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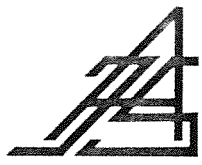

No. 3
Arabidopsis Information Service
Institut für Pflanzenbau und Pflanzenzüchtung
Universität Göttingen, Deutschland

March, 1966

A R A B I D O P S I S

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

No. 3 Göttingen March, 1966

CONTENTS

A. Brief Notes

B. Techniques

(continued)

A. BRIEF NOTES

After-ripening in Arabidopsis thaliana

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I n t r o d u c t i o n: The dormancy level of freshly harvested Arabidopsis thaliana seeds decreases during storage. Since germinability is dependent upon the extent of after-ripening and the induction of secondary dormancy, the control of external factors during seed storage may be of great importance in genetic studies of dormancy.

Few germinability studies have been reported on the effects of light, temperature, or seed age on dry seeds of A.thaliana. Low temperature storage of imbibed seeds of A.thaliana increases germinability. LAIBACH (1956) found that the germinability of 78 day old seed of A.thaliana was correlated with the length of cold treatment of imbibed seeds. Imbibed A.thaliana seeds are sensitive to light. SHROPSHIRE et al. (1961) determined the light action spectrum for germinability in the Estland ecotype; germination control by the phytochrome system was demonstrated. KUGLER (1951) reported that Hannoversch-Münden ecotype seeds have an obligate light requirement for germination. REHWALDT (1965) found that dark-stored dry seeds of the Landsberg ecotype are highly sensitive to incandescent light. LANGRIDGE (1957) found that seeds were germinable when taken from the fruit at 12 days after fertilization, but fully-ripened seeds were dormant for about two weeks. RATCLIFFE (1961) reported that 12 weeks of afterripening was sufficient for the removal of dormancy.

The after-ripening experiments here reported were conducted to determine the effects of light, humidity, and seed age on seed germinability. The experiments permitted comparisons of after-ripening patterns between the Landsberg ecotype and several closely related mutant lines.

M a t e r i a l and M e t h o d s : In the first experiment, single harvests of the Landsberg ecotype and the late flowering (co), narrow-leaf (an), reduced-chlorophyll b (ch^2), and the an ch^2 mutant lines were collected. The seeds were stored under light-humid conditions at 24 (±2)°C and at a relative humidity of 55%. The seeds were exposed to a 20 h day of 1000 ft-c white fluorescent light. Germination tests were conducted after 2 days and after 1, 3, 6, 9, and 12 weeks from the time of harvest. The seeds were imbibed with 10^{-3} KNO_3 and incubated in the dark at 25°C for 78 (±6) h (REHWALDT, 1965).

The second experiment consisted of light-dry, light-humid, and dark-humid storage treatments on aliquot samples from a single harvest of Landsberg seed. Dry storage was over indicating anhydrous $CaSO_4$. The other storage conditions were identical to those described in Experiment 1. Germination tests identical to that described in Experiment 1 were made at 1,6, and 12 weeks of seed age. Parallel germination tests using two days of cold imbibition (7.5°C) were conducted.

R e s u l t s : The data of the first experiment are shown in Table 1.

Table 1: Seed germinability of the Landsberg ecotype and the mutants an, ch^2 co, and an ch^2 at different seed ages during after-ripening under growthroom (light-humid) conditions. (Per cent germination based on 120 seeds per sample)

Line	Seed age (weeks) 1	3	6	9	12
Landsberg	0.0	5.0	3.3	2.5	1.7
an	0.0	19.2	27.5	23.3	14.2
ch^2	0.0	8.3	17.5	25.8	10.8
an ch^2	0.8	17.5	40.0	33.3	34.2
co	0.0	0.0	0.0	0.0	0.0

The an, ch^2, and an ch^2 mutant lines showed a greater increase in germinability during seed storage than the Landsberg ecotype. Seeds of the co mutant were completely dormant at all seed ages. In no case was dormancy completely removed by 12 weeks of seed storage. The highest level of germinability appears to have been reached by seeds of the an ch^2 mutant line.

The data of the second experiment are given in Table 2. For all seed storage treatments, except light-dry, germinability was increased by two days of cold treatment of imbibed seeds. The order of increasing effectiveness for after-ripening is light-dry, light-humid, and dark-humid seed storage. Light-dry storage was completely ineffective for after-ripening. A comparison of the germinability levels of the light-humid and dark-humid stored seeds shows that light exposure is inhibitory to the after-ripening process.

Table 2: Seed germinability of the Landsberg ecotype during after-ripening under light-dry, light-humid and dark-humid conditions

Treatment		Seed age 7 days		Seed age 42 days		Seed age 84 days	
Storage	Cold imbib. (days)	No.of seeds	% germin.	No.of seeds	% germin.	No.of seeds	% germin.
Light-dry	0	315	0.0	374	0.0	142	0.0
Light-humid	0	420	0.0	255	2.4	154	0.6
Dark-humid	0	329	0.6	246	51.2	180	55.6
Light-dry	2	377	1.1	282	1.4	147	0.0
Light-humid	2	330	46.1	247	64.4	146	40.4
Dark-humid	2	286	89.5	206	98.1	181	95.0

D i s c u s s i o n : The results of these experiments show that the rate of after-ripening of Landsberg seed is dependent upon storage conditions. Dark and humid (55% relative humidity) storage conditions were most effective for after-ripening though complete germinability was not reached by 12 weeks of storage. The findings of RÖBBELEN and KERSTEIN (1965) suggest that the humid storage at 55% relative humidity was near the optimum for after-ripening.

As pointed out by HONING (1930), studies involving the comparison of germinability levels of seed population should be based on specific seed storage conditions. This conclusion is supported by the results of the after-ripening experiments reported here. The striking differences in after-ripening of Landsberg seeds under different storage conditions (Table 2) and the differences between closely related lines in after-ripening under controlled conditions (Table 1) show that after-ripening is influenced by environmental conditions as well as by the genetic constitution of the seed. SEMENIUK and STEWART (1962) found that different after-ripening patterns may exist between species and cultivars of Rosa as tested under two sets of after-ripening conditions. The possibility that a change in the seed storage conditions may remove or reverse apparent germinability differences between seed populations should be considered in studies involving comparisons of seed dormancy.

S u m m a r y : A comparison of different seed storage conditions on the rate of after-ripening for Arabidopsis thaliana (L.) HEYNH. showed that dark storage was more effective than light (1000 ft-c 20 h per day) and humid (55% relative humidity) storage was more effective than dry (over anhydrous $CaSO_4$). Closely related lines exhibited different after-ripening patterns. Seeds of the narrow-leaf (an), reduced chlorophyll b (ch^2) and an ch^2 mutant lines were less dormant than these of the Landsberg ecotype, but seeds of the late-flowering (co) mutant line remained completely dormant during 12 weeks of light-humid seed storage.

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This work was performed while the author was supported by a NSF Science-Faculty Fellowship at the College of Forestry at Syracuse, N.Y. The author thanks Drs. F.A.VALENTINE, H.E.WILCOX and H.B.TEPPER for advice and Dr. G.P.RÉDEI for seed stocks of the ecotype and mutant lines.

The influence of seed storage in different atmospheric conditions on the rapidity of germination

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Experiments have been performed to test the influence of storage in air, in oxygen, or in nitrogen on the germination of Arabidopsis seeds. Two types of seeds were used for this experiments:

A: Arabidopsis thaliana, race En-2, harvested one year ago,

B: Arabidopsis thaliana, race En-2, harvested immediately before these experiments.

The seeds of group B, therefore, were within the period of dormancy, at the beginning of the experiment. These seeds have been brought into the different storage conditions (desiccators) for a period of 10 weeks. The germination has been tested by sowing the seeds on soil and counting the numbers of emerging seedlings 8 and 21 days resp. after sowing.

	Germination within 8 days	Germination within 21 days	Percentage of seeds with delayed germination*
A. Seeds harvested one year ago			
unstored	63.2%	74.6%	14.6
stored in air	71.5%	78.5%	9.4
stored in O_2	72.3%	80.3%	10.2
stored in N_2	69.5%	78.2%	10.7
B. Seeds freshly harvested			
unstored	3.3%	47.1%	87.8
stored in air	49.0%	72.7%	35.3
stored in O_2	52.7%	75.8%	31.6
stored in N_2	40.2%	75.2%	45.7

* Total of germinated seeds = 100

The results are summarized in the Table. Column 1 shows the germination percentages 8 days after sowing. It can be seen, that the germination of the freshly harvested seeds was not higher than 3.3% against 63.2% for the old seeds ("unstored"). The storage of the old seeds under laboratory conditions increased the germination to about 70%, but gave no significant differences by the different atmospheric conditions. On the contrary, the storage of freshly harvested seeds in either air, oxygen or nitrogen led to consident differences. The germination was highest after storage in O_2 and lowest after storage in N_2. If the germination is tested by counting the seedlings 3 weeks after sowing, no differences between the number of seeds from the different storage can be seen. Apparently the storage affects only the speed but not the ability of germination. As there is no delayed germination visible after storage of old seeds in N_2, we assume that the effect of storage in different atmospheric condition is due to interaction with the phenomenon of seed dormancy. From these experiments it may be reasonable to conclude, that oxygen storage shortens the seed dormancy whereas lack of oxygen leads to a prolongation of seed dormancy.

Promotion of Arabidopsis seed germination by blue, red, and far-red light

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The stimulation of germination in the dark by a short irradiation with red light (R) and the reversion of this effect by postirradiation with far-red (FR) has been described by SHROPSHIRE et al. (1961) in Arabidopsis thaliana, race Estland. Generally, the reaction of our race ST 56 of A.thaliana proved to be the same. For our irradiation experiments dry seeds were soaked for 24 or 48 h on 0,75% Agar with Knop solution in the dark. The following 15 min of red light irradiation successfully stimulated germination, while an addition of 15 min of far-red reverted this stimulation to the level of the dark control (Table 1). During soaking as well as during germination in dark after the irradiation treatments a standard temperature of 25°C was used. A detailed description of the light sources has been given elsewhere (VELEMÍNSKÝ and RÖBBELEN, 1966).

Table 1: Germination after irradiation with red and far-red light

Soaking	Irradiation	% of germination
24 h	15 min R	85.3
24 h	15 min R + 15 min FR	8.8
48 h	15 min R	95.3
48 h	15 min R + 15 min FR	10.3
Dark control		3.6

Using the same cultivation techniques, the dark germination could also be promoted by blue and far-red light. In these cases, however, several hours of irradiation were needed to reach the same percent of germination as after the action of 15 min of red light. Both, the blue and far-red effect were dependend on the duration of irradiation as it is typical for the so called High Energy Reaction (HER) (see Table 2).

Table 2: Germination after irradiation with blue and far-red light

Soaking	Irradiation	% of germination after blue light	% of germination after far-red light
0	3 h	12.1	3.3
0	12 h	30.1	18.7
0	24 h	78.0	34.6
48 h	3 h	56.1	47.2
48 h	6 h	89.6	63.3
48 h	12 h	90.1	80.5
48 h	24 h	97.4	89.4
Dark control		3.6	

This HER effect was intensified by a following 15 min of red light irradiation, regardless wether it was applied immediately or after a 24 h dark period (D). It could be reverted by 15 min of far-red, but only to the level of germination induced by the HER. Just so a short time irradiation with far-red, given after the HER stimulation + a 24 h dark-period, did not influence markedly the degree of the HER-induced germination (Table 3).

Table 3: Synergistic effect of red, blue, and far-red light resp. on germination (soaking = 0 h)

Soaking	% of germin.	Irradiation	% of germin.
24 h BL	67.0	24 h FR	65.7
24 h BL + 24 h FR	77.4	24 h FR + 15 min R	92.5
24 h BL + 24 h D + 15 min R	78.7	24 h FR + 15 min R + 15 min FR	67.0
24 h BL + 24 h D + 15 min R + 15 min FR	63.7	24 h FR + 24 h D + 30 min FR	53.8
24 h BL + 24 h D + 15 min FR	61.5	24 h FR + 24 h D + 120 min FR	51.2
24 h BL + 24 h D + 15 min BL	72.4	24 h FR + 24 h D + 12 h FR	61.8
		dark control	11.1

The HER stimulation of germination was very slight, if any, when the agar for germination was prepared without Knop-solution (Table 4). Moreover, the stimulation after 15 min of red light was slighter, when the seeds were pre-irradiated with far-red for 27 h, than after the same time of dark soaking. On the contrary, such a drastic effect of the Knop-solution was not observed after a similar irradiation of lettuce seeds (cultivar "Maikönig").

Table 4: Influence of Knop-solution on the HER in Arabidopsis and lettuce seeds.

Soaking	% of germination: Arabidopsis Knop	Arabidopsis H_2O	lettuce Knop	lettuce H_2O
Dark control	11.3	3.5	98.8	78.0
27 h FR	65.8	9.5	11.3	5.7
27 h FR + 15 min R	93.5	48.9	92.3	95.4
27 h FR + 15 min R + 15 min FR	67.0	7.1	7.4	5.8
27 h D + 15 min R		83.0		
27 h D + 15 min R + 15 min FR		9.0		

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This work was carried out at the Institute of Agronomy and Plant Breeding of the University Göttingen, Germany, and supported by the Alexander von Humboldt-Stiftung.

Seed aging causes mutant deficit

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With decreasing germination capacity old seeds from heterozygous Arabidopsis-plants sometimes produce only a few or no recessive seedlings. This was particularly true, when seedlings of our chlorophyll mutant stocks harvested as early as 1960 were sown on soil in October 22, 1965, in comparison to similar seed samples from 1962, 1964, and 1965. For example, the following mean germination percentages were gained from sowings of 3 x 100 seeds each after 10 days:

Designation	Mutant phenotype	Year of harvest							
		1960		1962		1964		1965	
		% germ-inated	%* mut-ants	% germ-inated	%* mut-ants	% germ-inated	%* mut-ants	% germ-inated	%* mut-ants
L 31	albina, lethal, alabaster cotyledons	18.7	11.6	56.8	22.4	94.3	27.3	98.2	33.4
L 34	albina, lethal, cream-white cotyl.	8.6	1.3	37.8	11.2	98.8	15.5	97.6	20.9
L 58	xantha, lethal, yellow cotyledons	7.4	0	24.3	9.5	90.6	18.1	89.0	21.4
V 81	chlorina (ch_3), viable, greenish-yellow rosette	12.1	6.2	64.5	18.2	95.1	22.8	91.7	24.7
V 75	viridis, viable, green veins, bright intercostal zones	15.8	3.1	30.9	19.1	93.4	24.6	96.9	23.8

* Total of germinated seeds = 100

In this experiment the emergence of mutant seedlings was reduced already after one year of storage while the normal green seedlings still appeared with about 100%. During all the years the seeds had been stored in a dry, cool cellar-room under conditions which kept the germinability rather well (cf. RÖBBELEN and KERSTEIN, 1965). Dr.VELEMÍNSKÝ from Prague told me that from segregating seeds after one year of storage in his laboratory in some cases no mutant plant could be received. Therefore, the above experiment was repeated with an equivalent sample of fresh, but fully after-ripened seeds stored for 104 and 175 days resp. in a desiccator over a concentrated KCl-solution giving a rel. humidity of 86%:

Mutant designation	Storage in 86% rel.humidity 104 days		175 days		Storage in dry, cool cellar-room (175 days)	
	% germinated	%* mutants	% germinated	%* mutants	% germinated	%* mutants
L 31	58.2	12.0	5.1	3.1	97.8	30.7
L 34	49.7	2.1	9.2	0.3	99.1	20.0
L 58	56.9	13.7	29.0	7.2	94.2	22.2
V 81	71.6	12.8	16.8	8.7	86.5	25.3
V 75	63.3	9.3	11.6	6.5	93.4	24.6

* Total of germinated seeds = 100

The results demonstrate that the mutant deficit drastically increases under storage conditions which damage seed vitality. The effect is different for the various mutants tested. The two albina types seem to be more sensitive than the more viable mutants, even though fresh seeds of L 31 heterozygotes usually show a significant surplus (against 25%) of mutant individuals. The finding may be of interest not only for the maintenance of seed stocks but also for mutation experiments.

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The leaf formation rate as a heritable character in Arabidopsis

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In Arabidopsis, there exists a close positive correlation between the flowering time and the number of leaves. From this fact, it might be infered that in different genotypes the leaf formation rate (LFR) is the same and that both the flowering time and the leaf number are controlled by the same genetic factors. However, NAPP-ZINN (1961) assumes that the genetic basis of LFR and the flowering time is different. Indeed, in a note (CETL, 1965a) it has been shown that there exist radical differences not only in the flowering time and in the leaf number but also in LFR as expressed by the leaves/days ratio.

Further experiments were carried out on 48 mutually related and unrelated early A_2 lines obtained by repeated natural autogamy from local populations (CETL, 1965b). Plants from control and vernalized seeds (2 weeks at +2±1°C) were grown under constant conditions (25±3°C, continuous illumination with 1250 lux) for 42 days.

The number of days from germination to appearance of the flower primordia (x), the number of rosette leaves (y), and the number of rosette leaves per day (y/x) as an index for LFR were counted. Corresponding means were estimated for the whole material and for individual lines. There were significant general differences caused by vernalization: a decrease of 17 and of 12 per cent for flowering time and leaf number respectively, and an increase of 6 per cent for the y/x ratio.

The analysis of variance for x, y, and y/x values (Table 1) shows in agreement

Table 1: Variability of x, y, and y/x values in 48 A_2 lines

Vernalization (weeks)	Variability	N	x		y		y/x	
			V	F	V	F	V	F
0	between lines	47	535.35	62.40*	64.50	57.08*	0.0216	21.60*
	within lines	1188	8.58		1.13		0.0010	
2	between lines	47	193.49	54.66*	16.30	37.05*	0.0246	22.35*
	within lines	1144	3.54		0.44		0.0011	
V_0/V_2	between lines			2.77*		3.96*		0.88
	within lines			2.42*		2.57*		0.91

*Exceeds the 1 per cent point

with the earlier note (CETL, 1965a) that there are significant differences between lines not only in the flowering time and in the leaf number but also in the number of leaves per day, i.e. in LFR.

The relations among the mean values of individual lines confirm that the flowering time and the leaf number are closely correlated (r = 0.80). On the other hand, a corresponding correlation between the y/x ratio and both preceding charakters has not been proved (r = -0.34 and 0.07 respectively). This means that LFR is practically independent on the earliness and that in both early and late lines, there may exist different leaf formation rates.

Within the pairs of lines, there is a greater variability in unrelated (non-sister) than in related (sister) pairs, with the exception of the y/x ratio in vernalized plants (Table 2).

Table 2: Variability of $\bar{x}$, $\bar{y}$, and $\bar{y}/\bar{x}$ values between and within the sister (S) and non-sister (NS) pairs of A_2 lines

Vernalization (weeks)	Variability Pairs	N	$\bar{x}$		$\bar{y}$		$\bar{y}/\bar{x}$	
			V	F	V	F	V	F
0	between pairs S	34	24.32	9.11*	4.23	24.88*	0.1822	18.98*
	within pairs S	35	2.67		0.17		0.0096	
	between pairs NS	54	22.64	0.83	3.25	0.87	0.1911	2.58*
	within pairs NS	55	27.16		3.75		0.0712	
V_{NS}/V_S	between pairs			0.93		0.77		1.05
	within pairs			10.17*		22.06		7.73*
2	between pairs S	34	12.40	7.90*	1.17	5.09*	0.1370	3.37*
	within pairs S	35	1.37		0.23		0.0406	
	between pairs NS	54	5.04	0.83	0.52	0.84	0.0455	0.83
	within pairs NS	55	6.06		0.62		0.0545	
V_{NS}/V_S	between pairs			0.41		0.44		0.33
	within pairs			3.86*		2.70*		1.34

* Exceeds the 1 per cent point

From these results it may be concluded that LFR as expressed by the y/x ratio, similarly as the flowering time and leaf number, represents a heritable character. This is confirmed both by the significant differences between lines and by the significantly greater variability within the non-sister than the sister pairs of lines. LFR is independent on the earliness although there is a close correlation between the flowering time and the leaf number; small deviations from the regression line represent considerable differences when expressed in terms of LFR.

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Effects of laser irradiation on the growth of Arabidopsis thaliana

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In a preliminary experiment seeds of Arabidopsis thaliana race Estland were exposed to 6943 Å ruby laser beam to study it's effects on the growth of M_1 plants.

A model 600 Laser manufactured by Maser Optics, Inc. giving laser pulses of 0.0005 sec. duration was employed in the experiments. Two different methods of laser exposure were used. Seeds were soaked in water for one hour and either subjected to the laser beam through a microscope (focused beam) or put directly in the path of the laser beam (unfocused). Untreated controls were also maintained. Subsequent to the exposure, the seeds were grown in aseptic test tube culture in continuous illumination at 78± 2° F.

Treatment	No. of seeds treated	No. of seeds germinated	Average height of plants at 30 days	Average No. of branches at 30 days	Average No. of flowers at 30 days
Control	6	5	4.9 cm	5.02	30.4
Unfocused beam	6	6	6.17 cm	6.67	49.2
Focused beam	7	3	5.17 cm	4.00	23.3

Effects observed are summerized in the table. Laser energy is detrimental to the viability of the seeds, the effect being very high in the focused beam. Vigorous and early flowering as well as increase in the average number of flowers, number of branches and height of plants at 30 days growth were evident in the unfocused treatment. Focused laser beam is very small in diameter as compared to the size of the seed and in all probability the area of impact in the different seeds was not the same. This would explain the high mortality as well as erratic results in this experiment. Unfocused beam would irradiate the seed more uniformly. The unusual results in the unfocused treatment may be due to several causes which are under investigation at present. It is also intended to study the genetic effects and the mutagenic properties of laser irradiation.

The vernalization requirement of "winter-annual" populations from Western Moravia

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Among 64 local populations collected in 1963 and 1964 there were found 18 with <10 per cent generative plants after 42 days when growing at 25±3°C under continuous illumination with 1250 lux (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1965; CETL, 1965). The original seeds of these "winter-annual" populations were vernalized 0 (control), 2, 4, and 6 weeks at +2±1°C. The vernalization requirement was expressed by means of a minimum vernalization rate which induces 100 per cent generative plants in the conditions indicated above.

Population	Vernalization requirement (weeks)	Population	Vernalization requirement (weeks)
Bí-2b	4	Ně	>6
Bí-4	4	Ro-1	4
Bo-3	2	Ro-2	4
Br-2	4	Ře	4
Hr	>6	Tr	4
Iv	4	Tv	4
MK-1	4	Vp-1	2
MK-2	4	Vp-2	>6
Ne	4	Vr-1	4

These data show that the vernalization requirement of moravian populations is nearly the same as reported by RATCLIFFE (1965) for races from Britain, but lower than for those from the Scottish Highlands.

The comparison of our populations with the samples kindly supplied by Dr.RATCLIFFE and by the Exchange Service of the botanical gardens is underway.

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Acceleration of flowering of the long-day plant Arabidopsis by 8-azaadenine

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The incorporation of 8-azaadenine (2×10^{-5} M) to the aseptic culture medium shortened the time required for developing microscopically visible flower primordia and the number of leaves appearing before the first flower buds to about half under 8 h daily illumination in both wild type and a late monogenic mutant. In the presence of equimolar amount of adenine the flowering was not accelerated by the analog.

Effects in incorporated radioisotopes in Arabidopsis thaliana

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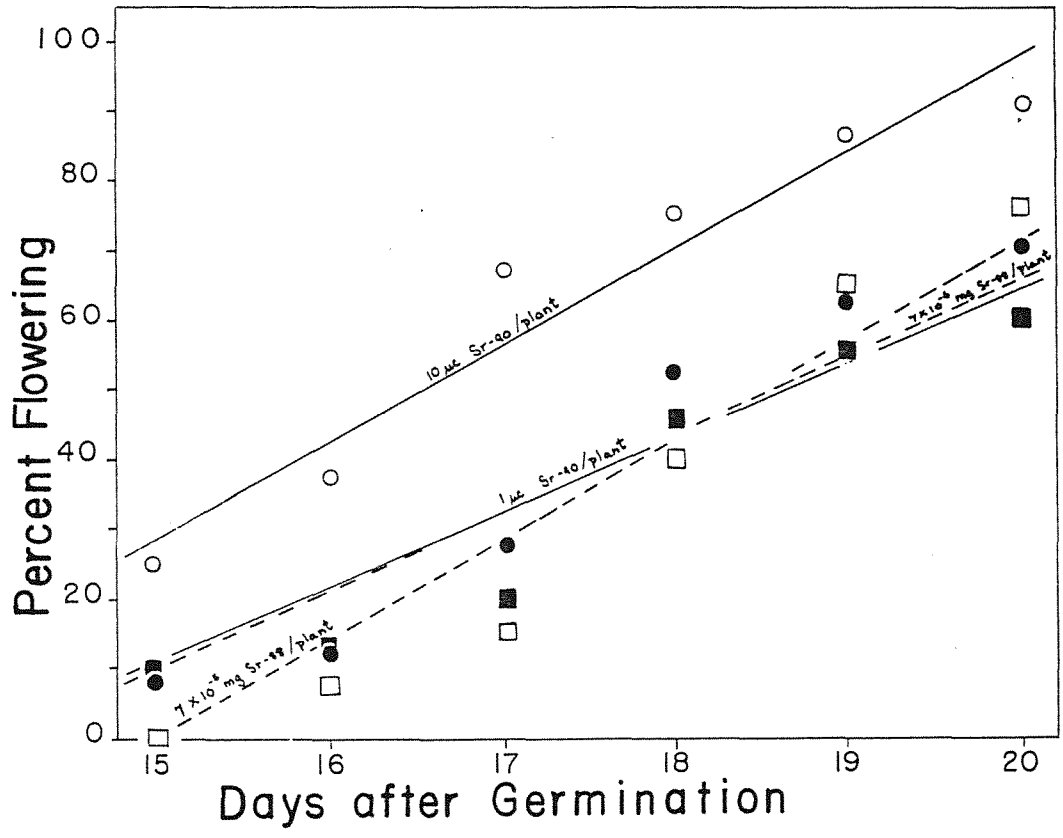
Low levels of radiation produced by the radioisotopes strontium-90 and cesium-137 in the culture medium and incorporated into the plant have initiated early flowering in Arabidopsis thaliana (L.) HEYNH. race Estland. The plants were grown in aseptic test tube culture in continuous illumination at a temperature of 78±2°F. The isotopes were added to the medium in concentrations of 1 µc and 10 µc per plant. Control plants were grown in media containing stable strontium or no isotope of either element. The plants grown in 10 µc of strontium-90 or cesium-137 flowered earlier than those grown in the 1 µc concentration or in any of the control treatments.

An experiment is now being carried out with plants growing in 20 µc of strontium-90 which indicates that 10 µc gives maximum stimulation of flowering. Another experiment in which the absolute concentration of strontium is constant but the activities are again 1 µc and 10 µc shows that the stimulatory effect is due to radiation rather than isotope effect since the plants in the more active medium are flowering earlier. Studies on the distribution of these isotopes also indicate that the stimulatory effect is due to radiation and is not an isotopic effect.

The distributions of strontium-90 and cesium-137 differ in the plant. Strontium-90, like calcium, remains in the older parts of the plant, the rosette, the primary shoot and first flowers. On the other hand, cesium-137 is redistributed to the growing parts of the plant like potassium. The distribution of the isotopes was determined by counting and by autoradiography. Relatively more isotope was taken up by the plants grown in 1 µc activity.



The seeds of these plants given various treatments will be grown to determine the genetic effects of the incorporated radioisotopes and chronic exposure to low level radiation in the M_1 generation.

The graph compares the flowering time of plants grown in 1 µc and 10 µc per plant of strontium-90 with plants grown in equivalent concentrations of strontium-88. The criterion for induction of flowering was the "1-cm shoot" given by MÜLLER (1961) which is the time that the shoot rises one cm above the rosette.

Reference :
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Der Einfluß von Gibberellinen auf die Apikaldominanz bei Arabidopsis thaliana

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In einer früheren Mitteilung (NAPP-ZINN, 1963a) war bereits davon die Rede, daß man durch Anwendung von Gibberellinsäure bei der Arabidopsis-Rasse Zürich die Bildung von Stengeltrieben weitgehend unterdrücken kann. In solche Versuche, die sich inzwischen auch auf andere, bei NAPP-ZINN (1963b) aufgezählte Arabidopsis-Linien erstreckten, wurden nun auch die Gibberelline A_1, A_4, A_5, A_7, A_8 und A_9 einbezogen. Dabei wurden bisher u.a. folgende Ergebnisse erzielt:

1. Bei der Mehrzahl der bislang benutzten Arabidopsis-Linien (14 von 22) ließ sich durch Giberellinsäure die Bildung von Seitentrieben (Stengeltrieben) in der Weise unterdrücken, daß es nicht einmal zur Bildung von Knospen in einer bestimmten Zahl von Blattachseln kam. Besonders stark ausgeprägt war diese Reaktion bei spätblühenden (winterannuellen) Rassen wie Kru, Te und St.
2. Bei empfindlicheren Rassen konnte dieser Effekt durch j e d e s der verwendeten Gibberelline hervorgerufen werden. Die Wirksamkeit der einzelnen Gibberelline in dieser Hinsicht entsprach in der Regel der Wirksamkeit bezüglich der Blütenbildung (s.NAPP-ZINN, 1963b).
3. Soweit das Alter der Pflanzen bei Behandlungsbeginn variiert wurde, war der Effekt um so größer, je früher die Behandlung begonnen hatte.
4. Soweit die Gibberellin-Konzentration variiert wurde, schien mit 25 oder 5o mg/l die optimale Konzentration erreicht zu sein.
5. Soweit die Frequenz der Gibberellin-Applikationen variiert wurde, war der Effekt umso größer, je öfter die Behandlung erfolgte.

Die vorstehend aufgeführten Ergebnisse lassen vermuten, daß Gibberelline auch normalerweise bei Apikaldominanz und korrelativer Hemmung eine Rolle spielen könnten. Damit soll natürlich keineswegs gesagt sein, daß Gibberelline hier die einzigen verantwortlichen Substanzen seien. Die Suche nach e i n e r verantwortlichen Substanz (JACOBS, 1959) mutet ohnehin nach allem, was man über die biochemischen Grundlagen entwicklungsphysiologischer Prozesse und Phänomene weiß, anachronistisch an. In Versuchen, die mit solcher Zielsetzung unternommen werden, pflegt man gewöhnlich allenfalls eine Substanz zu finden, die unter den jeweiligen Versuchsbedingungen den begrenzenden Faktor darstellt. So erscheint z.B. bezüglich der korrelativen Hemmung Auxin im einen Falle als die entscheidende Substanz (LIBBERT, 1964), in anderen Fällen dagegen nicht (JACOBS, DANIELSON, HURST und ADAMS, 1959).

Für eine Beteiligung von Gibberellinen an der Kontrolle der Anlegung und Weiterentwicklung von Achselknospen sprechen ferner:

1. gleichartige Befunde an Corchorus olitorius, über die STANT (1963) in einer inzwischen erschienenen Arbeit berichtet,
2. der Umstand, daß sterile Blattachseln bei Angiospermen vorzugsweise in der blühenden Region auftreten (HOLTHUSEN, 1940) und die Blütenbildung vielfach durch Gibberelline ausgelöst werden kann,
3. ein Befund von LIBBERT (1955a), demzufolge es sich bei dem bei Pisum nachgewiesenen Korrelationshemmstoff und seiner auxinfreien Vorstufe möglicherweise (ebenso wie bei Gibberellinen) um Laktone handelt und
4. die mutmaßliche Förderung der Blütenbildung bei Pisum durch diesen Korrelationshemmstoff (LIBBERT, 1955b).

Die Versuche über die Beteiligung von Gibberellinen an der Kontrolle der Apikaldominanz bei Arabidopsis werden in Gemeinschaft mit G.LEYRAL fortgesetzt. Eine ausführlichere Mitteilung wird später an anderer Stelle erscheinen.

S u m m a r y : In many Arabidopsis strains the formation of lateral shoots could be suppressed by application of several gibberellins.

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Mit Unterstützung der Deutschen Forschungsgemeinschaft.

Pollen tube growth in intra- and interspecific combinations

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In order to clarify the genetic affinity between Arabidopsis thaliana and related species, several cross-pollinations were tested. Except the confirmation of LAIBACH's results on hybridization between Cardaminopsis suecica x Arabidopsis thaliana (LAIBACH, 1958) until now seed set was only gained in the interspecific combination Arabidopsis pumila x A.thaliana. Therefore the question arose, wether some definite factors hold responsible for this defective fertilization or embryogenesis. Primarily the pollen tube growth has been tested in a few crossing combinations.

M a t e r i a l: The four species Arabidopsis thaliana (En), Arabidopsis pumila (A2), Cardamine hirsuta (C45) and Sisymbrium officinale (S132) were used. The plants were cultivated in the green-house from September to December with an additional illumination during 18 hours.

M e t h o d s: Flowers were pollinated two days after emasculation. From 10 to 500 min after pollination the pollen tube growth was examined periodically in unfixed preparations of pistils. Sections were placed on slides into a fluorescence dye and immediately afterwards observed by a fluorescence microscope. The dye specifically stains callose in plant tissues. As pollen tubes contain callose the "fluorochrome" contrasts them from the rest of the tissues by a characteristic yellow-green fluorescence (LINSKENS, 1957). The dye was isolated chromatographically from waterblue ("Wasserblau": MERCK No.1279) (ARNOLD, 1956).

P r e p a r a t i o n of the f l u o r o c h r o m e: A 7% solution of waterblue is heated up to boiling. Then a 10 mol KOH is added dropwise until the colour changes and a brown-red precipitate arises. The clear brown-red supernatant is passed through a cellulose-column and the chromatograph developed by water. The dye then separates into 7 components which can be sampled successively. The first fraction can be sold as ink (high quality)! The fluorochrome is gained from the 4. and 5. zone. It is ready for use without further preparation and can be stored for a long time without any loss of fluorescence. This fluorescence appears after irradiation with UV-light (OSRAM HBO 200, filter: SCHOTT OG4 + GG4).

The cellulose-column can be used for the preparation of the dye several times.

R e s u l t s: By this method the pollen tube growth of the following cross-combinations was tested:

Arabidopsis thaliana x Arabidopsis thaliana	(En x En)
Arabidopsis pumila x Arabidopsis pumila	(A2 x A2)
Arabidopsis pumila x Arabidopsis thaliana	(A2 x En)
Cardamine hirsuta x Arabidopsis thaliana	(C45 x En)
Sisymbrium officinale x Arabidopsis thaliana	(S132 x En)

Pollen grains just released from the anther do not fluoresce. But just before germination a yellow-green fluorescent region is discerned, at which the pollen tube appears. The tubes penetrate into the stigma and grow through the conductive tissues of the style and the septum. Not earlier than the first pollen tubes reach the basis of the silique, the tubes enter the cavity of the ovary. They grow towards the ovules and arrive at the micropyle along the funiculus; i.e., the fertilization is porogamous (Figure 1).

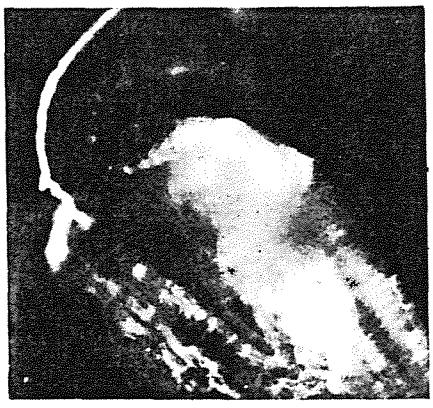

Figure 1

Figure 2 shows the development of pollen tubes per unit time in the tested combinations. The estimates are only qualitative. They are classified into the following cathegories:

0 Pollen grains not yet germinated
1 Beginning of germination (fluorescent region!) up to appearance of pollen tubes
2 Tubes arrived at the basis of the stigma papillae
3 Tubes penetrating the conductive tissue
4 Tubes passed through the style
5 Tubes passed through half of the septum
6 Tubes arrived at the basis of the silique
7 Tubes enter the micropyle of 50% of the ovules
8 All ovules fertilized

In case of the i n t r a s p e c i f i c c o m b i n a t i o n s A2 x A2 and En x En the pollen germinated 10 min after pollination. In En x En the tubes entered the micropyles already after 160 min. Meanwhile in A2 x A2 the tubes only passed through half of the septum; they arrived at the micropyles 500 min after pollination. Surely this difference in growth rate is not a constant characteristic of the two species, because it largely depends on environmental conditions and the physiological state of the plant.

Within the three tested i n t e r s p e c i f i c c o m b i n a t i o n s the pollen tube growth differs obviously. No inhibition of growth could be recognized, when A.pumila was pollinated with A.thaliana. The tubes passed through the style and the septum in the same time interval as pollen tubes of the own species. There seemed to be only a little retardation of growth, when the tubes arrived at the ovules and tried to pass through the micropyle. The seed set after this hybridization is about 45%. - The combination C45 x En represents a lower degree of compatibility. Though the pollen tubes reach the ovules their growth is inhibited during two stages: 1) at the penetration into the stigmatic tissue and 2) during their chemotropic orientation in the cavity of the ovary, which seemed to be disturbed. In the region of the micropyle sometimes the ends of the tubes were seen to be thickened and numerous windings were recognized. This combination failed to set seeds. - In the combination S132 x En the tubes apparently were unable to dissolve the cuticula of the stigma papillae. Therefore similar thickenings and bendings appeared as in the forementioned case, but already at this early stage.

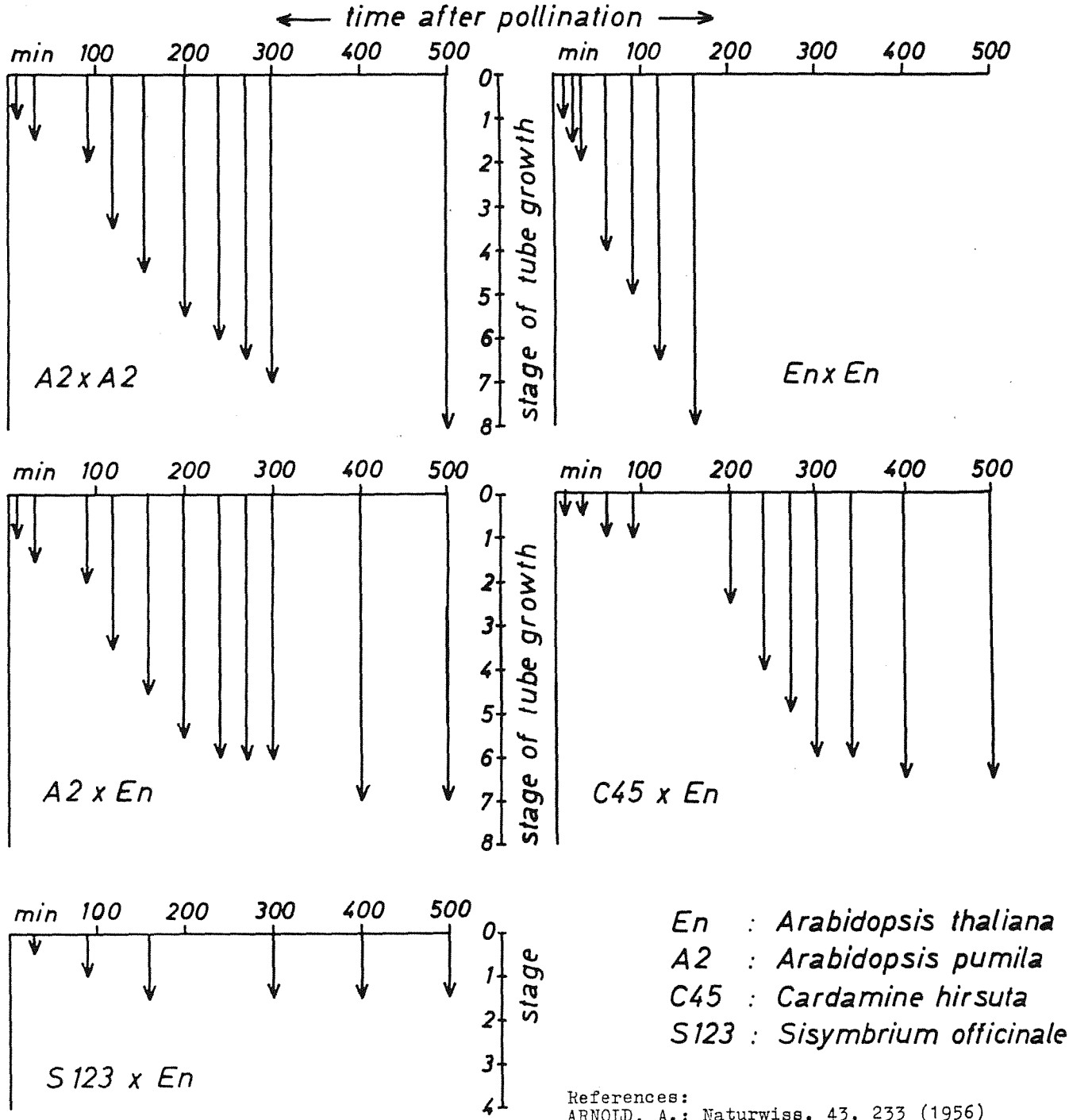

Figure 2

References:
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Preliminary studies on callus culture of Arabidopsis thaliana

Renu ANAND

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Seeds of Arabidopsis thaliana were germinated aseptically in petri-dishes to be transferred directly into different culture media. The seeds were sterilized with 5% Chlorox, washed twice with sterile double distilled water, and then transferred into sterile autoclaved petri-dishes containing three layers of filter paper soaked with sterile water. The dishes were wrapped with rubber petri-dish seals and kept at 4°C for 48 hours. They were than placed in a culture room kept at a constant temperature of 27± 1°C, with a 16 hour photoperiod. The light source was cool white flourescent tubes with a light intensity of 350 ft-c. at the petri-dish level. Under these conditions seeds germinated after 48 hours. A variety of media both solid and liquid, containing various growth hormones were used in an attempt to obtain callus growth of the seedlings. WHITE's medium (WHITE, 1943, here abbreviated as W), supplemented with coconut milk (CM) and 2,4-dichlorophenoxyacetic acid (2,4-D), was found to callus the seedlings. The resultant growth of the callus yielded free clusters of cells.

Next optimum concentrations of CM and 2,4-D, for maximum callus growth were sought. Combinations of CM at 10%, 15%, and 20% with 2,4-D at 10^{-4}M, 10^{-5}M, 10^{-6}M and 10^{-7}M were tried. A measure of the increase in fresh and dry weight of the callus after three weeks growth showed 2,4-D at 10^{-6}M and CM at 15% concentrations to be best. Also liquid medium supported greater growth than the solid. 125 ml flasks containing 50 ml of the medium shaken at 130 rotations per minute yielded only a non-friable type of tissue. These callus clusters contain modified cells like tracheids and cells rich in starch grains, in addition to parenchymatous cells.

Interesting results were obtained with 10^{-7}M 2,4-D and 15% CM. In this liquid medium the callus produced a large number of root primordia over the entire tissue surface. In the course of two weeks these primordia grew out as roots, and after two months of growth their length had increased tremendously. This observation is of particular interest since TORREY and SHIGEMURA (1957) reported previously that 2,4-D suppresses organisation of roots.

For histological study differentiating root primordia were fixed in formalin-acetic acid-alcohol (FAA) and dehydrated through butanol-ethanol-water series, embedded in paraplast and sectioned at 8 µ. Sections were stained with safranin fast-green. Examination of these slides revealed that the roots originated from a row or two of thin-walled cambial-like cells which were located between tracheidal cells within and parenchymatous cells rich in starch outside. Further study is being pursued and should elucidate the sequential development of roots from this unorganized mass.

Recent reports show that modified MURASHIGE and SKOOG-medium induced Arabidopsis thaliana callus to form roots and shoots (YOKOYAMA and Jones, 1965). However, I have been unable to obtain shoots in MURASHIGE and SKOOG-medium with kinetin and IAA in different combinations. Roots developed profusely from the undifferentiated callus with IAA 10^{-6}M and kinetin 2×10^{-8}M, 10^{-7}M, 2×10^{-6}M and 10^{-5}M. These roots, however, differ from the ones obtained with CM and 2,4-D in that they do not show root hair elongation although small hair initials are seen.

Subsequent work should throw more light on the nature and cause of these roots, which seem to be a unique feature of this tissue.

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Erfahrungen bei der Auslese von Trisomen

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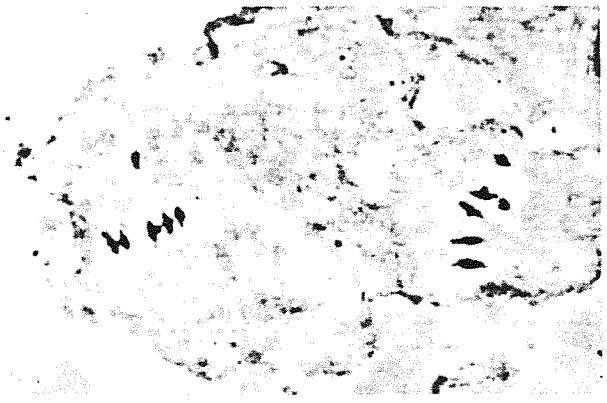
Ein vollständiges Sortiment der 5 möglichen Trisomen von Arabidopsis hat STEINITZ-SEARS (1963) erstmalig aufgestellt und beschrieben. Für genetische Arbeiten insbesondere an quantitativen Merkmalen ist es jedoch oft unerläßlich, ein Trisomen-Sortiment innerhalb der interessierenden Genotypen zur Verfügung zu haben. Da eine Trisomie nicht wie eine Monosomie durch Rückkreuzungen übertragen werden kann, ist das schnellste Verfahren zur Herstellung solcher Reihen die Neuauslese. Über Erfahrungen aus solchen Arbeiten mit einer größeren Anzahl von Rassen aus dem Sortiment von Professor LAIBACH (vgl. RÖBBELEN, 1965) sei kurz berichtet. Als Beispiel beziehen sich alle angegebenen Daten auf die Rasse En-2 als Ausgangsform.

Zunächst wurden durch Colchizinierung (0,2% mit Wattebausch oder Tragant-Schleim auf Rosetten kurz vor der Blütenanlage) Tetraploide hergestellt, diese mit Diploiden gekreuzt und die resultierenden Triploiden nochmals mit Diploiden zurückgekreuzt. In den Nachkommenschaften aus 15 derartigen 3n x 2n Kreuzungen fanden sich in den Pollenmutterzellen (Methode vgl. STEINITZ-SEARS, 1963) folgende Chromosomenzahlen:

2n =	10	11	12	13	14	15	16	
Anzahl Individuen	329	35	10	9	12	4	6	Σ 405

Unerwartet war zunächst, daß auch 16-chromosomige Formen auftraten. Wenngleich die Ursache dafür bislang noch nicht eindeutig geklärt werden konnte, wiesen doch Brückenbildungen in Anaphase I dieser Formen auf das Vorkommen von "non-disjunction" oder ähnliche meiotische Unregelmäßigkeiten hin, denen zufolge eine Bildung von 11-chromosomigen Gameten auf triploiden Pflanzen denkbar würde.

Für die vorliegende Aufgabe erwies es sich als vorteilhaft, daß die trisomen Individuen (deren Metaphase I vgl. nebenstehend) nach den diploiden die häufigste Klasse sind. Dieses Ergebnis läßt sich am leichtesten als Folge einer Gametenkonkurrenz deuten, da von vielen Pflanzenarten bekannt ist, daß eine steigende Anzahl von überzähligen Chromosomen zu vermehrten Störungen im Befruchtungsablauf führt.



Innerhalb der großen phänotypischen Variabilität der 15 ausgesäten Nachkommenschaften ließen sich schon bei oberflächlisher Betrachtung mehrere Gruppen von sehr ähnlich aussehenden Pflanzen erkennen. Ein Vergleich dieser Phänotypen mit den zugehörigen Chromosomenzahlen ergab, daß die zahlreichen Pflanzen mit zwergigem Wuchs fast ausschließlich Chromosomenzahlen von 2n=13 (oder 14) aufwiesen. Die 11-, 12- und 16-chromosomigen Pflanzen entwickelten Rosetten mit einem etwa normalen Durchmesser, während sämtliche 15-chromosomigen Pflanzen auffällig große Rosetten ausbildeten. Dieses Ergebnis ist insofern bemerkenswert, als die 15-chromosomigen Pflanzen ja keineswegs Triploide darstellen, sondern, wie auch die meiotischen Paarungsverhältnisse erkennen ließen, sehr verschiedene Chromosomenkombinationen enthielten. Die bisherigen Beobachtungen beruhen allerdings bislang noch auf einer zu geringen Individuenanzahl, als daß man bereits über einen Vorteil einer "zahlenmäßigen Euploidie" spekulieren könnte.

Die 5 Trisomen von En-2 glichen in ihren wichtigsten Merkmalen den von STEINITZ-SEARS beschriebenen Phänotypen. Je nach dem trisomen Chromosom vererbte sich die Trisomie auf die Nachkommen mit einer Häufigkeit von 4,9 bis 37,1%. Dieser Anteil ließ sich in einer Trisomen-Nachkommenschaft durch Auslese kleiner Samen oder durch Pikieren von verspätet auflaufenden Keimlingen wesentlich erhöhen, beispielsweise bei Aussaat einer Trisomen-Nachkommenschaft von Typ der Abb. 1c nach STEINITZ-SEARS am 19.Juli wie folgt:

Datum des Pikierens	29.Juli		2.August		5.August		9.August		Insgesamt	
	2n	2n+1	2n	2n+1	2n	2n+1	2n	2n+1	2n	2n+1
Unsortierte Samen	83	1 (1,2%)*	92	39 (29,8%)	67	43 (39,1%)	18	18 (50,0%)	260	101 (28,0%)
Kleine Samen	28	0 (0,0%)	32	37 (53,6%)			8	38 (82,6%)	68	75 (52,5%)

* Gesamtanzahl am gleichen Tage pikierter Keimlinge = 100

S u m m a r y : In Arabidopsis trisomics are the most numerous class in progenies of 3n x 2n crosses. The percentage of trisomics in progenies of trisomics varies from 5 to 37% according to the aneuploid chromosome. By selection of small seeds or lagging seedlings the portion of trisomic descendants can be doubled.

Literatur:
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Mit Unterstützung der Deutschen Forschungsgemeinschaft.

New experiments to induce plastomic mutations

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In contrast to the results of MICHAELIS (1958) in Epilobium and RÖBBELEN (1962) in Arabidopsis thaliana, we could get no plastomic mutants in Oenothera by ionizing radiation and mutagenic agents. We therefore intended to repeat the experiments of RÖBBELEN with Arabidopsis thaliana. Presoaked seed was irradiated with different doses of X-rays. Among the brought up plants a high percentage of spotted ones could be found. The leaves of these spotted plants were fixed according to RÖBBELEN. Frozen sections were made and stained with Rhodamin B. On the limit between white and green regions, in some cases cells were found which can be declared as "mixed cells". Full account will be given as soon as the total of the fixed material is worked up.

References:
MICHAELIS, P.: Planta 51, 600-634, 722-756 (1958)
RÖBBELEN, G.: Z.Vererb.lehre 92, 25-34 (1962)

Abnormal RNA metabolism of mutant im

G.P.RÉDEI

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It was demonstrated earlier that 6-azauracil or 6-azauridine modifies the expression of the variegation associated with the recessive gene im. This pyrimidine analog increased several fold the chloroplastid pigment content of the mutant. Similar observations were made with kinetin. Inorganic phosphorus content of the mutant, especially that of the white tissue, was also two-three times higher than that of the wild type. These findings indicated an anomaly of the RNA metabolism. A crude nuclease enzyme extracted from the mutant digested more RNA per time units. This increase of activity was highly dependent on the pH of the incubation mixture. From a purified yeast RNA, at pH 4.0 twice as much acid soluble products were made by the "mutant enzyme" than by that of the wild type. At the pH optimum of the RNA digestion (7.1) the difference is slight. This may be due to the fact that at neutral pH there is also a very high phosphodiesterase activity, while this latter enzyme did not hydrolyse appreciable amount of p-nitrophenyl-thymidine-5-phosphate below pH 5. The RNA digestion at pH 7 is about 10-12 times as fast as at pH 4. It is also conceivable that the mutation affects only one of the several components of the Arabidopsis nuclease. The activity of the two enzymes was compared on protein content. The amount of soluble protein per dry weight is only slightly lower in the mutant than that in the wild type. Since the differentiation of the plastids was abnormal in the white sectors of the mutant a comparison of crude enzymes on protein basis might be misleading because of the different composition of the total protein. Phospho-diesterase, 3'-nucleotidase, 5'-nucleotidase, phosphatase activity is not much different, however, in the mutant from that of the wild type. The crude enzyme of the wild type and the mutant hydrolyze the phophodiester bonds of all four nucleotides of RNA. The purine linkages, especially that of the guanosine phosphates, were split preferentially in both genotypes. From the three tested synthetic polynucleotides, however, polyuridylic acid was digested much easier than polycytidylic or polyadenylic acid. The information available at this time does not allow any firm conclusion whether the mutant makes only more ribonuclease or this enzyme would be qualitatively different from that of the wild type.

Distinguishing among certain py-heterozygotes by means of media containing a thiamine antagonist, oxythiamine

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In a previous communication, results of recombination experiments between genes py-1^2 and py-2^1 were mentioned (FEENSTRA, 1965). The amount of work, involved in crossing the heterozygous py-1^2/py-2^1 plants by the homozygous py-d tester line was the bottle-neck in testing a large number of gametes.

F_2-families on mineral medium segregate in deficient and semi-wild plants (1:1), the latter being only distinguishable from true wild type plants (recombinants) in a late growing stage. Since it was considered probable that semi-wild and true wild plants have different thiamine levels, experiments were set up to find a medium that would differentiate between them at an early stage, by means of a thiamine antagonist.

Since py-mutants are blocked in the synthesis of the pyrimidine part of the thiamine molecule, an inhibitor had to be chosen which does not contain this moiety, because otherwise it will be used as a nutrient (RÉDEI, 1960). Therefore oxythiamine, containing 4-hydroxy- instead of 4-amino-pyrimidine, was employed.

Genotypes py-2^1/py-1^2, + /py-1^2 and py-2^1/ + were obtained by appropriate crosses and tested on an oxythiamine concentration series of 0, 1, 2, 4 etc - 64 mg/100 ml mineral nutrient solution. Oxythiamine being a rather labile compound, the seeds got a pre-sowing cold-treatment, in order to start germination and growth on a freshly prepared substrate. As is shown in Figure 1, the py-1^2/py-2^1 type is strongly inhibited on the concentrations 4 and 8 mg/100ml, whereas the + /py-2^1 type still grows fairly well. At high antagonist levels both types are equally strongly inhibited. Type py-2^1/ + reacted like type + /py-1^2.

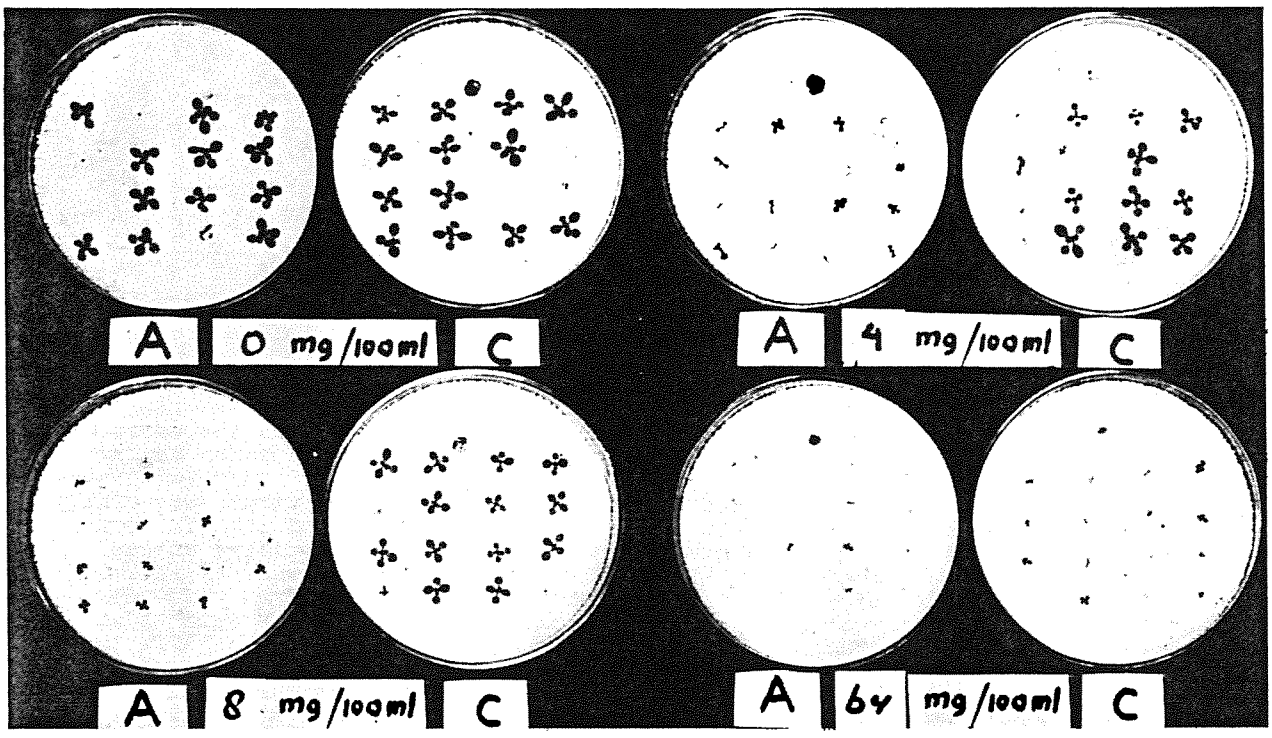

Figure 1: Ten days old seedlings of genotypes py-1^2/py-2^1 (A) and + /py-2^1 (C) on media of different oxythiamine concentration

This method is now being used for the detection of possible recombinants in the F_2 of the cross py-1^2 x py-2^1. If such plants can be found, testing of the segregation in the next generation will have to provide the final proof of the genotype of the plants isolated.

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RÉDEI, G.: Genetics 45, 1007 (1960)

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

Radiation induced mutants showing changed inflorescence characteristics

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The many mutants of Arabidopsis thaliana (race Enkheim) produced up to now predominantly show alterations of characteristics within the v e g e t a t i v e region. In my earlier mutation experiments I found only one form, the mutant seminosa, with a remarkably changed morphology of siliques (REINHOLZ, 1947).

Recently, ARNOLD (1965) and RÖBBELEN (1965) described some new inflorescence-mutants, one fertile and 3 sterile types. New investigations (REINHOLZ, 1965a) performed in order to test the influence of the temperature during irradiation of air-dried seeds on the mutation rate, gave rise to 2 new heritable variants for the inflorescence region.

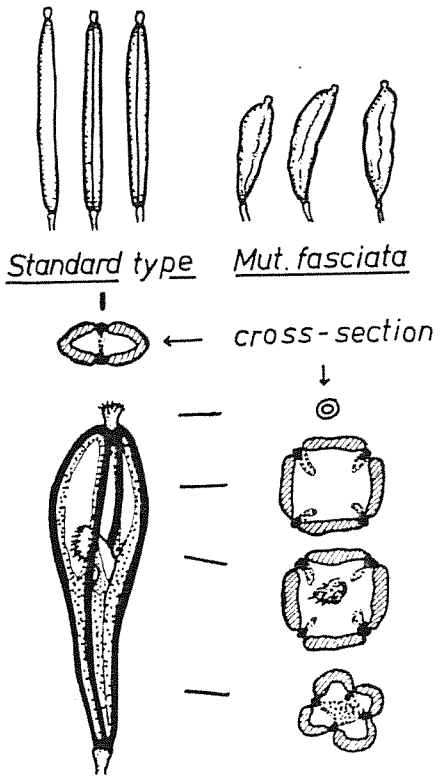

Figure 1: Mutant fasciata

● placentar carina ◎ septum
◎ valva ◎ stigma

1. Mutant fasciata (No. -10°C 242): This mutant exhibiting a multifoliaceous rosette and a fasciated stem has already been described (REINHOLZ, 1965b). The tendency to multiple organs is also expressed in each individual silique implying a new constant, heritable characteristic.

The numerous, short, thick, multiseminal and often crooked fruits of the strong plants have a cucumber-like appearance (upper part of Figure 1) accompanied with a changed structure (lower part of Figure 1). In contrary to the two-fan-shaped regular-slender siliques of the standard form, the mutant shows quadripartite blown-up fruits with a small and pointed base, and four placentar carinae. The latter are closely connected at the base of the fruit by the septae and an axillary tissue ending freely in the fruit part presenting a papillae-bearing stigma-tissue. In the upper part, below the pistil, where only small septal strips exist, the placentar carinae are bent apart. In accordance to the shape of the fruit, the four valvae at the pistil side are strongly enlarged.

2. Mutant digitata (No. -10°C 286): This vegetative normal mutant begins to shoot and to blossom a bit earlier than the standard type. But the growth-inhibited endflorescence is soon exhausted and the enrichment shoots put forth. These conflorescences form long-petioled individual siliques out of the axils of the bracts and umbels of two or more siliques (see Figure 2), more or less without any petiole, situated at the top of the shoots. Sometimes growth-inhibited malformed or three-fan-shaped siliques are to be found. On account of the high fertility of both mutants and of the complete penetrance and constant expressivity of the inflorescence-characteristics differing from the norm, the two mutated genes are expected to be good markers.

References:
ARNOLD, C.G.: Arabid.Inf.Serv. 2, 11 (1965)
REINHOLZ, E.: Fiat-Report 1006, 1-70 (1947)
– : "Neue Ergebnisse der biophysikalischen Forschung" Pp. 88-99, Thieme-Verlag, Stuttgart 1965a
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RÖBBELEN, G.: Arabid.Inf.Serv. 2, 12-13 (1965)

(Figure 2 see next page)

Induction of recessive lethals by X-rays

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I. In connection with the finding that after treatment of seeds with EMS or nitrosamides the frequency of recessive lethals increases exponentially ($n \approx 2$) with the dose (MÜLLER, 1966), it was interesting to study the dose-response-relationship in the case of X-rays. Seeds of Arabidopsis soaked in distilled water for 18 h were irradiated with X-rays (180 kV, dose rate: 410 R/min). Degree of sterility (s) and frequency of recessive lethals were determined by the embryo test. (For root length reduction induced by X-rays cf. MÜLLER, 1964).

Dose (kR)	Progenies scored	s (%)	m_b (%[b])	m_c (%[c])	m'_c (%[c])
0	1873	0	0.5	0.13	0.02
1	1154	1	1.4	0.28	0.06
4	682	2	8.6	2.10	0.20
8	481	9	15.3	3.83	0.79
12	594	30	24.9	5.40	0.97
16	481	51	30.0	7.08	1.12
20	387	52	32.1	9.79	1.22

The results show that the relationship between dose and frequency of M_2 mutants (m_c) is approximately linear.

II. In another experiment "dry" seeds were irradiated with 8 kR and 72 kR and hydrated in the presence of O_2 and N_2. The water content of the seeds was stabilized prior to irradiation by storing them for 4 days at room temperature in desiccators over dry $CaCl_2$ (~6%), saturated $CaCl_2$ (~9%), saturated $NaClO_3$ (~16%) or water (~22%). The frequency of recessive lethals was determined by the embryo test. (5 pods per M_1-plant were scored.)

Dose 8kR				Dose 72kR			
Water content %	Hydration	Progenies scored	M_2-mutants m_c (%)	Water content %	Hydration	Progenies scored	M_2-mutants m_c (%)
6	O_2	341	1.8	6	O_2	72	11.8
6	N_2	269	1.2	6	N_2	137	7.2
9	O_2	656	1.0	9	O_2	101	11.3
9	N_2	249	0.8	9	N_2	105	10.3
16	O_2	217	0.8	16	O_2	32	13.5
16	N_2	302	1.1	16	N_2	37	14.4
22	O_2	336	3.9	22	O_2	} no survival	
22	N_2	102	3.4	22	N_2		

References:

MÜLLER, A.J.: Kulturpflanze 12, 237-255 (1964)
- : Züchter, in the press (1966)

(Legend for symbols see next page)

Mutation experiments with EMS

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Seeds of Arabidopsis thaliana were treated with ethyl methanesulfonate (EMS). The dependence of the growth rate of primary roots and other somatic effects, fertility of M_1 plants, and frequency of resessive lethals (embryonic lethals and chlorophyll mutations) on the mutagen dose and various treatment conditions was studied. The main results are:

1. With increasing d o s e the frequency of chlorophyll mutations and the frequency of embryonic lethals increase exponentially ($n \approx 2$). This applies as well to variation of EMS concentration as to variation of treatment time.

2. Mutation frequency, sterility, and root length reduction increase by raising the t r e a t m e n t t e m p e r a t u r e . For the range tested (18°-36°C) a temperature quotient of Q_{10}=2.6 was found.

3. The increase of m u t a t i o n f r e q u e n c y is limited only by the decrease of fertility of M_1-plants and not by a decrease of survival. The relations between mutation frequency, degree of sterility, and root length reduction are not influenced by changing the treatment conditions.

4. Mutation frequency, sterility, and root length reduction are independent of the duration of s o a k i n g of the t r e a t e d s e e d s in water under partially anaerobic conditions. Delaying the start of germination up to 4 days after treatment does not influence the mutation frequency.

5. By raising the t e m p e r a t u r e during a period of 6 h a f t e r t r e a t m e n t mutation frequency, sterility, and root length reduction are increased.

6. The damage caused by d r y i n g s e e d s after EMS treatment is reduced completely by soaking the treated seeds in water for 11 h before drying.

7. The effectiveness of partially hydrolyzed EMS solutions corresponds to the concentration of EMS. Physiological damage caused by the h y d r o l y s i s p r o d u c t s is without practical significance.

8. The relations between various e s t i m a t e s of m u t a t i o n f r e q u e n c y (frequency of segregating plant progenies, frequency of segregating pod progenies, frequency of M_2-mutants) have been analyzed. It is shown that there is no direct proportionality between the frequency of M_2-mutants (m_c) and the initial mutation frequency.

A detailed publication on the subject is prepared in the journal "Züchter".

Increasing the effectiveness of mutagenic treatments by inhibitors of metabolism

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Simultaneous treatment with NaN_3 or KCN has been found to decrease the frequency of mutations induced by nitrosoalkylureas in seeds of Arabidopsis (VELEMÍNSKÝ et al., 1965; MÜLLER, 1965). Further studies showed that with other mutagenic agents these inhibitors of metabolism have the opposite effect: They enhance the mutagenic effectiveness. The following table includes data from simultaneous treatments (sim) and from posttreatments (post) with NaN_3 and KCN. In all cases presoaked seeds were treated. Degree of sterility (s) and frequency of recessive lethals were determined by the embryo test.

Treatment	Progenies scored	s (%)	Segregating progenies m_b (%)	m'_b (%)
Control (water)	500	0	0.4	0
500 mM KCN, 3 h, 24°C	400	0	0.3	0
2 mM NaN_3, 12 h, 24°C	400	0	0.5	0
N-nitroso-N-methyl-N'-nitroguanidine				
0.5 mM, 4 h, 24°C, pH 5	400	4	59.5	11.3
" + sim 2 mM NaN_3	400	70	93.0	16.5
" + sim 1 mM KCN	400	48	82.3	14.3
" + post 1 h 2 mM NaN_3	400	10	66.4	12.8
" + post 2 h H_2O, 8 h NaN_3	400	3	58.7	11.4
X-rays				
12 kR in 35 min	320	7	29.7	3.1
" + sim + post 24 h 2 mM NaN_3	150	25	49.3	4.8

Treatment	Progenies scored	s (%)	M_2-mutants m_c (%)	m'_c (%)
N-nitroso-N-methylurethane				
32 mM, 3 h, 24°C	96	40	39.2	5.3
" + sim 2 mM NaN_3	119	70	50.9	10.2
tri-2-chloroethylamine (data see below)				

Symbols: s = degree of sterility, m_b = frequency of segregating pod progenies, m_c = frequency of M_2 mutants, m'_b = frequency of pod progenies segregating for chlorophyll mutations, m'_c = frequency of M_2 chlorophyll mutations.

References:

VELEMÍNSKÝ, J., T.GICHNER, and V.POKORNY: Biol.Plantarum (Prague) 7, 325-329 (1965)
MÜLLER, A.J.: Naturwiss. 52, 213 (1965)

Mutagenic activity of tri-2-chloroethylamine

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Presoaked seeds of Arabidopsis were treated for 30 min at 36°C with the trifunctional alkylating agent tri-2-chloroethylamine HCl (HN3). After treatment the seeds were soaked either in water for 3 h at 36°C or in 2 mM NaN_3 for 1 h and after that in water for 3 h. Root length reduction (r), degree of sterility (s) and frequency of recessive lethals were determined.

Treatment	r (%)	Progenies scored	s (%)	Segregating progenies m_b (%)	m'_b (%)
control (water)	0	400	0	0.8	0.3
5 mM HN3	58	469	0	14.1	2.3
10 mM HN3	81	273	2	27.1	5.1
15 mM HN3	90	400	5	42.5	11.5
10 mM HN3, post NaN_3	85	225	1	42.7	10.2
15 mM HN3, post NaN_3	96	250	8	61.6	13.2
hydrolysate	3	250	0	0.7	0

The hydrolysate tested was obtained by keeping 15 mM for 1 h at 90°C. The results show that,
(1) the relation between root length reduction (inhibition of mitosis) and mutation frequency is considerably higher than after treatment with EMS,
(2) the relation between degree of sterility and mutation frequency is essentially the same as after treatment with monofunctional agents (e.g., EMS),
(3) hydrolysis products of HN3 are not toxic and not mutagenic,
(4) posttreatment with NaN_3 enhance both the frequency of chlorophyll mutations (m'_b) and the frequency of embryonic lethals.

Influence of pretreatment with EDTA on the mutagenic activity of nitrosomethylurea

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Pretreatment with the chelating agent ethylenediamine tetraacetic acid (EDTA) was reported to enhance the frequency of chromosomal aberrations induced by N-nitroso-N-methylurea (NMH) in growing root meristems of Vicia faba (MICHAELIS et al., 1965). We, therefore, studied the influence of pretreatments with EDTA (Na-salt) on the frequency of recessive lethals induced by NMH in dormant seeds of Arabidopsis.

Pretreatment	Treatment	Progenies scored	Segregating progenies m_b (%)	m'_b (%)
-	water	400	0.8	0.3
100 mM EDTA, 6 h, 36°C	water	350	1.1	0
water, 6 h, 36°C	0.2 mM NMH, 1 h, 36°C	350	38.9	6.8
100 mM EDTA, 6 h, 36°C	0.2 mM NMH, 1 h, 36°C	233	31.3	4.7
water, 12 h, 24°C	0.5 mM NMH, 1 h, 24°C	400	32.3	4.8
50 mM EDTA, 12 h, 24°C	0.5 mM NMH, 1 h, 24°C	245	23.7	3.7

In both experiments pretreatment with EDTA slightly diminished the mutation frequency. (The differences are only significant at P = 0.05.)

Reference:
MICHAELIS, A., J.SCHÖNEICH, and R.RIEGER: Chromosma 16, 101-123 (1965)

N-Nitroso-imidazolidon - an efficient mutagen

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Presoaked seeds of Arabidopsis were treated for 1 h at 36°C with aqueous solutions (pH 5) of N-nitroso-imidazolidon (NI). Posttreatment soaking: 2 h at 36°C in water. Root length reduction (r), degree of sterility (s) and frequency of recessive lethals were determined.

Concentration (mM)	r (%)	progenies scored	s (%)	m_b (%)	m'_b (%)	m_c (%)
0	0	400	0	0.5	0	0.1
2.5	2	200	1	17.5	2.0	4.1
5	11	231	8.2	63.7	7.8	19.6
10	47	116	55.8	95.4	23.4	58.2
20	(90)	-	60% germination, 8% survival			
hydrolysate	- 8	200	0	1.0	0	0.2

NI is unstable in aqueous solution (half-life time at pH 5: 4 h, at pH 8: 15 min). The hydrolysate was obtained by keeping 20 mM NI for 1 h at 90°C.

NI is known to be mutagenic also in Saccharomyces (MARQUARDT et al., 1964) and to induce chromosomal aberrations in Vicia faba (RIEGER, personal communication).

Reference:
MARQUARDT, H., F.ZIMMERMANN, and R.SCHWAIER: Z.Vererbungsl. 95, 82-96 (1964)

Fast neutron irradiation of dry and pre-soaked Arabidopsis seed

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Nine months old dry and 48 h pre-soaked Arabidopsis seeds (race Li-2) were given 9 doses of fast neutrons from reactor B.A.R.N. at this institute, from 0 up to the lethal dose for dry seeds; 100 seeds were irradiated per dose, for each of the two treatments.

Germination and root-length at the 10th day were recorded, after which the surviving plantlets were transplanted into soil for determination of final survival percentage, fertility and M_2-mutation frequency (screening for this latter parameter is still in progress). Furthermore, selection for early and late flowering is carried out in the M_2, in the control and in the irradiated material; this will be continued in further generations.

Germination, although delayed, still occurs at exceedingly high doses; it bears apparently no relation to survival (germination delay probably does, for a given kind of radiation, but seems a rather unsuitable parameter). The formation of leaves may be examined under the binocular 6-7 days after germination; the proportion of plantlets forming leaves at that time is a good measure of ultimate survival in this experiment. The shape of the dose/survival curves is typically sigmoid, with a broad shoulder even for soaked seeds. The differences in sensitivity between dry and soaked seed, in respect of survival, are connected more strongly with differences in shoulder width than in the slope of the curves. This stands in contrast to fertility reduction and reduction in root growth, in which the slopes of the curves are markedly different for dry and soaked seed. This illustrates the fact that with fast neutrons % survival is a very inadequate means of predicting fertility.

The first 40 capsules on the main stem were harvested on each surviving plant; date of harvest was recorded for each capsule. Mean harvest date was calculated per plant, for the first 10 capsules and for all 40 capsules. The corresponding dose/harvest-date curves ran entirely parallel and the correlation, for each dose, of (mean harvest date 10 capsules) and (mean harvest date 40 capsules) was almost perfect. Mean harvest date is delayed linearly with dose or dry seeds, and also for soaked seeds with the exception of the highest dose (which showed a relatively big delay). Maximum delay was 17 days for dry seed and 13 days for soaked seed.

Fertility was determined as the total weight of seeds in the 40 capsules harvested (husks having been carefully sieved out): this weight is 35-40 mg per unirradiated plant. With increasing dose, fertility decreases sharply; soaked seeds were as usual more sensitive than dry seeds. On linear scales, comparison of the curves is not easy. Very satisfactory linearity was obtained, however, when log(dose) is plotted against fertility-reduction expressed as a percentage of the control fertility, on log/probability paper. Consequently, the dose/fertility-reduction curves can be completely characterized by the log(dose) value at the median effective dose 50% = "log ED 50" and the slope (b) of the line. Dose may be expressed in neutrons/cm^2 or, in our case, where fast neutron fluxes are not yet precisely known, in units of exposure time for a rigorously standardised experimental setup. Sensitivity comparisons may thus be made by (1) calculating the ratio of the ED 50 for the comparative treatment against a standard, in the present case: ED 50 (soaked) / ED 50 (dry) = 0.63 and (2) by calculating the ratio of b (comparative treatment)/ b (standard treatment), which for this experiment was b (soaked) / b (dry) = 1.269. The latter value gives the ratio of the standard deviations of the distributions or, in other words, the relative increase in effect per unit dose for the comparative treatment (FINNEY, 1964). The confidence interval for both ratios can be calculated.

The transformation discussed here has been applied to many data from fast and thermal neutron irradiations, on several species, and was in all cases found to be very satisfactory, although it still seems impossible to explain satisfactorily the biological significance of the log(dose) transformation. The possibilities for application of techniques of probit analysis to radiation-dose/response relationships is at present being studied.

Reference:
FINNEY, D.J.: Probit Analysis, 2nd Edit. Cambridge Univ.Press 1964

from seeds of about 100 individuals of 8 different groups of viable laggards (3) and morphological variants (5). In no family there were any homozygosity nor clear-cut segregation ratios neither in progenies of laggards nor of morphological variants. BROWN, BHATIA, and SMITH (1965) tested their laggards for at least 7 generations and were, nevertheless, unable to stabilize an inbred line, breeding true for the selected phenotype. They therefore discussed these variant forms in terms of"many genes, heterozygosity, or low penetrance".

With this in view we were surprised to find the number of such variants increased over the control only in the S and E, but not in the P series (Table 1). As surely

Table 1: Frequency of laggards and morphological variants in the M2-generation after EMS treatment in different developmental stages

Stage	% of M_2-families with:					
	Lethal laggards		Viable laggards		Morpholog. variants	
	<10%	>10%	<10%	>10%	<10%	>10%
S	11.0	4.9	17.5	8.9	27.2	16.7
E	15.1	8.6	24.7	14.2	19.3	12.6
P	1.3	0	3.6	0.2	0.4	0
Untreated	0.5	0	1.8	0	0.9	0

in pollen the cytoplasm contributes less to the progeny, we at first were inclined to attribute the EMS induced variation in the M_2 to somatic changes within the cytoplasm, i.e., to "Dauermodifikationen". But in the M_3 generation of morphological variants not only the original M_2 plant phenotype, but also in a lower frequency forms were obtained which belong to quite different mutant groups (Table 2). This speaks in favour of polygenic variation within complex quantitative traits. It is,

Table 2: Number of phenotypes in segregating M_3 progenies of 5 morphological M_2-variants with short leaves

Serial No.	Normal phenotypes	Laggards		Morphological variants			
		lethal	viable	short leaves	narrow leaves	dense rosette	others
16	184	37	42	103	7	3	11
24	97	5	14	46	2	10	9
51	165	2	9	63	3	6	18
63	113	4	6	56	15	-	5
67	194	17	25	23	-	3	14

no doubt, highly speculative at present to discuss these findings at the level of primary mutagenous mechanisms. But we are going to continue this kind of experiment with other chemomutagens under varying treatment conditions in order to confirm whether we really treat with a different ratio of gross to small mutations after pollen versus egg cell and seed treatment, and what are the basic events. This will possibly contribute to a better theory of the effective factors in chemical mutagenesis within cells of higher plants.

References:

BROWN, J.A.M., C.R.BHATIA, and H.H.SMITH: Arabidopsis Research, Rep.Int.Symp. Göttingen 1965, pp. 171-183

RÖBBELEN, G.: Z.Vererb.lehre 93, 127-153 (1962a)

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- : Beitr.Biol.Pflanzen 41, 323-335 (1965b)

- : Proc."Symposium on the Mutational Process", Prague 1965. In press, 1966

New tested chemical mutagens

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Compound	Treatment	m_b	m_{bch}	S
Methoxyethyl methanesulphonate	$2x10^{-1}$ M	55.5	19	15.6
Di-sulphonoxydiethylester	$3x10^{-2}$ M	58.7	17.7	21.9
1,1,1-Methanesuphonyloxymethyl propane	$1.5x10^{-3}$ M, 24 h, 36°C	8.7	0	1
1-Butyl-1-nitrosourea	$5x10^{-3}$ M	81.8	30.7	37.4
1-Isobutyl-1-nitrosourea	$5x10^{-3}$ M	10.0	2.4	4.4
1-Allyl-1-nitrosourea	$5x10^{-3}$ M	64.6	6.6	15.2
1,2-Dimethyl-1-nitrosourea	$5x10^{-3}$ M	76.7	13.1	62.6
1-Ethyl-3-nitro-1-nitrosoguanidine	$5x10^{-3}$ M	84.0	27	68
1-Butyl-3-nitro-1-nitrosoguanidine	$2.5x10^{-3}$ M	2	0	1
1-Isobutyl-3-nitro-1-nitrosoguanidine	$2.5x10^{-3}$ M	3.6	0.3	1
1-Amyl-3-nitro-1-nitrosoguanidine	saturated	2.3	0	1
Ethyl-vinyl nitrosamine	10^{-1} M, 24 h, 25°C	73.0	21.9	54.6
Methyl-benzyl nitrosamine	$5x10^{-2}$ M, 24 h, 25°C	67.8	26.4	55.9
Control	H_2O	1.5	0.1	1

Mutation frequency and sterility calculated according to MÜLLERs method.
Treatment conditions (if not otherwise stated): duration 18 h, 24°C; 6 h posttreatment washing.
m_b = % of segregating siliques.
m_{bch} = % of segregating siliques for chlorophyll mutations.
S = sterility grade.

The effect of N-nitroso-N-methylurea on some quantitative characters in Arabidopsis

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Dry seeds of the early race Dijon were treated for 24 hours at 25°C with 0.05, 0.10 and 0.20 mM solutions of N-nitroso-N-methylurea. The M_2-plants were grown under constant conditions (25±3°C, continuous illumination with 1250 lux). Since the M_1-plants treated with 0.20 mM were highly sterile the corresponding M_2-population could not be studied. From each M_1-plant, 16 seeds were sown; in each M_2-population, there were 160 lines.

The frequency of induced chlorophyll abnormalities (alb+xa+ch) was found as follows:

Concentration	Segregating M_1-plants %	Mutant seeds %
0	0.0	0.0
0.05 mM	29.4	2.5
0.10 mM	41.1	4.8

The following changes of means and genetic variability were induced in five quantitative characters:

Concentration	Breadth of cotyledons mm		Height of plant at flowering cm		Days to appearance of fl.primordia		Number of rosette leaves		Number of rosette leaves per day	
	$\bar{x}$	V_G	$\bar{x}$	V_G	$\bar{x}$	V_G	$\bar{x}$	V_G	$\bar{x}$	V_G
0	1.69		1.79		12.5		4.0		0.32	
0.05 mM	1.69	0.0220	1.79	0.1075	12.6	2.4359	4.1	0.1162	0.33	0.0007
0.10 mM	1.70	0.0384	1.74	0.1174	12.8	1.9430	4.1	0.1294	0.32	0.0007

These results show that mutations can be induced by N-nitroso-N-methylurea not only in qualitative but also in quantitative characters. At the same time, the changes of means in all characters studied were quite small, while the induced genetic variance was considerable in all cases (cf. BROCK, 1964). It seems that the amount of the induced genetic variability is not increasing linearly with the concentration (cf. DALY, 1960, 1961).

References:
BROCK, R.D.: FAO/IAEA Technical meeting on use of induced mutations in plant breeding. Rome, 25 May - 1 June 1964.
DALY, K.: Genetics 45, 983 (1960)
-: Genetics 46, 861 (1961)

Reverse mutation of py-mutants

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Reverse-mutation frequency may be used as one way of characterizing mutants. All our mutants have been obtained after EMS treatment. Since there are indications that EMS preferably causes GC - AT transitions (KRIEG, 1963), nitrous acid and hydroxylamine would be suitable mutagens. MÜLLER (1965), however, states that the latter two compounds do not give mutants in Arabidopsis, which results were confirmed in our own experiments on forward mutations. Since EMS can also give AT - GC transitions (KRIEG, l.c.), though at a much lower frequency, reverse-mutation studies were initiated with different py-mutants, using this compound.

In preliminary experiments about 10.000 seeds per mutant were treated with 12.5 mM EMS at 24°C for 24 h, and sown on mineral medium. A reverse mutation will give rise to a plant of chimerical structure, part of which is able of synthesizing thiamine. Due to diffusion of the latter substance, no clearcut sectors can be expected. A number of green plants were obtained, some of them showing small diffuse yellow sectors. To stimulate seedling growth, which possibly may help mutated sectors in synthesizing sufficient thiamine to sustain development of the whole plant, besides completely mineral substrates also media containing small (0.003 - 0.006 mg/l) amounts of thiamine were used.

Our results up to now do not allow us to give quantitative data, but we can state that from one line, py-2^2, we obtained two wild-type plants, the offspring of which was analyzed. The c h i m e r i c a l s t r u c t u r e of these two plants could be established without doubt. The experiments are being continued.

References:
KRIEG, D.R.: Genetics 48, 561-580 (1963)
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This work was supported by a grant from the Netherlands Organisation for the Advancement of Pure Research (Z.W.A.)

Frequency of forward mutations and reversions in two chlorina-mutants after EMS treatment

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The monogenic chlorina mutants V 81 (ch_3) and V 89 (ch_4) of Arabidopsis thaliana (race Enkheim, En-2) were induced by mutagenic seed treatment with ethyl methanesulfonate (EMS) and diethyl sulfate respectively. Sown in the greenhouse during January and February without additional illumination they usually develop rather viable plants. (diameter of the rosette at the beginning of flowering about 2,5 to 3 cm). The leaves of V 81 show a homogenous deeply yellow colouring up to maturity (cf. RÖBBELEN and WEHRMEYER, 1966), those of V 89 turn somewhat more greenish at later developmental stages. Both phenotypes can be distinguished even under chimeric conditions. Therefore, dormant seeds of homozygous mutants and heterozygous plants were treated with 0,1-0.3% EMS solution (0.1 mol phosphate buffer in bidistilled water, pH 7, 20±0.1°C, 24 h, submerged), washed for 1 h in running tap water and sown on soil. From the segregating progenies of heterozygous plants in the case of V 89 the heterozygous individuals could be selected before being transplanted by their more bright green leaf colour. With V 81 all of the green plants had to be grown and their heterozygosity proved in a progeny test.

The EMS induced leaf sectoring was registered several times during the development of the rosette. Green spots or sectors were recognized on the yellow leaves of both mutant types, and yellowish, but also white sectors were determined on the green leaves of the heterozygotes:

Mutant	% EMS for seed treatment	No. of plants	No.of chimeras		% chimeras		No. of chimeras with mutated sectors in the inflorescence
Forward mutations (heterozygous plants investigated)							
		Sectors :	yellow	white	yellow	white	yellow
V 81	0.1	448	44	5	9.81	1.11	8
	0.2	504	119	10	23.62	1.98	41
	0.3	448	193	28	43.10	6.24	50
V 89	0.1	448	13	1	2.91	0.22	0
	0.2	448	77	5	17.2	1.16	17
Reversions (homozygous mutants investigated)							
		Sectors :	green	white	green	white	green
V 81	0.1	1200	36	0	3.0	0	7
	0.2	1200	110	3	9.16	0.25	17
	0.3	1000	131	5	13.10	0.42	17
V 89	0.1	1200	6	2	0.5	0.17	0
	0.2	1200	41	4	3.49	0.33	3

Quite a lot of yellow sectors in the heterozygotes had just the same colour as the homozygous mutant. Some of these sectors reached the inflorescence zone. The seeds produced in this area were separately harvested and sown. But from a total of 68 M_2-progenies from these sectors only 5 contained mutants which phenotypically were identical with the original strain. The rest of all was more or less deviating. Thus most of the yellowish sectors in the treated heterozygous resettes seem not to be produced by true forward mutations from $+^{ch}/ch$ to ch/ch, but by genetic changes at other loci. Test crosses have been initiated in order to elucidate the nature of the observed changes. (For similar experiments cf. GICHNER and VELEMÍNSKÝ, 1965.)

Just so the green sectors on the yellow mutant rosettes proved to give rise to M_2-plants which in all of the tested 26 progenies were different from the normal green wild type. There were no true backmutations, but only phenotypical reversions; most of the offsprings were lighter green and frequently changed in some other morphological or physiological characteristics. For genetic analyses crosses have been performed with these reverted types to the wild strain.

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The effect of temperature, cysteine and gibberellin on radiation damage of seed germination

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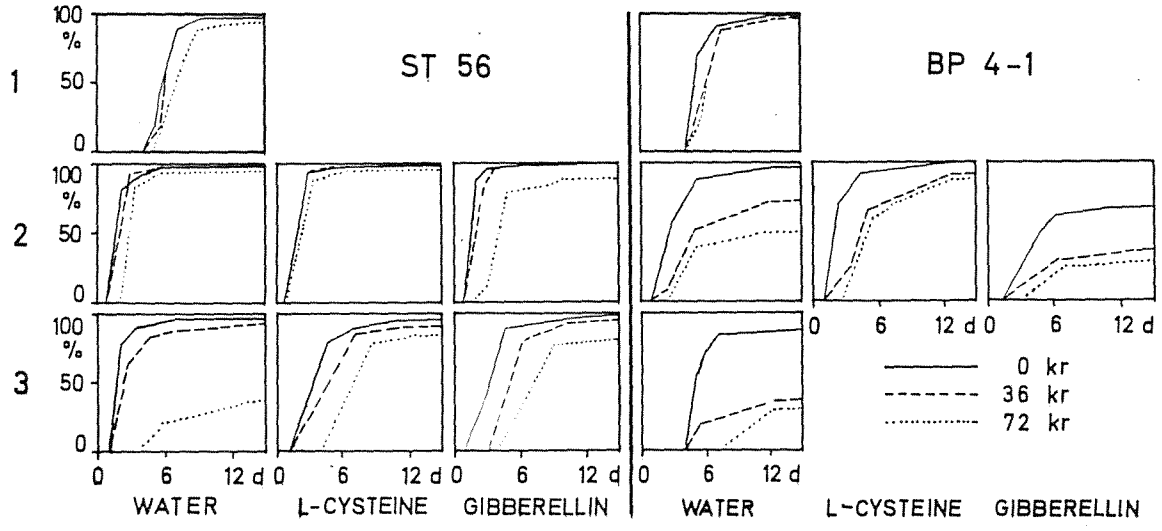
According to VELEMÍNSKÝ (1963) 72 kr of X-rays is a limiting dose for dry seeds of Arabidopsis thaliana. For γ-rays, however, it was found by DALY (1960a) that at 150 kr 76% of the plants still survive, when cultivated at a low temperature. Irradiated seeds are more sensitive to higher temperature, but the survival can be increased when the plants grow at lower temperature at least for the first 4 days (DALY, 1960b). HOLLAENDER et al. (1951) and LUČNIK and CARAPKIN (1959) proved that there is a protective of cysteine. According to GAUZE and NOTANI (1960), the germination of irradiated maize seeds can be increased by gibberellin.

Seeds of the Arabidopsis races ST 56 (maximum germination temperature 31.5°C) and BP 4-1 (29.0°C) were X-irradiated (180 kV, 0.05 mm Al) with 36 and 72 kr and placed for germination in the following various temperature conditions in the darkness:

1. 4 days at 4°C, then at 24°C,
2. at 24°C,
3. 4 days at 29°C, then at 24°C.

Some lots of seeds were presoaked for 12 h in a 0.1% solution of l-cysteine and in a 0.01% solution of gibberellin and washed for 1 hour. There were 3 x 100 seeds in each variant.



The Figure shows that the race ST 56 was generally more resistent to the X-irradiation than the race BP 4-1. The radiation damage was the greater the higher the temperature especially during the first 4 days. Presoaking in a l-cysteine solution caused an increase of germination compared with the control. The corresponding effect of gibberellin was not so clear as in the case of l-cysteine. Similar results were obtained with γ-irradiation.

From these findings it may be concluded that the radiation damage of the seed germination can be moderated when the seeds are kept for the first 4 days at a low temperature and/or in a solution of l-cysteine.

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B. TECHNIQUES

Arrangement of Arabidopsis plants in test tube aseptic culture

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In usual test tube culture after LANGRIDGE (1957) illuminated by fluorescent light tubes and equipped with longwise mounted test tube holders two sources of heterogenity in growth conditions may be revealed: firstly, non-uniformity of illumination of the outer and the inner rows of plants within the test tube holders, and secondly, somewhat differing intensity of light emitted by the middle and terminal portions of fluorescent tubes. These two sources cause rather small, but, nevertheless, detectable systematic differences in the rate of growth and development of plants located in corresponding portions of test tube holders, i.e., in the outer as compared to the inner rows, and in the middle as compared to the end parts of the holders.

It is obvious, that complete uniformity may be achieved only at the considerable expence of space and light utilization. But, it is well known that undesirable effects of excessive variation may be well compensated by adequate statistical design and analysis of experiments. In case under question the arrangement of plants in randomised blocks seems to be most profitable. At least two advantages of this method may be mentioned: firstly, the arrangement of material in randomised blocks increases the accuracy of experimental comparisons to be made, and secondly, the probability of undesirable local chance aggregation of plants of any experimental variant is materially reduced relative to completely random spatial distribution of plants.

Since the choice of randomised blocks should meet the demand of less variation in conditions within than between the blocks, then, on the basis of the above considerations on the sources of heterogenity, the four-rowed test tube holders (with test tubes arranged in chess-board order) may be subdivided into four portions: (1) the middle halves of the outer rows, (2) the same of the inner rows, (3) the terminal quarters of the outer rows, and (4) the same of the inner rows. Then, each block is comprised of similar portions of all the involved holders. In the simplest case, all the experimental variants are represented by equal numbers of plants in every block. At last, but not least, within each block the actual places to be assigned for plants of the certain experimental variants are chosen at random, i.e., with the aid of tables of random sampling numbers.

The details of the design of experiment in randomised blocks may be found in any comprehensive statistical manual on the planning of experiments.

Reference:
LANGRIDGE, J.: Austral. J. biol. Sci. 10, 234-252 (1957)

An increase of germination of dormant seeds by pricking

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It has been found that the dormant seed (about 5 months old) can germinate at normal conditions (25°C, continuous light, 1250 lux) when their testa is pricked with a fine needle by means of a single prick.

In an experiment, seeds of 30 A_1 lines from 5 local populations after 7 days gave only 11,9 per cent germination. At the same time, the remaining seeds were divided into halves and one part has been pricked. After further 7 days, 6.9 and 46.7 per cent of the seeds germinated in the control and the pricked part respectivly.

In another experiment the effect of pricking was studied in seeds of local populations HV-3 and Vra with different per cent of germination. One lot of seeds was pricked 2 hours after placing for germination, another 2 days later. There were 3 x 20 seeds in a variant.

Time of pricking after moistening	Per cent germination HV-3 $\bar{x}\pm s$	Vra $\bar{x}\pm s$
Control	3.3±5.8	85.0±0.0
2 hours	58.3±18.6	96.7±5.8
2 days	58.3± 7.6	96.7±5.8

The operation used increased the germination in a poorly germinating population and perfected it in a good germinating one. Since no visible differences in the imbibition of control seeds of these two populations were observed it is possible that the deciding cause of non-germination at least for the most part was the impermeability of the testa for gases.

Arabidopsis seedling growth for radiobiological studies

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Arabidopsis thaliana, race Li-2, is used in our studies on the radio-sensitivity of seeds in the course of germination under different environmental conditions. Suitable techniques were required for: 1) germination and growth of very young seedlings for root length measurements, and 2) growing large numbers of plants to maturity in order to evaluate survival, fertility and the frequency of recessive lethals (MÜLLER, 1963) and for progeny tests. Results of experiments on 1) are summarised below; those on 2) are reported seperately (CONTANT, 1966, this issue).

The technique used by MÜLLER (1964) formed the basis for the present experiments. However, MÜLLER's substrate, cotton-wool padding and filter paper, was replaced by a g a r , which for our purpose offers the following advantages: a) easy and rapid preparation, even of large numbers of petridishes; b) constant composition, also with regard to moisture; c) homogeneous density and moisture distribution within a dish, even if placed at an angle; d) very smooth surface, causing no obstacle to root tips ; e) transparence, so that germination can be observed with lids closed (avoidance of infection), whereas root length can be measured by placing the dishes on millimeter paper with clear ruling. The dishes must be incubated at an angle of 60° (MÜLLER, 1964) in order to obtain straight roots; even so, they are slightly curling and measurement with the dishes closed is only advisable if repeated observations are required on the same roots. For the last, or only, measurement, the dishes are opened and the roots straightened by gently pulling the plantlets by their cotyledons, still leaving them on the agar surface and measuring them with the aid of the millimeter paper on which the dish is standing; consequently the measurements are rapid. Although infection was generally not serious, seed sterilisation will be tried. The effect of several other factors on germination and root growth was studied in a series of replicated trials.

Optimum l i g h t i n t e n s i t y was found to be, at 24°C, in the range of 9000-13000 lux (Philips TL33RS; selenium cell measurements inside the dishes); speed and final rate of germination, root growth, speed of unfolding of the cotyledons and ultimate cotyledon length were all very significantly lower at 36000 Lux (P = 0.01 or better), and some reduction in respect of these parameters already occurred at 18000 lux. The addition of P h i l i n e a tubes (wolfram spiral) affected neither germination nor average root-length, but consistently and markedly reduced root-length variability.

At 13000 lux, a t e m p e r a t u r e of 28°C gave slightly better germination speed, root growth (both significant at P = 0.05), uniformity and general appearance than 24°C, whereas 20°C was very significantly suboptimal in all respects except for final germination percentage, which was not effected.

P o l y c a r b o n a t e petridishes promoted germination speed in comparison with glass dishes in some experiments but not in others. Root growth was not affected at medium light intensity but was very significantly more rapid in polycarbonate dishes at high light intensity (P = 0.001). These effects may of course depend on light source used.

Agar of 0.75% was preferable to either 0.5% or 1.0%. The addition of 0.1% KNO_3 promoted even germination (cf. Van der VEEN, 1965) and was highly benefical to all aspects of growth and early development. No extra benefit was derived from a complete nutrient medium (cf. MÜLLER, 1964). With the (very evenly moulded) polycarbonate dishes 2 1/2-3 mm agar was optimal for the combined requirement of good transparence and even agar distribution.

Placing dishes under an a n g l e of 60° increased root length by 12-25% at 9000 lux, in comparison with dishes placed horizontally. This difference is much smaller than in MÜLLER's experiments (1964), possibly because the smooth agar surface permits better root growth than filter-paper in the horizontal position. Using dishes at an angle much reduced the incidence of cotyledons sticking to the agar, which was occasionally serious in horizontal dishes, causing impaired vigour or even death. Lowering atmospheric moisture by means of filter-paper with glycerine was not beneficial in this respect and reduced speed and evenness of germination.

T w o r o w s , each containing 2x20 or 1x40 seeds, can be sown in a 9 cm polycarbonate dish, at 25 mm spacing. No difference was found between top- and bottom row, provided the bottom row was sufficiently far (3 1/2 cm) from the bottom of the dish (condensation water). Larger dishes would be of advantage but are not yet available. Large glass dishes will in the meantime be tried.

In most stage sensivity studies, p r e t r e a t m e n t s forming part of the culturing technique must be avoided. This is one reason why race Li-2 is very suitable; although a 5-day cold hydration period improves the evenness of germination, this treatment is not obligatory; after-ripening is short and dormancy only

moderate under our conditions. Experiments are planned to reduce after-ripening by low doses of X-rays (cf. BHATIA, 1965); in radiobiological experiments, however, this is a rather undesirable procedure. Anaerobic presoaking at room temperature before the 5-day cold period led to very rapid germination; neither the optimal period for such a treatment nor the ultimate benefit can yet be evaluated.

R o o t - l e n g t h measurements in units smaller than 1 mm led to highly significant personal bias ($P = 0.01$) and should be avoided (cf. MÜLLER, 1964). In analysing root length data the total or average length of all roots within a dish belonging to one treatment is generally regarded as one observation. However, the frequency distributions for root length within a petridish, in the case of low doses or of unirradiated material, often corresponded well with a normal distribution; in such cases there seems to be no reason to avoid using the t-test for establishing the significance of treatment-differences.

In view of the above results, the m e t h o d finally a d o p t e d consists of a) 5-day cold treatment if experimental requirements permit (which is often not the case); b) Sowing in 9 cm polycarbonate petridishes on 0.75% agar + 0.1% KNO_3; 2 rows at a distance of 25 mm, each containing 40 seeds; c) Incubation at 27° (for technical reasons preferred to 28°C) at a light intensity of 12000-13000 lux of Philips TL33RS + addition of Philinea red incandescent light; dishes placed at an angle of 60°; d) Observations on germination with lids closed; e) Root measurements with lids closed if repeated observations are required on the same plantlets, otherwise with lid removed and after straightening the roots on the agar surface by gently pulling the plantlets by their cotyledons; in both cases measuring to be done with the aid of millimeter paper on which the dishes are placed. Final recording normally takes place after 7 days.

References:
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MÜLLER, A.J.: Biol.Zbl. 82, 133-163 (1963)
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Full cycle Arabidopsis culture in radiobiological studies

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The requirement of uniformity in Arabidopsis culture is particularly stringent in studies on the segregation of radiation-induced mutations for not easily classifiable traits, and in our stage sensitivity studies. Van der VEEN's method (1965) for uniform culture in soil involves amongst other things the transplanting of seedlings with a small pre-cut cylinder of agar; a 48 h dark period is used for etiolating the seedlings in the course of germination, so that the hypocotyledons become 1-1 1/2 cm long.

As etiolation will only take place when the dark period is accurately timed in relation to the germination processes, this method leads to very heterogeneous results when applied to seeds in which these processes differ from normal in time or otherwise in an unknown manner, e.g., after irradiation. Lengthening the dark period produced very weak seedlings, whereas shortening this period increased the heterogeneity in hypocotyledon length and germination. When, however, 30-100 lux of P h i l i n e a (tubes with wolfram spiral) were given during the 48 h dark period, etiolation was reduced to 1/3 of that in complete darkness; the plantlets were more vigorous and of more even appearance: they had larger cotyledons which became green in spite of the extremely low light intensity, even before they had fully expanded. It appears thus possible to regulate the degree of etiolation, which may be useful in some applications.

However, in many radiobiological experiments it appears essential to have the least possible environmental changes shortly after irradiation (except those introduced as research variables). Moreover, the beneficial effect on vigour, of Philinea during the dark period, illustrates the fact that etiolation causes considerable weakening. After considerable experimentation the folowing procedure was found satisfactory and less time-consuming than the etiolation method.

1. Seeds are sown in polycarbonate petridishes and treated exactly in the manner described previously (CONTANT, 1966, this issue). A cold pre-treatment is given if it does not interfere with experimental requirements, but 3-months old seed of Li-2 germinates well without it. The seedlings are transplanted when their roots have attained 15-20 mm. The cotyledons are then well developed and in part of the plants the first 2 leaves are visible. In control plants this is after 6-7 days, but in irradiated material it may take longer (when the danger of infection arises they may be transplanted with shorter roots). Seed sterilisation may be beneficial, though infection is generally low.

2. Seed boxes or benches are sterilised and prepared in such a way that the top inch consists of a sterilised, finely sieved leaf-mould:compost or soil : compost mixture, well and evenly moistened and not compacted. A 2 1/2 cm deep wedge is drawn in the soil with a flat piece of metal or other suitable means. The seedlings are gently lifted from the agar (without adhering agar) and laid, roots hanging down, on one side of the furrow at the desired spacing, with their cotyledons just above soil level. When one row has thus been completed, the next wedge is drawn at a convenient distance (4-5 cm). This automatically closes the previous furrow. After planting the whole box or bench, it is kept covered with plastic foil for 1-2 days; watering is not necessary during this period if the pre-planting moistening has been well done. Fungi will be avoided if the soil has been well sterilised, if the temperature is kept at 20-21°C and if light intensity is restricted during these days. Subsequent watering should be by means of a fine spray or mist, or preferably from beneath by capillary action, as in the system used by C.W.LAWRENCE (pers.comm.), which is presently being tried. Algal growth can be kept in check by maintaining a light and porous soil structure. In growth-chambers, at 12000 lux of Philips TL33RS + Philinea during 16 h,a day temperature of 24°C and a night temperature of 20°C, and a relative humidity of 70%, excellent growth and development took place. Continuous light, however, was very unfavourable with this light source.

Direct sowing into shallow depressions in the soil, also suggested by C.W. LAWRENCE, probably constitutes a further simplification, but is neither applicable to many of our irradiation experiments on different stages during and shortly after germination, nor when root measurements are to be done. This method is, however, being tried for progeny tests.

References:
CONTANT, R.B.: Arabid.Inf.Serv. 3, 34-35 (1966)
VEEN, J.H.van der: Arabid.Inf.Serv. 2, 31-32 (1965)

Large scale culture of Arabidopsis

C.W.LAWRENCE

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Bench top trays, 3-5 metres x 2 metres x 15 centimetres deep, lined with polythene sheet in which a few wholes have been punched, are used. Approximately 5 cm of coarse gravel are placed at the bottom and covered by 10 cm of John Innes potting compost No. 1 which is firmed down lightly and evenly (important). The compost is covered by a thin layer of very finely sieved compost. Water is introduced directly into the gravel layer, allowed to soak up into the soil and then allow to drain away. The soil should be very moist at sowing time.

Seeds are placed on moist filter paper in petri dishes, allowed to imbibe for about an hour at room temperature and then stored at 1-2°C for 3-4 days. Often this cold treatment is not necessary, but if given routinely, it ensures even germination. After cold treatment, seeds are laid with the aid of a fine paintbrush on the surface of the soil at the bottom of small depressions (finger tip size). A spacing of 3-4 cm within rows and 5 cm between rows is usually adequate. Plot labels or similar means of identification of different families should be as short as possible since their shadows can cause uneven growth.

After sowing, the tray is covered with a frame draped with fine muslin. The muslin is kept damp during the germination period to ensure high humidity at the soil surface, and is removed after the cotyledons have expanded, to avoid etiolation. During the early stages of growth all watering is carried out as described above, but mature plants can be watered normally.

Germination under these conditions is even, and 95% or more seeds give rise to mature plants. Occasionally algae start to grow on the soil after sowing, especially if the soil is over wet, but are quickly killed by allowing the soil surface to dry, and therefore do not harm. It is unnecessary to water frequently and it is possible to maintain a fairly dry soil surface with adequate moisture for good growth at the bottom of the tray. Large trays take up to 4000 plants.

Fixation of cytological material

L.M.STEINITZ-SEARS

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Fixation of Arabidopsis buds for meiosis can be made in the usual 6 : 3 : 2 (methanol, chloroform, propionic acid) with the addition of 1% "Tween 20". This eliminates the need of evacuation. Good meiotic metaphase preparations have been obtained in this manner, but earlier meiotic stages are difficult to stain.

Kultur von Arabidopsis-Blattstecklingen

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Bei früheren genetischen Versuchen waren Arabidopsis-Pflanzen in Pikierkisten kultiviert worden; jeden Tag wurden die frisch aufgeblühten Pflanzen mit einem scharfen Messer abgeschnitten und entfernt. Am Ende solcher Versuche lagen gelegentlich (erstmals im Frühjahr 1955) einzelne frisch-grüne Blätter auf der in den Pikierkisten befindlichen Erde. Bei genauerem Zusehen zeigte sich, daß sich diese Blätter in der Regel bewurzelt hatten; in zwei Fällen waren dagegen nur Sproßknospen gebildet worden (hier konnte die nachträgliche Entwicklung von Wurzeln durch eine Wuchsstoffbehandlung ausgelöst werden); nur in einem Falle kam es zur spontanen gleichzeitigen Regeneration von Sprossen und Wurzeln (Abb. 1).

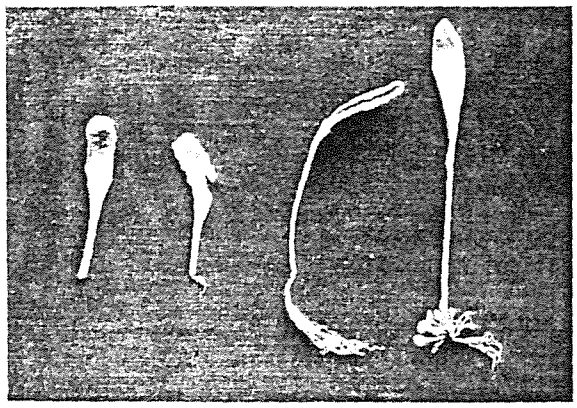

Abb. 1: Blattstecklinge von Arabidopsis thaliana. Photo Dr. EBERHARDT

Auf Grund dieser Beobachtungen war zu hoffen, daß es gelingen werde, die Regeneration von Arabidopsis-Blattstecklingen experimentell in die eine oder andere Richtung (Spross- oder Wurzelbildung) zu lenken und so ein Mittel zur Verklonung in die Hand zu bekommen. Unter diesem Gesichtspunkt wurden in den letzten Jahren mehrere Versuchsserien durchgeführt: über die Ergebnisse der letzten Versuchsreihe (die freilich in mancher Hinsicht unter den Unvollkommenheiten des alten Grenobler Botanischen Instituts zu leiden hatte) soll hier berichtet werden. Vorweg sei bemerkt, daß es bislang noch nicht gelungen ist, die Entwicklung von Sprossknospen an Blattstecklingen unter Kontrolle zu bekommen. Die nachfolgenden Mitteilungen beziehen sich daher ausschließlich auf die Wurzelbildung.

Als Material dienten Blätter von Pflanzen zweier winterannueller Linien (vor allem McKELVIE's Mutante F, daneben die Rasse St), die am 27. 10. 1964 ausgesät und fortan im Warmhaus bei natürlicher Tageslänge kultiviert worden waren. Für jedes Versuchsglied wurden gewöhnlich mindestens 100 Blätter zu je 10 in mit sterilem Sand gefüllte Petri-Schalen von 10 cm Durchmesser gesteckt; der Sand wurde mit 1/10 Knopscher Nährlösung befeuchtet, der gegebenenfalls Wuchsstoffe zugesetzt waren.

Bei der Mutante F steigerte eine Temperatur von durchschnittlich 17°C, verbunden mit natürlicher Tageslänge (Februar/März), die Überlebensrate vor sowie nach der Bewurzelung der Stecklinge, verglichen mit 23 bzw. 26°C und Dauerlicht (der Prozentsatz bewurzelter Stecklinge wurde durch diese Temperatur- und Lichtbedingungen nicht signifikant beeinflußt): bei 17°C und natürlicher Tageslänge waren Überlebens- und Bewurzelungsrate im vollen Tageslicht höher als im Schatten.

Was den Einfluß von Auxin (NES) betrifft, so war die Befeuchtung des Substrates mit NES-Lösung sehr viel wirkungsvoller als ein 24- und erst recht als ein 48-stündiges Bad der Stecklinge in der NES-Lösung (Mutante F, Gewächshauskultur März/April, volles Sonnenlicht). Bei der erstgenannten Applikationsart wurde der höchste Prozentsatz überlebender Blattstecklinge durch 2×10^{-6} proz. NES-Lösung, der höchste Prozentsatz bewurzelter Stecklinge durch 2×10^{-5} proz. NES-Lösung erzielt.

Bereits bewurzelte, NES-behandelte Stecklinge beider Linien wurden am 10. Juni (4 Wochen nach dem Stecken) einzeln in Töpfe mit Gartenerde pikiert und dann mit Lösungen von Streptomycin oder Tyrosin gegossen, die in Versuchen anderer Autoren die Bildung von Sproßknospen an Calli und regenerierenden Organen gefördert hatten. Es kam jedoch nicht nur zu keiner Knospenbildung, sondern vielmehr zu einer Verringerung der Überlebensdauer.

Bei langstieligen (3 cm) Blattstecklingen der Mutante F lagen Überlebens- und Bewurzelungsrate höher als bei kurzstieligen (1 cm). Bei der Rasse St erreichten diese Raten unter denselben Bedingungen erheblich höhere Werte (nach 4 Wochen über 80% lebend, über 40% bewurzelt) und unterschieden sich nicht bei lang- und kurzstieligen Stecklingen.

Ausgewachsene Blätter (der Mutante F) überlebten bei Stecklingskultur (langstielig) zu einem geringeren Prozentsatz als noch nicht ausgewachsene; bezüglich der Bewurzelung gab es dagegen keinen signifikanten Unterschied.

S u m m a r y : Under certain conditions, rooting of Arabidopsis leaf cuttings is favoured by high light intensity, irrigation with NAA (0.2 ppm), long petiole and some genes.

Mit Unterstützung der Deutschen Forschungsgemeinschaft.

C. MATERIAL

The editor would be highly pleased to have in the next issue of AIS under this heading contributions and discussions on the problem of how to coordinate the gene nomenclature as well as the maintenance of natural and mutant stocks of Arabidopsis. Some proposals have already been put forward during the Symposium in Göttingen, April 1965. There is urgent need to look for cooperators

1. who propagate natural races with avoidance of any severe selection pressure,
2. who maintain and compare selected lines from natural races, mainly those which are commonly used for experimental designs,
3. who hold mutant stocks for comparison with newly described types in order to coordinate naming and
4. who are engaged with gene mapping, e.g.,of only one linkage group each.

Applications are requested to be sent to the editor as soon as possible!

D. NEWS

Laboratory Research Communications

G.W.M.BARENDSE: Flower formation in Arabidopsis thaliana, strain Köln. Experiments on the effect of gibberellic acid, kinetin and α-tocopherol (Vitamin E) on flowering of the cold requiring strain Köln of Arabidopsis are in progress. It has already been found that gibberellic acid enhances flowering, but merely so because leaves are formed more rapidly. The number of leaves formed before flowering is hardly reduced by gibberellic acid and the latter therefore can not be considered as having substituted for the cold requirement. Also experiments on the effect of a growth-retardant ((2-chloroethyl) trimethylammonium; CCC) during vernalization are carried out.
(MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, USA)

A.D.McKELVIE: Linkage studies with Arabidopsis. A preliminary account of linkage in Arabidopsis has been published by McKELVIE (Arabidopsis Research, Göttingen, pp. 79-82, 1965) showing the degrees of linkage between a number of different genes. In order to fit this into a general pattern of linkage for Arabidopsis, a comparison is being made between mutants induced by McKELVIE, by RÉDEI and by RÖBBELEN. The mutants are being crossed in an effort to investigate both allelism and linkage. The mutants of RÉDEI are: an; as, gi^2; pa (er); gl^2; lu, co; vc_2 (er). Those of RÖBBELEN are V 18, 19, 21/1, 21/2, 24/1, 24/3, 24/5, 25/1, 26, 28, 29, 32, 36, 37, 40, 42, 43, 44, 47, 48, 49, 50, 51. The initial crosses are only being done at present but results will be announced as soon as possible. It may be possible to incorporate mutants from other research workers and a few such mutants would be acceptable. It will, however, be impossible to deal with a large number of new genes.
(Agricultural Botany Department, College of Agriculture, Crown Mansions, 41 1/2 Union Street, Aberdeen, Scotland)

Studies underway:

I.CETL and J.KUČERA, Brno: N a t u r a l p o p u l a t i o n s. Distribution of summer-annual and winter-annual types, variability in quantitative characters, frequency of homozygous and heterozygous plants, comparison with the sensitiveness to high temperatures . - I n d u c e d m u t a t i o n s affecting developmental and quantitative characters. Methods of investigations, effect of various mutagens in different genetic backgrounds.

J.VELEMÍNSKÝ, T.GICHNER, and JIŘINA ŠVACHULAVÁ, Prague: (1) Study of the action of nitrosamides and nitrosamines in combination with metabolic inhibitors. (2) Study of the normalization-phenomenon of chlorina mutants.

Back volumes of Arabidopsis Information Service

The AIS No. 1 and 2 as well as the Suppl.Vol. 1: "Arabidopsis Research" have been printed with 200 copies each. Because of the unexpected high number of orders only a few copies of No. 1 are still available; the supply of No. 2 and the Suppl. Volume is entirely exhausted. Photocopies of single (!) contributions from "Arabidopsis Research" will be provided at cost price by: Niedersächsische Staats-und Universitätsbibliothek, 34 Göttingen, Germany, or by the Library of Congress, Washington (refer to E-49-113).

E. BIBLIOGRAPHY

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BERGER, Brigitte: The taxonomic confusion within Arabidopsis and allied genera. Pp. 19-25
KRIBBEN, F.J.: Interspecific hybridization with Arabidopsis. Pp. 26-30
BOUHARMONT, J.: Fertility studies in polyploid Arabidopsis thaliana. Pp. 31-36
RATCLIFFE, D.: The geographical and ecological distribution of Arabidopsis and comments on physiological variation. Pp. 37-45
CETL, I.: Some developmental features in natural populations of Arabidopsis thaliana (L.) HEYNH. Pp. 46-52
REHWALDT, C.A.: Germination response to light on dry seeds of Arabidopsis thaliana. Pp. 53-55
NAPP-ZINN, K.: Theory of vernalization - New experiments with Arabidopsis. Pp.56-61
Van Der VEEN, J.H.: Genes for late flowering in Arabidopsis thaliana. Pp. 62-71
BARTHEIMESS, Ilse: Vom Blühalter abhängige positive oder negative Merkmalskorrelationen. Pp. 72-78
McKELVIE, A.D.: Preliminary data on linkage groups in Arabidopsis. Pp. 79-84
HIRONO, Y., and G.P.RÉDEI: Concurrent products of premeiotic recombination. Pp.85-90
RÉDEI, G.P.: Genetic basis of an abnormal segregation in Arabidopsis. Pp. 91-99
RÖBBELEN, G.: Plastom mutations. Pp. 100-105
JACOBS, M.: Isolation of biochemical mutants in Arabidopsis. Pp. 106-112
FEENSTRA, W.J.: Biochemical genetics of thiamine biosynthesis. Pp. 113-118
RÉDEI, G.P.: Genetic control of subcellular differentiation. Pp. 119-127
HOFFMANN, P.: Chlorophyll formation and photosynthesis in Arabidopsis thaliana (L.) HEYNH. Pp. 128-130
VELEMINSKÝ, J., and G.RÖBBELEN: Chlorophyll formation and fine structure of chloroplasts in a chlorina mutant of Arabidopsis thaliana. Pp. 131-137
RÖBBELEN, G.: Submicroscopic structure of mutant chloroplasts. Pp. 138-141
REINHOLZ, Erna: X-ray induction of embryonic malformations. Pp. 142-146
FUJII, T.: Effects of UV-rays on Arabidopsis seedlings. Pp. 147-151
MÜLLER, A.J.: On the chimerical structure of M_1 plants. Pp. 152-153
BONOTTO, S., M.JACOBS, and L.LEDOUX: A preliminary study on the penetration and the translocation of a highly polymerized heterologous ^{3}H-DNA in Arabidopsis thaliana (L.) HEYNH. Pp. 154-164
WALLES, Solveig, and G.AHNSTRÖM: Correlation between the mutation frequency and the alkylation of deoxyribonucleic acid upon treatment of seeds of Arabidopsis thaliana with ethyl methanesulfonate. Pp. 165-170
BROWN, J.A.M., C.R.BHATIA, and H.H.SMITH: Lethal, laggard, and flowering time variants in generations following thymidine analogue treatment of Arabidopsis buds. Pp. 171-183
JACOBS, M.: Mutagenicity of some monofunctional alkylating agents. Pp. 184-191
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G. ANNEX

Current Arabidopsis Research at Brookhaven

C.R.BHATIA and H.H.SMITH

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

Manuscript received at January 24, 1966

Growth of Arabidopsis thaliana on deuteriated media

A detailed study of the biological effects of the stable isotope of hydrogen was started in our laboratory using race "Estland" of Arabidopsis. The objectives are to gather information on the effects of partial or complete deuteriation of biologically important molecules on the development of plants, possible genetic effects of the resulting deuteriation of the hydrogen bonds in DNA and deuteriation effects on radiation sensitivity of seeds.

Surface sterilized dry seeds are planted aseptically on mineral media containing increasing amounts of deuterium oxide, 10, 30, 50, 70 and 90 per cent heavy water and rest H_2O, solidified with 0.78 per cent ion agar. Plants are grown under continuous illumination in controlled environment chambers. In the first experiment, there were no surviving plants at maturity in deuterium levels of over 10 per cent. Seeds obtained from survivors were grown on progressively increasing concentrations of heavy water, in subsequent generations. Results indicate that Arabidopsis can be gradually adapted to grow on deuteriated media. So far, we have obtained seeds from plants grown on media containing 50 per cent D_2O, and the experiment is being continued with increasing percentage of heavy water in the culture medium.

The results show that deuteriation delays and inhibits seed germination, retards growth, delays time to opening of the first flower, reduces plant height at flowering, increases the number of leaves, and reduces pollen and seed fertility. Germination percentage of seeds obtained from plants grown on partially deuteriated media is lower than that of controls. Plants grown on deuteriated media are lighter green in color and show lack of chlorophyll. A limited analysis of the progeny of plants raised on partially deuteriated media did not show any chlorophyll or morphological mutations. Details will be published later.

Dimethyl sulfoxide as a mutagen carrier

Recently there have been several reports that dimethyl sulfoxide (DMSO) enhance absorption through biological membranes, and it is being tested as a carrier for drugs in several laboratories (KLIGMAN, 1965). We have observed similar rapid transport of dyes dissoved in DMSO through plant tissue. Subsequently we started experiments to investigate whether this solvent can be used as a carrier for chemical mutagens in higher plants, employing Arabidopsis as the test material. If feasible, mutagens dissolved in DMSO can thus be transported to the meristematic region more rapidly and treatment time will be considerably reduced.

The results show that 100 per cent DMSO is toxic to Arabidopsis seeds and plants. However, we have found that up to 30 per cent DMSO applied as a single drop to the apical meristem is not lethal, though it retards growth slightly. Ten per cent DMSO did not show any appreciable retardation effect. We have used similar concentrations of IuDR, BuDR and FuDR dissolved in aqueous and 30 per cent DMSO solutions. The growth inhibiting effect of FuDR dissolved in 30 per cent DMSO was very marked. This could be due either to a synergistic effect of the two or to increased uptake of FuDR. The mutagenic effect of these treatments and others with EMS is under investigation.

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KLIGMAN, A.M.: J.Am.Med.Assoc. 193, 796-804, 923-928 (1965)

Modification of leaf shape in Arabidopsis by phenyl boric acid

Treatment of Arabidopsis seedlings of race "Estland" and erecta type of race "Landsberg" at the cotyledonary leaf stage, with 300-600 ppm of phenyl boric acid (PBA), changes the shape of leaves subsequently formed. Leaves that develop after the treatment are narrower and lanceolate with acute apices, in contrast to the broad leaves with round apices found in untreated controls. This effect is observed in all the treated plants. However, the effect is more marked in erecta as the control leaves are rounder in this type. Except for the occurence of lanceolate leaves, plants treated with this range of PBA concentrations are similar to controls in other morphological and physiological characters. At higher levels growth is retarded. The lanceolate shape induced by PBA is not inherited and the progeny of treated plants produce normal leaves. These results confirm the findlings of MATHAN (1965) in tomato where PBA simulates the effect of the lanceolate gene. To our knowledge there is no record of a gene producing lanceolate leaves in Arabidopsis but there are several which modify the form of leaves (McKELVIE, 1962).

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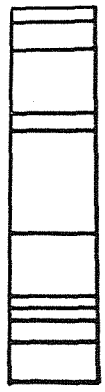
Esterases of Arabidopsis thaliana



With the application of histochemical techniques to the proteins separated electrophoretically on polyacrylamide gels, it is possible to locate different molecular forms of specific enzymes on the zymograms. Using this technique, we have observed the pattern shown in the Figure for esterases from the leaf extracts of race "Estland". In total we obtain nine different bands showing esterase activity. We have tentatively labeled these bands as Es 1 to Es 9. This pattern will be used as standard for a survey of the genetic variation in esterases.

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