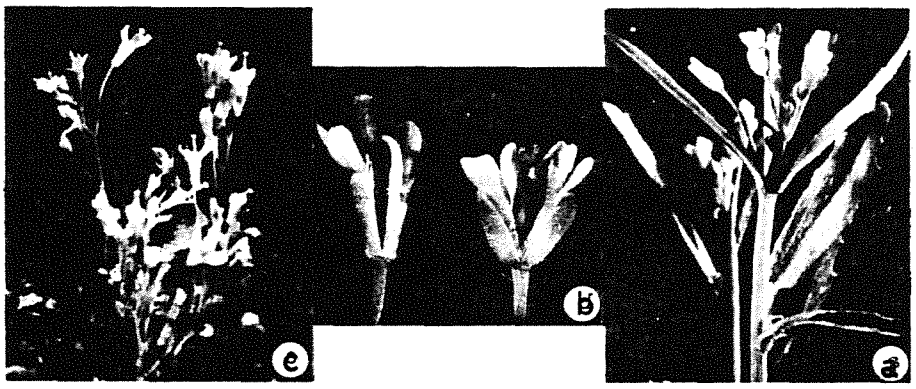

Figure: a) Normal wild type inflorescence, b) close up of flowers: ~~left~~ right, wild type; ~~right~~ left, Fragilis trisomic and c) Fragilis inflorescence

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Sulfhydryl protection against X-rays in Arabidopsis

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MALVAREZ et al. (1965) concluded for barley that cysteamine protects seeds from ionizing radiation by improving M_1-seedling growth and adult survival, while leaving the frequency of chlorophyll mutations unchanged. Recently, BICK and JACKSON (1968) reported a very effective sulfhydryl compound, viz. 1.4-dithiothreitol (DTT), which when applied to marsupial leucocyte cultures during X-irradiation, halves to chromosomal damage (in terms of hits per cell). We decided to test DTT, and also BAL (2.3-dimercaptopropanol), on Arabidopsis (Landsberg-'erecta'), using MÜLLER's embryotest to score for genetic effects. The underlying idea was that the sequence M_1-lethality, M_1-sterility, embryonic lethals, chlorophylls, can be roughly paralleled by a sequence going from gross chromosomal aberrations to "point" mutations. It was then hoped that the protective substances would lead to a more favourable spectrum of the different types of damage. Some of our preliminary results (which are not very promising) are reported here.

G e r m i n a t i o n t e s t s (on agar): Seeds were treated for 5 hrs with dilution series of DTT and BAL in deionized water, both anaerobically (submers) and aerobically (on saturated filterpaper). DTT (tested up to 1.2%) gives only a slight germination delay. BAL, however, gives between 0.2 and 0.4% a sudden and sharp decrease in germination percentage (scored at 40 hrs), although final germination (scored at 136 hrs) is not much reduced (however, seedlings are weak). The toxic effect was most pronounced under anaerobic conditions. In tapwater BAL polymerizes by oxidation (in contrast to DTT), and the decrease in germination percentage (at 40 hrs) becomes less.

X - r a y e x p e r i m e n t : In one of our experiments seeds were soaked for 5 hrs on filterpaper (1.2% DTT), X-rayed (430 Rad/min at 30 cm), left for 2 hrs (still in the sealed Petridishes), rinsed for 5 min and sown on agar. As a comparison, a 50 mM EMS-treatment (6 hrs, 24°C) was added. All items in the Table gave germination over 90%. We scored survival to seed-set, and (in 2 fruits per plant) resp. number of ovules, % of ovules non-fertilized and % embryonic lethals (among ov. fert.). The experiment suffered from several adversities, a.o. aphid infestation, resulting in heterogeneous embryo-development, so that our data for chlorophylls (notably chlorina's, etc.) were not representative. These have therefore been interpolated from other experiments. The Table gives the most illustrative comparisons.

	Control	22.5 kR	22.5 kR + DTT	15 kR	15 kR + DTT	50 mM EMS (6 hrs)
Survival (%)	84	0	55	35	65	65
Plants scored	30	-	63	49	83	78
No. of ovules/fruit	61	-	55	54	54	52
Ov. non-fertilized (%)	8	-	56	56	48	56
Embr. lethals (%)	1	-	16	16	10	34
Chlorophylls* (%)	0.1	-	4.6	3.0	2.1	20.0

* by interpolation from other experiments

The following points emerge:

1) DTT provides c o n s i d e r a b l e p r o t e c t i o n against X-rays. E.g. with 22.5 kR (a completely lethal dose), DTT gives even better survival than 15 kR without DTT.
2) For sterility and mutations 22.5 kR + DTT happens to give the same results as 15 kR without DTT. (The numbers of plants scored were too small to attach much weight to the difference 4.6 vs. 3.0 for chlorophylls).
3) From 1) and 2) follows that the only DTT induced "spectrum shift" between the different categories of damage refers to "survival". There was not, in going from M_1-sterility to M_2-mutations, a progressive retention of the latter (increase in efficiency), as was originally hoped for. Cf. also 15 kR with 15 kR + DTT.
4) Thus EMS remains by far the more efficient mutagen , as it gives (see Table) at equal level of M_1-fertility a far higher mutant yield in M_2 (cf. also MESKEN and VAN DER VEEN, 1968).

References:

BICK, Y.A.E., and W.D. JACKSON: Nature 217, 479-480 (1968)
MALVAREZ, E.N., E.A. FAVRET, and G.S. RYAN: Boletin Genetico (Argentina) 1, 3-16 (1965)
MESKEN, M., and J.H. VAN DER VEEN: Euphytica 17, 363-370 (1968)

An attempt to sensitize Arabidopsis seeds to gamma irradiation after BUdR treatment

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The increase of sensitivity to radiation injury after thymidine analogue incorporation has been shown by several authors in microorganisms, in mammalian and human cells (KAPLAN et al, 1960; DJORDEVIC and DJORDEVIC, 1965; DEWEY et al, 1966; DJORDEVIC and SZYBALSKI, 1960). KIHLMAN (1963) reported an increase in the rate of chromosome breaks due to X-rays in Vicia faba roots pretreated with 5-bromodeoxyuridine. Such a kind of experiment is described here with Arabidopsis and this also includes the mutagenic effects induced in M_2 generation.

Dry seeds of Arabidopsis thaliana, race Wil_2, were pretreated by immersing seeds in a 10^{-3}M BUdR solution for 24h at 24°C. Control seeds were soaked in water. Then, they were irradiated in a Gamma-Cell (3000 C, ^{60}Co) at 23°C with doses of 10, 25 and 50 krad. Seeds were immadiately sown in boxes in the glasshouse and transplanted in pots after full development of the first leaves. Various characteristics of the vegetative and reproductive development were studied and also the chlorophyll mutants in M_2 generation. The results are summarized in Table 1.

Table 1: (1) Percentage of germination 7 days after sowing. (2) Percentage of plants with 4 leaves after 13 days. (3) Percentage of flowering 30 days after sowing. (4) Percentage survival at maturity among transplanted plants. (5) Degree of fertility measured as number of seeds per pod. (6) Frequency of M_2 chlorophyll mutations

Treatment	(1)	(2)	(3)	(4)	(5)	(6)
H_2O	93.0	95.7	98	100	100	0.04
BUdR 10^{-3}M	83.8	100	98	100	91.3	0.09
H_2O + 10 krad	77.3	94.3	95	100	77.9	0.78
BUdR + 10 krad	76.9	95.0	98	100	57.6	1.83
H_2O + 25 krad	68.5	90.1	80	98.2	48.6	1.60
BUdR + 25 krad	65.0	72.2	90	86.0	44.2	1.93
H_2O + 50 krad	62.7	19.1	60	84.4	23.6	5.62
BUdR + 50 krad	57.2	5.7	70	75.8	13.5	5.23

Plants treated only with BUdR are not affected in their developmental cycle, except for a little decrease in germination rate and fertility. An increase to radiation sensitivity may be noted in these seeds for developmental characteristics as regards to leaf formation and survival rate, but dose reduction factors are very low compared to those obtained in human cell lines (DJORDEVIC and SZYBALSKI, 1960). On the contrary, the presence of the analogue decreases the inhibition of the flowering process due to gamma-rays. Fertility is clearly much affected and this fact can be related to the enhancement of chromosomal aberrations produced in the embryo cells as reported by KIHLMAN (1963) in Vicia faba. There is some evidence for an increase in mutational radiosensitization of the seeds treated with BUdR, but only at low doses of gamma rays. It may be possible that some radiation damages are made irreversible by BUdR treatment, but this effect is masked for doses like 25 or 50 krad by the amount of perturbations induced in presoaked seeds by irradiation alone.

As BUdR is uptaken and incorporated into seeds of Arabidopsis (JACOBS, 1968), it can be assumed that the mechanism of sensitization to gamma-rays by this thymidine analogue is related to its incorporation into DNA. Similar experiments with seeds harvested from plants grown on a medium containing BUdR are now in progress.

References:

DEWEY, W., B. SEDITA, and R. HUMPHREY: Science 152, 521-523 (1966)
DJORDEVIC, B., and O. DJORDEVIC: Nature 206, 1165-1166 (1965)
- , and W. SZYBALSKI: J.exp.Med. 112, 509-531 (1960)
KAPLAN, H., K. SMITH, and P. TOPLIN: Nature 190, 794 (1960)
JACOBS, M.: Mutation Res. 7, 51-62 (1969)
KIHLMAN, B.A.: Hereditas 49, 353-370 (1963)

Action of gamma-radiation on dormant, soaking and presoaked Arabidopsis seeds

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It is a well known fact that the processes of seed soaking and germination are usually accompanied by an increase in general radiosensitivity. Our experiments, the results of which are briefly summarized below, were carried out in order to compare the influence of seed soaking on various biological actions of irradiation on Arabidopsis thaliana (L.) HEYNH.

In preliminary experiments with seeds of the race Enkheim it was found that under room conditions the process of water uptake by seeds takes about 6 hrs, and that mass seed germination happened about 48 hrs from the beginning of soaking. As it is difficult to obtain more or less synchronous seed populations at the critical points of the soaking curve, only dormant, soaking, and presoaked seeds (0, 3, and 24 hrs of soaking, respectively) were subjected to ^{60}Co gamma-irradiation (doses: 0, 2.5, 5, 10, 20, and 40 kR, dose rate about 200 kR/hr). The influence of seed soaking on the increase in radiation induced seedling lethality, inhibition of vegetative and reproductive growth and sterility of plants was studied in usual test tube culture. Ten independent replicate experiments, each including twenty plants per treatment, were carried out. The obtained data were subjected to anovar and rank correlation analysis.

On the basis of the response to soaking the studied effects of irradiation may be crudely classified in the following way: (I) those affected already by 3hrs' soaking, this group includes the cotyledon stage lethality, the frequency of completely sterile plants among survivors, and the mean number of seeds per fertile plant; (II) those affected only by 24hrs' soaking, this group includes the rosette stage and total lethality, the mean root length at the 7-th day of the plant growth, and the fraction of seedlings, which formed the rosettes within the 1st week of experiment, and (III) those unaffected by soaking - the fraction of plants which formed the inflorescences within three weeks of experiment, and the duration of the life cycle. It may be seen from this classification that (a) in the respect of decreasing susceptibility to soaking the studied effects of radiation may be ordered as follows: induced sterility - lethal effects - growth inhibition, and (b) within the groups of characters, corresponding to lethality and growth inhibition the influence of seed soaking is lower for the effects of irradiation registered at the later stages of plant growth.

Rank correlation analysis of the data revealed the following: (I) a close correlation between the cotyledon stage lethality and practically all the other characters corresponding to lethal and growth inhibition actions of radiation, (II) a close correlation between the frequency of sterile plants and the decrease in the mean number of seeds per fertile plant, (III) some particular correlations within the group of characters corresponding to lethality and growth inhibition, and (IV) no correlation between the characters corresponding to induced plant sterility, on the one hand, and all the other characters corresponding to the lethality and growth inhibition, on the other hand.

A comparative consideration of the presented data suggests a diverse nature and complex interrelationship between the multiple biological actions of radiations on higher plants. In this respect certain particularities in the manifestation of radiation induced plant sterility seem to be especially interesting. A more detailed description and discussion of the above presented data will be published elsewhere.

Storage effect in gamma-irradiated Arabidopsis seeds

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Experiments are in progrees to determine the effect of room temperature storage of gamma-irradiated dormant seeds on survival, growth, development, fertility, and mutation rate in Arabidopsis thaliana (L.) HEYNH., race Enkheim. A summary of preliminary results of the root growth studies is presented below. The seedlings for root growth measurements were grown either in Petri dishes ("Keimwurzeltest" by MÜLLER, 1964), or in usual test tube culture by LANGRIDGE (1957).

In the first series of experiments (see Table 1) a single dose of irradiation was used, while the storage time was varied. A marked (storage time dependent) increase in irradiation induced root growth inhibition was observed.

Table 1: Root length at the 7-th day of growth (^{60}Co gamma, 70 kR, dose rate about 180 kR/hr, test tube culture)

Storage time	hours					days	
	0	1.5	3	12	24	4	16
Mean root length in mm	10.0	9.5	8.9	7.5	5.0	1.9	1.9

In the second series of experiments (see Table 2) the effect of two weeks' storage was studied at a number of irradiation levels.

Table 2: Root length at the 7 th day of growth (^{60}Co gamma, dose rate about 180 kR/hr, 2-weeks' storage

Dose, kR			0	10	20	40	80
Mean root length in mm	Petri dishes	no storage	12.7	11.4	10.4	8.7	5.9
		storage		10.1	7.7	4.9	3.0
	test tubes	no storage	15.3	-	14.8	13.3	9.0
		storage		-	14.9	11.2	6.4

Here again, a heavier radiation injury was observed in seeds subjected to storage. More detailed description and discussion of the obtained data will be published elsewhere.

References:
LANGRIDGE, J.: Aust.J.biol.Sci. 10, 243-252 (1957)
MÜLLER, A.J.: Kulturpflanze 12, 237-255 (1964)

Relative effectiveness of X- and neutron-irradiation in inducing growth inhibition in Arabidopsis

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In the course of investigation of various biological actions of diverse kinds of ionizing radiations on higher plants the experiments were carried out in which the effectiveness of fast neutrons relative to X-rays in inducing root growth inhibition ("Keimwurzeltest" by MÜLLER, 1964) in Arabidopsis thaliana (L.) HEYNH., race Enkheim, was estimated. Seed irradiation and dosimetry were carried out by the staff of Außenstelle des IfB im ZfK-Roßendorf. Neutron beam was obtained at Y-120 cyclotron (D-Be reaction, average LET about 30-35 keV per micron , dose rate about 6 kRad per hr). Parameters of X-irradiation were as follows: I=20 mA, U=245 V, filter 1 mm Cu + 2 mm Al, dose rate about 6 kRad per hr.

Five experiments were carried out each consisting of at least a hundred root measurements per treatment. The obtained data in per cent of respective control values are tabulated below.

Dose, kRad		0	4.9	10.0	15.3	20.0	20.2	30.0	40.0
Mean root length, %	Neutrons	100	54.2	20.9	10.9	-	8.7	-	-
	X-rays	100	-	98.6	-	91.2	-	79.9	69.6

Crude estimation of relative effectiveness of the radiations applied has shown that the fast neutrons were by about an order of magnitude more effective than the X-rays. This RBE value is in good agreement with respective data obtained by other authors for biological actions of similar radiations on higher plants.

Reference:
MÜLLER, A.J.: Kulturpflanze 12, 237-255 (1964)

Concentration - mutagenic relation following dimethylnitrosoamine (DMNA) treatment

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Given a 3 hr treatment with DMNA (25°C), a maximal frequency of segregating M_1 siliquae was found following 300 to 600 mM and then with an increasing concentration the mutation frequency decreases. The decrease in the mutation frequency at higher concentrations is accompanied with a pronounced decrease in germination. A possible explanation could be a selective DMNA action, which at higher concentrations causes a lethality of seeds, which could give mutated plants.

DMNA mM	Scored plants	% of segregating M_1 siliquae	% of M_1 siliquae segregating chlorophyll mutants	Sterility degree	Germination %
1600	87	14.7	1.6	15.7	2.2
1400	137	13.7	1.7	7.3	9.4
1200	136	29.6	4.5	15.5	31.2
1000	136	27.0	8.3	9.1	39.6
800	137	34.7	6.5	24.4	71.4
600	137	46.8	8.4	26.8	88.5
400	137	40.3	13.9	19.9	92.1
300	136	47.0	10.3	24.6	96.4
200	140	16.4	4.3	4.9	94.9
100	140	12.9	1.4	7.5	95.3
50	136	8.1	0.5	7.5	95.8
0	104	2.6	0	0.2	98.3

Absence of chlorophyll mutations in Arabidopsis treated with LSD

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Reports indicating that absorbed LSD may cause some chromosome breakage in animal cells have appeared (COHEN et al., 1967; IRWIN and EGOZCUE, 1967; and SKAKKEBAEK et al., 1968). To test the mutagenic capacities of this compound we treated Arabidopsis seeds with LSD in water solutions to determine wheter chlorophyll mutations would be induced.

Seeds of Arabidopsis thaliana (L.) HEYNH., race Landsberg, have been used in two experiments. The lysergic acid diethylamide (LSD) was obtained from the Connaught Laboratories, Toronto, Canada. In the first experiment lots of approximately 100 seeds were soaked in LSD solutions at concentrations of 1, 10 and 100 µg/ml for 24 hours at 24°C. In the second experiment concentrations of 1, 10 and 50 µg/ml were used for 17 hours at 26.5°C followed by concentrations of 0.5, 5 and 25 µg/ml for the following 8 hours. One ml of solution was used for 100 seeds. - For comparison one batch of seeds was treated with 1% ethyl methanesulfonate (EMS) for 2 hours and another batch was irradiated with 10 kR of X-rays (200kVp, 20mA, 0.27mm Cu filter). - After treatment all seeds were washed three times (5 minutes) with tap water and placed on VELEMINSKYs complete agar medium. The seeds were then subjected to cold temperature (4°C) for two days and subsequently transferred to the growth chamber (25°C, 68% humidity and 8,640 lux). At maturity seeds from each M_1 plant were collected individually. The M_1 seeds were sown on agar medium. All chlorophyll mutants detected in M_2 populations were recorded. In the Table the mutations have been assigned to M_1 plants that produced mutant offspring.

Table: Chlorophyll mutation frequencies in plants treated with LSD, EMS and X-rays

Treatment	Duration of treatment	No. of seeds treated	No. of M_1 plants tested	M_1 plants with mutation		Total M_2 seedling numbers
				No.	%	
Experiment 1						
Dist. H_2O	24 hrs	236	58	0	0	2320 estim.
LSD 1 µg/ml	24 hrs	98	9	0	0	198
LSD 10 µg/ml	24 hrs	77	11	0	0	286
LSD 100 µg/ml	24 hrs	100	13	0	0	364
Experiment 2						
LSD 1 µg/ml	25 hrs**	109	54	0	0	2322
LSD 10 µg/ml	25 hrs**	100	28	0	0	728
LSD 50 µg/ml	25 hrs**	101	50	0	0	2500
EMS 1%	2 hrs	242	240	74	30.8	5520
X-rays 10 kR	50 min	233	160	11	6.9	5120

** First 17 hrs concentration as given, last 8 hrs concentration reduced to one-half of original.

In agreement with reports by various workers, we found high chlorophyll mutation frequencies in EMS treated populations and moderate mutation frequencies in populations subjected to X-irradiation. No mutations appeared in the progenies of the 165 M_1 LSD treated plants. As used here, LSD has not been shown to have any mutagenic effects. Negative results with LSD as a mutagen have also been reported by GRACE et al. (1968) who found no evidence of either chromosome breakage or gene mutation in treated Drosophila.

References:

COHEN, M.M., M.J. MARINELLO, and N. BACK: Science 155, 1417-1419 (1967)

GRACE, D., E.A. CARLSON, and P. GOODMAN: Science 161, 694-696 (1968)

IRWIN, S., and F. EGOZCUE: Science 157, 313-314 (1967)

SKAKKEBAEK, N.E., F. PHILIP, and O.F. RAFAELSEN: Science 160, 1246-1248 (1968)

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The influence of dimethyl sulfoxide on the mutagenic activity of two alkylating agents

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Dimethylsulfoxide (DMSO) is known as a penetrant carrier of drugs through biological membranes (MARTIN et al., 1967). BHATIA (1967) has shown that 5% DMSO enhances the mutagenic effect of ethyl methanesulfonate (EMS) when simultaneously applied to flower buds. This result is explained by a more rapid uptake of the mutagen. It seemed interesting to test the influence of DMSO on the effectiveness of seed treatments with mutagens. Presoaked seeds of Arabidopsis thaliana were treated for 3 hrs at 24°C with EMS and N-nitroso-N-methyl-N'-nitroguanidine (NMG) dissolved in water or 5% DMSO respectively. After treatment the seeds were washed and stored in water for 6 hrs. The frequencies of embryonic lethals and chlorophyll mutations were determined by the embryo test.

Treatment	No. of M_1-plants scored	Frequency of segregating silique progenies (in %)	
		embryonic lethals	chlorophyll mutations
Water	300	2.3	0.3
5% DMSO	300	3.0	0
50 mM EMS	300	53.3	12.7
" + 5% DMSO	300	42.7	9.7
1.2 mM NMG	300	62.3	17.0
" + 5% DMSO	300	60.7	15.7

We found (see table) that 5% DMSO does not enhance the mutagenic effect. In the case of EMS a decrease of mutation frequency was observed. It is not known whether in the combined solution reactions occur which reduce the concentration of the active EMS. After 3 hrs treatment with 50 % DMSO (without mutagen) germination of the seeds was delayed for 7 hrs, but germination rate and survival rate were normal. Therefore, it is possible to use DMSO concentrations higher than 5 % which was chosen because of its effectiveness with bud treatment.

References:
BHATIA, C.R.: Arabid.Inf.Serv. 4, 39 (1967)
MARTIN, D., A. WEISE und H.J. NICLAS: Angew.Chem. 79, 340 (1967)

Mutation induction by fractionated doses of isopropyl methanesulfonate in Arabidopsis

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Isopropyl methanesulfonate (iPMS) is a monofunctional alkylating agent which reacts so fast that mutation experiments without after-effects can easily be arranged (MÜLLER, 1967). The rate constant for the reaction with water is 0.71 per hr at 25°C (EHRENBERG et al., 1966).

In e x p e r i m e n t I anaerobically presoaked seeds of Arabidopsis thaliana, race 'Dijon G', were treated with 80 mM iPMS (buffered, pH7, prepared at the start of treatment) for 1 hr at 24°C. This treatment was repeated either immediately or after 3 hrs, 12 hrs, 23 hrs and 47 hrs storage in N_2-water at 24°C. Other variants were exposed only to the first or the second treatment respectively (see table). 51 hrs after the first treatment all variants were transferred to aerobic conditions allowing germination. In e x p e r i m e n t II presoaked seeds were treated with 40 mM iPMS at 24°C for 1 hr, 2 hrs, 3 hrs and 4 hrs. (After each hour the solution was renewed.) After treatment the seeds were washed and submerged in water for 3 hrs. The frequencies of embryonic lethals and chlorophyll mutations were determined by the embryo test (MÜLLER, 1966). The results are summarized in the table.

First treatment	Interval (water storage) hrs	Second treatment	No. of M_1-plants scored	Frequency of segregating silique progenies (in %)	
				embryonic lethals	chlorophyll mutations
E x p e r i m e n t I					
Water	-	-	2634	1.8	0.19
80 mM, 1hr	-	water	400	8.5	1.3
"	0	80 mM, 1hr	400	59.2	14.8
"	3	"	375	61.9	15.8
"	12	"	300	55.3	14.7
"	23	"	400	58.6	14.0
"	47	"	400	52.1	9.3
Water	23	"	400	12.5	3.0
Water	47	"	400	12.8	2.3
E x p e r i m e n t II					
Water			400	2.5	0.5
1 x (40 mM, 1hr)			400	6.8	1.3
2 x (40 mM, 1hr)			400	16.2	2.8
3 x (40 mM, 1hr)			400	39.8	9.0
4 x (40 mM, 1hr)			400	64.2	17.5

From the results the following conclusions may be drawn: (1) Mutation induction proceeds at an increasing rate as treatment time increases. Therefore, the dose-response relation is described by a sigmoid curve, if the f r e q u e n c y of mutated cells (or silique progenies) is considered and by a shoulder curve, if the mean n u m b e r of mutations per cell (or progeny) is considered. (2) The mutagenic response is not significantly influenced by the dose rate (4 x 40 mM vs. 2 x 80 mM). (3) Dose fractionation does not significantly change the mutation frequency, at least, if the interval between the treatments does not exceed 24 hrs. The first treatment yields relatively few mutations, but sensitizes in some way the seeds to the second treatment. The sensitized state is stable under anaerobic conditions.

As pointed out previously (MÜLLER, 1966) the shape of the dose curve does not indicate a definite mechanism. Even if the results do not significantly differ from the equation $M = aD^2$ there is no reason to consider only a two-hit mechanism and to ignore other explanations. One of these involves the progressive inactivation of a repair system by the mutational treatment. But is also possible that mutations are far more probable after a double alkylation within a DNA region than after a single alkylation. These explanations could account equally well for the results of the dose fractionation experiment because both, the alkylation of DNA and the inactivation of the repair system, could be preserved in anaerobically stored seeds.

All dose curves obtained with chemical mutagens in Arabidopsis (e.g. GICHNER, 1965; GICHNER and VELEMÍNSKÝ, 1967; MÜLLER, 1964, 1966, and unpubl.) are shoulder curves, i.e., they differ significantly from a linear curve. This is true also for the results published by Van der VEEN (1968). The special interpretation proposed by this author is based on the supposition that there is a constant threshold dose for all seeds (and cells) of the treated population. (Note, that Van der VEEN cites the results of MÜLLER (1966) incompletely and incorrectly and that indeed his interpretation cannot be applied to these results.)

References:
EHRENBERG, L., U. LUNDQUIST, S. OSTERMAN and B. SPARMAN: Hereditas 56, 277 (1966)
GICHNER, T.: "Arabidopsis Research", Rep.Int.Symp. Göttingen 1965. P. 200
- , and J. VELEMÍNSKÝ: Mutation Res. 4, 207 (1967)
MÜLLER, A.J.: Züchter 34, 102 (1964)
- : Züchter 36, 201 (1966)
- : Arabid.Inf.Serv. 4, 43 (1967)
VEEN, J.H.Van der: Arabid.Inf.Serv. 5, 49 (1968)

Two allelic chlorophyll mutations of Arabidopsis thaliana

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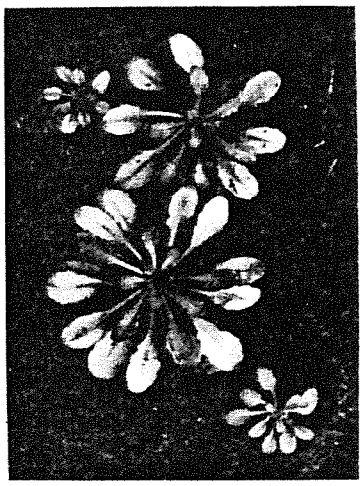

The new powerful mutagens induce a very wide spectrum of chlorophyll mutations. Of special interest are allelic mutations. Genetic analyses of viable chlorophyll mutations induced in our laboratory by γ-rays and EMS and of mutations kindly supplied by Prof. Röbbelen revealed that our mutation viridoalbina 40/3, induced by irradiation of seed (40 kR), is allelic to Röbbelen's mutation "weiss ausbleichend", V-155/2. The F_1 hybrids are all alike V-155/2. The F_2 segregates monofactorially. Reciprocal crosses give the same results.

The responses of these allelic mutations to environmental conditions, however, are very different. In the beginning of the development they are phenotypically alike, later viridoalbina 40/3 grows very slowly. In the greenhouse, during the autumn-winter season, plants of V-155/2 are almost fully green, while viridoalbina 40/3 is green white, small and weak. In the spring-season in the greenhouse they are phenotypically alike, but differ in size.

Figure: Allelic viridoalbina mutants:
large plants = V 155/2; small plants = 40/3

A chlorina mutation of Arabidopsis thaliana induced by gamma rays

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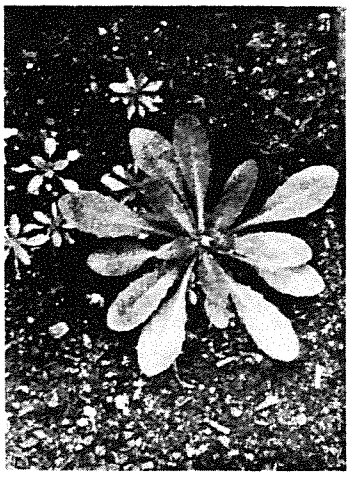

Figure:
Small yellow homozygote recessive plants and a large yellow-green heterozygote of the mutant chlorina 80/2

In previous papers a mutant form chlorina 80/2 was described which was induced by gamma-rays of ^{60}Co (dose 80 kR) on dry seeds. The crossing of this form with race Enkheim shows monofactorial segregation (KASYANENKO, 1967; KASYANENKO and NASYROV, 1968). Detailed investigations of this mutant have revealed that it is heterozygous. When grown in the greenhouse it segregates 2:1 - two parts of large, yellow-green plants and one part of small yellow plants with leaves early dying off. Silique analysis shows that in large plants 25% of all embryos are of the embryo lethal type "vana". The silique of small yellow plants contain only normal embryos (KASYANENKO and USMANOV, 1968). Thus it was established that the described mutant chlorina 80/2 is heterozygous. The dominant homozygotes are not viable and dye in an early embryonic stage. This gives 25% of vana lethals in siliques of large plants. The recessive homozygotes are phenotypically small yellow plants with leaves early dying off and normal embryos. Heterozygotes are large, yellow-green plants described earlier as chlorina 80/2. Reciprocal crosses between the heterozygote and the recessive homozygote were carried out and their results support the above scheme.

References:

KASYANENKO, A.G.: In "Issledovaniya po photosynthesu", Dushanbe, 1967
- , and Yu.S. NASYROV: Physiologiya rasteniy, 15, No. 3, 1968
- , and P.D. USMANOV: DAN academii Nauk Tajikskoy SSR, XI, No. 5, 1968

B. TECHNIQUES

EMS treatment of plants

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It is possible to treat young plants (3 to 12 days old) with EMS. The leaves are very sensitive to the EMS (probably on account of the low pH)*. The best results are obtained by treating the roots and avoiding contact with the leaves. Even then a long treatment damages the plants and a short severe treatment(240 mM 1 hr, or 480 mM 1/2 hr, unbuffered EMS) was found preferable.

By treating the upright plants on the medium on which they are growing (filter paper or agar), the solution can be spread over the medium to reach the roots without touching the leaves. After the treatment the plants must be transplanted to a fresh medium. Plants on filter paper can be "washed" on the paper (to remove the EMS) before transplanting. On agar the EMS may be replaced by water, but, as some will have diffused into the agar, the plants must be transplanted as soon as possible. When transplanting no EMS-contaminated medium may be included. Some root damage is thus sure to occur. By transplanting into perlite the plants recover quickly, even after complete removal of the roots. The plants can then be transferred, with a clump of perlite, to soil. They can be scored in the usual way.

Similar treatments gave good survival and mutant frequencies for chlorophylls, of 2% to 6% (embryo test for 2 consecutive fruits).

*(Addition of the Editor: EMS gives aqueous solutions with a pH of 7, if the substance is undecayed and the solution is fresh. Because of their high toxicity, low pH solutions of EMS have a drastically decreased mutagenic effectiveness, which can be improved only partially by buffering. Cf.: C.F. KONZAK et al., in "Induction of Mutations and the Mutation Process". Pp. 123-132. Czech.Acad.Sci.Publ.,Praha 1965.)

C. MATERIAL

Some new mutants induced by N-nitroso-N-methylurea

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Following well defined mutants of Arabidopsis were produced by N-nitroso-N-methylurea:

autumnalis (aut): Cotyledons smaller, yellow green. Germination delayed and not uniform. Rosettes loose, smaller. Leaves light green, narrower, pointed. Petioles shorter. Very early flowering, especially in early autumn. Inflorescences loose, pods short and cylindrical.
F_1 after crossing with the standard type: normal.
F_2 yet unknown.
Origin: Dijon G after 0.05 mM NMU. From M_2 to M_6 constant, in the M_3 new aberrant types appeared.

delicata (da): Cotyledons smaller. Rosettes somewhat smaller, delicate. Leaves bright green. Flowering slightly delayed.
F_1 after crossing with the standard type: normal.
F_2 yet unknown.
Origin: Dijon G after 0.10 mM NMU. From M_2 to M_6 constant.

deltoidea (dl): Cotyledons smaller. Germination not uniform. Rosettes small. Leaves deltoid, pointed, dentate, decurrent, grayish. Very late flowering. Inflorescences loose, pods thin, subfertile.
F_1 after crossing with the standard type: normal.
F_2 117 normal : 35 dl.
Origin: Dijon G after 0.10 mM NMU. From M_2 to M_6 constant, in the M_4 new constant aberrant type appeared (see rotundifolia, rf).

grisea (gr): Cotyledons grayish green. Rosettes flat, compact, small. Leaves roundish, broadest on the basis, grayish green. Inflorescences looser.
F_1 after crossing with the standard type: normal.
F_2 18 normal : 8 gr.
Origin: Dijon G after 0.05 mM NMU. Detected in a M_3 line derived from a weak M_2 plant. From M_3 to M_6 constant.

lucida (lc): Cotyledons yellow green. Rosettes compact, slightly smaller. First leaves roundish, the following ones normal, shinning yellow green. Young leaves greener. Very late flowering.
F_1 after crossing with the standard type: normal.
F_2 100 normal : 35 lc.
Origin: Dijon G after 0.05 mM NMU. From M_2 to M_6 constant.

lanceolata (ln): Cotyledons narrower, with visible petioles. Rosettes loose, smaller. Leaves pointed, narrower, lance-shaped, extremely decurrent. Petioles long. Very early flowering. Inflorescences looser.
F_1 after crossing with the standard type: normal.
F_2 yet unknown.
Origin: Dijon G after 0.10 mM NMU. From M_2 to M_6 constant.

quasinormalis (qn): Cotyledons larger. Rosettes small. Leaves with necrotic edges. Inflorescences loose. Subfertile. Expression of all these characters very variable, in certain experimental conditions resembling the standard type.
F_1 after crossing with the standard type: normal.
F_2 115 normal : 36 qn.
Origin: Dijon G after 0.10 mM NMU. From M_2 to M_6 constant, in the M_3 and M_4 new aberrant types appeared.

rotundifolia (rf): First leaves roundish, edges rolled up.
F_1 after crossing with the standard type: normal.
F_2 yet unknown.
Origin: Dijon G after 0.10 mM NMU. Detected in the M_4 line, derived from the mutant line deltoidea, dl (see above). From M_4 to M_6 constant.

In many other mutants, pure lines were as yet not obtained and numerous new aberrant types continue to appear during the breeding experiments. In some M_3 lines, often six or more different aberrant types could be found. Experiments have been carried out to explain this behaviour.

D. LABORATORY RESEARCH COMMUNICATION

Resistant mutants to certain antimetabolites

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A programme of study aimed at the production of resistant mutants in Arabidopsis thaliana is under way. For the production of mutations, different mutagens are being used and at present EMS treatment has given rise to a few "presumptive" mutants showing remarkable resistance to some antimetabolites. Presence of these substances in "adequate" quantities could not in any way inhibit the developmental aspects, including fertility, of these plants. Confirmation tests are in progress.

E. BIBLIOGRAPHY

(First addition to the list compilated in A.I.S. No. 5)

BERGER, Brigitte: Entwicklungsgeschichtliche und chromosomale Ursachen der verschiedenen Kreuzungsverträglichkeit zwischen Arten des Verwandtschaftskreises Arabidopsis. Beitr.Biol.Pflanzen 45, 171-212 (1968)

BHATIA, C.R., and H.H. SMITH: Adaptation and growth response of Arabidopsis thaliana to deuterium. Planta 80, 176-184 (1968)

BOELPAEPE, R. de: Etude des mutations létales récessives induites par le MNG chez Arabidopsis thaliana (L.) HEYNH. Agricultura (Louvain) 16, 48-55 (1968)

BOUHARMONT, J.: Evolution of the chromosome numbers in Arabidopsis polyploids. In: Chromosomes Today, Proc. 1.Oxford Chromosome Conf. 1964. Plenum Press, New York 1966

BROCK, R.D.: Quantitative variation in Arabidopsis thaliana induced by ionizing radiations. Radiat.Bot. 7, 193-203 (1967)

FEENSTRA, W.J., and Beatrix I. Van den BERG: Continuously segregating revertants of pyrimidineless mutants of Arabidopsis thaliana. In "Proc. XII Int.Congr.Genet.", Tokyo, Aug. 19-28, 1968. Vol. 1, p. 114

GICHNER, T., L. EHRENBERG, and C.A. WÄCHTMEISTER: The mutagenic activity of β-hydroxyethyl methanesulfonate, β-methoxyethyl methanesulfonate and diethyl 1.3-propanedisulfonate. Hereditas 59, 253-262 (1968)

- , J. VELEMÍNSKÝ, and J. KŘEPINSKÝ: Strong mutagenic activity of Streptozotocin - an antibiotic with an alkylnitroso group. Molec.Gen.Genetics 102, 184-186 (1968)

HABERER, G., K. EICHHORN, H. ABEL, und V. IVANOV: Strahlenbiophysikalische Untersuchungen an Arabidopsis thaliana. 1. Physikalische Grundlagen. Studia biophysica (Berlin) 6, 113-120 (1968)

HIRONO, Y., and H.H. SMITH: Mutations induced with a DNA base analog in higher plant, Arabidopsis. In "Proc. XII Int.Congr.Genet.", Tokyo, Aug. 19-28, 1968. Vol. 1, p. 117

- , - , and J.T. LYMAN: Tumor induction by heavy ionizing particles and X-rays in Arabidopsis. Radiat.Bot. 8, 449-456 (1968)

HUSSEIN, H.A.S.: Genetic analysis of mutagen-induced flowering time variation in Arabidopsis thaliana (L.) HEYNH. Ph. D. Thesis Wageningen 1968

IVANOV, V.I., A.V. SANINA, and H.A. TIMOFEEFF-RESSOVSKY: Effect of seed gamma-irradiation on survival, growth, development, and seed setting in Arabidopsis thaliana (L.) HEYNH. (Russ.) Radiobiologia 8, 118-123 (1968)

- , - , - , und G. HABERER: Strahlenbiophysikalische Untersuchungen an Arabidopsis thaliana. 2. Vorläufige Versuche über Bestrahlung ruhender Samen mit Röntgenstrahlen, Protonen und Alphateilchen. Studia biophysica (Berlin) 10, 149-154 (1968)

JACOBS, M.: Effets induits chez Arabidopsis thaliana (L.) HEYNH. par les colorants à l'acridine sur les plantes traitées et leurs générations successives. Bull.Soc.Roy.Bot.Belg. 102, 1 (1968)

- : Un mutant chlorophyllien d'Arabidopsis déficient pour la synthèse de thiazole. Bull.Soc.Roy.Bot.Belg. 102, 2 (1969)

- : Studies on the genetic activity of thymidine-base analogue in Arabidopsis thaliana. Mutation Res. 7, 51-62 (1969)

KAMRA, O.P.: Effects of incorporated radioisotopes and acute radiation exposure on Arabidopsis thaliana. In "Proc. XII Int.Congr.Genet.", Tokyo, Aug.19-28, 1968. Vol.1, p.112

KRANZ, A.R.: Endogene und exogene Beeinflussung der apparenten Strahlungsenergienutzung annueller Pflanzen. Angew.Bot. (Berlin) 41, 271-278 (1968)

KUČERA, J., and I. CETL: The relation of the hypocotyl growth rate to the vernalization requirement in Arabidopsis thaliana (L.) HEYNH. In "Differentiation of Apical Meristems and Some Problems of Ecological Regulation of Development of Plants". Proc.Symp. Praha-Nitra, Aug. 30 - Sept. 6, 1964. Pp. 309-312, Academia, Praha 1966

- , - : Über die Entwicklung der Lokalpopulationen von Arabidopsis thaliana (L.) HEYNH. in der Abhängigkeit von Jarowisation und Photoperiode. (Czech, with German summary) Publ.Fac.Sci.Univ.of J.E. PURKYNĚ, Brno.No. 479, 1-13 (1967)

LAWRENCE, C.W.: Radiation induced polygenic mutation in Arabidopsis thaliana. I. Selection for flowering time. Heredity 23, 321-337 (1968)

- : Radiation induced polygenic mutation in Arabidopsis thaliana. II. Analysis of lines selected for flowering time. Heredity 23, 573-589 (1968)

MĚSÍČEK, J.: The chromosome morphology of Arabidopsis thaliana (L.) HEYNH. and some remarks on the problem of Hylandra suecica (Fr.) LÖVE. Folia geobot.phytotax. (Praha) 2, 433-436 (1967)

MESKEN, M., and J.H. VAN DER VEEN: The problem of induced sterility: a comparison between EMS and X-rays in Arabidopsis thaliana. Euphytica 17, 363-370 (1968)

NIKOLOV, Ch.V., and V.I. IVANOV: Effect of gamma-irradiation and heat treatment on seeds of Arabidopsis thaliana (L.) HEYNH. (Russ.). Radiobiologia 8, 442-447 (1968)

- , - : Influence of heat treatment and gamma-irradiation of seeds of Arabidopsis thaliana (L.) HEYNH. on the M_2 mutation frequency. (Russ.). Genetika 5, 166-168 (1969)

PEDERSON, D.G.: Environmental stress, heterozygote advantage and genotype-environment interaction in Arabidopsis. Heredity 23, 127-138 (1968)

RÉDEI, G.P.: Antimetabolites and differentiation in mutants of Arabidopsis. In "Proc. XII Int.Congr.Genet.", Tokyo, Aug. 19-28, 1968. Vol. 1, p. 134

REHWALDT, C.A.: Filter paper effect on seed germination of Arabidopsis thaliana. Plant and Cell Physiol. 9, 609-611 (1968)

RÖBBELEN, G.: Reversions indicating efficient mutagenesis. In "Proc. XII Int.Congr.Genet.", Tokyo, Aug. 19-28, 1968. Vol. 1, p. 114

- : Genbedingte Rotlicht-Empfindlichkeit der Chloroplastendifferenzierung bei Arabidopsis. Planta 80, 237-254 (1968)

SANKHLA, N., and D. SANKHLA: Interaction between growth regulators and (±)-abscissin II in seed germination. Z.Pflanzenphysiol. 58, 402-409 (1968)

ŠVACHULOVÁ, J., and J. VELEMÍNSKÝ: N-metabolism and chlorophyll content in a chlorophyll mutant supplied by sucrose. Studia Biophysica (Berlin) 5, 137-142 (1967)

VELEMÍNSKÝ, J., and T. GICHNER: The mutagenic activity of nitrosoamines in Arabidopsis thaliana. Mutation Res. 5, 429-431 (1968)

- , V. POKORNY, and T. GICHNER: The effect of visible light on mutagenic activity of 1-methyl-3-nitro-1-nitrosoguanidine and 1-methyl-1-nitrosourea. Biol.Plantarum (Praha) 10, 85-94 (1968)

VEEN, J.H. VAN DER, and P. WIRTZ: EMS-induced genic male sterility in Arabidopsis thaliana: a model selection experiment. Euphytica 17, 371-377 (1968)

WIBAUT, Christiane: Analyse quantitative des synthèses nucléiques dans l'apex de l'Arabidopsis thaliana (L.) HEYNH. au cours des diverses phases de son développement, en photopériode inductive. C.R. Acad.Sci. Paris 263, 343-346 (1966)

- : Examen histoautoradiographique des modifications du fonctionnement apical de l'Arabidopsis thaliana en photopériode défavorable à la floraison. C.R. Acad.Sci. Paris 263, 1582-1585 (1966)

(In the press)

BESNARD-WIBAUT, Christiane: Modifications des synthèses nucléiques dans l'apex de l'Arabidopsis thaliana (L.) HEYNH. lors du passage à l'état reproducteur. Analyse histoautoradiographique de l'action de la photopériode et de l'acide gibbérellique sur le fonctionnement apical. Coll.Intern. sur les "Aspects cellulaire et moléculaire de l'induction florale". Liege 1968

GICHNER, T., and J. VELEMÍNSKÝ: Factors influencing the mutagenic activity of 1-methyl-3-nitro-1-nitrosoguanidine and 1-ethyl-3-nitro-1-nitrosoguanidine in Arabidopsis thaliana. (Czech with English summary). Genetics and Plant Breeding (Praha

HIRONO, Y., and H.H. SMITH: Mutations induced in Arabidopsis by DNA nucleoside analogs. Genetics

JACOBS, M.: Comparaison de l'action mutagénique d'agents alkylants et de radiations gamma chez Arabidopsis thaliana. Radiat.Bot.

RÉDEI, G.P.: Arabidopsis thaliana (L.) HEYNH. A review of the genetics and biology. Bibliographia Genet.

VELEMÍNSKÝ, J., J. ŠVACHULOVÁ, and W. STARZECKI: Photosynthesis in two chlorophyll mutants of Arabidopsis thaliana growing on a sucrose medium. Photosynthetica (Praha)

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