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Arabidopsis Information Service

The newsletter is intended to cover all aspects of research with Arabidopsis and allied species. It provides a forum for the publication and discussion of current research news, especially in genetics, but also in ecology, morphology, development, physiology, and biochemistry. The newsletter is also open to all informations on methods, materials and stock-exchange as well as to laboratory research communications dealing with tentative experimental results and research programs underway. It is hoped that by such a policy the newsletter will extend the international communication on Arabidopsis research.

At present one number is issued annually in March. Contributions should be submitted not later than January 15, each year. For preparation of the manuscript see inside the back-cover. It is understood, that the contents of the contributions are the sole responsibility of the authors.

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

No. 4 Göttingen March, 1967

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(continued)

A. BRIEF NOTES

Diminution of chromosome number in Arabidopsis polyploids

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Hexaploid and octoploid plants of Arabidopsis thaliana had been induced by colchicine treatment of a tetraploid strain. After several generations most of the plants had only 20 somatic chromosomes instead of 30 or 40, and only a few individuals showed a chromosome number higher than 20. Observations were made to see whether a progressive loss of chromosomes occurs in these high polyploids.

Chromosome counts are difficult to obtain in polyploid Arabidopsis. The most reliable method appeared to be counting at meta- or anaphase II in both nuclei of the pollen mother cells. Flower buds were fixed according to STEINITZ-SEARS (1963) but without evacuation. After a quick staining in acetocarmine, smears were observed in phase contrast.

Chromosome numbers were determined (a) in the offspring of a plant with 26-28 somatic chromosomes and in the progeny of two of these plants, and (b) in the offspring of a plant with 2n = 36-38 arisen from a colchicine induced tetraploid as well as in the progeny of two of these plants. These counts gave the following numbers, some of which being only approximate:

Parental chromosome number	Chromosome numbers in the offspring
(a) 26-28	22, 20-22, 22-24, 24, ±24, ±20, 24, 24, 25
22	±21, 20, 20, 22, 21, ±22, ±22, 21, ±21, ±22, 20-22, 26, 22, 20-22
25	21, ±22, 26, ±22, 23, 20, ±20, 23
(b) 36-38	±36, ±36, ±36, ±36, ±32, 34, 36-38
±36	32-34, 32, ±34, ±34, 34, 32
34	34, 31, 35, 36, 33, ±35, ±32, 33, 33

Many plants had a lower chromosome number than their parent, while an increase was rare. No single plant with 40 chromosomes was recovered. On the other hand, the tetraploid strains were ± stable, and within their progeny only a few aneuploids occurred. Meiotic studies revealed several causes of chromosome loss in these high polyploids.

(1) In plants with more than 20 chromosomes some PMS's in the first meiotic division showed a more or less complete splitting of the spindle and of the equatorial plate in two generally unequal parts, one of them frequently including only 2-4 chromosome pairs. During the second division all the nuclei divided at the same time, but the microspores containing only a few chromosomes were abortive.

(2) In all plants multivalents and univalents were frequent in metaphase I causing an uneven distribution of the chromosomes during anaphase. This irregularity produces a large variability in the chromosome numbers during the second division and in the microspore nuclei; it may lead to an increase as well as decrease of somatic numbers in the offspring.

(3) Frequently 1-3 univalents remained in the spindle median as laggards, being the reason for a decrease in the chromosome number of the microspores. Nevertheless, some laggards divided during the anaphase I and both strands went to the opposite poles; not undergoing a second splitting they gave an uneven distribution at anaphase II.

These 3 irregularities sufficiently explain the variability of the chromosome numbers in progenies of high polyploids and the general tendency towards their decrease. This diminution apparently stops at the tetraploid level, though it seems possible that the 20-chromosome plants so produced do not possess the four complete sets of a true autotetraploid.

Indeed, two of these 20-chromosome plants gave progenies with a significantly later blooming than the true tetraploids. However, their meiotic behaviour appeared not to be different, and seed and pollen sterility were not increased. Further comparison of true and "secondary" tetraploids are thus necessary.

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

The location of linkage groups on the chromosomes of Arabidopsis by the trisomic method

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Trisomics in Arabidopsis (Columbia wild type) were first described by L. M. STEINITZ-SEARS (1963). They were unnamed and are termed here according to their phenotype: R (round leafed), Y (yellow or light), Nc (narrow cut leaf), and Ns (narrow serrated) respectively. In the race En-2 RÖBBELEN and KRIBBEN (1966) selected four trisomics which were numbered I-IV. McKELVIE (private communication) also found some trisomics in his stocks.

Trisomics were crossed to homozygous mutant marker lines representing the six linkage groups established by G. P. RÉDEI (RÉDEI and HIRONO; cf. also Arabidopsis Research, Göttingen, p. 210) and kindly provided by him. Some additional material was used in Göttingen provided by A. D. McKELVIE. F_2 segregations from trisomic F_1 plants were observed and some of the same markers were used at both institutions. The data obtained independently at the two laboratories are presented here in two tables from which some conclusions may be drawn about the location of the linkage groups and the correspondence of the trisomic lines. Detailed reports will follow.

Table 1: Information obtained from F_2 segregation data in Columbia, Mo.

Linkage group after RÉDEI	Marker used	Trisomic tested R	Y	Nc	Ns	II
1	an	-	-		-	-
2	py	+		-		
2	hy	+	-			
3	gl	-	+	-	-	-
4	ch	-	-	-		+*
5	lu		-	+		
5	tz	-		+	+	

*probably on II; needs further confirmation

Table 2: Information obtained from F_2 segregation data in Göttingen

Linkage group after RÉDEI	Marker used	Trisomic tested I	II	III	IV
1	an	-	-	-	-
2	er*	-	-	+	-
3	gl	+	-	-	-
5	lu	-	-	-	+
after McKELVIE					
2	g'	+	-	-	-
3	q'	-	-	-	+

*this marker may be hard to classify with the trisomic III phenotype

Form the phenotypic appearance of the trisomic as well as from the genetic data it can be stated that:

R is the same as III and carries RÉDEI's linkage group 2.

Y is the same as I and carries RÉDEI's linkage group 3, which seems to be the same as McKELVIE's linkage group 2.

The two narrow leafed trisomics (Nc and Ns), formerly regarded as two different types, are the same and identical with IV; it carries linkage group 5 of RÉDEI and 3 of McKELVIE.

Trisomic II differs from R, Y, and N and carries RÉDEI's linkage group 4.

The fifth primary trisomic (STEINITZ-SEARS, 1963) was lost because of its weak nature and poor transmission. Efforts are being made to recover this type.

References:
McKELVIE, A. D.: in "Arabidopsis Research", Göttingen, pp. 79-84, 1965
RÉDEI, G. P., and Y. HIRONO: Arabid. Inf. Serv. 1, 9-10 (1964)
RÖBBELEN, G., and F. J. KRIBBEN: Arabid. Inf. Serv. 3, 16-17 (1966)
STEINITZ-SEARS, L. M.: Genetics 48, 483-490 (1963)

This work was supported by NIH grant GM-12400 to L. M. Steinitz-Sears and by a grant of the Deutsche Forschungsgemeinschaft to F. J. Kribben.

A new segregation distorter factor in Arabidopsis

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An abnormal progeny was obtained from x-ray irradiated seeds (50,000 r) of the Columbia wild type. In the second selfed generation it segregated into 69 green and 57 yellow-semilethal plants. The progenies of 20 green sibs were further analyzed. All repeated the abnormal ratio. In the subsequent generation 112 normal phenotype plants were further studied; only seven of them did not segregate. The majority of the segregating families was abnormal though some displayed approximately normal ratios.

Normal phenotype individuals from abnormally segregating families were crossed reciprocally to normal testers with recessive markers. When the testers were used as males, 46.4% (13/28) of the individuals did not segregate for the yellow-semilethal factor; in the reciprocal crosses the corresponding value was 12.9% (4/31).

It appears that the abnormal ratio is caused by two factors: a yellow-semilethal factor and a segregation distorter which were induced in the same cell, and involving homologues of the same chromosome. In the original cell these two factors were apparently in repulsion. The yellow-semilethal factor is normally transmitted through both egg and sperm, but the distorter factor is generally not transmitted through the sperm. Their relative distance can be determined by the segregation ratio and transmission data (HIRONO, 1964).

In the second selfed generation 57 (45.2%) yellow-semilethal plants were observed in a population of 126. If the segregation distorter is not transmitted through the male, and the female transmission is nearly normal as the limited data indicate, one would expect 43.6% recessives if the recombination between the distorter and the yellow-semilethal is 12.9%. (The frequency of non-segregating progeny is a measure of recom-

bination between these two factors.) In another experiment 112 normal phenotype plants from the abnormal families were progeny tested; only seven (6.3%) failed to segregate for the yellow-semilethal factor. When the double heterozygotes were outcrossed to female testers, 87.1% (27/31) of the progenies segregated normally for the yellow-semilethal. The reciprocal crosses, however, indicated equal transmission of both strands (15:13). Furthermore, five families (18.5%) out of 27 segregated with a definite deficiency of the recessive class, indicating that the distorter and the yellow-semilethal were in coupling phase. The difference among 6.3%, 12.9% and 18.5% could probably be due to the size of the small populations studied.

Crossing over is quite a variable phenomenon. Exact information on recombination can be obtained in large populations only. It appears safe to conclude that these two factors are in the same chromosome and the map distance is in between 6 and 18 units. So far it was not successful to locate these two factors as to the linkage group established in our laboratory. The markers an, er, gl^1, gi^2, and co are independent of these two factors.

Information obtained in our laboratory indicates that gametophyte factors (HIRONO and RÉDEI, 1965; RÉDEI, 1965) are frequently produced by mutagens. The estimation of mutation rate by the use of the mutant frequencies in the second selfed generation after treatment may be loaded with different but definite systematic errors because of the presence of gametophyte factors. The poor male transmission may lead to an over- or underestimate of recessive frequencies in the second selfed generation depending whether the gametophyte factor occurred in repulsion or in coupling with the induced mutant factors.

References:

HIRONO, Y.: Ph.D. thesis, University of Missouri, 1964
HIRONO, Y., and G. P. RÉDEI: in "Arabidopsis Research", Rep. Int. Symp. Göttingen, pp. 85-90, 1965
RÉDEI, G. P.: Genetics 51, 857-872 (1965)

The variability of developmental characters in natural populations of Arabidopsis thaliana (L.) HEYNH.

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It is known that in Arabidopsis the developmental characters are the most variable ones. NAPP-ZINN (1964) has shown that there may be considerable variation not only among different populations and different plants within a population but also in the progenies of individual plants. In our experiments it has been found that a high variability exists both between and within populations, which appears to be distributed into determinable categories (CETL, 1965). In this communication the variability within progenies of individual plants is presented in addition to the earlier data.

The experiments were carried out on five populations, two of which were "summer annual" (Kr-4, Pi) and three "mixed" (Bo-1, Dou, He-2) (Cf. CETL, 1965, pp. 47-48). The seed was sown in the experimental garden of the Department on May 3, 1965. Under these conditions an elimination of late types, especially in the "mixed" populations, and also a possibility of crossing were not excluded.

The progenies of all harvested plants were grown at 25+ 3°C under continuous illumination (1250 lux) for 42 days. In this case the variability within progenies in the number of days to appearance of the flower primordia ($\underline{x}$) and in the number of rosette leaves (y) was expressed in terms of the variation coefficients ($\underline{v}$) as these are practically independent of the absolute values. At the same time $\underline{v}$ values of four homozygous races (S-96, Di, Li-5, and H-55) were calculated for comparison (see table).

Populations	Distribution of variation coefficients in the progenies 0------5-----10-----15-----20-----25-----30-----35-----40-----45-----50										
	x										
Bo-1			3	12	4	2	1			1	
	%		13.0	52.2	17.4	8.1	4.3			4.3	
Dou			7	10	5	2	2			1	
	%		25.9	37.0	18.5	7.4	7.4			3.7	
He-2		1	13	27	14	1	1				
	%	1.8	22.8	47.4	24.6	1.8	1.8				
Kr-4			10	12	7	4	2	2	1		
	%		26.3	31.6	18.4	10.5	5.3	5.3	2.6		
Pi			26	30	27	4	2				
	%		29.2	33.8	30.4	4.5	2.2				
Races			4								
	%		100.0								
	y										
Bo-1			6	11	3	2	1				
	%		26.1	47.8	13.0	8.7	4.3				
Dou		1	5	7	5	5	3				1
	%	3.7	18.5	25.9	18.5	18.5	11.1				3.7
He-2		1	14	24	13	2	3				
	%	1.8	24.6	42.1	22.8	3.5	5.3				
Kr-4			10	15	5	2	2	1	1	1	1
	%		26.4	39.5	13.1	5.3	5.3	2.6	2.6	2.6	2.6
Pi		1	47	36	5						
	%	1.1	52.8	40.4	5.6						
Races		2	2								
	%	50.0	50.0								

It is surprising that a great majority of progenies shows a higher variation in x as well as in y than the homozygous races. If the value v = 10.0% in both characters is conventionally chosen as a limit between the homozygotes and heterozygotes then in the material presented here only 25.6 and 36.4% progenies appear to be homozygous in the two characters. As it can be seen from unpublished data referring to A_3 lines obtained from similar natural populations, the percentage of homozygous progenies can be increased by means of a repeated autogamization in the two characters to 43.3 and 57.4%.

These results confirm, in agreement with the data presented by NAPP-ZINN (1964), that in natural populations of Arabidopsis there is an apparent heterozygosity at least in the characters studied. This heterozygosity in such an autogamous plant, its origin and causes, must be examined by special methods.

References:

CETL, I.: in "Arabidopsis Research", Rep. Int. Symp. Göttingen, pp. 46-52, 1965
NAPP-ZINN, K.: Beitr. zur Phytologie, Festschr. H. WALTER, pp. 39-49, 1964

The behaviour of "summer annual", "mixed", and "winter annual" natural populations as compared with early and late races in field conditions

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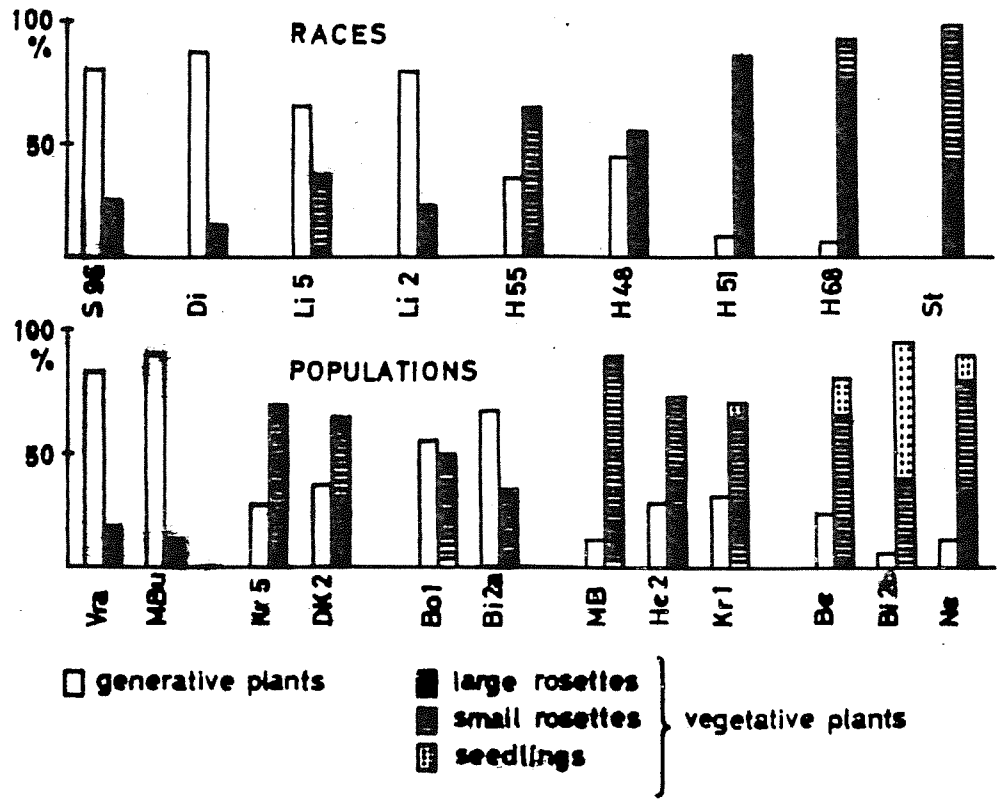
In earlier experiments (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1965; CETL, 1965) the developmental characters of natural populations were studied under artificial conditions. Thus, it appeared desirable to know the behaviour of these populations in field conditions when the usual time limits of the seed shedding were maintained.

For this purpose twelve different populations were chosen according to the percentage of generative plants and to the earliness of the generative plant fraction ascertained in laboratory experiments (25± 3°C, continuous illumination 1250 lux). Among these populations there were four "summer annual" (two early: Vra, MBu, and two late: Kr-5, DK-2), five "mixed" (two with about 50% generative plants: Bo-1, Bi-2a, and three with about 25% generative plants: MB, He-2, Kr-1), and three "winter annual" (Be, Bi-2b, Ne). For comparison 9 homozygous early and late races were used (S-96, Di, Li-5, Li-2, H-55, H-48, H-51, H-68, and St. Cf. VAN DER VEEN, 1965; NAPP-ZINN, 1957, 1963).

All these samples were sown in 10-inch pots with 15 seeds each in three replications on March 3, 1966. The pots were placed in the experimental garden of the Department. The early sowing was necessary for removing the differences in the earliness: in the first few weeks after sowing the vernalization requirement was fully covered in winter annual types while the summer annual ones were inhibited in development by low temperatures and by short days. In consequence, the development was almost simultaneous in all samples. Immediately before flowering the pots were covered with polyethylene bags for prevention of crossing and mixing of seeds. The majority of seeds was shed between June 20 and 23. At the same time the mother plants and bags were removed. Every week generative plants (with visible flower primordia or already flowering) were scored and removed. At the end of the vegetation period (October 19) the remaining vegetative plants were counted and classified according to size of rosettes. Since the germination could not be pursued quantitatively during the experiment it was only possible to judge its course indirectly with the aid of this classification.



The results in the figure show that in races as well as in populations the total percentage of generative plants was diminishing from the earliest types to the latest ones. In early races, and populations without a vernalization reaction, practically all plants flowered during summer and fall while in the late ones almost all plants remained vegetative because of their vernalization requirement. This means that the conclusions on the developmental character according to the laboratory results correspond to the situation in nature.

It must be taken into account that the seeds of all generative plants had to germinate in a short time after shedding as it is in agreement with the visual observation of germination. For this reason these plants could develop in favourable conditions of temperature and photoperiod. On the other hand, the very small part of vegetative plants in early types had to germinate considerably later than the generative ones.

In late types there was a conspicuous difference between races and populations: the majority of plants in races was represented by large rosettes which indicate that they germinated relatively early, while in the populations the plants occured mostly as small rosettes or seedlings and showed that they germinated only at the end of the summer period. Therefore, in natural conditions the after-ripening requirement of late (winter annual) populations seems to be greater than those of the races in which selectional changes in the direction to the shortening of the after-ripening period are possible.

Generally, it can be concluded from the given data that in field conditions the behaviour of a type is dependent not only on the earliness or lateness determined by an interaction of the vernalization and photoperiodic requirement but also on the after-ripening conditions.

References:

CETL, I.: in "Arabidopsis Research", Rep. Int. Symp. Göttingen, pp. 46-52 (1965)
CETL, I., J. DOBROVOLNÁ, and E. EFFMERTOVÁ: Arabid. Inf. Serv. 2, 3 (1965)
NAPP-ZINN, K.: Z. Vererbungslehre 88, 253-285 (1957)
- : Ber. D. Bot. Ges. 76, 77-89 (1963)
VAN DER VEEN, J. H.: in "Arabidopsis Research", Rep. Int. Symp. Göttingen, pp. 62-71, 1965

The author thanks Prof. K. NAPP-ZINN and Dr. J. H. VAN DER VEEN for furnishing us with seeds of the races

The role of the temperature factor in the geographical distribution of "summer annual" and "winter annual" natural populations of Arabidopsis thaliana (L.) HEYNH.

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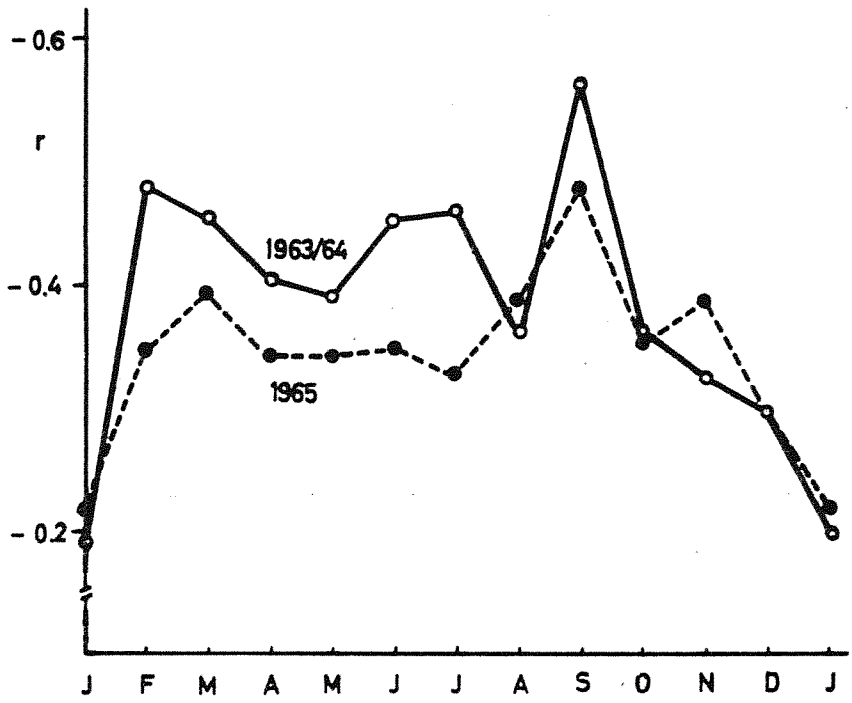
In earlier communications (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1965; CETL, 1965) it has been shown that there exists a relation between the geographical-climatic conditions of localities and the developmental character of populations growing in West-Moravia: the "winter annual" populations seem to be bound to the more warm and dry lowland, the "summer annual" populations to the more cold and wet highland, while the "mixed" ones occupy intermediate positions.

In experiments carried out in conditions inducing a rapid flowering in the summer annual types and inhibiting it in the winter annual ones (unvernalized post-dormancy seeds grown on soil at 25±3° C under continuous illumination with 1250 lux, duration of the experiment 42 days), it was possible to distinguish between the two main developmental types. The percentage of the generative plants was chosen as a measure for the developmental properties of a population. With the aid of this characteristic, the correlations were estimated between it and the geographical-climatic conditions expressed by (1) the height above sea level, and (2) the average temperature (macroclimatic data for 1901-56 read from SYROVÝ, 1958).



Significant positive correlation coefficients were found for the height above sea level and the percentage of generative plants ($\underline{r}$ = 0.6197, $P < 0.0001$ in the 64 populations collected in 1963/64, and $\underline{r}$ = 0.4710, $P = 0.0001$ in the 45 populations collected in 1965). For the average yearly temperature and the percentage of generative plants, the correlation coefficients were significant and negative ($\underline{r}$ = -0.3996, $P < 0.01$, and $\underline{r}$ = -0.3068, $P < 0.05$, respectively). Thus the more elevated the geographical position and the lower the average yearly temperature, the more a population consists of generative (=summer annual) plants, and vice versa.

Interesting results were also obtained when the correlations were estimated between the monthly average temperatures and the percentage of generative plants (Figure 1). In both of the series, the maximum values were found for September ($P < 0.0001$). A second maximum could be shown in February-March ($P < 0.0001$, and $P < 0.01$, respectively). On the other hand, for the winter months (December, January) the correlation coefficients were low and mostly unsignificant. For the spring and summer months (April to August) the values of correlation coefficients were near to those for the yearly average. This means that the autumnal and also early spring temperatures appear to be mainly responsible for the above indicated relation.

These results seem to be in agreement with the hypothesis (CETL, 1965) that the lack of summer annual types in the lowland can be connected with the danger of their premature passing into the generative state and of a subsequent loss of the wintering ability, and that the temperature conditions of the autumnal period appear to be decisive. In other words, at least in the territory studied, there seems to exist an effective selection pressure against the summer annual types in the lowland localities where the autumnal temperatures are relatively high. A similar explanation may be valid also for the early spring period.

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CETL, I.: in "Arabidopsis Research", Rep. Int. Symp. Göttingen, pp. 46-52 (1965)
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SYROVÝ, S. (Edit.): "Atlas podnebí Československé republiky", Praha (1958)

Influence de la température et de la lumière sur la germination d'Arabidopsis thaliana

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Dans cette courte note, nous nous proposons de décrire l'état de nos recherches concernant la germination chez deux lignées d'Arabidopsis thaliana (L.) HEYNH. Dans les travaux ayant trait aux rapports entre germination, température et lumière, on constate que quelques lignées seulement ont été testées (KUGLER, 1951).

Pour les deux lignées, nous avons comparé la germination en fonction de la température avec et sans lumière. Notre première lignée est issue de la race Limburg$_5$ (L 32 s_2 = Li$_5$), la seconde résulte d'un croisement entre la race Stockholm et la race Limburg$_5$ (K 7841 s_2 = H$_{55}$ früh = St x Li$_5$). Pour cette étude, les graines sont régulièrement réparties dans des boites de Pétri en plastique de 9 cm de diamètre contenant de l'ouate de cellulose humidifiée avec de l'eau distillée. Chaque boite contient cent graines. Pour chaque pourcentage que nous donnons, nous avons utilisé huit boites. Les tableaux 1 et 2 donnent pour les températures de 10°C, 15°C, 20°C, 25°C, 30°C, les pourcentages de germination soit à la lumière soit à l'obscurité. Ces pourcentages ont été calculés six jours après le début de la mise en culture.

Tableau 1: Race Li$_5$

	10°C	15°C	20°C	25°C	30°C
Lumière 6 jours	55%	100%	100%	100%	43%
Obscurité 6 jours	11%	100%	8%	1%	0%

Tableau 2: Lignée H$_{55}$ früh

	10°C	15°C	20°C	25°C	30°C
Lumière 6 jours	100%	100%	100%	80%	15%
Obscurité 6 jours	83%	100%	85%	8%	0%

Pour ces deux lignées, on constate que les graines germent à la lumière à toutes les températures. Les graines de la lignée Li$_5$ germent plus lentement que celle de l'autre lignée. A l'obscurité, le besoin de lumière apparait aux températures supérieures à 15°C pour la lignée Li$_5$ et supérieures à 20°C pour la lignée H$_{55}$ früh.

Les tableaux 3 et 4 donnent pour les températures de 10°C, 15°C, 20°C, 30°C, les pourcentages de germination à la lumière et à l'obscurité pour des graines ayant subi un prétraitement au froid humide (+2°C, eau en excès) pendant sept jours. Les pourcentages sont calculés au bout de 3 jours.

Tableau 3: Race Li$_5$

	10°C	15°C	20°C	25°C	30°C
Lumière 3 jours	96%	98%	100%	100%	94%
Obscurité 3 jours	40%	96%	100%	100%	66%

Tableau 4: Lignée H$_{55}$ früh

	10°C	15°C	20°C	25°C	30°C
Lumière 3 jours	17%	88%	100%	100%	90%
Obscurité 3 jours	20%	48%	100%	93%	57%

Pour les deux lignées considérées, le prétraitement au froid humide a un double influence:

(1) La stratification accélère la germination à toutes les températures. En effet les pourcentages maxima définitifs sont atteints en 3 jours au lieu de 6 jours, pour les températures supérieures à 20°C.

(2) La stratification supprime ou tout au moins diminue la photosensibilité aux températures les plus élevées. KUGLER a mis en évidence un besoin absolu de lumière pour la race Hm: A toutes les températures (7°C, 14°C, 20°C, 23°C, 25°C, 29°C, 32°C), elle n'a obtenu aucune germination à l'obscurité après 12 jours.

Nous tentons de vérifier si un éclairement de rouge clair (656 nm) permet d'obtenir des pourcentages élevés de germinations avec des graines ayant subi des prétraitements divers. SHROPSHIRE et Coll. (1961) ayant mis en évidence l'importance de la durée de l'imbibition et de la composition de la solution imbibante, nous avons réalisé des prétraitements de 20 h, 24 h et 48 h avec de l'eau distillée et avec une solution de nitrate. Les premiers résultats que nous avons obtenus sont en contradiction avec ceux de SHROPSHIRE et Coll. Deux explications sont possibles:

(1) Différences d'âge des graines (1 mois dans les expériences de SHROPSHIRE et Coll., 6 mois pour nos expériences).

(2) Différence de lignée (ces auteurs n'ont pas mentionné le nom de celle qu'ils employaient.

S u m m a r y : The germination behaviour of two strains of Arabidopsis thaliana has been studied. The seeds of one strain (Li_5) require light at temperatures above 15°C, those of the other (H55 früh) at temperatures above 20°C. As in many other cases, the light requirement could be annulled or largely reduced by a seven days' cold treatment of imbibed seeds.

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Nous adressons nos remerciements au Professeur Dr. NAPP-ZINN qui a bien voulu nous fournir les graines des lignées que nous avons utilisées.

Selection for seed dormancy in Arabidopsis thaliana

C. A. REHWALDT

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In any partially after-ripened seed population there exists a wide range in the degree of dormancy as exhibited by individual seeds. If this reflects genetic variation, rapid initial changes in the dormancy level under high selection pressure are expected. The experiments reported here were conducted to test the effects of selection for dormancy on one line of Arabidopsis thaliana (L.) HEYNH.

Selection for high and low dormancy were carried out on germinating seed populations for three successive generations on the basis of the first and last 3 to 5 seeds to germinate in a sample of 200 or more seeds. The first selection was conducted on seed harvested from a single plant of a line (race Landsberg) obtained from Dr. G. P. RÉDEI, Columbia, Missouri. There was no control of light during the selection process. All plants were grown at 24°C, 55% relative humidity, and in a 20 hr light period of 1500 ft-c white fluorescent light. Following selection, the germinabilities of the low dormancy (LD_3) and the high dormancy (HD_3) lines were compared by dark germination tests (REHWALDT, 1965) on seeds of three plants of both lines. Two seed harvests provided two replicates. In a second experiment, self-pollinations and reciprocal cross-pollinations were carried out between plants of the LD_3 and HD_3 lines. The same plant was used as the maternal and pollen parent for each cross. This was replicated four times. The significance of the differences was determined by an F test based upon arc sin transformed values.

The average germination of the LD_3 line and the HD_3 line was 90.1% and 88.6% respectively (Table 1). The small difference follows the expected pattern but is not significant. In the second experiment, the average germination for seeds from the LD_3 plants when self-pollinated and when cross-pollinated with HD_3 was 81.3% and 81.2% respectively, while that of the HD_3 line was 61.4% and 68.9% (Table 2). Though germinability appears to follow the expected pattern resulting from selection for dormancy with possible maternal transmission, the differences are not significant at the 5% level.

Table 1: Germinability after three generations of selection for high and low dormancy

Line	Replicate	Plant	Total seeds	% germination plants	% germination lines
Low dormancy (LD_3)	1	1	122	95.1	
		2	353	79.3	
		3	336	88.1	
	2	1	259	99.6	
		2	375	89.3	
		3	424	88.9	90.1
High dormancy (HD_3)	1	1	264	92.0	
		2	311	88.7	
		3	298	90.9	
	2	1	438	85.4	
		2	276	79.7	
		3	449	94.7	88.6

$F_{(df = 1.9)} = 0.34$; $P > 0.10$

DOXTATOR and FINKNER (1958) and SNYDER (1953) in studies of Beta vulgaris, a cross-pollinating species, found small changes in the speed of germination following selection. Selection for dormancy may be even less effective for self-pollinating species which tend to be highly homozygous. The data reported here indicate that the experimental material was highly homozygous or that heritability for dormancy is relatively low.

Table 2: Germinability of seed from self-pollination of lines selected three generations for low (LD_3) and high (HD_3) dormancy and from reciprocal crosses of these lines

Line	Total seeds	% germination replicates	% germination lines
LD_3 selfed	253	68.0	
	352	88.9	
	195	74.4	
	215	94.0	81.3
HD_3 selfed	249	34.1	
	309	91.3	
	331	53.2	
	249	67.1	61.4
LD_3 x HD_3 (pollen)	222	69.8	
	279	80.0	
	221	77.4	
	134	97.8	81.2
HD_3 x LD_3 (pollen)	322	45.7	
	322	80.7	
	337	61.4	
	284	87.7	68.9

$F_{(df = 3.9)} = 3.76$; $0.10 > P > 0.05$

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An albina mutant without seed dormancy

J. VELEMÍNSKÝ and T. GICHNER

(Institute of Experimental Botany, Prague, Czechoslovakia)

A lack of seed dormancy of some chlorophyll mutants has been reported by several authors (WALLACE and HABERMANN, 1958). This phenomenon is generally explained by the absence of carotenoids.

In our experiments a similar mutant, albina-50 (according to the numeration of our laboratory), was observed. The seeds of this mutant gained from heterozygous plants germinated immediately after the harvest, whereas the normal seeds from the same plants germinated only after a certain dormancy period.

% of seeds in the siliques		% of germinating seeds sown immediately after the harvest	
green	white	green	white
75.24	24.76	0	23.96

The dormancy period of normal seeds and also the germinability of albina mutants was influenced by the storage conditions.

Conditions of storage		Days after the harvest: 8	15	20	38	58	82	144	158
		% of germination							
$Ca(NO_3)_2$ - 24°C	green	10.7	47.6	-	35.6	74.4	-	-	-
	white	13.7	2.4	-	1.4	0	-	-	-
$Ca(NO_3)_2$ - 5°C	green	0.2	-	0.4	0.7	-	7.47	14.8	-
	white	10.0	-	13.3	21.5	-	12.1	13.5	-
P_2O_5 - 24°C	green	0	-	-	0.4	-	0.6	-	0.3
	white	16.7	-	-	14.0	-	12.3	-	15.3
P_2O_5 - 5°C	green	0	-	0	0	-	0.7	28.6	-
	white	21.9	-	13.6	18.8	-	14.4	18.1	-

Seeds from heterozygous plants were stored in desiccators with saturated solutions of $Ca(NO_3)_2 \times 4\ H_2O$ (relative humidity at 20°C = 55.9%) resp. with P_2O_5 (relative humidity at 20°C = 5%) under the temperature of 5 and 24°C resp. After various times of storage, seeds were sown on Petri-dishes with wet blotter paper and after 8 days the germination was scored. (Germination conditions: 20 to 25°C, 12 hrs light, 7,000 lux.)

In conditions of storage, inducing according to RÖBBELEN and KERSTEIN (1965) a short dormancy period of normal seeds, the albina seeds lost their germinability approximately after two months. Conditions of storage prolonging the dormancy period prolonged also the germinability of albina mutants. The germination of both albina mutants seeds and of the normal seeds was inhibited by coumarin and thiouracil, the action of the latter could be, however, reverted by uracil (VELEMÍNSKÝ, 1967). The seeds of albina mutants are able to germinate in dark, but normal seeds cannot. This character is influenced by the storage conditions in the same way as germination by light.

It seems that the loss of germinability is irreversible, as a cold treatment (5°C, in dark; Van der VEEN, 1965) which stimulated the germination of the normal seeds did not have an effect on albina seeds.

Chromatographically and under a UV-lamp no chlorophylls and carotenoids could be found in the cotyledons of albina 50. We observed a similar lack of dormancy in the case of another albina mutant, but this did not germinate in the dark.

It is not clear, however, whether there is a causal relation between the absence of carotenoids and a lack of dormancy, since we also found an albina mutant without carotenoids and chlorophylls with the same dormancy period as the normal seeds.

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Inhibition of seed germination in Arabidopsis by coumarin and 2-thiouracil

J. VELEMÍNSKÝ

(Institute of Experimental Botany, Prague, Czechoslovakia)

The inhibitory effect of 2-thiouracil (TU) on germination and the reversion of this effect by uracil (U) was described by KAHN (1966) for lettuce seeds and by SHIMENO and KINOSHITA (1963) for leaf mustard seeds. The same is true for Arabidopsis seeds, race Dijon (harvested in June 1965 and stored at room conditions). The seeds were sown in Petri-dishes on filter paper soaked with 20 ml of aqueous solutions of thiouracil, uracil, or a mixture of both. Germination proceeded in a 12 hour photoperiod at 25 ± 3°C, under 7,000 lux and was scored after 8 days. The results summerized in the table indicate that already 5×10^{-4}M TU inhibited the germination entirely, but this effect could be reverted by 10^{-3}M uracil.

Treatment	% of germination after 8 days	Treatment	% of germination after 8 days
10^{-3}M TU	0	10^{-3}M TU + 10^{-3}M U	34.7
5×10^{-4}M TU	0	5×10^{-4}M TU + 10^{-3}M U	81.6
H_2O	99.6	10^{-3}M U	99.3

The inhibition by TU is reversible when the seeds treated with TU are transferred after 8 days to Petri-dishes with filter paper and distilled water.

To entirely inhibit the germination by coumarin, a concentration of 5×10^{-4}M had to be applicated on the seeds. As in the case of TU, this inhibition is reversible when the seeds treated with coumarin are transferred to the filter paper with distilled water.

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The influence of gibberellic acid on the germination of irradiated and non-irradiated Arabidopsis seeds

Erna REINHOLZ

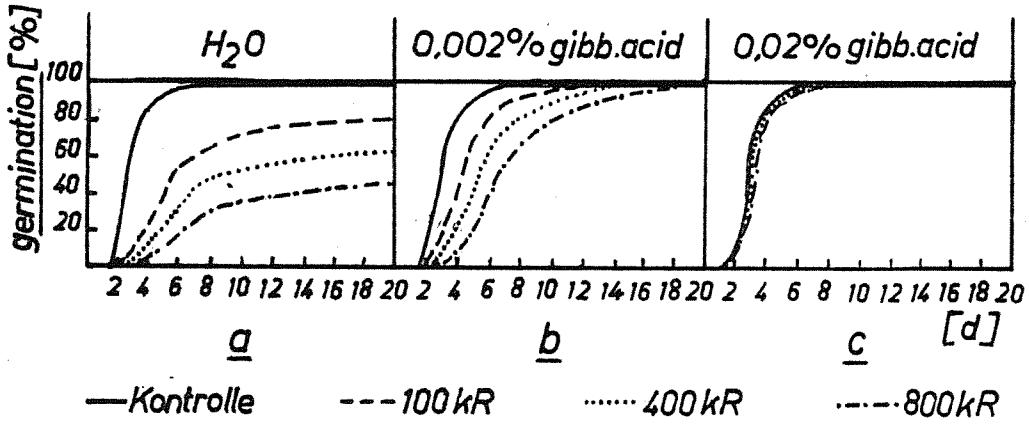
(Max-Planck-Institute for Biophysics, Frankfurt/M., Germany)

In No. 3 of this newsletter KUČERA described that Arabidopsis seeds (race ST 56 and BP 4-1) pre-irradiated in an air-dried state with X-ray doses as high as 36 and 72 kR, respectively, showed a significant improvement in germination percentage and germination rate after treatment with cystein, whereas the effect of gibberellin was not equally clear cut. BP 4-1, the race being more sensitive to radiation, even showed a reduction in its germination ability if the seeds were soaked in a 0.01% solution of gibberellin for 12 hours. This was found for irradiated series as well as for the non-irradiated controls.

For this reason some of our results concerning germination responses of Arabidopsis seeds (race Enkheim) after treatment with gibberellic acid may be of interest. In those experiments gibberellic acid in the whole concentration range tested showed promoting effects; but in no case any evidence for an inhibition of germination was obtained. Stimulation of germination after treatment with gibberellic acid was also observed by KRIBBEN (1957) in seeds of the bastard A. suecica x A. thaliana requiring after-ripening.

For the germination, the seeds (at least 500 per series) were kept in Petri-dishes on filterpaper soaked with water and gibberellic acid solutions (MERCK), respectively. Light and temperature conditions were varied for the different series.

Figure 1: Day light - room temperature



As shown in Figure 1a, the curves for the rate of the daily germination, at room temperature (20± 2°C) and during a natural day (June 1966), were lowered when doses as high as 100 to 800 kR were applied. Treatment with 0.002% aqueous solution of gibberellic acid increased the germination rate up to 100% in all cases. The germination rates, however, still lagged significantly behind the controls. But with a saturated solution of gibberellic acid (0.02%) a germination rate of at least 95% was reached for both irradiated samples and their controls on the sixth day after soaking.

In continuous light, En-seeds show less germination than in a light-dark change (REINHOLZ, 1947). Non-irradiated seeds of the above mentioned En samples at 16°C and 500 lux continuous light (incandescent bulbs) showed a germination rate as low as 57%, while less than 25% germination was recovered after irradiation with doses ranging between 60 and 160 kR. A 0.002% solution of gibberellic acid improved the germination ability (Figure 2b); with the saturated solution, which proved to be the

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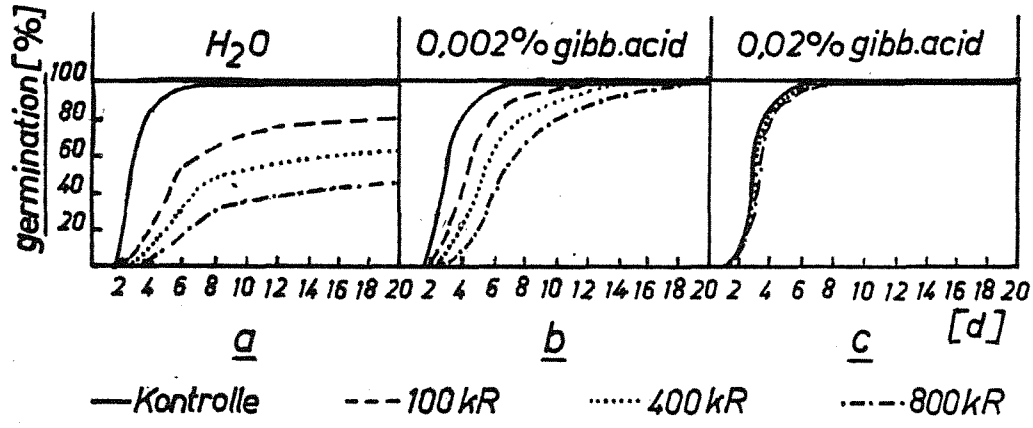

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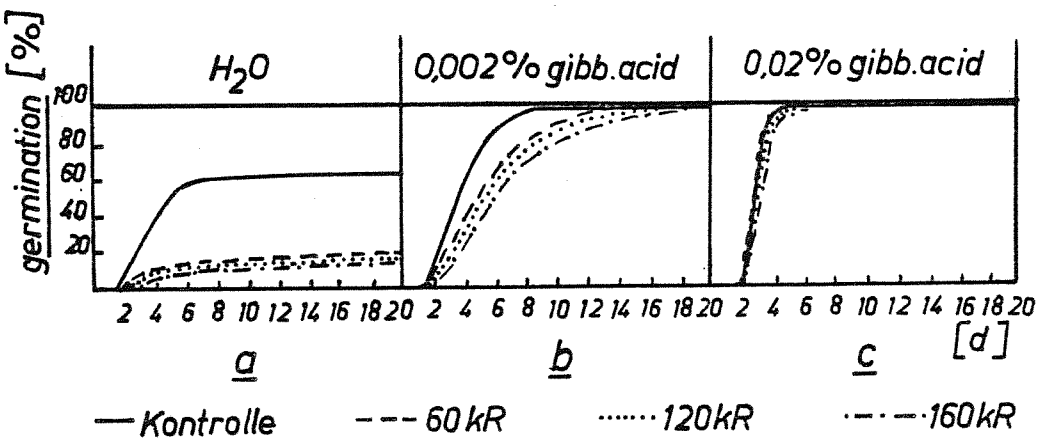
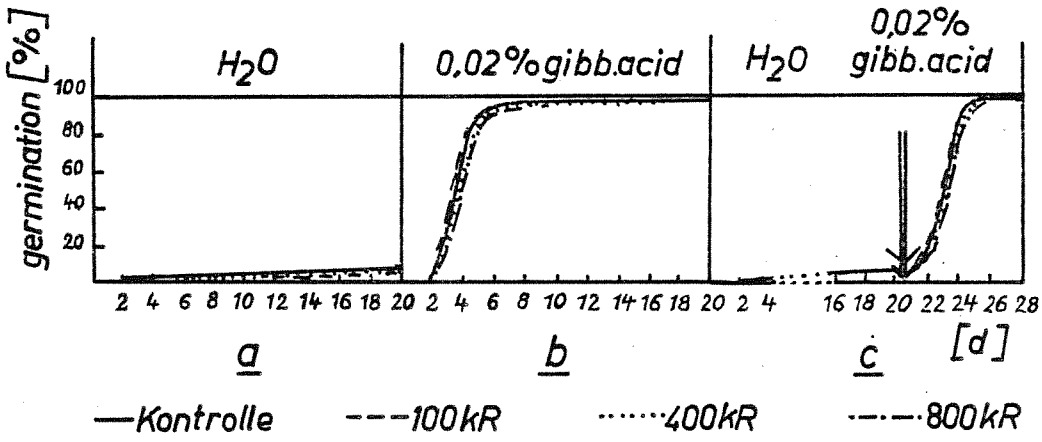
Figure 2: Continuous light - 16°C



most efficient concentration, the rates of the daily germination were identically high for both irradiated samples and their controls (Figure 2c). These data indicate that an inhibition of germination caused by pre-irradiation with X-rays and amplified by continuous illumination can be overcome by an application of gibberellic acid.

Figure 3: Darkness - room temperature



Even in complete darkness where non-irradiated En-seeds show a germination rate below 4% (REINHOLZ, 1947 and Figure 3a), the application of gibberellic acid results in the normal germination of non-irradiated and irradiated seed samples. Gibberellic acid had the same efficiency when applied 20 days after soaking of seeds in water. 48 hours after application germination started with the same rate as if applied at the beginning of soaking.

The gibberellic acid response of the race Hm, having an obligate light requirement for germination, revealed a quite analogous behaviour. KRIBBEN (1957) already reported on a promoting effect of gibberellic acid on the germination of non-irradiated seeds of Hm kept in darkness. With a concentration as low as 0.0001%, which was used by KRIBBEN, we found, however, no response. The concentrations showing good response in our experiments were one to two orders of magnitude higher.

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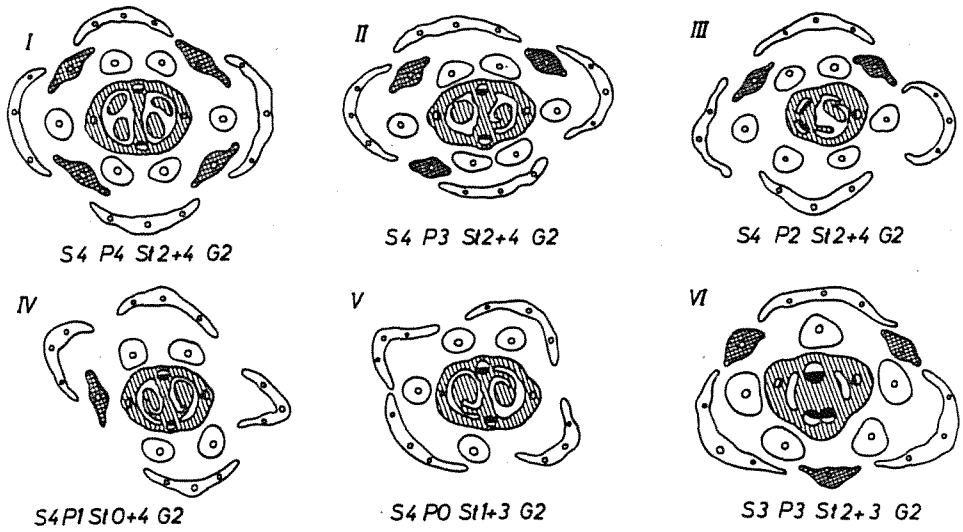
Occurrence of oligo- or apetaly and other malformations in Arabidopsis flowers as a result of phenylboric acid (PhB) treatment

Barbara HACCIUS

(Institute of Special Botany, University of Mainz, Germany)

As shown in previous publications (HACCIUS et al., 1959-1966), a single PhB-treatment of plant vegetative points produces severe malformations or total inhibition of only those organs which were in the earliest induction stage at the time of treatment. The present communication deals with flower abnormalities in Arabidopsis thaliana as a result of PhB-application. The same effect can not be obtained by using boric acid solutions.

The seeds of A. thaliana (race Stockholm) used in our experiment were kindly placed at our disposal by Prof. Dr. K. KNAPP-ZINN (Grenoble). One drop (0.1 ml) of a 300 ppm solution of PhB was placed on the shoot apex of young plants at the early rosette stage. Inflorescences of treated as well as of untreated plants were collected and examined. In the former, the frequency of flowers with a reduced number of floral parts was striking.



Transverse section of normal (I) and PhB-induced abnormal (II-VI) flowers of Arabidopsis thaliana

Microscopical observations of more than 100 anomalous flowers yielded the following results (S sepals, P petals, St stamens, G gynoecium):

t r i p e t a l y = S 4; P 3; St 2+4, 1+4 or 0+4; G 2 (Fig. II) was found in 37% of the examined anomalous flowers,

d i p e t a l y = S 4; P 2; St 2+4, 1+4 or 0+4; G 2 (Fig. III) in 27%,

m o n o p e t a l y = S 4; P 2; St 2+4, 1+4 or 0+4; G 2 (Fig. IV) in 16 %,

a p e t a l y = S 4; P 0; St 0+4 or 1+3; G 2 (Fig. V) in 5% and

t r i m e r y = S 3; P 3; St 2+3; G 2 or 3 (Fig.VI) in 15%.

In 3 flowers one carpel was open with the ovules exposed and 21 flowers possessed a pistil with only one functioning carpel.

McKELVIE (1962) in his list of mutant genes in A. thaliana describes 3 mutations with apetalous flowers (angulosa-2, apetala and clausa), and 3 mutations with a reduced number of petals (grandiflora-1, languida and serrata-1). Hence, this is our third case of PhB-induced malformations simulating well-known mutations: (1) lanceolate leaf in tomato (HACCIUS and GARRECHT, 1963), (2) tripistilly and pistilloidy in Pisum (HACCIUS and WILHELMI, 1966), and (3) oligo- or apetaly in Arabidopsis.

MATHAN (1965, 1966), as a consequence of his biochemical investigations on normal and on lanceolate tomato plants, concludes that the primary action of PhB as well as of the lanceolate gene "is to cause the increase in the level of activity of four oxidative enzymes and the latter in turn, among other factors, determine the shape and size of the leaf in tomato". However, the question arises if it is probable that the same biochemical mechanism accounts for gene induced lanceolate leaves in tomato as well as for flower mutations in Pisum and Arabidopsis.

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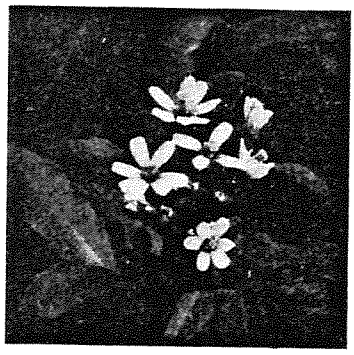
Variation in petal number

G. P. RÉDEI

(Department of Field Crops, University of Missouri, Columbia, Missouri, USA)

Dr. HACCIUS made very interesting observations on chemically induced phenocopies. She successfully reduced the number of several floral parts by phenylboric acid.



In our laboratory an i n c r e a s e in petal number was occasionally observed. It is not known which environmental factor has the major role in the induction of polypetaly. Certain genotypes are specially susceptible to these factors. In one winter, on mutant an nearly 60% of the flowers displayed polypetaly. Sometimes not a single abnormal flower is found on the same genotype plants. The attached picture (taken a few years ago) illustrates the extent of the anomaly.

Genetic and ontogenetic correlations between leaf pigment and assimilation efficiency in mutants of Arabidopsis thaliana

A. R. KRANZ

(Institute of Botany, University of Frankfurt-M., Germany)

Last year HOFFMANN (1965) published preliminary results on chlorophyll formation and photosynthesis in Arabidopsis thaliana race En-1 under continuous light (300 ftc.). His experiments represented no direct dependence of CO_2 assimilation on total chlorophyll content. The pigment was in excess in relation to photosynthetic intensity. But this statement was only valid for the final ontogenetic stages (ripening husks, rosette leaves dead). At that time there were no special studies about the genetic variation of this possible physiological correlation in Arabidopsis. The older experiments of RÖBBELEN (1957) showed that there was no significant interaction between chlorophyll reduction and growth in Arabidopsis mutants containing more pigment than 35% of the normal type. With other plants similar results were obtained (GOTTSCHALK and MÜLLER, 1964).

At present there is no exact and overall accepted method for separating the amount of photosynthesis from respiration in light. For that reason we applied methods introduced in plant ecology by WALTER (1948). We have defined the assimilation efficiency as the mean increase in dry weight or calory yield per day and plant (KRANZ, 1966).

Studying the leaf pigments, chlorophyll a and b, and the carotenoids in various leaf colour mutants of Arabidopsis thaliana (L.) HEYNH. we have confirmed the significant differences in quantity and quality compared with the standard line En-2 at all ontogenetic stages of development. There is four times more pigment in the tetraploid line En-4n than in the gene mutants ch^1, ch^2 and V 81. In ch^1 no chlorophyll b and in ch^2 a reduced content of b can be measured (HIRONO and RÉDEI, 1963). In V 81 (= ch^3, see VELEMÍNSKÝ and RÖBBELEN, 1966) a relative high amount of carotenoids is found. Flowering plants yield the highest chlorophyll a content per leaf area.

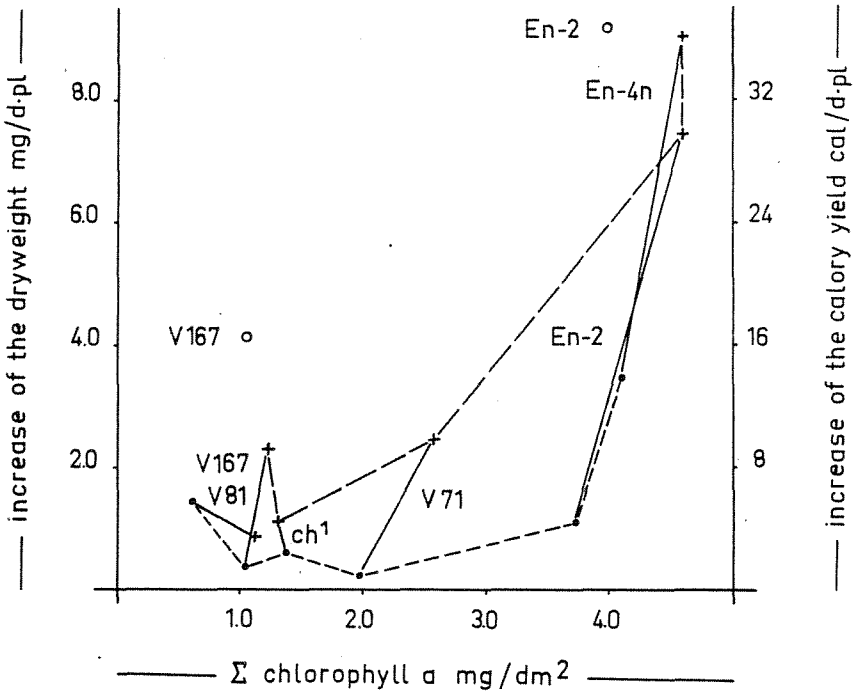


Figure 1: Correlations between chlorophyll a-content and assimilation efficiency in mutants of Arabidopsis thaliana. Experiment No. '66-1
• = leaf formation (rosette phase)
+ = stem formation (flowering time)
o = seed formation (ripening husks)

On constant conditions in temperature (20 ± 1°C), humidity (65 ± 3 p.c.) and light (24 hrs, 490 ftc) our experiments result in positive o n t o g e n e t i c c o r r e l a t i o n s between chlorophyll a content and dry weight increase during the phases of leaf formation and flowering time (see Figure 1, solid lines). Later on, the pigment quantity decreases whereas the assimilation efficiency increases (see V 167 and En-2).

The g e n e t i c c o r r e l a t i o n of these physiological correlated characters in the tested material seems to be curvilinear; e. g. during the early phase of leaf formation (see densely broken line) there is a lower assimilation efficiency per unit of chlorophyll a than in the following flowering time (see sparsely broken line above). The curvatures of the genetic correlations differ with the ontogenetic stages. These preliminary results demonstrate that the genotypes of the tested Arabidopsis material are fitted to a positive physiological correlation between chlorophyll content and assimilation efficiency.

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Arabidopsis thaliana (L.) HEYNH. a suitable object to study genotype-environmental interactions

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Since MATHER (1955) pointed out that in general the date of flowering in plants interacts strongly with the environmental conditions this has been proved in many species. It means that the difference between certain phenotypes associated with a given difference of genotypes varies with the environment.

In Arabidopsis thaliana as a winterannual plant (LAIBACH, 1965), not only does the time of flowering but also the number of leaves and side branches on the main stem interact with varying temperatures (NAPP-ZINN, 1961; LANGRIDGE, 1962) as well as with changes in the photoperiod (HÄRER, 1950). Papers by Van der VEEN (1965), NAPP-ZINN (1962) and BARTHELMESS (in preparation) show that the number of genes governing flowering time is limited and that it should be possible to isolate the factors exhibiting major influences and to study their special behaviour towards different environmental conditions in detail. Thus Arabidopsis could be a very good model with which to study the interactions which exist for instance in all our winter cereals between the length of the photoperiod and the cold requirement on one hand and the resulting cold resistance on the other, relations which are very difficult to study under field conditions. To this concept the author wishes to add a few further details.

Out of the cross between the races Catania st. beh. and Catania w. beh. two lines have been selected for early flowering and either many (A) or few (B) side branches (BARTHELMESS, 1964). After one generation of outcrossing inbetween these two lines a set of F_2 populations (12 populations in each line) was derived which again were studied for the expression of the two characters, but this time under spring/summer conditions and autumn/winter conditions* as well. It turned out that the same set of F_2 populations behaved completely different under the chosen environments as is demonstrated in the following table:

	Summer		Winter	
	Age of flowering	No. of side branches	Age of flowering	No. of side branches
A (many side branches)	34.10	3.34	52.17	3.39
B (few side branches)	35.65	3.09	70.90	5.40

Under summer conditions the difference between A and B is not very obvious in these F_2 populations (though the number of side branches is significantly different when compared within flowering-age classes). But the A and B groups react entirely different when brought up under winter conditions: Besides the much higher flowering age in both groups, B is about 50% later than A and has nearly twice as much side branches as A.

In addition, selection has been practised for early flowering and many (A) or few (B) side branches respectively on these F_2 populations under the different conditions. This gave rise to entirely different offspring groups. When grown together under spring/summer conditions in the following year, none of the 74 winter selections was as early as the earliest control population (= selection lines without interscrossing) while one third of the 70 populations selected under summer conditions flowered earlier than the earliest control population.

*in a temperated greenhouse with natural day length

The next table shows the average means:

		Age of flowering		No. of side branches	
		$\bar{x}$	Var.	$\bar{x}$	Var.
Controls:	A	37.07	2.61	3.35	0.02
	B	35.21	5.60	2.43	0.07
Selections:					
spring/summer	A	33.60	4.34	3.11	0.18
	B	33.08	5.30	2.73	0.14
autumn/winter	A	36.43	2.66	3.03	0.29
	B	36.00	3.65	2.53	0.19

Thus selection under different environments out of the same set of F_2 populations led to different results in respect of flowering time according to genotype-environment interactions. The result in respect of the number of side branches also proved to be different though not very easily describable as the surpassing of the control level happened in different flowering age classes. (Further details see BARTHELMESS, in preparation, 1967.)

C o n c l u s i o n : Populations as the ones described above or even more appropriately selected genotypes should be very helpful to clarify the following questions:

(a) Do all the different genes concerning flowering time show interactions, and when, will they exhibit them either with the photoperiod or low temperatures alone or with both; and which genes will influence in addition via pleiotropy the behaviour of the side branches on the main stem? (The different reactions of group A and B under winter conditions demonstrate that entirely different behaviour is possible.)

(b) This should be an excellent experimental material to specify by means of single genes the theory of correlated characters by FALCONER (1960) who establishes that a character selected under different environmental conditions should be regarded as a separate character in each environment because selection may act on an entirely different set of genes in each of the environments under consideration.

(c) In the special case where a race which exhibits heterosis when crossed to others like Catania w. beh. is involved one could check not only the epistatic relations between different genes but at the same time analyse which of the genes influencing flowering time are also involved in creating heterosis. Preliminary data suggest that some of the genes are inactive in this respect while others display quantitatively varying effects in the induction of heterosis.

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Seed production and phenotypic plasticity of Arabidopsis thaliana at different densities and mineral nutrient levels

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The seed output of Arabidopsis thaliana race "Estland" was scored from populations grown in a factorial experiment with four plant densities and three mineral nutrient levels. The experiment had a six-fold replication.

The populations were raised on "acid-washed" sand (99% 2-0.2 mm) in polythene pots 5 cm high and 6 cm internal diameter at the top. Seeds were sown on the surface of the sand with regular spacings at the following densities: 1 seed/pot (30 sq cm/seed); 10 seeds/pot (3 sq cm/seed; seed-seed distance 1.75 cm); 100 seeds/pot (0.3 sq cm/seed; seed-seed distance 0.55 cm); 1,000 seeds/pot (0.03 sq cm/seed; seed-seed distance 0.175 cm).

After the seed had been sown the sand was moistened with distilled water. Six days later the seedling cotyledons were expanded and Hoagland's solution (HOAGLAND and SNYDER, 1933), with iron supplied as the ferric sodium salt of EDTA, was applied at normal strength (H); 1/10 dilution (H/10); and 1/100 dilution (H/100). Thereafter at weekly intervals the pots were flushed with distilled water and recharged with the appropriate solution. The experiment was carried out in a glasshouse with a 15 hours/9 hours light/dark alternation. From the populations seed was collected daily over a fruiting period of six weeks by cutting off ripe fruits. The seed was subsequently counted and expressed as the total seed production of each population over the $2\frac{1}{2}$ month duration of the experiment.

R e s u l t s a n d d i s c u s s i o n: Seed production of the population is shown in Figure 1. Populations supplied with the H and H/10 solutions showed a homeostatic plateau of seed production at densities greater than 10 seeds/pot. Similar plateaus of seed output with increasing density have been shown to occur in other annual plant species (HARPER, 1961). However, populations supplied with the H/100 solution showed a peak seed output at the sowing density of 100 seeds/pot. At the sowing density of 1,000 seeds/pot seed output was markedly reduced to a level below that of even the single plant/pot population.

Figure 2 shows seed output as seed production per plant. Homeostasis of seed output shown by populations supplied with the H and H/10 solutions was achieved by adjustment in the form and seed output of individual plants rather than by mortality of the plants in the populations. This adjustment of form included overall plant size, the development of side branches, the number and size of fruits produced, and the number of seeds/siliqua.

Figure 2 demonstrates that in the populations supplied with H/100 solution and at a sowing density of 1,000 seeds/pot seed production of individual plants was below the critical level of 1 plant giving rise to 1 seed. In fact, mortality occurred in these populations. This was sudden and extensive after the plants, which were minute, had been at the early leaf stages for nearly two months. However, no pathogenic cause was detected and death was attributed primarily to starvation. After this wave of mortality a few survivors flowered and fruited amongst which the limiting condition of 1 seed/plant appeared to predominate.

Ecologically, the results suggest that field populations growing at low levels of available nutrients are more density-dependent in seed output than populations growing at higher levels of available nutrients. In this way, population density at low nutrient levels is potentially more sensitively controlled by inherent population freed-back mechanisms.

Implicit in the phenotypic plasticity exhibited by Arabidopsis thaliana in this experiment is the necessary precaution of standardising growth conditions in any critical comparative study of variation within the species.

Details and a fuller discussion of the experimental findings are to be submitted for publication elsewhere.

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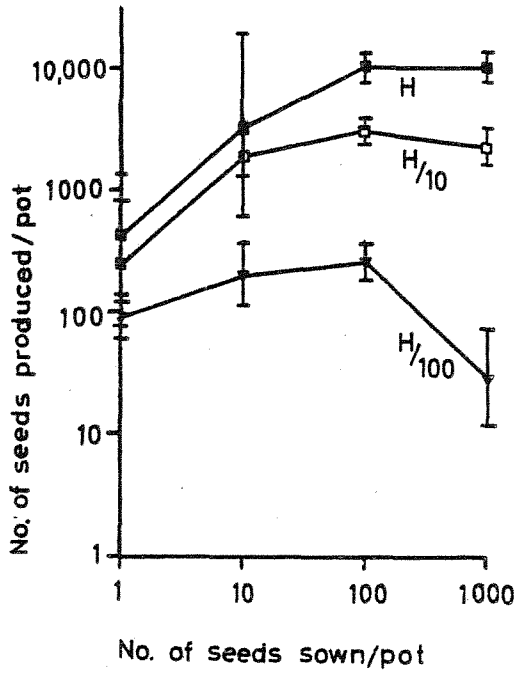

Figure 1: Seed output of populations of Arabidopsis thaliana race "Estland" from three sowing densities at three nutrient strengths. I is 4 X Standard Error.

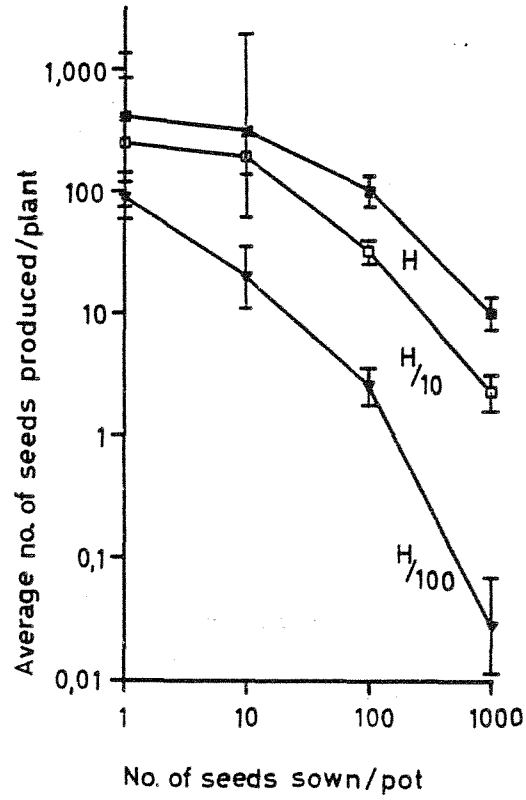

Figure 2: Mean seed production per plant (per seed sown) in population of Arabidopsis thaliana race "Estland" from different sowing densities at three nutrient strengths. I is 4 X Standard Error.

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Use of Arabidopsis for mineral nutrition experiments

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Arabidopsis thaliana, grown in aseptic culture on chemically defined agar media, is an ideal tool for mineral nutrition research. A great diversity of races and mutant types is available for study, and the media is easily altered to produce any complete or partial mineral deficiency desired.

In a class experiment, "Estland" wild type was grown under 24 hour photoperiod in a media in which $NaNO_3$ replaced KNO_3. The composition of this media per plant is as follows:

Anhydrous formula weights in Arabidopsis growth media per 4 ml tube

Macronutrients		Micronutrients	
$MgSO_4$	1.44×10^{-5} gm	$FeSO_4$	33.8×10^{-3} mg
$Ca(NO_3)2$	2.86×10^{-3} gm	$MnSO_4$	5.28×10^{-3} mg
NaH_2PO_4	1.92×10^{-3} gm	$CuSO_4$	0.614×10^{-3} mg
KNO_3	2.42×10^{-3} gm	$ZnSO_4$	0.664×10^{-3} mg
or		H_3BO_3	7.44×10^{-3} mg
$NaNO_3$	2.03×10^{-3} gm	Na_2MoO_4	0.14×10^{-3} mg
Organic Compounds			
Difco Agar	32.0×10^{-3} gm	EDTA	96.0×10^{-3} mg

Every two days ten plants grown in the potassium deficient media and ten control plants were harvested, and the fresh and dry weight was obtained. At the final harvest, the dry plant material was bulked and ashed. The ash was dissolved in 5% HCl, and the potassium content determined with a BECKMAN flame spectrophotometer at 767 millimicrons with reference to a standard curve. Germination rate, flowering time, and the visible appearance of the plants was also noted.

95% of the seeds sown on the potassium free media germinated, but the potassium deficient plants grew more slowly than the controls (Fig. 1). The floral meristem elongated only about 1 cm, the older rosette leaves died before flowering, and all leaves had a red color. The results of the photometric analysis are:

Potassium analysis using BECKMAN flame spectrophotometer 767 mμ

	Number plants	Dry weight gm	Ash		K		
			gm	% of dry weight	mg	% of dry weight	% of ash weight
Control	126	0.2691	0.0409	15.2	1.005	0.38	2.46
K-less	214	0.1570	0.0213	13.6	0.0694	0.044	0.325

However, these severe visible and chemical symptoms of potassium deficiency are strongly contrasted by the normal life cycle of the stressed plants. The experimental plants had a mean flowering time of 22 days after sowing, only one day later than the control.

The only source of potassium was that provided by the seed and contamination in the media, which was evidently limited to very low levels. Despite such a severe environmental stress, and the severe restriction on growth imposed by this, the potassium deficient plants were reserved; all flowered producing small pods. From these twenty plants, however, only two seeds were obtained.

(Fig. 1 see next page)

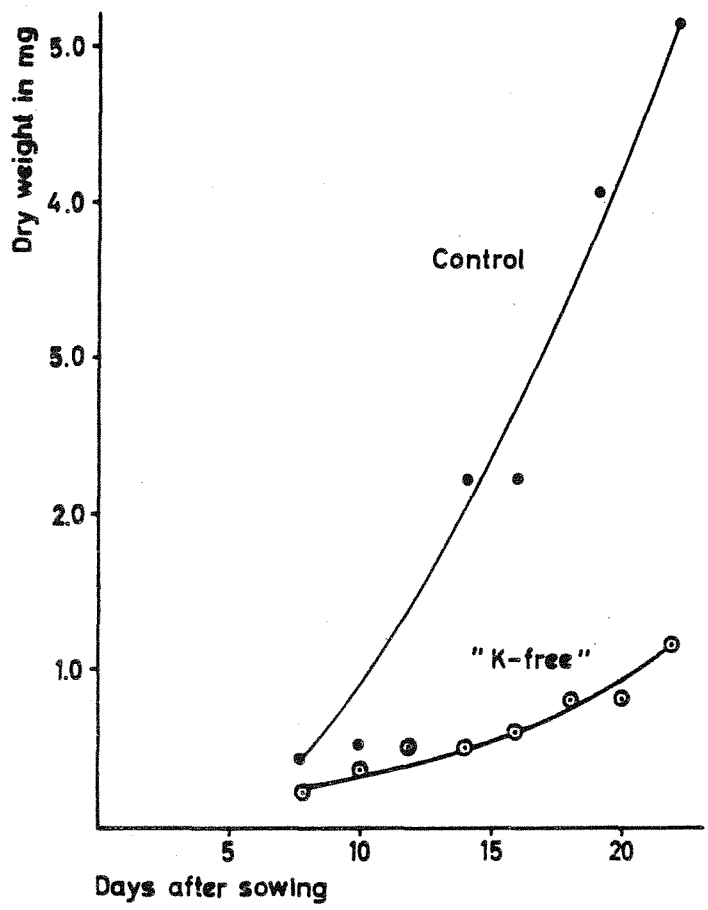

Figure 1: Growth curves of Arabidopsis thaliana, aseptically cultured in mineral media and "potassium free" media

The assistance of Professor T. ASANO in performing the flame spectrophotometry is gratefully acknowledged. Work was carried out under the direction of Professor J. A. M. BROWN.

Arabidopsis as an indicator of soil fertility

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A few years ago it was observed that on the different soil batches, brought to the greenhouse, the growth of Arabidopsis greatly varied with apparently no association with the visible structure, color or other obvious characteristics of the samples. Later six other samples were simultaneously collected. Four of the samples included soil from the maize breeding nursery. One was taken from the continuous corn plot (corn since 1889) of the Sanborn Field of the University (historical site from which S. A. WAKSMAN isolated the fungus producing the antibiotic Aureomycin). One sample was obtained from the never cultivated field known as the Tucker Virgin Prairie.

Maize and Arabidopsis were seeded in pots using the six soil sources. Dry matter of seedling plants was determined for each species on each lot.

Dry weight of Arabidopsis and maize seedlings expressed in percent of sample 4 which had the greatest weight

	Sample No.	Dry weight in percent of sample No. 4	
		Arabidopsis	Maize
Rollins Bottom	4	100	100
Rollins Bottom	3	61	102
Rollins Bottom	2	43	100
Rollins Bottom	1	36	68
Sanborn (continuous corn)	5	5	63
Tucker (virgin prairie)	6	2	64

The extremely low weight of Arabidopsis on the Tucker Virgin Prairie Soil was not expected. The relative response of the maize growth on the six samples was in line with previous knowledge of their respective levels of fertility. The varied response of Arabidopsis on these six soils suggest some element was limiting its growth which cannot readily be detected by the growth of maize.

A recent experiment demonstrated that with phosphorus application (5 ml 0.2 M Sörensen buffer per each 3-inch pot) normal yield of Arabidopsis can be obtained even on soil samples No. 5 and No. 6. It appears that Arabidopsis is a very efficient indicator of readily available phosphorus content of the soil.

It is interesting to note the dry weight of maize on sample No. 5 is in excellent agreement with the data collected on Sanborn Field during the first 49 years of the experiment, where continuous corn (no fertilization) had a forage yield 68.6% relative to continuous corn with 6 tons manure applied annually (SMITH, 1942).

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A note on the growth response of Arabidopsis in culture media containing deoxyriboside base analogues

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The recent report by HIRONO and RÉDEI (1966) concerning the effect of deoxyriboside base analogues on flowering in Arabidopsis is of particular significance since in contrast to earlier work (BROWN, 1962; JACOBS, 1964) these authors elicited a striking morphogenetic response when base analogues were incorporated into the culture media on which the plants were grown, without inhibition of growth at the reported analogue concentration of 1×10^{-5}M.

In attempting a confirmatory experiment, 5-iododeoxy-uridine (IUdR) at a concentration of 5×10^{-4}M in the agar media was used. This concentration when in the media caused complete inhibition of seedling growth in both wild type and late flowering mutants. SMITH (1962) reported that 5×10^{-5}M IUdR in the agar media for Arabidopsis was a threshold concentration "which barely permits plants to mature". However, both 5×10^{-4}M and 5×10^{-5}M are morphogenetically active and non-inhibitory when applied topically to the vegetative plant axil in conjunction with 0.1% "Tween 20".

The base analogues IUdR and BUdR were applied originally directly to the apex because of the effects reported by SMITH, and also because autoradiographic studies using ^{3}H-thymidine in the culture media (BROWN unpub.) indicated that while heavy incorporation of this nucleoside occurred within 4 hours in the root meristem, plants grown throughout their life cycle on mineral agar containing 2.5 µc/ml of ^{3}H-thymidine showed no autoradiographic evidence of incorporation into the bud tissue. This is supported by work reported by GIFFORD (1960). Incorporation of IUdR into root meristem DNA analogously to thymidine has been demonstrated by SMITH et al. (1963). Thus, using ^{3}H-thymidine as an indicator for the incorporation and transport of IUdR and BUdR, it was expected that these analogues would not be transported to the shoot apex a s s u c h, when they were supplied in the agar media and entered via the roots. SEBESTA (1962 pers. comm.) stated that BUdR in the germinating solution did not effect growth of cucumber seedlings, though bromo-uracil was morphogenetically active (SORMOVA et al., 1960), while EVANS and AXELROD (1961) demonstrated that C^{14}-labelled uracil and thymine in the germinating solution of rape seedling was mostly degraded to β-amino-isobutyrate and very little was incorporated as metabolically active pyrimidine.

The questions posed by these results are several.

(1) What is the reason for the differential inhibitory threshold in shoot vs. root modes of application?
(2) Is the metabolic fate and mode of action of these analogues the same in both shoot and root modes of entry?
(3) If the deoxyriboside analogue itself, analogously to the case with ^{3}H-thymidine, is n o t transported via the root to the shoot apex, what is the morphogenetically active molecule that acts on the shoot meristem?

Further researches on the metabolism and mode of action of the competitive DNA base analogues in higher plants are indicated and are continuing at this laboratory.

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Temporary suppression of H^3-thymidine incorporation by 5-bromodeoxyuridine in the shoot meristem of Arabidopsis

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In previous experiments it has been shown that application of either 5-bromodeoxyuridine (BUdR) or 5-iododeoxyuridine (IUdR) to the vegetative meristem of Arabidopsis thaliana (L.) HEYNH. grown under short days results in an acceleration of floral morphogenesis (BROWN, 1962). It was of interest to determine what effect the application of these analogues had on DNA synthesis within the meristem at various times after treatment.

Seeds of Arabidopsis thaliana, race "Estland", were sown on 0.78% mineral agar in test tubes and placed in an environment of 16 hour photoperiod, 28°C + 1°C day temperature, 22°C night temperature. The light period was from 6 A.M. to 10 P.M. Ten day old plants, selected for uniformity, were treated with 10 µl of 5 x 10^{-4}M solution of BUdR applied by syringe to the axil of the rosette. H^3-thymidine (specific activity 4.4 curies/mM, Radiochemical Centre Amersham U.K.) in solution at a concentration of 4 µc/ml was applied in the same manner either simultaneously or 3, 6 or 14 hrs after the analogue treatment. Control plants were given only the H^3-thymidine treatment at equivalent times. Both isotope and analogue solutions also contained 0.1% "Tween 20" and 1/4 strength mineral nutrients.

Plants were fixed either 3 or 6 hrs after addition of the isotope, using CRAF fixative. Plants were dehydrated, embedded in paraffin, and sectioned at 4 microns. Sections mounted on gelatin subbed slides were deparaffinised and autoradiographed using Kodak NTB emulsion. The slides were stored in light tight boxes containing silica gel at 4°C for 14 days before developing with Kodak D19 at 23°C for 6 minutes. After fixing the developed slides were passed through a water-ethanol-xylene series and coverslips mounted with euparal. Slides were searched for median longitudinal sections of meristems using phase-contrast illumination.

Meristem sections were drawn in outline using the Wild drawing apparatus on a Wild M20 microscope and reduced silver grains over the tunica and corpus were plotted. The results are summarised in the table.

Treatment and fixation times			Grain count in meristem	
10 µl 5 x 10^{-4}M BUdR	10 µl H^3-thymidine	fixation	number of sections	mean grains per section
nil	11 A.M.	+ 3 hrs	8	38
nil	11 A.M.	+ 6 hrs	2	57
11 A.M.	11 A.M.	+ 3 hrs	7	9
11 A.M.	+ 3 hrs	+ 6 hrs	5	2
11 A.M.	+ 6 hrs	+ 12 hrs	4	0
11 A.M.	+ 24 hrs	+ 30 hrs	8	33
nil	+ 24 hrs	+ 30 hrs	6	45

The grain counts are corrected for background, which was less than 6 grains per field with the 100x objective. It is not possible in the small meristem cells of Arabidopsis to distinguish clearly between nuclear and cytoplasmic label, and the counts given thus represent the total label found over the tissue.

These results and those of previous studies (BROWN et al., 1964) indicate that H^3-thymidine is present in the meristem after 3 hours; the degree of labelling is increased after 6 hours. However, BUdR treatment drastically reduces the amount of labelling within 3 hours, and in the presence of BUdR, there is no significant label due to H^3-thymidine incorporation in the meristem after 6 hours.

Meristem pre-treated with BUdR for 24 hours before application of H^3-thymidine for a six-hour period, once again show labelling though the intensity is slightly less than the control.

The apparent inhibition of H^3-thymidine incorporation may be due to the inability of a tracer amount of thymidine to compete with the large excess of BUdR for phosphorylation on the pathway for nucleotide tri-phosphate formation and incorporation into DNA. It is possible that DNA synthesis may be blocked during the first 24 hours, though evidence for the incorporation of IUdR into shoot and root meristem cells during this period (SMITH et al., 1963; BROWN and SMITH, 1964) indicates that DNA synthesis does proceed in these tissues in the presence of a large exogenous concentration of the analogue. Moreover, tracer amounts of labelled IUdR are incorporated in the presence of a large excess of "carrier" IUdR.

The reappearance of labelling due to H^3-thymidine after 24 hours suggests that BUdR induced inhibition is of short duration, probably because the analogue is degraded rapidly and the normal biosynthesis of DNA re-established.

It has not been possible as yet to detect any change in the distribution of H^3-thymidine incorporated in Arabidopsis meristems following incorporation of BUdR or IUdR. An ontogenetic significance to the relatively inactive sub-apical cells of Arabidopsis (BROWN et al., 1964) and other long-day plants, e.g. Sinapsis alba (BERNIER and BRONCHART, 1963) is suggested by the effects of BUdR and IUdR on floral development. It has also been reported that 5-bromouracil treatment increased the frequency of mitoses by 60% in root meristems of Allium cepa after 4 days (SORMOVA et al., 1960) which suggests that a wave of synchronous divisions may result from a temporary analogue-induced alteration in the rate and pattern of DNA synthesis in the meristem.

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Chlorophyll and free amino acid content in normalizing and not normalizing pigment mutants feeded with sugar

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In No. 2 of this newsletter the authors reported about lethal chlorina mutants, some of which became greener with increasing age when feeded with 2% glucose or sucrose. Two of these chlorina mutants, one normalizing and one not normalizing on agar with sugar, were compared with regard to their content of chlorophylls (Table 1) and free amino acids. The aim was to determine whether the content of free amino acids is influenced by the normalization. It was already demonstrated (VELEMÍNSKÝ et al., 1965) that in cotyledons of chlorophyll-lethals grown on soil a higher amount of free amino acids, especially of amides, asparagine, and glutamine, accumulates.

Table 1: Chlorophyll content in a not normalizing (chlorina 42) and a normalizing (chlorina 1467) mutant grown on agar with 2% sucrose or glucose

chlorina 42			chlorina 1467		
Age (days)	Stage	Chlorophyll (% of green plants)	Age (days)	Stage	Chlorophyll (% of green plants)
10	cotyledons	10.3	7	cotyledons	24.7
19	2nd leaves	10.4	21	4th leaves	49.7
28	6th leaves	12.4			
61	inflorescence stem	17.5			

The feeding with 2% glucose or sucrose enables the mutants to develop rosettes and flowers and, although differently influencing the chlorophyll synthesis, did not influence essentially the free amino acids content of both mutants in comparison to the nonfeeded mutants. In the chlorina 42, where the chlorophyll content remained nearly constant at 10%, and in the chlorina 1467, where the chlorophyll content increased from about 25% in cotyledons to 50% in plants 21 days and older, the amides accumulate distinctly more than in corresponding green plants (see Table 2).

Table 2: The content of free amides (asparagine and glutamine) in the leaves of mutants grown on agar with 2% sucrose resp. glucose

chlorina 42				chlorina 1467			
Age (days)	Stage	Amides (γ/100 mg f.w.) mutant	green plant	Age (days)	Stage	Amides (γ/100 mg f.w.) mutant	green plant
6	cotyledons	157.0	39.0	7	cotyledons	92.3	30.2
34	6th leaves	143.0	77.0	21	4th leaves	351.9	47.0
90	inflorescence stem	1091.0	163.0				

We assume that the accumulation of amides in the mutant 1467, inspite of sucrose feeding and increase of chlorophyll, indicates some instability of structural proteins or of the chlorophyll-protein complex. The proteinic part of this complex is then steadily decomposed, which results in the high amount of free amides. Sugar serves as a source for the synthesis of new proteins so that the higher content of chlorophyll can be maintained. This assumption is supported by the fact that the normalized mutant plants bleach after being transplanted back to a medium lacking sucrose.

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Normalisation of nutritional mutants on supplemented media

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By means of the r e s c u e m e t h o d presumed biochemical mutants have been obtained as reported earlier (JACOBS, 1965). As an amino acid deficient type, one arginine-less mutant has been studied more extensively. On mineral medium, the plant showed a variegated pale green color and during the development, death often occurred. Arginine doses of 1 to 20 mg/100 ml of culture medium restore normal vegetative growth. Another but uniform viridis mutant responds to the addition of tryptophane (0.15 to 3 mg/100 ml of culture solution) by greening at the level of rosette leaves. Among vitamin deficiencies, in addition to thiamine-less mutants, para-aminobenzoic acid enhances growth of a leaky mutant, but only in conditions of discontinuous illumination (12 hrs of light). Normal phenotypes have also been obtained with addition of uracil in the case of a morphological mutant with a compact rosette and narrow leaves. Irregularity in the response towards the concentration gradient has been noted, however, for this mutant. In the case of some other mutants in which the biosynthesis of nicotinic acid, cysteine, and methionine was altered, instability appeared through repeated selfing.

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Intracellular distribution of ribonucleases

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Arabidopsis contains at least three enzymes capable of digestion of RNA. The non-particulate cytoplasm contains an acidic ribonuclease, the organelle fraction harbors a neutral ribonuclease. These are genuine ribonucleases producing 3'-monophosphates of purines and pyrimidines through 2':3' cyclic phosphates. In addition a phosphodiesterase occurs both in the soluble cytoplasm and in the particulate fractions. This latter enzyme yields nucleoside-5'-phosphates.

Distribution and activity of these enzymes among the cellular protein fractions is indicated below. The data do not give information on the absolute amount of RNase in these fractions.

Cellular fractions	Acid soluble nucleotides (O.D./protein)	
	pH 4.8	pH 7.0
2,000 x g	.595	2.182
25,000 x g	.112	.480
112,000 x g	.087	.260
Non-particulate	.662	.765

Penetration of ^{3}H-DNA in seeds of Arabidopsis without nucleic acid synthesis

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We have previously observed (BONOTTO, JACOBS, and LEDOUX, 1965) that Arabidopsis seeds take up exogenous ^{3}H-DNA and that the label is partly found in the seedlings after germination. In order to know whether the penetration of exogenous nucleic acids is in some way related to endogenous DNA synthesis the following experiment was done.

Presoaked seeds (16 hrs in 0.01 M phosphate buffer, pH=7.0) were irradiated with 10^5 rad of ^{60}Co gamma rays, a dose which stops completely the ^{3}H-thymidine incorporation into the DNA (BONOTTO, JACOBS, and LEDOUX, 1966). At different times after irradiation the seeds were incubated during two hours in 0.01 M phosphate buffer containing Escherichia coli ^{3}H-DNA (1.5×10^6 dpm/µg) or ^{3}H-thymidine (3 C/mM). Suitable controls were treated in the same way.

The incorporation of ^{3}H-thymidine into the DNA and the uptake of ^{3}H-DNA by the seeds were followed by means of a technique utilizing centrifuged DEAE-cellulose paper pulp columns (DAVILA, CHARLES, and LEDOUX, 1965) and results refer to the sum of radioactivity found in the acido-insoluble fractions.

It was observed that for at least 3 hours after irradiation DNA synthesis was stopped or strongly reduced (Table 1). Under the same experimental conditions, the seeds were able to take up exogenous DNA (Table 2).

Table 1: Incorporation of ^{3}H-thymidine by control and by gamma irradiated (10^5 rad, ^{60}Co) seeds

Time after irradiation (hrs)	Time of presoaking (hrs)	Radioactivity (cpm/250 seeds)		% inhibition
		control	irradiated	
1	17	46	2	95.66
5	21	88	23	73.87
10	26	103	37	64.08
24	40	113	104	7.97

Table 2: Uptake of ^{3}H-DNA by control and irradiated seeds

Time after irradiation (hrs)	Time of presoaking (hrs)	Radioactivity (cpm/250 seeds)	
		control	irradiated
1	17	180	239
5	21	189	170

These results seem to suggest that (a) the uptake of exogenous nucleic acids by the seeds takes place even in absence of endogenous synthesis of DNA, and (b) gamma rays inhibit the DNA synthesis but not the uptake of exogenous macromolecules.

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BONOTTO, S., M. JACOBS, and L. LEDOUX: in "Arabidopsis Research", Rep. Int. Symp. Göttingen, pp. 154-164, 1965

BONOTTO, S., M. JACOBS, and L. LEDOUX: Arch. Int. Phys. Bioch. 74, 311-312 (1966)

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*Boursier-EURATOM

Increased uptake of ^{3}H-DNA by Arabidopsis with EDTA

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In previous investigations on the penetration of heterologous ^{3}H-DNA in Arabidopsis (BONOTTO, JACOBS, and LEDOUX, 1965) it was found that the labelled macromolecules were taken up either by the plant in toto or by its isolated organs. The results reported here indicate that the presence of disodium ethylenediamine tetraacetate (EDTA) in the incubation mixture increases the penetration of the exogenous labelled DNA.

Rosette leaves cut from 49 day-old plants were incubated for different periods of time in 0.01 M NaCl or in 0.01 M NaCl plus 0.066 M EDTA, both containing 40 µg/ml of ^{3}H-DNA (^{3}H-DNA of Escherichia coli having a specific activity of 2.2 x 10^{6} dpm/µg). The leaves were then washed nine times (3 times with 0.01 M NaCl, 3 times with unlabelled DNA in 0.01 M NaCl, and 3 times with distilled water) in order to eliminate contaminant ^{3}H-DNA, and assayed for total radioactivity by liquid scintillation after a two-hour hydrolysis at 60°C with hydroxide of hyamine. The results of this experiment are given in Figure 1.

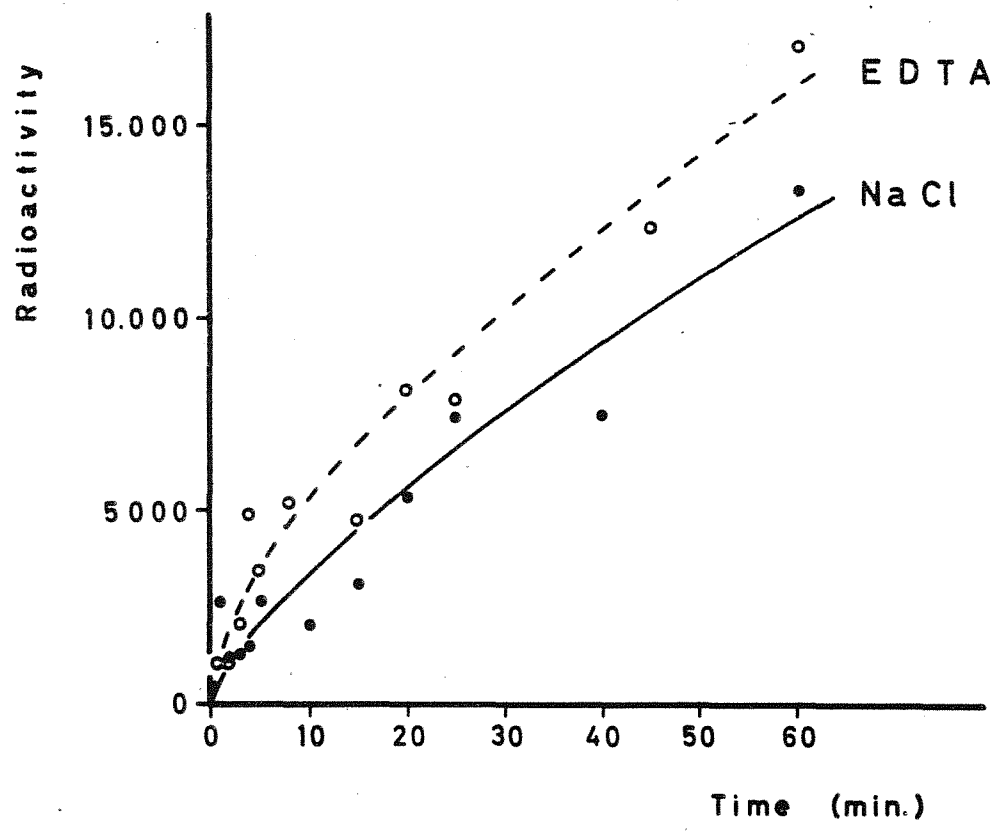

Figure 1: Kinetics of the ^{3}H-DNA uptake by isolated rosette leaves. The continuous line shows the control, the broken one corresponds to the treated leaves. Radioactivity : dpm/rosette leaf

In two other experiments the roots of 34-day-old flowering plants (15-25 cm long) were placed 20 hours in contact with labelled DNA dissolved in 0.01 M NaCl plus 0.066 M EDTA respectively. The plants were then washed as mentioned for the leaves and cut

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into 1 cm long segments which were assayed for total radioactivity. In order to eliminate the error due to contamination, the roots which were in contact with the labelled DNA were cut and discarded. The results of these experiments are reported in Table 1.

Table 1: Radioactivity (cpm) found in the different segments of plants supplied with ^{3}H-DNA-NaCl or with ^{3}H-DNA-EDTA

No. of plant's segment	Experiment No. 1 (cpm/1 cm segment)		Experiment No. 2 (cpm/1 cm segment)	
	NaCl	EDTA	NaCl	EDTA
1 (I)	230	705	442	3735
2	102	139	95	302
3	60	125	93	115
4	60	134	96	138
5	60	127	87	135
6	46	110	70	182
7	51	389 (II)	58	198
8	60	187	60	91
9	57	88	56	472 (IV)
10	84 (III)	88	68 } (IV)	
11	51	96	66 }	
12	51	135	1191	5368
13	43	160		
14	56	157		
15 } (IV)	44 }	326 }		
16 }	60 }	225 }		
	1115	3191		

(I) rosettes
(II) 2 stem leaves
(III) 1 stem leaf
(IV) flowers and flower buds

Our findings indicate that EDTA increases notably the uptake of a heterologous ^{3}H-DNA into the isolated rosette leaves (Figure 1) and the transport from the roots to the stem and to the flowers (Table 1).

It was reported that EDTA enhances the action of some chemical antibacterial agents against Pseudomonas aeruginosa NCTC 8203 and NCTC 7244 and against Escherichia coli (BROWN and RICHARDS, 1965). Moreover, EDTA enhanced the action of lysozyme on Ps. aeruginosa (REPASKE, 1958) and the absorption of heparin from the gastro-intestinal tract of rats (WINDSOR and CRONHEIM, 1961).

Pretreatment with EDTA caused an increase in the frequency of chromosomal aberrations induced by the polyfunctional alkylating agent triethylenemelamine (TEM) or by N-nitroso-N-methylurea (NMH) in root meristems of Vicia faba (MICHAELIS and RIEGER, 1965; MICHAELIS et al., 1965). No sensitization with EDTA, however, was observed in treatments with 1-methyl-3-nitro-1-nitrosoguanidine in Vicia faba (GICHNER et al., 1963) and with EDTA and X-rays in Drosophila melanogaster (ONDŘEJ, 1965). In Arabidopsis EDTA did not influence the mutagenic activity of EMS (GICHNER and VELEMÍNSKÝ, 1965) and only slightly diminished the mutation frequency by NMH (MÜLLER, 1966).

In our experiments the effect of EDTA might be related to its well known chelating properties. The removal of Mg++ or Ca++ or both from the cell wall may affect its physico-chemical properties so that permeability barriers to foreign macromolecules were strongly reduced. It is known in fact from electron microscopic investigations that after EDTA treatment the structure of cell walls in pea roots becomes loosened (KLEIN and GINZBURG, 1960).

Another hypothesis to explain the action of EDTA (TIZIO, 1965) is that the observed stimulation of the permeability to ^{3}H-DNA might depend on the inhibition or activation of enzymes involved in the absorption process.

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Biological effects of an heterologous DNA on Arabidopsis

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As reported in a previous paper (BONOTTO, JACOBS, and LEDOUX, 1965) Arabidopsis seeds incubated in the presence of a labelled heterologous DNA (Escherichia coli ^{3}H-DNA) absorbed an amount of radioactivity increasing linearly with the time. It was observed that the absorbed DNA had a sufficiently high molecular weight to induce eventual biological effects (SCHAEFFER, 1964). In another paper LONI and BONOTTO (1966) reported a statistically significant inhibition of the growth of primary roots of Arabidopsis following a treatment of seeds with calf thymus or commercial DNA. In this paper further results are presented on the effect of an heterologous DNA on the growth of the primary roots.

The seeds of Arabidopsis (Wil-2) were incubated for 42 hours at 24 ± 1°C in the dark in a 0.01 M NaCl solution containing 2 mg/ml of calf thymus DNA. The control groups were incubated in a 0.01 M NaCl solution. The seeds were then washed three times in a 0.01 M NaCl solution and placed on Petri-dishes (cf. MÜLLER, 1964) for germination at 24 ± 1°C under constant illumination (Tubes Phytor, 5,000 lux) and in 60% relative humidity. The root length was measured after 5 days. Under these experimental conditions the maximum r o o t l e n g t h observed in the control was 25 mm, but only 14 mm in seeds incubated in the heterologous DNA solution; i.e., the mean root length of the treated series was 47.55% as compared to the control. Moreover, Figure 1 demonstrates that the treatment with DNA induced an important shift of the distribution towards the classes with shorter roots. A statistical test was done on the two groups of roots. A logarithmic transformation was necessary in order to obtain the homogeneity of the two variances. The analysis of variance of logarithms gave the following result:

Source of variation	Sum of squares	d.f.	Mean Square	F*
Treatment	4.3093	1	4.3093	93.27
Error	10.4553	226	0.0462	

*$F_{0.01} = 6.76$

We observed, however, no effect of the DNA treatment on the s t e m l e n g t h which appears to be very similar in both the control and treated plants (Figure 2). This might be related to the fact that the apical meristem grows very slowly compared to the root meristem (MÜLLER, 1964) and that, therefore, it could represent a poor material to reveal an eventual early effect of DNA.

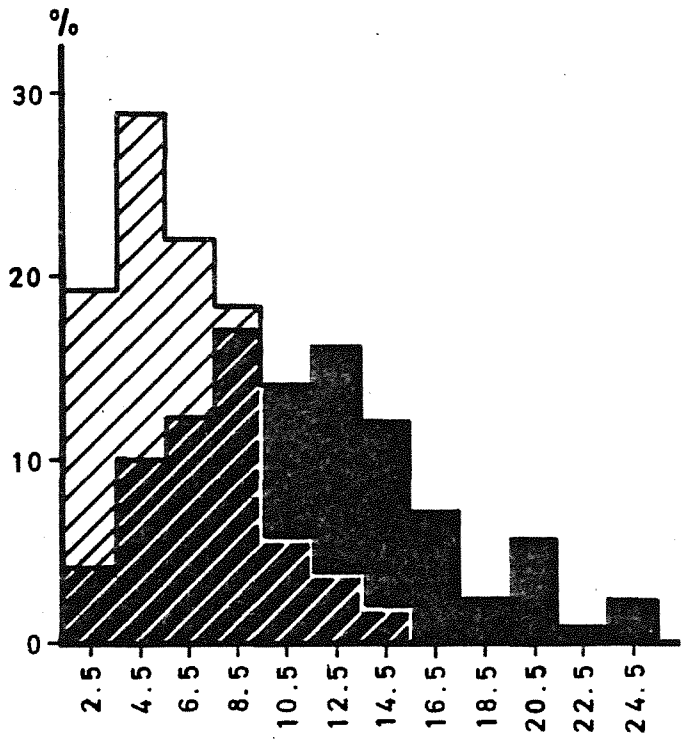

Figure 1: Frequency distributions of the length of roots from control (in black) and DNA-treated seeds (cross-hatched). The number of observations was 124 and 104 respectively.

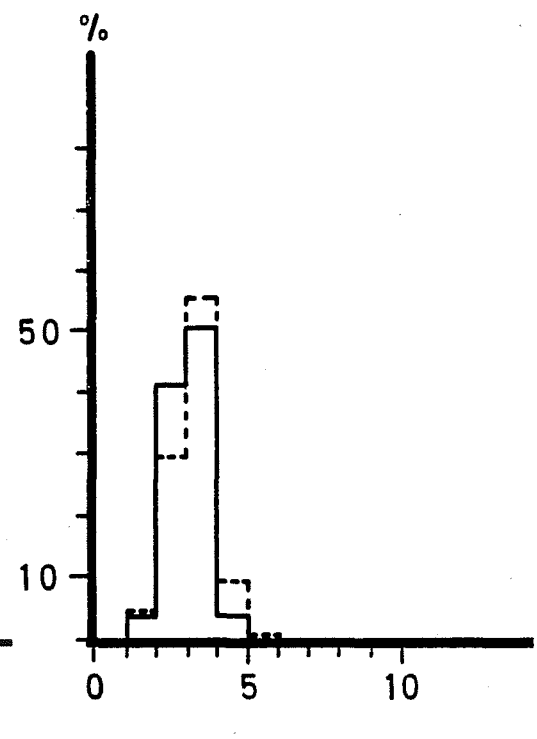

Figure 2: Frequency distributions of stem length. The continuous line shows the control, while the broken line represents the treated plants.

The observed growth inhibition of the primary roots of Arabidopsis could be due to a mutagenic effect of the absorbed DNA (OEHLKERS, 1953; WOLL, 1953). We can not, however, exclude the presence of a more complicated mechanism as, for instance, some interaction at the molecular level between the DNA of the plant and the absorbed heterologous DNA.

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Dimethyl sulfoxide as a carrier for chemical mutagens

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Some preliminary observations on the effect of dimethyl sulfoxide (DMSO) alone and some other chemical mutagens dissolved in DMSO were reported earlier (BHATIA and SMITH, 1966). The object was to investigate the feasability of using DMSO as a carrier for chemical mutagens to utilize the rapid absorption and transport of DMSO and substances dissolved in it through biological membranes. This report includes some additional data on the use of DMSO for this purpose.

Use of 30% DMSO reported earlier was later found to increase lethality of treated seedlings, therefore, for further work only 5% DMSO was used. Two kinds of mutagens were tried, the widely effective and potent EMS and the thymidine analogues IUdR, BUdR, and FUdR in aqueous and 5% DMSO solutions, either singly or in combination. The thymidine analogues IUdR and BUdR though reported to increase the frequency of lethals and laggards in the M_2 generation (BROWN, BHATIA, and SMITH, 1965), have not yet been demonstrated to induce distinct chlorophyll or morphological mutants in Arabidopsis or other higher plants.

Eleven-day-old seedlings, raised aseptically on mineral agar were treated with aqueous or 5% DMSO solutions of the mutagen by placing a drop at the growing point with a microsyringe. At this age, the early flower buds are at premeiotic stages. Details of the given treatments are listed in Tables 1 and 2. Each M_2 population was the selfed progeny

Table 1: Frequency of albina mutants in the M_2 generation following treatment of flower buds at premeiotic stages with EMS alone and EMS dissolved in 5% DMSO

Treatment	Number of M_1 families		Number of plants		Mutation rate %	
	total	segregating	total	mutant	per M_1 families segregating	per 100 M_2 plants
10 mM EMS in 0.1M phosphate buffer, pH 7	292	28	18653	118	9.59	0.63
10 mM EMS + 5% DMSO in 0.1M phosphate buffer, pH 7	257	46	18844	246	17.89	1.30
5% DMSO alone in 0.1M phosphate buffer, pH 7	275	0	19723	0		
Phosphate buffer, 0.1M, pH 7	300	0	26517	0		
No treatment, control	300	0	24813	0		

of a single M_1 plant and was raised in a separate petridish. The frequency of albina mutants was used as a measure of the mutagenic effect of each treatment. These mutants, unlike other chlorophyll mutants, can be recognised with certainty in the seedling stage of the M_2 generation. Pooled results of three independent experiments with EMS and two with base analogues are shown in Tables 1 and 2.

The results indicate that 5% DMSO alone is not mutagenic in Arabidopsis but enhances the effect of EMS, approximately two-fold. This most likely is due to an increased and more rapid uptake of the mutagen. With the thymidine analogue treatments, no albina type

Table 2: Number of M_1 families scored for albina mutants in the M_2 generation following treatment of flower buds at premeiotic stages with thymidine analogues in aqueous or in 5% DMSO solution

Treatment	Number of M_1 families		Number of M_2 plants
	total	segregating	
Aqueous:			
IUdR 5 x 10^{-4} M	78	0	As none of the families segregated for mutants, the number of M_2 seedlings were not counted, but on an average each family scored had 60-80 seedlings
BUdR 5 x 10^{-4} M	81	0	
FUdR 10^{-4} M	57	0	
FUdR+IUdR (combined)*	69	0	
FUdR+BUdR (combined)	73	0	
FUdR followed by IUdR after one hour	59	0	
FUdR followed by BUdR after one hour	62	0	
5% DMSO:			
IUdR 5 x 10^{-4} M	81	0	
BUdR 5 x 10^{-4} M	79	0	
FUdR 10^{-4} M	48	0	
FUdR+IUdR	57	0	
FUdR+BUdR	64	0	

*The same concentrations of FUdR and IUdR were used as in the single treatments in all combined and followed-by treatments

of mutants were observed in the M_2. Two chlorina mutants occurred in one family and another M_2 family had a seedling with a mutated sector. However, these mutational events seem to be of spontaneous origin and quite unrelated to the treatments given. These results seem to confirm that treatment of flower buds at premeiotic stages with the thymidine analogues IUdR and BUdR do not yield any chlorophyll mutants.

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Induction of laggards by cultivating Arabidopsis plants on medium with acridine orange and proflavine

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Acridine dyes, which are known to react with nucleic acids, have received increasing interest as mutagens in microorganisms, but relatively little attention has been given to acridine-induced mutations in higher plants except in barley (D'AMATO, 1950), tomato (BUIATTI and RAGAZZINI, 1966), and Arabidopsis (OVERBECK, 1952/53). This paper deals with an investigation of the mutagenic activity of proflavine and acridine orange. The plants were cultivated in Petri-dishes on perlite moistened by mineral solutions which contained the dyes in a concentration range of 1.25 to 62.5 mg/100 ml. This mode of treatment included conditions for photoactivation by visible light.

In the M_1-generation a slight decrease of germination was observed in the highest concentrations and a delay in development correlated with the given doses. Table 1 shows one aspect of this last effect, with the date of the first flower opening as criterion. The fertility measured by number of seeds per silique was not very much affected.

Table 1: Number of plants (in %) with first flower opened, cultivated on a medium containing proflavine and acridine orange, and under illumination conditions of 12 hrs dark/12 hrs light

Treatment	Concentration (mg/100 ml)	Days after sowing				
		40	46	51	58	65
Control	-	65.9	79.5	-	95.4	100.0
Proflavine	1.25	26.0	46.0	56.0	79.0	90.0
	2.50	11.6	23.2	34.8	62.6	67.2
	3.75	0	0	2.8	24.7	44.3
	5.00	0	0	0	3.1	12.5
	6.25	0	0	0	0	3.2
Acridine orange	1.25	0	6.8	20.4	56.8	68.1
	2.50	0	0	0	0	6.8
	3.75	0	0	0	0	0

In the M_2-generation the germination data were of special interest due to the emergence of laggard variants of both lethal and viable types that looked quite similar to those induced by 5-bromodeoxyuridine (JACOBS and BONOTTO, 1967) (Table 2). By the selection for viable phenotypes we were able to recognize that the ratio of laggards to normal types increased from the M_2 to the M_5 (Table 3). The same kind of instability as observed after BUdR treatment. Some typical mutants, e.g. viridis, were also present at a very low frequency (0.3 to 1.2%). Morphological variants were observed in successive generations, in families containing laggards. The modifications are essentially concerned with the shape of the leaves and their disposition within the rosette.

Table 2: Frequency of normal and laggard plants in M_2 after cultivation of the M_1 plants in media with proflavine or acridine orange

Treatment	Number of seedlings observed	Number of laggards	Percentage of seedlings		
			lethal laggard	viable laggard	normal
Control	530	9	1.3	0.4	98.3
Proflavine	1735	108	3.8	2.5	93.7
Acridine orange	848	99	3.2	8.5	88.3

Table 3: Segregation in progenies of viable laggards induced by proflavine or acridine orange

Number of generations after treatment	Number of seeds sown	Number of seedlings	Percentage of seedlings		
			lethal laggard	viable laggard	normal
2	822	635	26.0	3.7	70.3
3	291	148	26.4	16.9	56.7
4	442	361	45.7	7.8	46.5

It has been reported (LERMAN, 1964) that acridine dyes may act by intercalation between adjacent nucleotides and that they may induce deletions or insertions of nucleotides in the DNA. They have also been claimed to be very effective in producing cytoplasmic modifications in fungi (JINKS, 1963). It appears to be very interesting, therefore, that these two acridine derivatives induce the very same type of variants which were found after BUdR treatment and that these variants arise apparently in the same non-random manner.

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Changes in the EMS sensitivity of the shoot meristem of seeds during imbibition and germination

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After various periods of presoaking (0 to 48 hrs) seeds of Arabidopsis thaliana (Dijon G) were treated with ethyl methanesulfonate (EMS) for 1 hr. The mutagenic effect of this treatment was measured by the frequency of recessive lethal mutations (embryonic lethals and chlorophyll mutations).

It was found that the sensitivity of the shoot meristem changes considerably in the course of water imbibition and germination:

(1) The increase of sensitivity during the imbibition phase was not influenced by anaerobic conditions. Therefore, the lower sensitivity of dry or incompletely hydrated seeds is obviously only due to the slower penetration of EMS into the meristem cells. A first maximum of sensitivity was reached after about 5 hrs of presoaking at 24°C (or after 2 hrs at 36°C). Prolongation of soaking under anaerobic conditions did not significantly change the sensitivity.

(2) Under aerobic conditions which enable germination, the sensitivity decreased again and reached a minimum value after 24 to 30 hrs of soaking. Simultaneously, the proportion of chlorophyll mutations increased from 12% to 21%.

(3) With proceeding germination and beginning of the DNA replication in the shoot meristem, a second increase of sensitivity occurred. The sensitivity of seeds soaked for 48 hrs under aerobic conditions was found to be higher than the first maximum of sensitivity reached after 5 hrs of soaking.

A detailed publication on the subject is prepared in the "Biologisches Zentralblatt" (Supplement, 1967).

The influence of post-treatment-storage of seeds on mutation induction with alkyl methanesulfonates, nitrosamides, and X-rays

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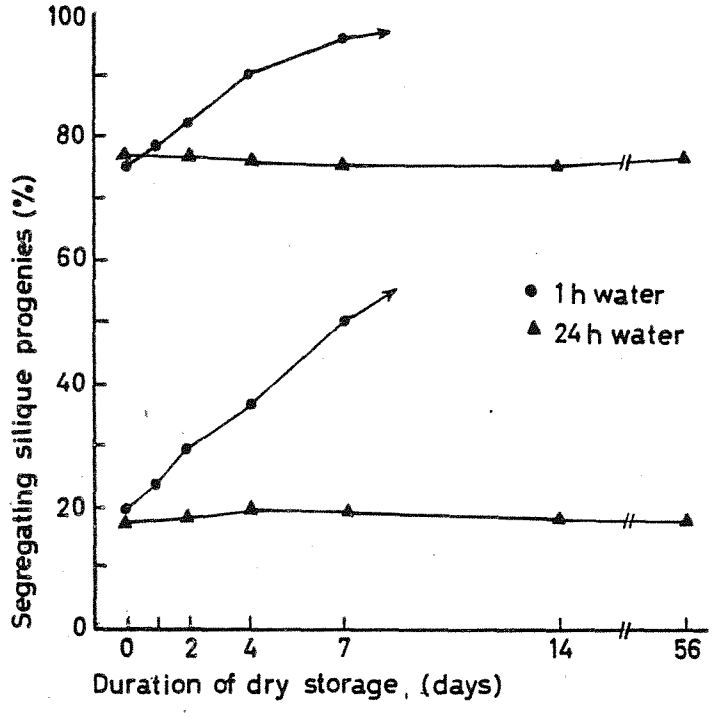
The germination of soaked seeds can be inhibited by various means, e. g., by anaerobiosis or by drying. This gives the possibility to interpose a storage period between mutagenic treatment and replication of DNA. Submersion of Arabidopsis seeds in water creates partially anaerobic conditions which are sufficient to inhibit germination. At 24°C this "w a t e r s t o r a g e" may be continued up to 5 or 7 days without loss of germinability (cf. MÜLLER, 1965b, 1967). "D r y s t o r a g e" of Arabidopsis seeds may be continued for years without loss of germinability provided the soaking before drying has been carried out under anaerobic conditions or, if under aerobic conditions, only for a period not exceeding 20 hrs (at 24°C).

Experiments with E M S*: Anaerobically presoaked seeds of Arabidopsis thaliana (Dijon G) were treated for 3 hrs at 24°C with 50 mM EMS and then washed and submersed in distilled water for 1 hr or 24 hrs respectively. After the "water storage" the seeds were either germinated immediately or dried over silica-gel at 24°C and germinated after various periods of "dry storage". The frequency of recessive lethals was determined by the embryo test. The results are summarized in the figure (upper curves: embryonic lethals and chlorophyll mutations; lower curves: chlorophyll mutations only; 14 hrs of dry storage after 1 hr of water storage led to almost completely sterile M_1 plants). In another experiment (see table) the effect of posttreatment with 20 mM cysteine on the EMS-induced mutation frequency was tested.



Experiments with o t h e r m u t a g e n s are summarized in the table. (In each case anaerobically presoaked seeds of A. thaliana "Dijon G" were treated.)

From the results the following conclusions may be drawn: (1) Delaying the germination by posttreatment storage in water (anaerobiosis) does not influence the fixation of premutational lesions induced by EMS, iPMS, NMH or NMG. This confirms previous results with NMH (MÜLLER, 1965b) and EMS (MÜLLER, 1966). (2) Cysteine given after the mutagenic treatment is expected to react with the mutagenic agent still present in the seeds at the post-treatment-storage period. Accordingly, cystein post-treatment reduced the mutation frequency in the case of the slowly reacting EMS, but not in the case of the rapidly reacting iPMS and NMG. (3) The increase of mutation frequency while storing EMS-treated seeds in a redesiccated condition has to be ascribed to reactions of EMS still present in the seeds after 1 hr of "water storage". It is prevented completely by soaking the treated seeds in water for 24 hrs. Previous results (MÜLLER, 1966) indicated that 11 hrs of post-treatment soaking at 24°C is sufficient for this purpose. Accordingly, with the rapidly reacting iPMS a "storage effect" could not be demonstrated. (4) Drying of soaked seeds immediately after treatment with X-rays increases the frequency of induced mutations. (5) In addition to previous results

Mutagenic treatment	Post-treatment storage	Segregating silique progenies (%) recessive lethals	chlorophyll mutations only
EMS 100 mM, 1 hr, 24°C	24 hrs water	56	13.2
	7 hrs cysteine, 17 hrs water	27	4.8
iPMS 80 mM, 2 x 1 hr, 24°C	30 min water	66	16.3
	" , 4 days dry	71	16.2
	" , 60 days dry	66	17.9
	1 hr water	52	12.8
	24 hrs water	53	13.5
	" , 3 days dry	51	11.4
	8 hrs cysteine, 16 hrs water	52	11.1
X-rays 12 kR	without	27	4.3
	75 days dry	39	6.1
NMH 1 mM, 1 hr, 24°C	2 hrs water	78	24.3
	" , 3 days dry	60	12.4
	24 hrs water	75	22.8
	" , 3 days dry	58	15.2
NMG 5 mM, 15 min, 24°C	4 hrs water	24	2.5
	1 hr cysteine, 3 hrs water	24	3.1
NMG 1 mM, 30 min, 36°C	2 hrs water	39	8.1
	" , 40 days dry	19	3.4
	48 hrs water	38	8.8
	" , 40 days dry	20	3.9
NMU 0.08 mM, 3 hrs, 36°C	19 hrs water	91	27.3
	" , 1 year dry	35	7.5

with NMH (MÜLLER, 1965a) it is shown that drying of seeds treated with various nitrosamides drastically decreases the frequency of induced mutations. The finding that this effect of drying is not influenced by the duration of "water storage" interposed between treatment and drying indicates that the nitrosamides induce a specific kind of premutational lesions which are stable under the conditions of "water storage", but reversible by drying. The premutational lesions induced by alkyl methanesulfonates or X-rays are obviously not reversible by drying.

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MÜLLER, A. J.: Biol. Zbl. 84, 759-762 (1965a)
- : Mutation Res. 2, 426-437 (1965b)
- : Züchter 36, 201-220 (1966)
- : Biol. Zbl. 86, Suppl., in press (1967)

*Abbreviations: EMS = ethyl methanesulfonate; iPMS = isopropyl methanesulfonate; NMH = N-nitroso-N-methylurea; NMG = N-nitroso-N-methyl-N-nitroguanidine; NMU = N-nitroso-N-methylurethane

Non-recessive embryonic lethals as revealed by reciprocal crosses between EMS treated and control plants

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After 5 days at 1°C on wet filterpaper (to break dormancy) and 24 hrs drying at 24°C, seeds of Landsberg-'erecta' were treated with 10 mM EMS (unbuffered, 24 hrs, 24°C), followed by 5 min rinsing. This dose leads to about 50% non-fertilized ovulae (control 10%), but it gives little if any reduction in germination % (agar) and survival, while conspicuous somatic effects up to the time of flowering are rare.

MÜLLER's embryo-test was applied to the 4th and 5th fruits (for ease of handling on one side of the septum only), and consequently also the different crosses to be reported had to be made on flowers 4 and 5. Mutant frequency is given as % of seeds which contain embryonic lethals and as % chlorophyll mutants among non-lethal embryos (Cf. MÜLLER's m_c and m'_c). Greyish white, greyish green, etc. embryos, which often go with smaller and/or deformed seeds, were classified by me as embryonic lethals. The results of one of the experiments (August 1966) are given in the table:

	Number of fruits	% ovules non-fertilized	% embr. lethals	% chlor. mutants
Control (C) (natural selfing)	140	11.2	1.1	0.0
Control x EMS	238	64.0	1.7	0.2
EMS x Control	229	48.1	7.1	0.2
EMS (natural selfing)	432	45.2	19.0	12.8

Additional data are: (1) C x C (between plants) gives the same results as C selfed, except for a very small increase in non-fertilized ovulae, (2) EMS x EMS (between plants) equals EMS x C (except for some increase in sterility), which shows that allelic mutations (e.g., included in overlapping transmissable deletions) are infrequent, and (3) artificial within-flower selfings, closely mimicking the mechanical damage in crossing, gave for both C and EMS plants the same results as the corresponding natural selfings. It follows that the reciprocal difference in embryonic lethals between C x EMS and EMS x C c a n n o t be ascribed to susceptibility of EMS flowers to mechanical damage. The difference in mutant %'s between C and C x EMS is probably due to sampling fluctuations, and there is no reciprocal difference in % of chlorophyll mutants. The high sterility of C x EMS is caused by the reduced availability and quality of EMS-pollen (per pollination, pollen from only one flower was used).

The class of embryonic lethals in EMS x C (after correction with the C-level still over 30% of the lethals in EMS-selfed) are not due to recessive mutations, and their spectrum shows, compared with EMS-selfed, a relative decrease of late embryonic lethals and a considerable rel. increase in early ones (such a shift is not induced by mechanical damage). Possible explanations are, apart from autonomous effects of the embryo cytoplasm, and apart from endosperm-embryo incompatibility connected with a phenotypic contrast AA+ versus A++:

(1) dominant embryonic lethals which are not transmitted by the male gametes (N.B. dominant chlorophyll mutations are rare), and
(2) a maternal physiological effect, such that, e.g., the feeding function of the nucellus is impaired by nuclear and/or plasmatic damage in the EMS mother plants.

The latter explanation seems somewhat more attractive, as the reciprocal difference is restricted to embryonic lethality, and the lethals do not occur in some plants only. It might then be possible to reduce this class of non-recessive lethals by modifying the environment. However, winter experiments (low light intensity) gave similar figures (6.0-8.0%) as the summer experiments. Non-recessive embryonic lethals might affect comparisons between different mutagen dosages, between different treatment modifications, and between different mutagens. Further attempts to distinguish between the different hypotheses are in progress.

Revertants of pyrimidineless mutants

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In a previous communication (HEYTING and FEENSTRA, 1966) purpose and method of our studies on reversion of pyrimidineless* mutants were briefly described. We now can report that after EMS treatment we obtained a number of lines showing in each consecutive generation a segregation in wild (or semi-wild) type and clearly deficient plants when grown on mineral medium, and after propagation by means of non-deficient plants. From eight different originally deficient lines one or more of such "revertant" lines were isolated (see table). Although wide fluctuations occur, in many families a one to

py-mutant	Number of seeds treated	Number of M_1-plants from which a "revertant" line could be established	Latest generation grown	Number of families tested in latest generation
V 43	40 x 10^3	1	M_3	2
V 163	27 x "	1	M_4	190
V 433	30 x "	1*	M_2	2
V 446	40 x "	2	M_5	29 and 141
V 462	30 x "	1	M_3	74
V 595	30 x "	1	M_3	20
V 632	17.8x "	1	M_4	31
V 643	30 x "	1*	M_2	4

*due to the small number of families only tentative

one ratio between growing and non-growing plants is approached.

The results of crosses between revertants and mutants confirm the non-homozygous character of the former. Possibly, in the explanation of these phenomena a role will have to be ascribed to interallelic complementation. This hypothesis is now being tested.

Selecting M_1 plants with a revertant sector proved to be difficult, due to the variable and often small effect of such a sector on the growth of the plant as a whole. Many suspected revertant M_1 plants therefore were grown to maturity, if necessary with thiamine added, in order to allow M_2-testing. Only in a relatively small number of cases the occurrence of M_2 plants growing clearly better than the original deficient type could be established. From 25 of the 33 pyrimidineless mutants tested so far no revertants could be obtained. 10,000 to 40,000 seeds per line were treated, and these numbers probably are too low to allow conclusions. With one mutant, V 131, however, even after treatment of 78 x 10^3 seeds no positive result was obtained so that here possibly the conclusion may be drawn that EMS cannot induce reversion.

Reference:
HEYTING, J., and W. J. FEENSTRA: Arabid. Inf. Serv. 3, 30 (1966)

This work was supported by a grant from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.)

*pyrimidine here short for 4-amino-5-hydroxymethyl-2-methyl-pyrimidine, i.e., the pyrimidine moiety of thiamine

The different action of 1-methyl-3-nitro-1-nitrosoguanidine in Arabidopsis and barley

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1-methyl-3-nitro-1-nitrosoguanidine (MNG) induces a high frequency of mutations in Arabidopsis (MÜLLER and GICHNER, 1964) but no mutations in barley (EHRENBERG and GICHNER, 1967). There are also differences in the M_1 generation. In Arabidopsis the M_1 root length decreases with the increasing MNG concentration, but the germination remains unchanged up to very high concentrations (Figure 1). After the action of MNG on barley both the germination and seedling height decreases with the increasing MNG concentration (Figure 2).

1-methyl-1-nitrosourea (MNH), which induces mutations in Arabidopsis (VELEMÍNSKÝ et al., 1964; MÜLLER, 1964) and barley (EHRENBERG and GICHNER, 1967), influences the M_1 germination of both species only at extremely high concentrations (Figure 1 and 2).

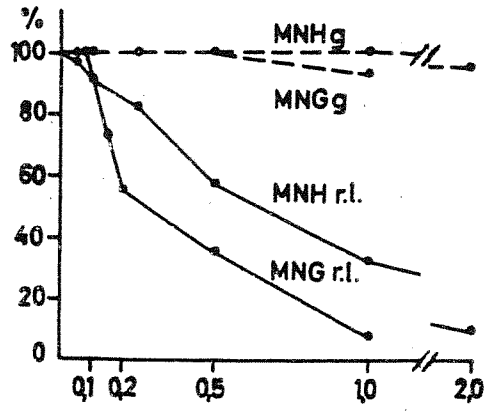

Figure 1: Percent of germination (g) and relative root length (r.l.) in Arabidopsis

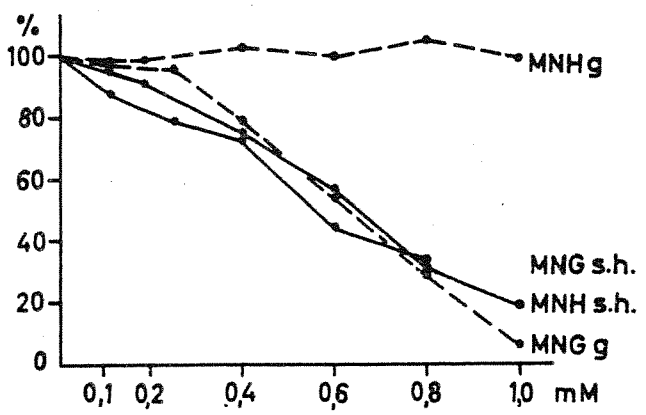

Figure 2: Percent of germination (g) and relative seedling height (s.h.) in barley

Considering that in both species the seeds were treated with MNG, one may assume that the differences in the M_1 generation are caused by differences in seed metabolism. These results show that although Arabidopsis is an excellent model plant for theoretical mutation studies the results obtained may sometimes be different from those with other plant species. Detailed results are in press (Biologia Plantarum, 1967).

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The effect of visible light on the mutagenic activity of 1-methyl-1-nitrosourea and 1-methyl-3-nitro-1-nitrosoguanidine

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Nitrosoamides as well as some other nitroso-compounds are known to be photolabile, e.g., under the action of UV-light they decompose and release nitrous acid (DAIBER and PREUSSMANN, 1964). This decomposition proceeds also under natural daylight or artificial visible light.

Seeds of Arabidopsis, race Dijon, were immersed for 24 hours in fresh solutions of 1-methyl-1-nitrosourea (MNH) and 1-methyl-3-nitro-1-nitrosoguanidine (MNG) in the light and in the dark. The temperature of treatment (25°C), the amount of the solution (6 ml), as well as its height and surface was constant for both light and dark treatment. Fluorescent tubes with the intensity of about 7,000 lux were used as source for artificial light. The results in Table 1 demonstrate that under the influence of light the activity of both mutagens is decreased. The mutagenic activity, expressed as the frequency of segregating M_1 siliques (m_b) is lower, and the same is true for the degree of sterility and root length reduction.

Table 1:

Compound	Concentration	Conditions of treatment	Root length % of control	Degree of sterility	m_b
MNH	0.5 mM in water	dark	80.97	95.2	92.5
		light	84.24	72.1	81.6
MNH	0.5 mM in buffer pH=5	dark	37.98	100.0	-
		light	76.45	81.7	88.4
MNG	0.5 mM in water	dark	24.34	98.2	85.0
		light	79.82	44.9	45.8
MNG	0.5 mM in buffer pH=5	dark	18.95	100.0	-
		light	73.22	35.8	40.3

The difference between light and dark treatment is higher in the case of MNG. This is in accordance with the rate and amount of HNO_2 release (Table 2). The amount of nitrous acid in the 0.5 mM MNG solution reached the concentration of about 0.2 mM already in 8 hours after the onset of light treatment. The maximal amount of nitrous acid, however, released from 0.5 mM MNH was only 0.1 mM which was reached after 24 hours.

Table 2: Increase of HNO_2 (in mM) during the action of light on the solutions of nitrosoamides

Solution	Conditions	0	1	2	4	8	24	48 hrs
0.5 mM MNH in water	dark	0.002	0.002	0.002	0.001	0.001	0.001	0.001
	light		0.007	0.015	0.021	0.051	0.074	0.057
0.5 mM MNH buffer pH=5	dark	0.001	0.001	0.001	0.001	0.001	0.002	0.003
	light		0.012	0.030	0.043	0.066	0.112	0.102
0.5 mM MNG in water	dark	0.004	0.005	0.004	0.004	0.003	0.004	0.006
	light		0.038	0.052	0.146	0.212	0.174	0.172
0.5 mM MNG buffer pH=5	dark	0.003	0.004	0.004	0.006	0.010	0.022	0.032
	light		0.058	0.064	0.157	0.200	0.157	0.163

As nitrous acid does not induce any embryonic and chlorophyll lethals in Arabidopsis (MÜLLER, 1964; and confirmed in our experiments) even at concentrations of 10 mM, it can be assumed that due to the photodecomposition of both nitrosoamides the amount of the active part of the mutagen is lowered.

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DAIBER, D., and R. PREUSSMANN: Ztschr. analyt. Chem. 206, 344-352 (1964)
MÜLLER, A. J.: Arabid. Inf. Serv. 2, 22-23 (1965)

About mutagenesis with a thymidine base analogue

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In No.2 of this newsletter, MÜLLER (1965), noting the fact that no recessive lethals can be induced by 5-bromodeoxyuridine (BUdR), points out that either the analogue had not reached the apical meristem or that an incorporation had not induced an increase of the number of recessive lethals. In order to elucidate this alternative we used tritiated BUdR (14 mc/mM) in three kinds of treatment. First, seeds were immersed for 47 hrs 15 min in a solution of ^{3}H-BUdR (222×10^{5} dpm/ml) and after washing sown on mineral medium. The amount of the analogue uptaken was estimated at different stages of the plant development. Fig. 1 shows that no significant radioactivity could be found in flowering buds

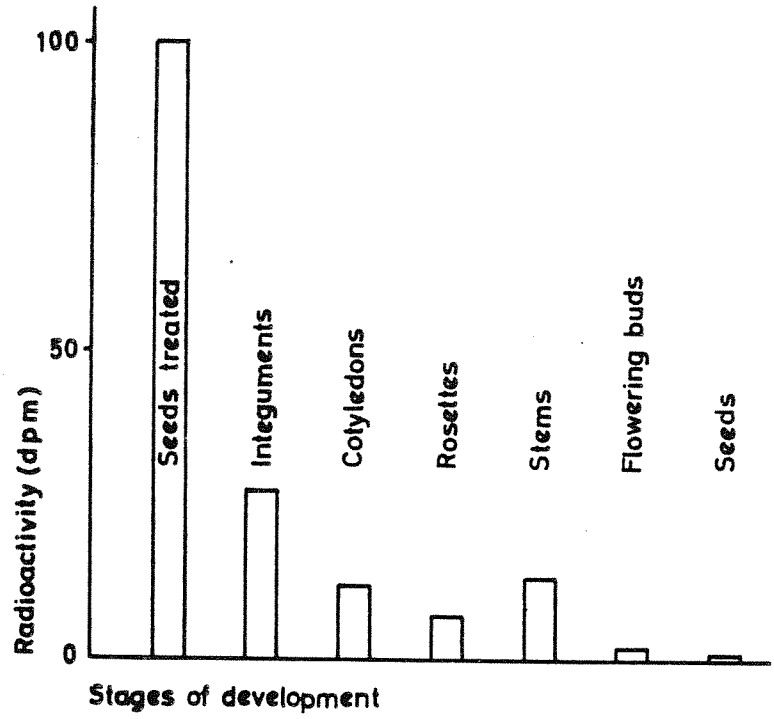

Figure 1: Fate of ^{3}H-BUdR uptaken by seeds during the following stages of plant development

*Boursier-Euratom

Table 1: Radioactivity* found in successive stages of development of Arabidopsis plants

Supplements to the mineral medium	Fractions	Cotyl.	Cotyl. + 2 leaves	Cotyl. + 4 leaves	Rosettes	Stems	Flowering buds	Siliques	Seeds
		dpm/100 mg fresh weight					dpm/100 units		
BUdR 1.25×10^{-5}M	AS	208.691	220.692	228.528	428.558	114.209	8.796	50.981	9.746
	AIS	19.977	30.441	38.906	12.284	156.861	2.055	1.784	756
	AS/AIS	0.096	0.138	0.189	0.029	1.373	0.234	0.035	0.078
BUdR 1.25×10^{-5}M + aminopterin 1.25×10^{-7}M	AS	313.525	331.381	618.187	149.070	68.155	17.092	41.612	9.648
	AIS	24.935	29.588	50.558	6.607	68.487	630	2.516	755
	AS/AIS	0.078	0.089	0.082	0.044	1.005	0.037	0.060	0.078
BUdR 1.25×10^{-5}M + FUdR 1.25×10^{-7}M	AS	377.943	309.163	369.413	214.175	99.483	15.777	4.711	10.225
	AIS	36.761	21.084	11.788	30.057	100.621	2.752	-	1.225
	AS/AIS	0.097	0.068	0.032	0.140	1.011	0.174	-	0.120

*1 ml of ^{3}H-BUdR (14 mc/mM) was added on the basis of 40 ml of the culture medium

or in seeds. Secondly, ^{3}H-BUdR ($2{,}22 \times 10^{5}$ dpm/ml) was applied to the axils of 18 day-old plants (50 µl per plant in 0.1% "Tween 20") and the seeds were collected. In this instance a certain percentage of the radioactivity appeared to be localized in the seeds. By the same method of treatment, BROWN, BHATIA and SMITH (1965) isolated laggard plants and one of us (JACOBS, 1964) chlorophyll mutants but with a low frequency. The third mode of treatment consisted in cultivating plants on a solution containing BUdR. Tritium labelled BUdR was added to the culture medium (555×10^{3} dpm/ml) and in two series a definite amount of aminopterin or fluorodeoxyuridine (FUdR) was also present in the medium. A modification of the SCHMIDT-TANNHAUSER procedure (1945) was used for the extraction of the nucleic acids.

The radioactivity present in the acidosoluble (AS) and in the acidoinsoluble fractions (AIS) was measured in a Tri Carb scintillation Spectrometer. The results in Table 1 show that:

(1) Penetration and incorporation occurs throughout the vegetation cycle, but the absolute amount of incorporation is very low in the reproductive parts. This may result from a dilution of the label initially incorporated in the growing shoot by cell division in the daughter cells. In fact, flowering buds represent a small part by weight with regard to the whole shoot.

(2) The presence of aminopterin and of FUdR, two inhibitors of pyrimidine synthesis, does not influence significantly the rate of incorporation, at least with the concentrations used. These findings suggest that BUdR can replace thymidine even in the presence of a normal pool of thymidilate.

(3) A sudden increase of incorporation versus penetration appears in growing shoots. A possible explanation for the rise of the incorporation ratio may result from the mitotic activity of the inflorescence apex. A higher rate of DNA synthesis could explain the increased incorporation of the label.

By this last mode of treatment, we observed in F_2 laggard variants of lethal and viable types. The viable phenotypes are recognizable by relatively poor growth and seed set. The frequency of the laggards in F_2 varies between 5 and 11% according to the concentration used (JACOBS, in press). Thus, from this last experiment it may be concluded that a certain amount of BUdR reached the shoot meristem and that its incorporation induced only one particular type of laggards with a high frequency and specificity. These characteristics, however, together with the behaviour of the variants in the following generations provide some presumptive evidence for an extrachromosomal basis of the product activity (JINKS, 1963). This possibility shall be tested in the near future.

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Growth of Arabidopsis thaliana in heavy water (D_2O)

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A short life cycle and a meagre water (D_2O) requirement by Arabidopsis thaliana should make this plant suitable for investigating the effects of partial or complete deuteriation of biologically important molecules on the growth and development of plants.

The method of cultivation was mainly after LANGRIDGE (1957). The mineral requirements were provided as Knop's solution containing an increased quantity of KH_2PO_4 (0.002 M). Iron when incorporated in the nutrient solution was found to bring about earlier germination and better growth at the 5 ppm level than at lower concentrations. The medium containing increasing amounts of D_2O, viz., 0.0, 12.5, 25.0, 37.5, 50.0, 62.5, 75.0, and 87.5% was adjusted to pH 6.0. The solution containing 0.75% Bacto-agar was autoclaved at 15 lbs for 15 minutes and then allowed to solidify at room temperature.

Dry surface-sterilized seeds, one seed per test tube, were inoculated under sterile conditions. To induce uniform germination the test tubes were kept overnight at a temperature of 0-5°C and later transferred to a growth chamber maintained at 25°C and 75% relative humidity. The test tubes were kept in continuous illumination of 750 f.c. light intensity throughout the growth period. The photosynthetic supply of carbohydrates was supplemented by the addition of 2% sucrose in the nutrient medium (1500 f.c. is essential for full photosynthetic efficiency).

Various growth and development criteria such as the germination, the appearance of leaf-pairs, floral initials and first open flower and the number of flowers, seeds, etc., were examined. Viability tests were carried out for the seeds obtained from various treatments by germinating on nutrient medium with normal water. The results are presented in Tables 1 and 2 and the salient findings briefly described.

Table 1: Effect of D_2O on the germination and development of Arabidopsis thaliana. Time (days) taken to attain different growth stages

Organogenetic development	Control	D_2O concentrations (%)				
		12.5	25.0	37.5	50.0	>50.0
100% germination	4	5	5	7	8	no germination
1st leaf pair	5	5	5	7	8	"
2nd leaf pair	5	5	6	8	9	"
3rd leaf pair	6	8	10	10	13	"
4th leaf pair	10	13	14	15	Nil	"
5th leaf pair	13	14	16	18	"	"
Floral initials	16	17	19	26	"	"
1st open flower	23	23	26	34	"	"

A delay in germination was a function of increasing D_2O concentration being maximal in 50% D_2O. Higher concentrations proved to be too toxic to give any germination (Table 1). Similarly, appearance of leaf-pairs was retarded by D_2O which at 50% concentration completely inhibited growth beyond third leaf-pair stage. This complete inhibition of growth could have resulted due to very high degree of chlorosis induced by D_2O.

There was a general delay in the appearance of various leaf-pairs, floral initials and first open flower (Table 1). The extent of delay increased not only with increase in D_2O concentration but also generally with progressive growth stages of plant.

There was a reduction in the number of open flowers per plant in the treated plants, a reduction which was more or less a function of the concentration of D_2O (Table 2). There was hardly any difference in seed weights between seeds obtained from H_2O and D_2O grown plants (Table 2). Similarly, the seeds from D_2O cultures were found to be just as viable as those obtained from normal (H_2O) cultures.

Table 2: Developmental data. (The figures in parentheses give percent of control.)

Characters examined	D_2O concentrations (%)			
	Control	12.5	25.0	37.5
Number of open flowers per plant	40 (100%)	31 (78%)	26 (65%)	17 (43%)
Number of seeds per plant	135	124	121	117
Ratio of number of seeds to number of open flowers	3.4	4.0	4.7	6.9
Seed weight (µg) per 10 seeds	222	220	214	215
Viability of seeds (%)	100	100	100	100

Looking to the number of seeds obtained per plant in different treatments and comparing them with the corresponding flower number (Table 2), it appears that although the total seed number was less in any D_2O treatment, yet seed / flower ratio steadily rose from about 3.4 in controls to 6.9 in 37.5% D_2O. This could be the result of an increase in number of fertilized flowers and/or of seeds per fertilized flower in D_2O treated plants. Further investigations are planned to obtain more definite information on this question.

Reference:
LANGRIDGE, J.: Austral. J. biol. Sci. 10, 243-252 (1957)

Effect of gamma radiation on permeability of Arabidopsis seeds

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The study of the effects of ionizing radiations on biological membranes has received particular attention in the actual biophysical research. Numerous examples of permeability changes induced by ionizing radiations have been reported in the literature both for animals and plants (SRB, 1964; VAN CANEGHEM and LACHAPELLE, 1965). Moreover, the release of a number of compounds by irradiated microorganisms (MANIL, 1959) and of dipicolinic acid by irradiated Clostridium spores (MILIC and BONOTTO, 1966) could be due partly to certain permeability modifications induced by ionizing radiations in cellular membranes in addition to eventual induced metabolic disorders. We have studied the effect of low doses of gamma radiation on the permeability of Arabidopsis seeds to EMS.

Seeds of Arabidopsis thaliana (L.) HEYNH., Wil-2, presoaked for 16 hrs in distilled water were irradiated in a Gamma-Cell (3.000 c, ^{60}Co) at 22-23°C with a dose of 5.000 rad. After the irradiation the seeds were placed during different periods of time in contact with a solution of ^{3}H-EMS (52.32 µg/ml), having a specific activity of 3.91 mc/mM. The seeds were than washed with distilled water three times during 10 minutes in order to eliminate contaminant ^{3}H-EMS, and digested 2 hrs at 60°C in a solution of hydroxide of hyamine Packard and methanol (1:1, v/v). The total tritium radioactivity was determined by liquid scintillation counting with a Packard Tri-Carb spectrometer.

In the table the amount of ^{3}H-EMS taken up by control and by irradiated seeds is reported. It appears that gamma radiation activates the uptake of ^{3}H-EMS in presoaked seeds. We have not observed a similar phenomenon in gamma irradiated dry seeds (BONOTTO and JACOBS, 1966). The results reported here could be explained by a permeability change induced by gamma radiation in the cellular membranes of the seeds leading to a higher absorption of the ^{3}H-EMS molecules.

Table: Uptake of ^{3}H-EMS by presoaked seeds of Arabidopsis non-irradiated or irradiated with 5.000 rad of ^{60}Co gamma rays

Time of ^{3}H-EMS incubation (hrs)	Amount of ^{3}H-EMS/150 seeds (µg x 10^{-2})	
	controls	irradiated
0.5	5.25	6.14
1	6.25	6.83
2	9.16	14.18
3	10.87	16.13
4.5	17.17	24.99
6	22.77	25.96
8	26.15	38.58
10	26.36	36.31

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Relation between radioresistance and thermoresistance of seed germination of Arabidopsis populations from Moravian localities

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Differences in radioresistance of seed germination in dependence on locality were found in Andropogon by MEWISSEN et al. (1959). KHVOSTOVA and NEVZGODINA (1960) showed considerable differences in radioresistance of pea varieties. In an earlier communication (KUČERA, 1966) it was demonstrated that in Arabidopsis the radioresistance of germination depends on the temperature and that its modification by chemicals is possible. The present report deals with further experiments on these relations between radioresistance of germination and temperature.

Seeds of 18 populations from the collection of this Department (cf. ČETL, 1965) were chosen for the experiment. Dry seeds were irradiated with gamma-rays (^{60}Co, 72 kR and 144 kR). The germination at 20°C in Petri-dishes was evaluated on the 7th day. Parallel tests were run without irradiation but with germination temperatures of 20°C and 30°C, respectively. There were 3 x 50 seeds in each variant. The relative values for the germination at 30°C (compared to 20°C) indicate the thermoresistance and those for the irradiated samples the radioresistance of the germination.

	Number of germinated seeds				
	unirradiated			irradiated	
		30°C			
Population	20°C		in % of 20°C	72 kR	144 kR
Bi-1	99	75	76	67	16
Bi-2a	86	41	48	58	35
Bi-4	83	26	31	78	74
Bo-1	83	91	110	7	3
Bo-2	100	83	83	95	40
Bo-3	100	91	91	60	52
Boh	28	63	224	0	0
He-2	85	90	106	8	4
Iv	46	6	12	26	21
Kl-2	97	77	79	21	14
Kr-2	100	73	73	52	34
MB	81	34	42	50	5
MBu	87	92	105	50	10
Me	95	60	63	17	12
MK-1	99	77	78	65	54
On	66	44	67	6	2
Pi	99	81	82	56	52
Ro-1	94	89	94	32	27

As can be seen from the table there were large differences in the germination of the different populations by the higher temperature as well as by the gamma-irradiation. The most striking observation, however, was that those populations with an increasing germination percentage at 30°C showed the lowest germination values after irradiation. The radioresistance and the thermoresistance of seed germination were inversely proportional to each other with a significant negative correlation coefficient ($r \pm 3 \cdot s_r = -0.84 \pm 3 \cdot 0.01$; $P < 0.01$ -for 72 kR).

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Survival and fertility of Arabidopsis thaliana (L.) HEYNH. following gamma-irradiation of seeds

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As a precursory to experiments on radiation mutagenesis in Arabidopsis certain dose-response relationships for survival in successive stages of plant development and for fertility were studied.

Air-dry seeds (about 5% water content) of Arabidopsis thaliana (L.) HEYNH., race Enkheim, stored at room temperature for 2 to 4 months after harvest, were irradiated with various doses of ^{60}Co gamma-rays (10, 20, 40, 70, 110, 160, 220 and 290 kR) at a dose rate of about 3.7 kR per minute, and then planted immediately into test tubes containing usual mineral nutrient agar; no cold treatment was employed. The following growth conditions were kept: photoperiod 20 hrs light (fluorescent tubes) plus 4 hrs darkness; temperature about 25°C in the light and 19°C in the dark; relative air humidity about 80%. Fourty seeds were sown per dose level in each of five independent replicated experiments with the plants in randomized blocks. The data on plant survival in successive growth stages were based on weekly phaenological records, and those on fertility on respective daily harvest records and seed counts. Methods of analysis of variance were applied to the obtained results.

The data on plant survival and fertility, presented in the table below, may be briefly summarized as follows: (a) seed germination (total percentage, but not the germination rate) was not inhibited by gamma-irradiation within the studied dose range; (b) plant lethality occurred mainly in the cotyledon and rosette stages; (c) completely sterile plants were found among survivors at doses of 40 kR and higher, and (d) regular decrease in mean number of seeds per fertile plant was observed in the entire dose range studied.

	Control data %	Dose (kR) 10	20	40	70	110	160	220	290
		Survival in % of control							
Germination	98.5	95	99.5	98	96.5	97.5	100	98	98.5
Cotyledon stage	95.4	96	96	95.5	98	95	83.5	38	15
Rosette stage	100	98	98.5	77	84.5	60	30	1.5	0
Inflorescence s.	100	100	100	92.5	96.5	94	83	0	-
Flowering stage	100	100	100	96	100	97	96.5	-	-
Total	94	89	93.5	64	77	50.5	14.5	0	0
		Fertility in % of control							
% of fertile* plants among survivors	100	100	100	76.5	81.5	62.5	48	-	-
Mean number of seeds per fertile* plant seeds	133	85.5	73.5	46	40.5	28	13.5	-	-

*fertile = producing any seeds

On applying analysis of variance to the obtained data, it was found that, even within relatively narrow limits of fluctuation of environmental conditions, the expression of a number of characters of plant survival and fertility as well as of growth and development was considerably variable, especially if some not-extreme effects of irradiation (e.g. growth and/or development inhibition) were studied.

Einfluss einer gamma-Bestrahlung ruhender Samen auf Wachstum und Entwicklungsgeschwindigkeit von Arabidopsis thaliana (L.) HEYNH.

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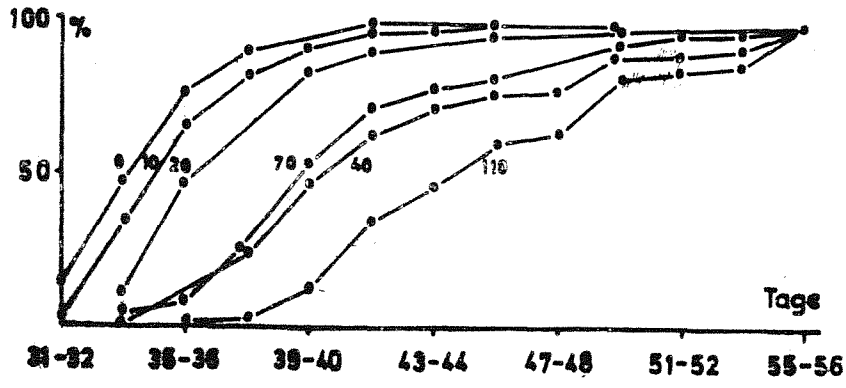
Als Grundlage für strahlengenetische Versuche wurde eine Reihe von radiobiologischen Reaktionen der Arabidopsis thaliana untersucht. In dieser Mitteilung werden zwei Wachstumsmerkmale (Länge der Wurzel am 7. Tag und Höhe der Pflanzen am 21. Tag nach der Aussaat) und drei Charakteristiken der Entwicklungsgeschwindigkeit untersucht (Anzahl Pflanzen, die am 7. Tag eine Rosette gebildet haben in Prozent aller gekeimten Pflanzen; Anzahl Pflanzen mit Infloreszenzen am 21. Tag in Prozent aller lebensfähigen Pflanzen und Vegetationsdauer bis zur Bildung der ersten reifen Schoten).

Ruhende Samen wurden mit Dosen von 10, 20, 40, 70, 110, 160, 220 und 290 kR bestrahlt. Die Anzahl der Varianten einschliesslich der unbestrahlten Kontrolle betrug somit 9. Fünf derartige Versuche wurden durchgeführt mit 40 Samen pro Variante, insgesamt also je 200 Samen in Kontrolle and 8 Bestrahlungsdosen. Die Aussaat erfolgte randomisiert in Reagenzgläsern mit üblichem Agarmedium. Alle Versuchsergebnisse wurden mittels Varianzanalyse verrechnet. Eine ausführlichere Beschreibung der Methodik ist in der Notiz von IVANOV und SANINA in diesem Heft enthalten.

Tabelle 1: Wachstum und Entwicklung der Pflanzen bei verschiedenen Bestrahlungsdosen der Samen

		Dosis (kR)								
		Kontrolle	10	20	40	70	110	160	220	290
Länge der Hauptwurzel am 7. Tag	mm	13.6	10.2	7.7	4.3	3.6	2.1	1.6	1.4	1.2
	% Kontrolle	100	75	56.5	31	26.5	15.2	12	10.5	9
Höhe der Pflanzen am 21. Tag	mm	70	61	40	11	15	6.8	4.9	-	-
	% Kontrolle	100	87	57	15.5	21.5	9.5	7	-	-
% Pflanzen mit Rosette am 7. Tag	abs.	88.6	75.2	74.2	60.8	53.4	26.9	16.3	0.5	0
	% Kontrolle	100	85	84	68.5	60.5	30.5	18.5	0.5	0
% Pflanzen mit Infloreszenz am 21. Tag	abs.	91.8	86.8	79.5	31.4	47.9	12.8	1.5	0	0
	% Kontrolle	100	94.5	86.5	34	52	14	1.5	0	0
Vegetationsdauer	Tage	35.3	36.4	38	42.8	41.8	45.4	-	-	-
	Abw. von Kontrolle, %	0	3	7.5	21	18.5	28.5	-	-	-

Die Ergebnisse sind in Tabelle 1 angeführt. Es ist daraus zu ersehen, dass die Abweichungen von der Kontrolle bei allen Merkmalen ziemlich gleichmässig mit der Erhöhung der Dosen ansteigen. Nach einer Bestrahlung mit Dosen über 110 kR werden spätere Vegetationsstadien kaum erreicht. Die zeitliche Verteilung, in der die volle Reife der Schoten in der Kontrolle und den Bestrahlungsserien erreicht wird, zeigt nebenstehende Abb.



Die Ergebnisse unserer Untersuchungen zeigen, dass in strahlengenetischen Versuchen mit Arabidopsis Bestrahlungsdosen für ruhende Samen im Bereich bis zu höchstens 100 kR verwendbar sind.

S u m m a r y: As a basis for radiation genetical investigations, the effect of gamma-irradiation of dry seeds on some growth characters of Arabidopsis (root length, plant height, rate of vegetative and reproductive development, duration of life cycle) was studied. The obtained results, presented in Table 1 and Figure 1 have shown that a dose range up to 100 kR may be used for experiments in radiation mutagenesis.

Multiple shoot formation in Arabidopsis induced by gamma-irradiation of seeds

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After irradiation of Arabidopsis seeds the formation of additional generative shoots in the normally single shooted plants is frequently observed. Therefore, an attempt was made to study some peculiarities of such a radiation induced "multicaulity".

Air-dry seeds of Arabidopsis thaliana (L.) HEYNH., race Enkheim, were irradiated with various doses of ^{60}Co gamma-rays and planted immediately into test tubes. The experiments were designed in randomised blocks. For further technical details see IVANOV and SANINA in this issue. During the generative stage of plant development the numbers of shoots were recorded in survivors. The obtained results are tabulated below:

	Dose (kR)						
	0	10	20	40	70	110	160
Multicaulous plants %	0	4.8	14.2	31.9	56.7	58.7	42.4
Mean number of additional shoots per multicaulous plant	-	1.13	1.24	1.18	1.26	1.36	1.36

The figures indicate a considerable increase in the percentage of multicaulous plants up to 70 kR followed by a plateau at 70 to 110 kR and a decrease at 160 kR. At the latter two doses a selective lethality of seedlings capable of additional shoot formation may be suspected, since at doses below 110 kR less than 20% of the seedlings died before maturity, while at 110 kR at least 40% and at 160 kR more than 80% of seedlings did not survive to the generative stage. The general pattern of this radiation induced multicaulity, namely the increase in percentage of multicaulous plants with increasing dose and the essential non-linearity of the dose-response curve was also confirmed by means of an analysis of variance (P below 0.0005), which revealed that the response under question, unlike some other effects of radiation on plant growth and development, is only moderately variable, both between the randomised blocks (P between 0.7-0.8) and between the independent replicated experiments (P between 0.8-0.9).

It is noteworthy that the considerable increase in the percentage of multicaulous plants (about 12-fold in the dose range of 10 to 110 kR) was accompanied by a very slight increase, if any, in the number of additional shoots per multicaulous plant (only about 1.2-fold in the same range). On these grounds it seems likely that the occurrence of a certain number of additional shoots is determined mainly by the structure of the embryonal shoot apex and its ontogenetic abilities, but not by the radiation level per se, the latter being responsible mainly for some kind of multicaulity inducing damage to the apical meristem, the probability of which increases with dose. So far, our data offer no conclusive indication as to the possible nature of such damage.

Comparison of the killing effect of gamma-rays and thermal neutrons

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An atomic reactor was built in 1964 in Kyoto University and it was named Kyoto University Reactor (KUR). Studies in higher plants of RBE values of neutrons for several characters have been investigated by our research group using KUR (MATSUMURA et al., unpubl.). Among them the killing effect of thermal neutrons in Arabidopsis was studied by the author. Dry and 48 hour steeped seeds were used and exposed to thermal neutrons by the pneumatic tube which was provided in the reactor; the length of irradiation time was 7 sec in minimum and 180 sec in maximum at the output of 1 MW. The 7 sec irradiation of dry seeds showed merely 19% of survival rate, and no survival of plants was observed in other lots.

After the above experience, the output of the reactor was decreased to 50 kW in the second experiment and observations were repeated; the details of irradiation and survival rates are given in the table with the results of gamma-ray irradiations. Gamma-ray irradiations for dry seeds had almost no effect, but a slight decrease of survival rate was observed in 100 kR lot. Decrease of survival rate was marked in wet seed lots. In the neutron lots, killing effects were severe and no survival of dry seeds was observed following 180 sec irradiation with neutrons contaminated with 45 kR of gamma-rays.

Higher killing effects in the wet seeds were also seen in the neutron lots, but this might be the result of the contaminating gamma-rays since the environmental modification of radiation effect is very small in high LET radiations, while it is rather large with sparsely ionizing radiations. These results show that the killing effect of thermal neutrons was very high. RBE of thermal neutrons on somatic mutations will be examined in the next step of the experiment and RBE versus LET relations should be determined.

Killing effects of thermal neutrons and gamma-rays for dry and wet seeds

Dose (sec)	Total neutron flux* (N_{th}/cm^2)	Contaminating gamma-rays** (kR)	Survival rate (%) dry	wet
0	-	-	93.9	-
5	1.39 x 10^{12}	1.25	-	80.6
10	2.44 x "	2.5	97.1	68.0
20	4.58 x "	5.0	69.5	63.5
30	7.48 x "	7.5	88.9	37.8
40	9.48 x "	10.0	71.9	43.3
60	14.5 x "	15.0	70.6	0.0
90	22.4 x "	22.5	59.0	0.6
120	26.6 x "	30.0	17.2	0.0
180	51.5 x "	45.0	0.0	0.0
300	71.9 x "	75.0	0.0	0.0
Dose of gamma-rays from ^{137}Cs (kR)				
	10		91.6	92.7
	30		90.8	75.6
	50		92.8	21.7
	70		93.3	0.0
	100		82.4	0.0

*Estimated from the activation of Au-foil

**Calculated from the measurement of gamma-rays at 1 MW

On the determination of absorbed dose in heavy ionizing particles

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Dry F_2 seeds obtained from F_1 hybrids between a hairless mutant and the wild strain of Arabidopsis were subjected to gamma-rays from a ^{137}Cs source and to ^{4}He, ^{12}C and ^{40}Ar-ions from the Hilac. From the experiments we could recognize a severe killing effect of heavy ionizing particles, and also dramatically high mutation rates and marked growth inhibition. RBE for somatic mutation at 0.5% frequency was roughly estimated as 10, 35 and 5 for He-, C- and Ar-ions, respectively (FUJII et al., 1966). The range of heavy ionizing particles is very short. The average seed volume was calculated roughly from the weight and measurements of length and width of the seeds, to be 20.5×10^{-3} mm^3. From this calculation it was concluded that Ar-ions must be stopped within the seeds but He- and C-ions certainly could penetrate and pass through seed tissue. Moreover the energy transfer varies according to the position of the range or tissue which may be seen from the Bragg Curve (BRUSTAD et al., 1960).

A further experiment was undertaken to determine whether the killing efficiency of heavy ionizing particles depends upon the amount of absorption in the seeds. Dry seeds were irradiated with C-ions in five different lots. Each lot received the same number of particles per cm^2, but the energies of the ions were varied for the different lots by a different thickness of absorbers. Lot 1 received a surface dose of 400 rads.

	Absorber (mg/cm^2)	Range (μ)	Survival rate (%)
Control	-	-	71.6
Lot 1	0	525	66.0
2	10.2	400	64.8
3	19.7	315	57.6
4	29.9	215	46.7
5	39.3	125	46.8

Survival rates decreased with the increase of the absorber's thickness. The results suggest that irradiation with C-ions with the maximum stopping power will show the most severe killing effect. The maximum of stopping power is 6×10^3 Mev/g/cm^2 at the range of 45 mg/cm^2. Namely the RBE value of C-ions should be larger than that obtained in the previous experiment if the embryo were attacked by ions with maximum stopping power. Further studies are now under way.

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Comparison of the mutagenic effects of fast neutrons with X-rays

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Arabidopsis thaliana, heterozygous for ch, produces gene specific yellow-green sectors on leaves of plants when the seeds are irradiated with X-rays (HIRONO and RÉDEI, 1965). Utilizing this system, the relative biological effectiveness (RBE) of fast neutrons to X-rays was investigated concerning this mutagenic event.

Since the mass production of F_1 seeds by crossing is burdensome in Arabidopsis, a F_2 population from the F_1 gi pa/ch was used. As the distances among these three genes are known (gi-24.8-ch-7.9-pa), the theoretical percentage of the heterozygote for ch in the population can be calculated. The expected percentage of plants which are heterozygous for ch in the wild phenotype and gi phenotype was determined to be 96.6 and 78.4 respectively. However, since the ratio of wild to gi phenotypes in the population is 38.7 to 11.5, the expected percentage of plants which are heterozygous for ch in the combined wild and gi phenotype will be 92.4.

Prior to irradiation, seeds were stored in a desiccator at room temperature over a saturated solution of chromic acid which maintained an atmosphere of 35% relative humidity. They were allowed to remain in this environment to equilibrate for moisture content for a period of 10 days. The moisture content of the seed was determined to be 7.7%. Seeds were exposed to both, fast neutrons generated from the Brookhaven National Laboratory Graphite Reactor and 250 kVp X-rays. Immediately after irradiations, seeds were sown in 4-inch pots and kept under short-day condition in a greenhouse where the temperature was between 20°C and 25°C. The number of wild and gi plants which had yellow-green sectors was scored at six weeks after the treatments.

	Dose of radiation (kR)	Number of the expected heterozygotes	Number of sectorial plants	% of sectorial plants
	0	358	0	0
Fast neutrons	4	573	22	3.8
	8	478	45	9.4
	12	498	60	12.1
	16	418	43	10.3
	20	147	17	11.6
X-rays	20	344	0	0
	40	534	15	2.8
	60	454	24	5.3
	80	467	35	7.5
	100	420	43	10.2

The results are shown in the table. The regression lines were calculated in the range where the number of sectorial plants increased proportionally with the dose. The regression line for X-rays is expressed as $y = -2.0900 + 0.1220x$ and the one for fast neutrons, $y = 0.1333 + 1.0375x$, where y is the percentage of the sectorial plants in wild and gi plants which are expected to be heterozygous for ch and x is the dose expressed in kR. Since both equations do not have the same y intercept, the RBE can not be obtained by the slopes of the two lines. The three points of y; 4, 7 and 10 which fall in the linear range were arbitrarily selected and the RBE values were calculated to be 13.4, 11.3 and 10.4 respectively. Taking the average of these three values, the RBE of fast neutrons, employing the heterozygous ch system in Arabidopsis thaliana, is 11.7.

Reference:
HIRONO, Y., and G. P. RÉDEI: Genetics 51, 519-526 (1965)

Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

B. TECHNIQUES

Harvesting large quantities of seed

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For our recombination and reverse mutation studies, large quantities of seed are required. Such quantities can be obtained only from plants grown on soil. Harvesting the seeds by cutting off ripe siliques involves much labour, since, due to shedding of the seeds from overripe fruits, several rounds of harvesting have to be carried out in order to obtain the maximal yield. Moreover, repeated harvesting increases the possibility of errors.

It turned out, however, that putting sleeves around the plants prevents seed shedding, and, at the same time, makes harvesting much easier. The sleeves, 30 cm long and ±7 cm wide, are cut from a tube of "Visqueen" polyethylene foil (0.008 mm) and put around the plants when the latter are about to produce their first flowers. The sleeves are supported by tripods made of metal wire. The tripods are put over the plants with their legs pushed in the soil. In every pot a tripod is placed, the sleeve may go either over or inside the tripod. The sleeves are fastened to the tripods with paperclips in such a way that between pot and sleeve a slit remains open, allowing air circulation through the sleeves. To promote this circulation, holes may be punched in the sleeves at about 5 cm from the bottom end.

For this method it is necessary that the plants are watered from below. As a routine, in our cultures 7 day old seedlings, after germination on perlite in Petri-dishes in a growth chamber, are transplanted with a dip of perlite to soil in 6 cm plastic pots, which are placed on plastic trays. For irrigation the trays are temporarily filled with water, or, since many of our lines are thiamine deficient, with a thiamine solution.

In summer, ripe siliques on sleeved plants remain closed; in winter they may open, but the seeds remain attached to the replum.

Harvesting is very simple: completely ripe plants, after removal of the tripods, are cut from their base, after which gentle squeezing of the sleeves makes the seeds drop from the siliques.

Unfortunately, there is one drawback to this method. Controlling an aphid or other insect infestation is much more difficult in sleeved plants than in plants grown normally in the open. A prophylactic spray with a systemic insecticide, e.g., systox, before the plants were sleeved sometimes had a favourable effect, but in a number of cases serious damage was inflicted upon the plants too.

Uniform cultures in soil: a comment

J. H. VAN DER VEEN

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In systematic methodological experiments with race Li-2, CONTANT (1966a) has, with respect to optimal germination procedures, confirmed our conclusions from experiments on a collection of races (VAN DER VEEN, 1965). However, instead of transplanting from the agar to the greenhouse very young seedlings with etiolated cotyledons (due to 48 hrs dark after 24 hrs light), CONTANT (1966b) prefers to transplant older non-etiolated seedlings (7 days old). This procedure is simpler and he is right in saying that the dark period may considerably weaken seedlings after different kinds of irradiation and that irregular (increased range of) germination, i.e., radiation induced, interferes with accurate timing of the beginning of the dark period. To our experience with non-mutagen-treated material, transplanting after 7 days-on-agar gives a pronounced increase in variance of, e.g., flowering time, and therefore we prefer to transplant younger seedlings. However, Dr. FEENSTRA in this Department, has very good results with transplanting 7 day old seedlings grown on perlite and into soil imbibing water from below (see elsewhere in this issue).

Additional data are given here on etiolation after mutagen treatment (though not applied with the aim of studying early somatic effects):

(1) From w e t seeds of Landsberg-'erecta', X-rayed with up to 16 kR, seedlings recover very well during the 2 or 3 days light between the dark period and transplanting. Seeds pretreated for 5 days at 1°C (to break dormancy) and X-rayed immediately after assuming room temperature are first given 24 hrs light as usual. Non-dormant dry seeds first imbibe for 24 hrs under light, and after X-raying are directly transferred to the dark.

(2) EMS treatment (in the dark) of dry seeds or of seeds dried after cold treatment, induces germination delay but not irregular germination. Thus, cumulative germination curves based on hour to hour scorings run parallel. With, e.g., 10 mM, 24 hrs, 24°C, the points of 20%, 50% and 80% germination all show a delay of about 8 hours as compared with the control. Therefore, prior to etiolation, EMS treated seeds are given 24 + 8 hrs light. After etiolation, the 2 or 3 days light give good recovery.

A final comment refers to LAWRENCE (1966) who puts pre-imbibed seeds directly on soil by means of a fine point brush. This is perhaps the most time-saving method. However, to our experience, in a greenhouse densely occupied with Arabidopsis experiments in all stages of plant development, it proved more or less to our surprise virtually impossible to avoid stray seeds, which can be a source of confusion. This, in fact, was one of our arguments for adopting the transplanting technique. Stray seedlings following-up can then be easily spotted.

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Planting seed suspension

G. P. RÉDEI

(Department of Field Crops, University of Missouri, Columbia, Mo., USA)

It is often necessary to plant large numbers of seeds. Single seed planting with forceps gives excellent results but it is extremely time-consuming. It is easy to broadcast the seed with the aid of a spoon or a piece of cardboard paper. The distribution is far from equal, however. Furthermore, only dry seed can be planted this way.

Recently a new planting technique has been employed in our laboratory with very satisfactory results. Given weight of seed (1000 seeds weigh ca. 17 mg) is suspended in 0.1% liquified, chilled agar. The number of seed is adjusted to one seed per drop and distributed by a 10 ml pipette. This way 10,000 seeds can be easily planted within one hour in any arrangement.

Improved method of leaf pigment chromatography

G. P. RÉDEI

(Department of Field Crops, University of Missouri, Columbia, Mo., USA)

With Dr. HIRONO we reported a paper chromatographic technique for the separation of chlorophylls. Far better separation in shorter time (10 min) can be obtained on 20 x 20 thin layer plates (MN 300 cellulose powder, 0.25 mm deep layer, solvent petrolether : acetone 10:1, development in refrigerator). There is practically no tailing. Extremely small amounts are detectable with the use of an ultraviolet Mineralamp UVS-12.

Reference:
HIRONO, Y., and G. P. RÉDEI: Nature 197, 1324-1325 (1963)

Extraction of Arabidopsis RNA

S. BONOTTO and M. JACOBS

(Laboratoire de Morphologie animale and Laboratoire de Génétique des Plantes Supérieures, Faculté des Sciences, Université libre de Bruxelles, Belgium)

Arabidopsis thaliana, a small autogamous Crucifer (2n = 10) with a short life cycle, can be grown on an inert substrate (perlite) soaked with mineral medium (JACOBS, 1964) under sterile conditions (LANGRIDGE, 1957), and in a relatively small area. As LAIBACH (1943) pointed out, it thus seems to be a very useful tool for experimental studies on plant development. By means of the numerous morphological and biochemical mutants recently obtained (LANGRIDGE, 1965; RÉDEI, 1962; JACOBS, 1965; FEENSTRA, 1964, etc.) it is moreover possible to follow the temporal succession of differentiation (RÖBBELEN, 1965 a) or to study the relations between nuclear and cytoplasmic genes (RÖBBELEN, 1965 b; RÉDEI, 1965).

Despite this great number of genetical investigations the development of methods for the isolation and the characterization of Arabidopsis nucleic acids has received little attention. DNA and ribosomal RNA were first isolated by one of us (BONOTTO, 1964 and 1965) following the method of MARMUR (1961) and that of LYTTLETON and PETERSEN (1964) respectively. At the same time WALLES and AHNSTRÖM (1965) isolated DNA from the seeds, purifying it moreover from contaminant polysaccharides.

On the basis of previous work on Acetabularia mediterranea (BALTUS and QUERTIER, 1966) we developed a suitable method for the extraction of both ribosomal (r-RNA) and soluble (s-RNA) ribonucleic acid from Arabidopsis. This method has furnished good results for the leaves or the cotyledons and for the whole plant, but when seeds were extracted it gave an RNA preparation which showed contamination, probably with polysaccharides.

Groups of 100 plants of Arabidopsis, Wil-2, 8 to 13 days old, were homogenized at 4°C by hand in a glass homogenizer fitted with a Teflon pestle using 2.5 ml of the following medium: Na acetate buffer, 0.01 M, pH 5.0; glucose, 0.54 M; EDTA, 0.001 M; and naphtalene disulfonate, 1 mg/ml. Non-disrupted membranes were again homogenized with 2.5 ml of the same medium and the homogenates were pooled. Homogenization was carried out as rapidly as possible.

Recrystallized sodium dodecylsulfate (SDS) was then added to a concentration of 1%. Freshly redistilled phenol (5 ml : phenol 85-H_2O 15 v/v) was then added to the homogenate, which was shaken at 4°C for 5 min. The aqueous layer was recovered after centrifugation for 10 min at 10.000 rev./min in a refrigerated centrifuge (International) and again treated with phenol in the same way. The recovered aqueous layer was then shaken with 5 ml of a mixture of chloroform and isoamyl alcohol (35:1, v/v) for 5 min at 4°C and re-centrifuged in the same way.

The nucleic acids were precipitated from the aqueous phase by the addition of 0.1 vol. of 10% NaCl and 2.5 vol. of absolute alcohol at -20°C for at least 3 hrs, although an overnight precipitation at -20°C was preferable for a quantitative recovery.

The precipitate, collected by centrifugation and washed twice with cold absolute alcohol in order to eliminate eventual contaminant phenol, was dissolved in 0.3 ml of the following buffer: Na acetate buffer, 0.01 M, pH 5.0; EDTA, 0.001 M; and naphtalene disulfonate, 10 µg/ml. The extract, clarified by centrifugation for 10 min at 2.500 to 3.000 rev./min was layered on top of 4.6 ml of a 5-20% sucrose gradient and centrifuged for 5 hrs at 37.500 rev./min in the SW 39 rotor of the Spinco ultracentrifuge. The bottom of the tubes was punctured and 2 drop-fractions were collected. To each fraction 250 µl of water was added and the absorption at 260 mµ was read in a Beckman DU spectrophotometer.

Figure 1 shows the centrifugation pattern of the RNA extracted from 10-day-old plants, from which primary roots were cut before extraction. Since sedimentation constants have not been determined, we have designated the two ribosomal RNA's as heavy RNA (hr-RNA) and light RNA (lr-RNA). The r-RNA (hr-RNA + lr-RNA) can be easily isolated from s-RNA by standing overnight at 4°C in 2M NaCl.

Figure 2 shows ultraviolet spectra of hr-RNA, lr-RNA, and s-RNA. The s-RNA showed a maximum of absorption at 270 mμ, possibly due to phenol contamination or to the fact that it may be particularly rich in aminoacyl-s-RNA.

In conclusion, the method reported here should be useful in studying both normal and abnormal (RÉDEI, 1966) RNA metabolism in this plant.

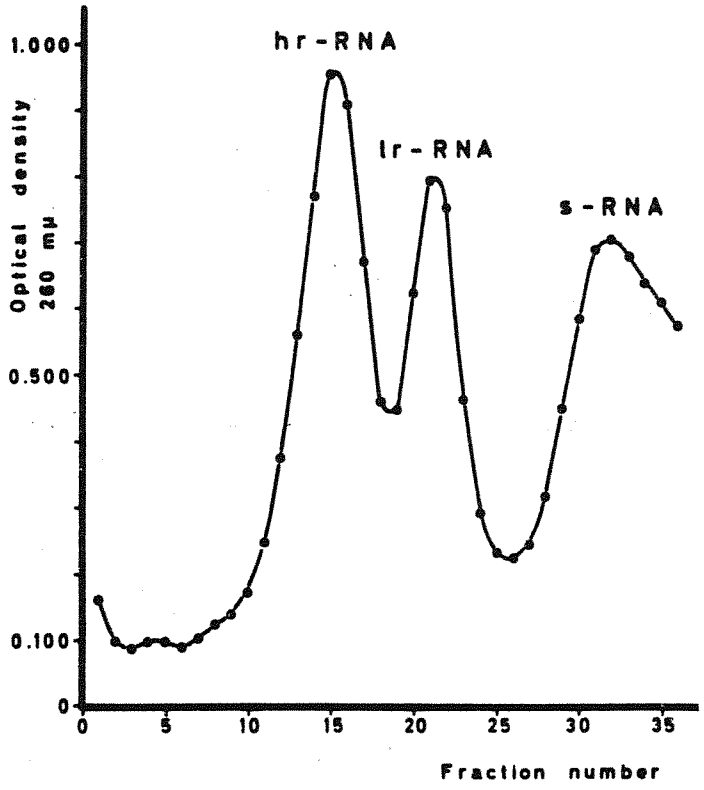

Figure 1: Sedimentation pattern of RNA from 250 Arabidopsis plants, 10 days old.
hr-RNA = heavy ribosomal RNA
lr-RNA = light ribosomal RNA
s-RNA = soluble RNA

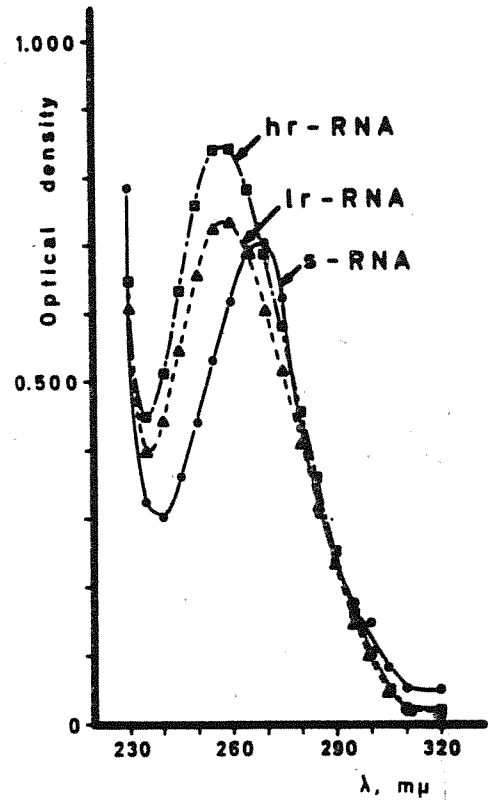

Figure 2: Ultraviolet spectra of the same RNA's as in Figure 1

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Electrophoretic separation of leaf proteins and the visualization of enzyme activity on gels

C. R. BHATIA

(Biology Division, Brookhaven National Laboratory, Upton, N.Y., USA)

Following the publication of a brief report on the esterases of Arabidopsis (BHATIA and SMITH, 1966), some requests were received for details of the procedure used. Therefore, it was thought that the technique may be of interest to other workers.

E x t r a c t i o n : Young leaves were collected, washed and immediately frozen at -20°C. Five grams of frozen tissue were used for each extraction. All subsequent steps were carried out in a cold room. From green tissues, it is important to get rid of the chlorophylls and other pigments which otherwise interfere with the separation of the proteins during the electrophoresis. To remove these pigments, the tissue was homogenised in cold acetone at -5 to -10°C; the acetone was removed by vacuum filtration and the residue again washed with cold acetone. The dried residue was dissolved in 5 ml of 0.1 M sodium pyrophosphate buffer containing 10^{-4}M EDTA and 0.7% 2-mercaptoethanol. The suspension was centrifuged for 10 min at 5000 x g and the supernatant again centrifuged at 35,000 x g for 30 min. The supernatant was dialysed against 100 x volume of 0.01 M phosphate buffer, pH 7.7, containing 0.2% 2-mercaptoethanol for 48 to 72 hrs. After the dialysis, the extract was again centrifuged at 35,000 x g for 30 min.

An improvement over this procedure was worked out in our laboratory by Dr. G. E. HART by modifying the procedure outlined by MORTON, 1955 (Procedure F). This has not been tried with Arabidopsis leaves, but with other green tissues it certainly yields much better results. The tissue was homogenised in 0.1 M sodium pyrophosphate buffer (buffer volume in ml = weight of tissue in gm). The homogenate was added slowly to ten volumes of acetone at -15°C with rapid stirring and held at this temperature for 15 min; the sediment then was collected by vacuum filtration. The sediment was washed twice by resuspending in cold acetone at -15°C and then dried for an hour. The dried material was ground to a fine powder and then suspended in 3 x volume of n-butanol at -15°C for 15 min. The sediment was washed again with acetone and dried. The rest of the procedure was the same as described earlier.

P r o t e i n e s t i m a t i o n s : Protein estimations on the extracts were made by the method of LOWRY et al. (1951).

E l e c t r o p h o r e s i s : Electrophoresis was performed using the method of disc electrophoresis as outlined by ORNSTEIN (1964) and DAVIS (1964).

V i s u a l i z a t i o n . P r o t e i n s : Gels were stained in 1% amido black in 7% acetic acid for one hour and then destained electrophoretically.

E s t e r a s e s: Esterase activity was visualized by incubating the gels at 37°C in a mixture of:

1 ml of 1% alpha naphthyl acetate
20 mg fast blue R. R.
50 ml phosphate buffer pH 5.9, 0.1 M

L e u c i n e a m i n o p e p t i d a s e : Gels were incubated at room temperature in a mixture of 25 mg Black K salt and 20 mg L-leucyl-β-naphthylamide in TRIS-maleate buffer, pH 6.

P e r o x i d a s e s : A nearly saturated solution of benzidine was prepared by dissolving 0.5 gm in 25 ml of 20% acetic acid. After filtration, this was added to 25 ml of 3% hydrogen peroxide, just before putting the gels. This reaction proceeds very rapidly and the gels have to be photographed immediately as the color fades on storage.

A l c o h o l d e h y d r o g e n a s e : Gels were incubated in the following mixture:

50 ml TRIS - HCl buffer, 0.1 M, pH 8.5
5 ml 95% ethyl alcohol
10 mg diphosphopyridine nucleotide
10 mg p - nitroblue tetrazolium
4 drops of 0.5% methylene blue

Staining is completed in 1 to 2 hours.

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Nutritional requirements of callus from Ei-6

J. R. LOEWENBERG and Paula J. THOMPSON

(Department of Botany, University of Wisconsin, Milwaukee, USA)

LOEWENBERG (1965) and ZIEBUR (1965) independently reported the culture of Arabidopsis callus on complex media. SHEN-MILLER and SHARP (1966) grew their cultures on a simple, completely defined medium. A callus culture from the strain Ei-6 that grows on a completely defined medium requires for optimum growth urea, a more potent auxin and a lower salt concentration than the callus that SHEN-MILLER and SHARP studied.

The tissue grows much better on mineral media with a total mineral concentration of approximately 30 mM than at higher and lower concentrations (Tables 1 and 2). Similarly the callus grows better on 1.5% glucose than at higher and lower glucose concentrations (Table 3). The results indicate that a medium osmotic pressure of about 2.5 atm is optimal.

Vitamins and urea can replace the undefined organics previously included in the medium (LOEWENBERG). This strain of Arabidopsis callus which grows poorly on the simple medium of SHEN-MILLER and SHARP at the two auxin-kinin combinations tested grows better with p-chlorophenoxyacetic acid than with NAA (Table 4).

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SHEN-MILLER, J., and W. R. SHARP: Torreya 93, 68-69 (1966)
WETMORE, R. H., and J. P. RIER: Am. J. Bot. 50, 418-430 (1963)
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(Tables 1-4 see next pages)

Table 1: Macronutrient composition of several media
(Millimoles per liter medium)

	BRAUN (1962)	HELLER*	NITSCH**	WHITE*
Na^+	24.9	7.9	1.8	3.0
K^+	12.2	10.0	40.0	1.7
NO_3^-	22.4	7.0	20.0	2.4
NH_4^+	6.0	-	-	-
PO_4^{---}	2.3	0.9	1.8	0.14
Ca^{++}	1.2	0.5	0.2	1.2
SO_4^{--}	10.4	1.0	1.0	2.9
Cl^-	12.2	11.0	20.4	0.9
Mg^{++}	3.0	1.0	1.0	1.5
Total mM	94.6	39.3	86.2	13.7

*after GAUTHERET (1954)
**after WETMORE and RIER (1963)

Table 2: Callus growth on several mineral media*
(Average fresh weight/callus after 4 weeks)

BRAUN	full strength	276 mg
	1/3 strength	433
	1/9 strength	346
NITSCH	full strength	174
	1/3 strength	491
	1/9 strength	300
HELLER		396
WHITE		278

*All media contained the entire organic supplement previously used (LOEWENBERG, 1965) and the microelements and iron at the concentrations listed in Table 4

Table 3: Callus growth on different glucose concentrations*
(Average fresh weight/callus after 4 weeks)

Glucose (w/v)	0.5%	1.5%	3.0%	5.0%	7.0%
Tissue wt (mg)	325	440	194	191	84

*The basal medium contained the NITSCH macroelements at one-third strength, the organics previously used (LOEWENBERG, 1965) and the microelements and iron at the concentrations listed in Table 4

(Table 4 see next page)

Table 4: Callus growth on various media
(Average fresh weight/callus after 7 weeks)

Basic medium* + 500 mg/liter each of yeast autolysate, trypticase, and soy peptone	695 mg
Basic medium* + 200 mg urea	419 mg
Basic medium* + 200 mg urea, 0.1 mg thiamine, 0.5 mg nicotinamide, and 0.1 mg pyridoxamine	1084 mg
SHEN-MILLER and SHARP medium, 0.64 mg furfuryl adenine and 2.0 mg naphthaleneacetic acid	155 mg
SHEN-MILLER and SHARP medium, 0.2 mg benzyladenine, and 1.0 mg p-chlorophenoxyacetic acid	319 mg

*Basic medium (mg/liter): KNO_3 674, $MgSO_4.7H_2O$ 82, $NaH_2PO_4.H_2O$ 83, KCl 498, $CaCl_2.2H_2O$ 29, Na_2FeEDTA 25, KI 0.75, $MnSO_4$ 4.4, $ZnSO_4$ 1.5, H_3BO_3 1.6, $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.006, glucose 15000, inositol 100, adenine 50, benzyladenine 0.2, and p-chlorophenoxyacetic acid 1.0.

All media were adjusted to pH 6.0 and solidified with 0.8% agar

C. NEWS

Laboratory Research Communications

The information presented in this chapter should not be used in publications without the consent of the author!

M.J. LAWRENCE and D.F. MATZINGER: Some knowledge of the inheritance of quantitative characters in Arabidopsis is a desirable preliminary to broader studies concerning response to selection, the genetic structure of wild populations, and the expressivity of genes determining such characters. Accordingly an experiment designed to provide some information about the technically convenient characters flowering time, height, and leaf number has recently been carried out. The breeding design used was a complete diallel using nine inbred lines as parental material and F_1, F_2 and backcross generations of all families in a completely randomized experiment in agar. The results are being analyzed at present.
(Department of Genetics, North Carolina State University, Raleigh, USA)

M.E. DENEEN and M.J. LAWRENCE: The genetic structure of wild populations with reference to quantitative characters. Experimental analyses of the distribution, amount and nature of genetic variation in wild populations begun some years ago in this Department with other species, have been extended recently to include Arabidopsis. The early results indicate that populations differ markedly in respect of both, the mean expression and their amounts of genetic variance for a character. Part at least of this genetic variance is caused by segregation, although part also may be due to differences between different homozygotes.
It has become clear also that it is necessary to vernalize much of the material in order to be able (1) to make contemporary comparisons between progenies and thereby avoid the need for scale transformation, (2) to make crosses between widely different flowering types, and (3) to be able to raise progenies on agar culture.
(Department of Genetics, The University, Birmingham, 15, U.K.)

J. ASHRAF: Ecological resistance of Arabidopsis. Studies on the physiology of resistance to various unfavourable climatic factors are underway. Of special interest are the relationship of this resistance to the formation of ecotypes, the molecular basis of increase in resistance and the inheritance of resistance to various unfavourable factors. Preliminary tests were run to standardize the criteria and methods for determining a plant to be resistant.
(Laboratory of Cytophysiology and Cytoecology, V.L.Komarov Botanical Institute, Academy of Sciences USSR, Prof. Popov street 2, Leningrad)

B.A. LEVENKO: Study on the protective effect of cysteine against gamma-rays in Arabidopsis. Seeds of the races Enkheim and Limburg were soaked in solutions of cysteine (0.005 and 0.05 M respectively) for 24 hrs. For control seeds were soaked in water. Immediately after soaking the seeds were irradiated with gamma-rays (10 and 20 kR resp.; 49 R/sec) and sown in soil. No differences were observed in speed of development, time of flowering and maturity, plant height and number of siliques between plants, which were grown from seeds soaked in water or cysteine solutions. Seeds were collected separately from individual plants for the determination of the number and the spectrum of chlorophyll mutations, which is now underway.
(Institute of Botany, Repin street 4, Kiev 4, USSR)

O.P. KAMRA: Report from Dalhousie University, Department of Biology, Halifax, N.S., Canada: Three graduate students from this Department have submitted theses for their M.Sc. degree in Biology with Arabidopsis used as an experimental organism. Their findings can be summarized as follows:

M.D. CASSELMAN: A study on regeneration in leaves of Arabidopsis, 1966. Plants of race Estland were grown under aseptic conditions in an inorganic nutrient - agar medium under 16 hrs illumination a day and at a constant temperature of 25°C. The ability of the laminae of Arabidopsis to regenerate on a basal inorganic medium supplemented with carbohydrates, vitamins, amino acid, and growth regulating substances was studied. A quantitative investigation of this ability was carried out, and the effects of such factors as type of laminae, initial size, and age were described. Moreover the ability of various parts of the laminae to regenerate was studied. It was found that there was a differential ability or gradient of regeneration in basal, middle and apical portions of the laminae. The effect of various factors such as age, type of laminae, light, the presence of naphthalene acetic acid, sucrose and auxin in the nutrient medium, on the gradient of regeneration was investigated.

Wen-Haw WANG: Some observations on regeneration in Arabidopsis, 1965. Plants were grown under sterile conditions on nutrient - agar medium in test tubes under at least 16 hrs illumination in a constant temperature (25°C). Calli and adventitious roots were formed from cut ends of nearly all explants cultured on basal medium containing proper supplements. Adventitious buds, however, were formed only from the very basal portion of excised leaves cultured in darkness. Light conditions were found to be important for the initiation of regenerated tissue and organs from explants. Subsequent development of organized tissue or organs was promoted by the addition of coconut milk in the medium. The synergistic combination of growth substance (NAA) with vitamins, amino acid, and urea or with coconut milk was found to be an important factor in the stimulation and support of regenerated tissue and organs. - Anatomical observations on intact plant and on regenerated tissue and organs, with special reference to the leaves have shown that all regenerated tissue and organs are endogenous in origin and that callus tissue has a great potentiality to differentiate into organized tissue and organs. - Polarity in regeneration of isolated parts of the plant has been found. - Development of reactivated axillary buds on intact plants as well as on excised leaves cultured in vitro was described and discussed.

Dorothy P.JEFFREY: Somatic and genetic effects of incorporated strontium-90 and cesium-137 on Arabidopsis, 1966. Somatic and genetic effects of incorporated strontium-90, cesium-137, phosphorus-32 and acute gamma ray exposure of seeds were investigated. Incorporated radioisotopes can cause early flowering in A. thaliana. Just so can acute 300 R and 3000 R X-ray treatment of dry seeds bring about earlier vegetative as well as floral development. - Strontium-90 remains in the stems and leaves of plants which have been grown on medium containing it while cesium-137 is relocated during the development of the plant finally to the flowers and fruits. Both are retained by the seeds of M_1 plants in concentrated form and are redistributed to M_2 plants giving low chronic exposures to the M_2 generation as well. This, however, is not sufficient to induce earlier flowering of the M_2 plants. - Incorporation of radioisotopes in M_1 seeds can cause physiological changes such as loss of apical dominance, dwarfism, gigantism and death in the M_1 plants. In the M_2 generation gene mutations appeared although an equivalent amount of gamma ray exposure given as an acute treatment to seeds failed to produce any genetic changes.

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(In the press)

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GICHNER, T., and J. VELEMÍNSKÝ: The mutagenic activity of 1-alkyl-1-nitrosoureas and 1-alkyl-3-nitro-1-nitrosoguanidines. Mutation Res. 1967
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VELEMÍNSKÝ, J., T. GICHNER, and V. POKORNÝ: Influence of concentration, pH of the solution, duration of treatment and hydrolysis of the solution on the activity of 1-alkyl-1-nitrosoureas and 1-alkyl-3-nitro-1-nitrosoguanidines in M_1 generation. Biol.plant. (Praha) 9, 1967

Post-Doctoral AEC Fellowships

Post-doctoral AEC fellowships are available in the Radiation Laboratory at Notre Dame, Indiana, USA, during 1967/68 and 1968/69. Anyone interested in post-doctoral work in radiation- or photobiology of Arabidopsis (or other organisms) is encouraged to enquire about these fellowships by writing for a descriptive brochure and application form to:

Prof.Dr. J.A.M. BROWN, Dept.of Biology, Univ. of Notre Dame, Indiana 46556
or The Director, AEC-Radiation Laboratory, Univ. of Notre Dame, Ind. 46556,USA

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