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

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I would like to express my gratitude to my wife for her assistance in reading the manuscripts and also to Mrs.Brigitte KIRCHHEIM for the editing and typing of this volume of AIS.

A. BRIEF NOTES

A mechanism of action of Bromodeoxyuridine.

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received February 1975

5-Bromodeoxyuridine and some other halogenated deoxyribonucleosides induce early flowering in several ecotypes and late mutants of Arabidopsis (BROWN, 1962; HIRONO and RÉDEI 1966). In some related species (BROWN, 1968) or in certain ecotypes or in mutants at a particular gene locus, these compounds fail to evoke the same response (HIRONO, and RÉDEI, 1966; RÉDEI 1969; REDEI, ACEDO and GAVAZZI, 1974).

BROWN (1968, 1972) applied the BrdU to the apical meristem of Arabidopsis in the prefloral stage and followed mitotic activity by autoradiography in the developing apex. His conclusions were that BrdU or the related compounds are incorporated readily into the nuclei of the prefloral flank meristem. The incorporation of the analog caused a transient cessation of division in the cells concerned. This suppression of mitotic activity in the flank meristem lead to an activation of the cells in the central initiation zone, free of the analog. Subsequently - in the absence of continued BrdU supply - the flank meristem cells eliminated the previously obtained analog and the entire apex now resumed mitotic activity. Thus BrdU triggered the precocious activity of the central zone just as it is necessary for the transition from the prefloral to floral stage under normal conditions of flower initiation.

This attractive interpretation could not be applied to our experiments. The plants were fed with BrdU (10^{-6} M) throughout their life cycle in aseptic test tubes. Then 6 weeks old plants, grown on 0.33 uCi 14-C-BrdU (47.4 mCi/mmole) were extracted and the DNA was purified with a procedure similar to that of LEDOUX, HUART and JACOBS (1971), followed by additional phenol and alcohol treatments. Subsequently the DNA was subjected to density gradient centrifugation in CsCl.The BrdU treatment caused a 14-20 mg increase in density compared to thymidine controls, indicating that under the conditions of these experiments approximately 1/5 to 1/4 of the thymine residues were replaced by the analog. Even with the most conservative estimates this indicates that the DNA contained about 70 million bromouracil residues. This figure is much too large to account for substitution only in a few structural genes. Mutation was not observed in these experiments. Thus it appeared most likely that BrdU affected quantitatively certain genes which are either rich in A-T or those where the substitution can easily lead to functional changes.

Recent years the regulatory role of BrdU in the differentiation of various animal cells and tissues has been recognized and intensively studied (RUTTER, PICTET and MORRIS, 1973). In Escherichia coli where both the operator site and the lac repressor can be isolated, it has been shown that BrdU-DNA competes with very high efficiency for and then it binds very tightly to the repressor protein (LIN and RIGGS, 1971, 1972, 1974). In higher organisms also the acid protein fractions of the chromatin have been found to control the activity of the DNA (STEIN, STEIN and KLEINSMITH, 1975).

We have grown Arabidopsis plants on 14-C-arginine and 14-C-valine in the absence and presence of BrdU. Crude chromatin was then extracted from the plants with saline-EDTA-sodium lauryl sulfate and trapped on dacron webs in the presence of high molecular weight calf or salmon DNA. The filter passed through all free proteins, including the histones split from the DNA by the detergent, but it retained a highly radioactive fraction tightly bound to DNA. The BrdU-chromatin was found to contain in every case substantially higher radioactivity (protein) than the appropriate control. This observation indicated that the analog acts apparently on the chromosomal level.

Other experiments revealed that flowering in Arabidopsis is actually suppressed in light (RÉDEI and ACEDO, 1974), and it proceeds with maximal efficiency in complete darkness. Under the latter conditions BrdU-treatment is ineffective. Thus it seems that BrdU-DNA can effectively bind a protein fraction controlling flowering. This is an acid protein like the repressor of bacteria or the regulatory chromosomal proteins of higher organisms. The simplest assumption to explain the physiological role of BrdU in flowering is that it facilitates the binding of a repressor to DNA which prevents the transcription of the chromosomal regions producing the flowering inhibitor under illumination.

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Effect of ozone on Arabidopsis

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received May 1975

Little is known about the mutagenic activity of ozone. DAVIS (1961) found increases in mutation frequencies of streptomycin dependence to non-dependence and of phage susceptibility to resistance in E. coli following ozone treatment as well as decreasing survival of the colonies. In 1971, ZELAC, CROMROY, BOLCH, DUNIVANT and BEVIS found chromosome breakage in lymphocyte cells of Chinese hamsters exposed to 0.2 ppm ozone for 5 hours. Chromosome bridges and fragments at anaphase were noted by FETNER (1958) in the rootips of Vicia faba following ozone fumigation of seed.

Seeds of Arabidopsis thaliana L. were sown in a soil:peat:perlite (1:2:1) mix in a growth chamber at a light intensity of 2000 f.c., day temperature of 23°C, night temperature of 17°C and R.H. 60 to 70%. To test the mutagenic capacity of ozone plants were fumigated during the bud stage in plexiglass chambers with the temperature and humidity controlled by an Aminco Aire H-T Controller. The air was mixed with ozone produced electrolytically by an Elcar Viva model ozone generator. The

mixture was then blown into a mixing chamber and then into the fumigation chamber. The air mixture moved past the samples to a false floor and was drawn through a bottom exhaust. Ozone levels were determined by monitoring the fumigation chamber with a Mast ozone meter, Model 724-2. Control plants were placed in a second chamber which was identical to the one described through which air, without added ozone, was circulated. Plants were exposed to a concentration of 100 pphm of ozone for 4 hours and 8 hours, following which they were returned to the growth chamber until ready for analysis.

MULLER's (1963) embryo-test was used on siliques 4 and 5 of treated and control plants, and seeds to produce the next generation were collected from siliques 1 and 2 (MESKEN and VAN DER VEEN, 1968). Plants were examined during many stages of growth for morphological abnormalities.

The degree of sterility, measured as the number of unfertilized ovules in the siliques, and the number of lethal embryos in the siliques increased significantly in fumigated plants (Table 1). Seeds produced from treated plants showed reduced germination (Table 2).

Table 1. Frequency of embryonic lethals and unfertilized ovules in treated, M_1 and M_2 plants.

Duration of Fumigation	Treated			M_1				M_2	
	No. of ovules	% embryonic lethals	% unfert. ovules	No. of ovules	% embryonic lethals	% unfert. ovules	No. of ovules	% embryonic lethals	% unfert. ovules
0 Hours	3,005	5.58	6.82	10,000	7.0	11.3	5,480	3.0	18.0
4 Hours	3,144	19.12	22.61	9,000	7.0	12.0	5,472	2.0	17.0
8 Hours	3,328	37.75	10.01	7,000	5.0	11.4	5,489	1.7	15.6

Table 2. Percent germination of seeds from treated and M_1 plants.

Duration of Fumigation	Treated		M_1	
	No. of seeds	% Germination	No. of seeds	% Germination
0 Hours	100	71.0	120	65.0
4 Hours	100	50.0	120	66.6
8 Hours	100	30.0	120	66.6

However these effects of ozone were found only in the treated generation and it may be presumed to be due to physiological effects or chromosomal damage rather than to the induction of lethal or sterility genes. Ozone did not induce morphological abnormalities such as chlorophyll, leaf, stem, flower or silique mutants. Although a leaf abnormality characterized by a slight notching in the first true leaf was found in M_1 and M_2 generations we have no definite proof that this is due to a gene mutation.

Apparently ozone, at the relatively high concentrations of this experiment, lowered fertility in A. thaliana but did not have any morphological mutagenic effects.

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New data on double mutations in Arabidopsis thaliana

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received 6/11/74

By means of treating Arabidopsis seeds with X-rays (7 to 28 kr) and methyl-nitroso-urea (MNU)(.04 to .12 mM) high frequency of lethal and vital chlorophyll and morphological mutants was induced and scored in M_2 : 4.5 per cent for X-rays and 24.3 per cent for MNU when the highest dose or concentration was used. Studying individual M_1 families in the M_2 we stated that some of them segregate more than one mutant type (in X-rays up to 3 different mutant types, in MNU even 6), the average number of mutations per segregating M_1 family being 1.88 and 2.34 for these highest dose and concentration. After RELICHOVÁ (1972) and RELICHOVÁ and CETL (1972) these mutations might be distributed in the apical initials of the vegetative shoot apex in different ways. Distinguishing between two basic alternatives (i.e. [2/0] and [1/1]) is possible by analysing single M_3 families, derived from single M_1 individuals (HÄNSEL, 1967). Table 1 (see page 7) shows some typical results of this analysis.

In this survey, cases are given where only two mutations appeared in the same M_1 family as the analysis becomes difficult if more than two mutations appear in the same M_1 family.

The frequency of both basic types of distribution ([2/0] and [1/1])were tested. Complete analyses of M_3 showed, that the observed frequency of real double mutations (i.e.of two mutations in the same initial cell, [2/0])was twice higher than the other case ([1/1]). The reason for this fact may be in the organization of vegetative shoot apex itself. Many authors report with agreement with our considerations the average number of initials to be 2 to 3 and the average size of mutated sector about 30 to 60 per cent. If we take into considerations also the variable fate of apical initial cells, then, studying a wide spectrum of mutation types, the number of mutations per one M_1 plant is higher than the number of genetic effective initial cells. It should mean that more than one mutation was frequently induced in these cells.

References:

HÄNSEL, H.: Genetika 9, 49-57 (1967)
RELICHOVÁ, J.: Arabid.Inf.Serv. 9, 28-29 (1972)
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Table 1: The analysis of individual M_1 families in M_2 and M_3.

M_1 family Mutagene tested and No. of M_1 plant	Generation	Analysis of M_2 and M_3 Numbers of standard /+/ and mutant /m_1,m_2/phenotypes			Distribution of mutations in the shoot apex
		+	m_1	m_2	
X 7 - 48	M_2	78	11 lethal	10	
	M_3	+ m_1 m_2 2 - - 2 - -		+ m_1 m_2 - - 24 - - 24 - - 24 - - 23 - - 24 - - 24	[1/1]
X 14 - 66	M_2	72	10 lethal	3 lethal	
	M_3	+ m_1 m_2 71 - 17 58 18 12 63 23 - 44 17 17 52 17 9			[2/0]
X 21 - 24	M_2	80	1 lethal	-	
	M_3	+ m_1 m_2 14 2 3 16 1 5 19 1 - 20 - 2 14 3 3			[2/0]
X 28 - 29	M_2	68	22	3	
	M_3		+ m_1 m_2 - 13 5 - 6 - - 6 - - 8 - - 4 1 - 13 1	+ m_1 m_2 - 9 1	[2/0]
MNU .04 - 11	M_2	73	9 lethal	7 lethal	
	M_3	+ m_1 m_2 16 1 6 11 2 2 15 1 1 14 1 5 17 2 2			[2/0]
MNU .04 - 29	M_2	76	2	2	
	M_3	+ m_1 m_2 51 11 - 77 11 - 55 19 14 74 6 14 57 3 26 95 - 2			[2/0]
MNU .08 - 101	M_2	51	1	4 lethal	
	M_3	+ m_1 m_2 76 16 - 63 6 20 73 - 26 79 21 - 78 - - 74 - 15			[2/0]
MNU .08 - 148	M_2	84	13	2	
	M_3		+ m_1 m_2 - 15 - - 6 - - 24 - - 3 -	+ m_1 m_2 - - 24 - - 1	[1/1]

On the chimerism in generative tissues of different inflorescences of Arabidopsis thaliana plants after irradiation of seedlings.

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received 4/11/74

The aim of this study is to find out that the initials of the main and lateral first order inflorescences of the shoot apex of A.thaliana seedlings which have developed one pair of leaves are the same.

Nine day old seedlings of A. thaliana (race En) grown on minimal agar medium in Petri dishes were irradiated by 1, 3 and 5 kr γ-rays. Then the seedlings with the first pair of leaves were transplanted into soil. They were grown there under continuous illumination at $24^{\circ} \pm 2^{\circ}C$. Both control and 1 kr-irradiated plants began to flower on the 32^{th} day after sowing, 3 and 5 kr ones on the 34^{th} and 35^{th} day respectively. Using MÜLLER's embryo test chlorophyll deficient mutants were scored irrespectively wether their embryos were of normal size or somewhat undeveloped. 20 pods were scored on the main inflorescences and not less than 5 pods on the lateral ones.

Table 1: Correlation between normal and mutant pods in inflorescences of different order in mutants of A.thaliana.

Types of inflorescences			pods				
			total		normal	mutant	
			number	%	number	number	%
Main			283	100	224	59	20.8 ± 2.42
Lateral	cauline	order I	744	100	574	170	22.8 ± 2.23
		order II	354	100	293	61	17.3 ± 2.02
		total	1098	100	267	231	21.0 ± 1.23
	rosette	order I	202	100	179	23	11.4 ± 1.00
		order II	90	100	77	13	14.4 ± 1.17
		total	292	100	256	36	12.4 ± 1.95

The data obtained after studying the effect of all doses were summarized in table 1. Of 144 plants 14 showed mutations ($8.4 \pm 2.16\%$). Mutations found out in the different inflorescences of a plant were identical. As can be seen in the table 20.8 ± 2.42 mutant pods were observed on the main inflorescences and $21.0 \pm 1.23\%$ on the lateral ones formed in the axils of cauline leaves. Lateral inflorescences formed in the rosette leaf axils contained 12.4 ± 1.93 mutant pods. The data obtained show that there were a few initial cells for generative tissues (about 5-8) in the shoot apex of the seedlings having one pair of leaves.

Results being available in literature in respect to irradiation of seeds of A.thaliana and other plants (including data obtained when studying dose dependence) indicate that in the shoot apex of a seed there are only 1 to 3 initial cells giving the material for generative tissues (GRINIKH et al.,1974). If these calculations are right it can be supposed that in our experiment initial cells devided not more than 1 to 2 times for a 9-day period after sowing.

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GRINIKH,L.I., SHEVCHENKO, V.V., GRIGORIEVA, G.A., DRAGINSKAYA, L.Y.: Genetika 10,7,18-28 (1974)

Fasciation studies in Arabidopsis thaliana (L.) HEYNH.

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received 20/5/75

Apart from very few constantly fasciated taxa like Celosia cristata and one pea cultivar already grown for several centuries, fasciation studies have normally been limited to single fasciated specimens of various species, which appeared then and when spontaneously. Only since the discovery of artificial induction of mutations, sufficient material of this kind may be easily obtained. Also McKELVIE's (1962) mutant clavata-1 (clv_1)has been induced by chemical mutagenes.

This mutation originally appeared within an early flowering summer annual strain, and is primarily characterized by club shaped pods (to which its name refers, this shape being caused by central proliferation). The responsible gene has pleiotropic effects; under all conditions blossoming is delayed, and it causes fasciation. In order to make the manisfestation of fasciation independent from the growing conditions, we introduced the gene clv_1 into two late flowering strains (florens-2 (f_2) and florens-5 (f_5) which regularly produced fasciated shoots even under long day conditions. The morphological comparison of late flowering fasciated plants ($f_2f_2clv_1clv_1$ and $f_5f_5clv_1clv_1$) with the corresponding normal ones (f_2f_2+ + and f_5f_5+ +) shows the following results:

1. Already in the rosette stage fasciated individuals have a higher leaf formation rate than normal ones (cf. PLANTEFOL 1974: Hedera). With increasing plant age the leaf number difference increases absolutely and relatively.
2. As the leaves of fasciated plants are smaller, their total dry weight is approximately the same as in normal plants.
3. The increase of the leaf number in clv-plants is connected with an increasing enlargement of the growing point to a kind of growing line (or growing edge); only in clv-plants there is, simultaneously, an increase of the number of parastichies. At a place where one parastichy branches into two, a forked (dichotomous, "double") leaf may be found (cf.PLANTEFOL 1968a,b: Hedera). Such forked leaves have only been seen in fasciated plants.
4. Among shooted clv-plants four types of fasciated stems, probably pure modifications could be distinguished, no.1 and 2 corresponding to GEORGESCU's (1927) classification:
   1. the bilateral stem (with two planes of symmetry),
   2. the dorsiventral stem (with only one plane of symmetry),
   3. the multi-edged fasciated stem (asymmetrical), and
   4. the serpent-like fasciated stem (asymmetrical).

Anatomical investigations in shoot apices yield some further results as follows:

1. The distal part of the shoot apex which is still devoid of leaf primordia, is much higher (and broader at its base)in fasciated plants than in normal ones. Fasciated plants thus differ from normal ones in the same way as water shoots of amphibic plants do from aerial shoots (McCULLY and DALE, 1961:Hippuris). When points of leaf initiation keep equal distances from each other, more leaves may thus be initiated at the same height in fasciated individuals than in normal ones.
2. In fasciated plants, procambium is formed at a greater distance from the growing than in normal ones. This fact is probably related to the "delayed" leaf formation.

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Thanks are due to Dr.A.D.McKELVIE for seed samples.

On the genetical basis of light requirement in seed germination of Arabidopsis

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In the last AIS issue KRANZ (1974) communicated some genetical aspects of P_{fr} - and GA_3-induced seed germination. On this occasion I report briefly about the actual situation of some series of our experiments which are still in progress. One of these series concerns the genetical basis of the light requirement of seed germination in the race Hannoversch-Münden (Hm) of Arabidopsis thaliana. With the same intention, KUGLER (1951) had crossed this race with several others which, however, germinate not only under light but also in constant darkness. But KUGLER did not draw any conclusions with regard to kind and number of genes involved because of the behaviour of the F_1 generations and the segregations in back crosses and F_2. As far as reciprocal F_1 hybrids were concerned, a maternal influence at least (probably of the plasmon, but hardly of the testa) could not entirely be excluded.

Hm is a summer annual race; in the context of certain vernalization experiments it was desirable to transfer its light requirement of seed germination into a winter annual race (Stockholm = St). A number of F_1 plants resulted from a cross St x Hm (H10 x H5). Seeds were separately harvested from 55 descendants (obtained by spontaneous selfing) of the F_1 plant, H176. A test sowing of 18 to 36 days old seeds from 17 of these 55 F_2 plants under light or darkness, respectively, showed that there was still an important requirement of after-ripening (also originating from Hm) in many of the F_3 families. Therefore, another sowing was done when the seeds of those 55 F_2 plants were 308 to 526 days old, i.e.when after-ripening, according to KUGLER, should already have been completed for a long time. The seed was sown, as usual, in Petri dishes of 9 cm diameter upon filter paper moistened with 2 ml of distilled water. Then it was kept at 20°C in darkness for 7 days. Only in one of those 55 F_3 families - H226s - not a single seed (among 192) germinated within a week. This result suggests that three gene loci might be involved in the light requirement of Hm, - a conclusion that, for the rest, SHIFRISS and GEORGE (1965) had also drawn from their investigations in two cucumber cultivars.

In order to test whether the "aim of breeding" had been attained, plants of the just mentioned F_3 family, H226s, were grown until flowering. Their average age at flowering equalled that of the summer annual parent, Hm (approximately 44 days from sowing), but the variation was much greater. Again, seed was harvested from 70 H226s plants (K11 through K80). Among the resulting F_4 families the earliest ones flowered 9 days earlier and the latest ones 9 days later than Hm. The flowering age of all these 70 F_4 families was far from that of the winter annual parent, St (approximately 120 days). Therefore, it may be supposed that the major gene loci for vernalization requirement (NAPP-ZINN, 1957) could be situated in the same chromosomes as those loci competent for the light requirement of seed germination.

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Mit Unterstützung der Deutschen Forschungsgemeinschaft

Different kinds of light requirement in seeds from wild populations of Arabidopsis thaliana?

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A further number of local populations of Arabidopsis thaliana from the Rhineland have been included in our recent studies on the population genetics (cf.NAPP-ZINN, 1964) and on the geographical distribution of "vernalization genes". Seeds have been harvested separately from 8 to 150 single plants of each population concerned. Seed was stored at room temperature. In the following paragraphs I report preliminary investigations of the germination behaviour in selected individual progenies from the following populations (breeding no. and dates of harvest are indicated):

I. Köln-Braunsfeld, Aachener Str.458, terrain of the Clarenbach Church: L3244s (May 28,1974)

II. Odenthal-Scherf, slopes at the way out of the village towards Schallemich: L3256s (May 14, 1974)

III. Eberbach/Rheingau monastery, between paving-stones: L3264s and L3273s (May 14, 1974)

IV. Lohmar-Geber, slopes at the way to Inger, shortly before the Federal Road no.507: L3372s (May 22, 1974), L3424s (May 28, 1974), and L3457s (June 12, 1974)

V. Köln-Lindenthal, Kerpener Str.and Universitätsstr., terrain of the University Library: L3491s and L3492s (June 12, 1974)

100 seeds per Petri dish of 9 cm diameter were placed on filter paper that was moistened with 2 ml of distilled water. There were two variant of treatment: one (A) was kept at light (natural day length), the other one (B) at darkness for 39 days, then light for 8 days, afterwards +7 to 8°C and darkness in a refrigerator for about 3 weeks, and finally again 20 to 23°C (first sowing) or 23 to 25°C (second sowing) and light 8 days. Some results are summarized in tables 1 and 2.

Table 1. Sowing of July 12 (12.7.), 1974

Pop.	Breeding no.	n	A: LIGHT germinated until 26.7.	A: LIGHT germinated 27.7.-20.8.	A: LIGHT remainder	n	B:DARKNESS germinated until 26.7.	B:DARKNESS germinated 27.7.-20.8.	B:DARKNESS remainder	LIGHT germ. until 28.8.	COLD germ. until 16.9.	LIGHT germ. until 24.9.	remainder
I	L3244s	100	90	0	10	100	14	0	86	0	0	3	83
II	L3256s	100	97	0	3	100	1	0	99	0	0	84	15
III	L3264s	100	97	0	3	100	58	0	42	0	0	42	0
	L3273s	100	71	0	29	100	8	0	92	0	7	85	0
IV	L3372s	100	3	8	89	100	0	0	100	0	0	79	21
	L3424s	100	1	4	95	100	0	1	99	0	0	94	5
	L3457s	100	6	19	75	100	0	5	95	0	11	62	22
V	L3491s	100	66	22	12	100	0	0	100	23	8	64	5
	L3492s	100	60	16	24	100	4	0	96	18	22	19	37

Table 2. Sowing of February 14 (14.2.), 1975

Pop.	Breeding no.	n	A: LIGHT germinated until 28.2.	1.3.-2.4.	remainder	COLD germ. until 24.4.	LIGHT germ. until 2.5.	remainder	n	B:DARKNESS germinated until 28.2.	1.3.-25.3.	remainder	LIGHT germ. until 2.4.	COLD germ. until 24.4.	LIGHT germ. until 2.5.	remainder
I	L3244s	100	100	0	0	0	0	0	100	5	1	94	0	0	0	94
II	L3256s	100	96	0	4	0	0	4	100	4	0	96	0	0	19	77
IV	L3372s	100	25	6	69	0	27	42	100	4	1	95	0	2	76	17
	L3424s	100	12	7	81	0	35	46	100	0	0	100	1	1	22	6
	L3457s	100	55	10	35	5	15	15	100	4	1	95	18	4	47	26
V	L3491s	100	71	10	19	3	4	12	100	6	5	89	14	4	12	59
	L3492s	100	64	24	12	0	1	11	100	11	10	79	15	2	26	36

Obviously the seeds of population IV plants only had a strong requirement of after-ripening which had not been overcome even 8 or 9 months after harvest. On the other hand, all families considered here but L3264s, showed a pronounced light requirement with regard to germination; but they reacted, at the first sowing, in three different ways after 39 days of darkness (interrupted every third or fourth day for a few minutes only when counting the germinated seeds):

1. In population I darkness induced an inhibition that even persisted after cold treatment and exposure to light.
2. In the populations II, III(L3273s), and IV darkness induced an inhibition of germination that only was removed to a considerable degree by the cold treatment.
3. In population V part of the seeds already germinated as soon as they had been transferred from darkness to light.

At the second sowing, the families L3256s (population II) and L3491s (population V) approached to the first type, whereas they formerly had corresponded to the third or second types, respectively.

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Thanks are due to Mrs.Angela ALTHÖFER for careful assistance.

Spectrophotometric evidence for differences in R/FR-reactions of the phytochrome in seedlings of monogenic mutants

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Differences in the phytochrome mediated photomorphosis of certain mutants of Arabidopsis thaliana (L.)HEYNH.have been observed (HEHL and KRANZ 1971, DIEKMANN and KRANZ 1972, KRANZ 1974). We had supposed that these differences resulted from defects in the red/far red-mechanism and its primary effect on the differentiation of the seedling (KRANZ 1974 b). But at this time there was no direct evidence for the defect of the phototransformation of the phytochrome itself.

For measuring the in-vivo transitions of this pigment induced by R/FR-radiation, at present we can only use the method of the spectrophotometric determination of the extinction differences in the seedlings. Our measuring equipment consists of an one-beam spectrophoto-

meter (ZEISS PMQ II) with monochromatic grid illuminator, automatic drive for the wavelength, specific sample holder connected by an optical lens system, sensitive photomultiplier, a/d-computer, printer and recorder. Both, the actinic light (R : λ = 660 $\pm$ 5.7 nm 280 pE . cm^{-2}. s^{-1}, FR : λ = 730 $\pm$ 5.7 nm 347 pE . cm^{-2} . s^{-1}) and the measuring beam (λ = 660 $\pm$ 0.6 nm 9 pE . cm^{-2} . s^{-1}, λ = 730 $\pm$ 0.6 nm 6 pE . cm^{-2} . s^{-1}) were produced by the monochromatic illuminator. The samples measured were seedlings of the mutants ch^1/ch^1, ch^2/ch^2, ch_3/ch_3 and the wildtype ch^+ch_+/ch^+ch_+ grown up in absolute darkness (14 $\pm$ days old, hypocotyl length 7 $\pm$ 2 mm). Germination has been induced by GA_3 plus c-AMP (10^{-3}M aqueus solution). In order to get stationary concentrations of the phytochrome (apparent photosteady state) the base values ($\bar{E}$) of the extinction at λ = 660 and 730 nm have been measured five times alternatively; between the measurements the sample has been kept in the dark. Then the extinction has been recorded again in five repetitions each treating with 5 min.R- and FR-radiation in order to get the difference ΔE after the actinic light treatment.

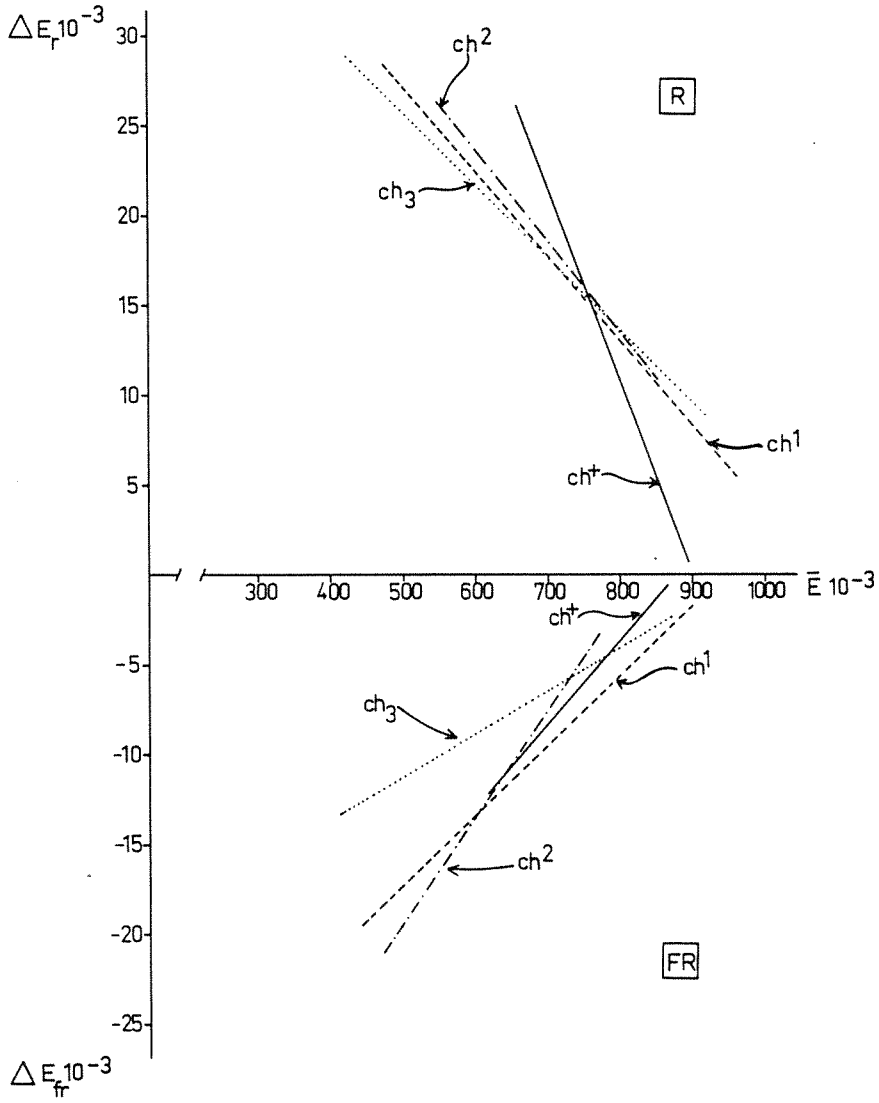
Preliminary tests had been shown, that the standard deviation of ΔE for one sample treated was low ($\bar{s}$ = 0.92 x 10^{-3}) but high between different samples ($\bar{s}$ = 7.54 x 10^{-3}) for one genotype (ch_3). Apparently the higher deviation depends on the sample amount (number and height of the seedlings). But it is impossible to control these factors in Arabidopsis because of its small seedlings and the requirement to keep them absolutely dark up to measurement. However $\bar{E}$ may be used as parameters for the sample amount. Thus we have calculated a significant correlation between $\bar{E}$ and ΔE. Figure 1 shows that this correlation (r) is negative after treatment with R(ΔE_r) and positive with FR(ΔE_{fr}) for all the genotypes studied, i.e.the decrease and increase respectively of the regression line depend on $\bar{E}$ which is nearly equal to the sample amount. This is only true for the measuring range of $\bar{E}$ and ΔE.

Table 1. Coefficients of the correlation (r) and the regression (b) with the Y-intercept (a) for different actinic light treatments (R,FR) in the wildtype (ch^+/ch^+) and three mutants of Arabidopsis thaliana seedlings (F_s-test for differences between two b's, α = probability, d.f.= degrees of freedom)

	after 5 min. R λ = 660 nm					after 5 min. FR λ = 730 nm				
genotype	r (α,FG)	a 10^{-3}	b	F_s (α, d.f.)		r (α,FG)	a 10^{-3}	b	F_s (α, d.f.)	
ch^+/ch^+	-0.950 (<<0.001,23)	97.005	-0.107	216.73 (<<0.001, 46)	153,04 (<<0.001, 46)	0.862 (<<0.001,18)	-41.724	0.048	5.07 (<0.05, 36)	12.55 (<0.005, 36)
ch^1/ch^1	-0.921 (<<0.001,23)	50.599	-0.047			0.866 (<<0.001,18)	-36.861	0.039		
ch^2/ch^2	-0.826 (<<0.001,23)	53.979	-0.050	3.54 (<0.05,41)	0.55 (α~0.45,46)	0.878 (<<0.001,18	-49.224	0.059	8.04 (<0.01, 32)	
ch_3/ch_3	-0.867 (<<0.001,18)	46.312	-0.041			0.626 (≈0.01, 14)	-23.576	0.024		

Further the statistical F_s-test (SOKAL and ROHLF 1973) yields significant differences of the regression coeffecients b between the genotypes (table 1). After treatment with the actinic light R the b of the mutants are significantly smaller than the b of the wildtype (ch^+) and the b of ch_3 too in relation to the other mutants. This signifies a smaller increase of the extinction after R in those genotypes, which we can interpret as the synthesis of the phytochrome P_r from a precursor P_v and/or the transition of P_r to P_{fr} because the absorption of both intermediates is measured at λ = 660 nm. Nevertheless it is common to the two reactions that the second step only is a p h o t ochemical one (MOHR 1972). Therefore we may conclude that the transition $P_r \xrightarrow{HR} P_{fr}$ is reduced in the mutants. After 5 min.FR other differences of b are obtained. All the genotypes are significantly different in their regressions; i.e.the decrease of the extinction after FR is smaller in genotypes having low coeffecients. This signifies the decay of P_{fr} by reversion to P_r in a thermal reaction or by destruction in an irreversible enzymatic reaction, the second of which seems allways to be the dominant process in a seedling.

Figure 1. Different regressions between the ground value of the extinction ($\bar{E}$) and the extinction differences (ΔE) after treatments with 5 minutes red (R) or far red (FR) actinic light in three mutants (ch^1, ch^2, ch_3) and the wildtype (ch^+) of Arabidopsis thaliana seedlings.



As mentioned above the prerequisite for quantitative studies, the photosteady state of the phytochrome, have been fulfilled aproximately. Therefore we suppose, that the absolute rate of the P_r formation equals the rate of the P_{fr} decay, that means the quotient of the regression coefficients $Q = b_r/b_{fr}$ is near 1.0. This is true for the mutants ch^1 and ch^2, but for the wildtype and the third mutant this value is significantly higher ($Q = 2.3$ and 1.7 rsp.). Consequently we may conclude, that the transitions of the phytochrome from P_r to P_{fr} in ch^+ and the P_{fr}-decay in ch_3 are probably defective dependent on their genotype. This supposition is supported by the known photomorphogenetic defects of ch^+ and ch_3 in contrast to ch^1 and ch^2 and by the fact that these genotypes are mutants of one gene but ch_3 of another gene locus (KRANZ 1971).

The experiments have been supported by a grant of the Deutsche Forschungsgemeinschaft.

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Nitrate uptake in Arabidopsis thaliana

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The uptake of water and ions is, together with photosynthesis the only way for higher plants to obtain their basic material for biosynthesis. Many data have been obtained regarding the uptake of kations, especially sodium, potassium and rubidium (NISSEN 1974). Much less is known about the uptake of anious, especially of nitrate. In the latter case the study of the uptake process is complicated by the rapid disappearance of nitrate after it has been taken up by the plant, due to its reduction, via nitrite, to ammonia, which subsequently is incorporated into amino acids. This conversion of nitrate may influence its uptake. Therefore, uptake experiments using mutants in which uptake and/or processing of nitrate is altered might yield more information about the uptake mechanism.

For the isolation of nitrate reductase-less mutants, selection for resistance to chlorate may be used (OOSTINDIER-BRAAKSMA and FEENSTRA 1973a). However, by this method, also mutants can be isolated with a normal to above normal level of nitrate reductase, but showing a lower chlorate and chloride content, after exposure to chlorate (chl-1 type mutants, see Van der LAAN et al 1971 and OOSTINDIER-BRAAKSMA and FEENSTRA 1973a).
A decrease in the uptake of chlorate might explain the chlorate resistance of such mutants. Since reduction of both nitrate and chlorate is brought about by the same enzyme, uptake of these ions might be mediated by one carrier system, and thus mutants with a lowered uptake of chlorate might concurrently show an altered uptake of nitrate. If this hypothesis came true, mutants of the chl-1 type could be profitably used for the study of the uptake of nitrate. Mutant B25 (OOSTINDIER-BRAAKSMA and FEENSTRA 1973b and 1974) could be useful studying the uptake of nitrate since this mutant is nitrate reductase-less and thus unable to process absorbed nitrate. A line with both the chl-1 mutation and the B25 mutation has been constructed, to study the interaction between the genes.

Methods were developed for the growth of large numbers of Arabidopsis thaliana seedlings in waterculture and for the measurement of the uptake of nitrate and chlorate by these plants. Time curves showed that the uptake of chlorate in wild type plants was comparable to the uptake of nitrate. The chlorate uptake was strongly reduced in the presence of nitrate. This may indicate a competitive inhibition and thus a common uptake system. Wild type plants, grown on an ammonium medium, showed a lower uptake of chlorate and nitrate than plants grown on a nitrate medium. This may point to an influence of the induction of nitrate reductase by nitrate on the uptake of nitrate and chlorate, or an induction by nitrate of the nitrate carrier itself. Gifts of nitrate and chlorate after N-starvation during a few days resulted in higher uptake rates of nitrate as well as of chlorate.

Wild type plants, chl-1 and B25 mutants were grown on an ammonium nitrate medium. After two days of N-starvation, the nitrate uptake was determined. Uptake of nitrate by chl-1 indeed turned out to be smaller compared to wild type. This implies that a mutation has been induced in a gene playing a role in the control of the uptake of nitrate. The uptake of nitrate by the B25 mutant was higher than by wild type plants. The same observation was made with chlorate as substrate.

type	relative uptake of nitrate per gram fresh roots	relative uptake of chlorate per gram fresh roots
wild	100%	100%
chl-1	76%	45%
B25	140%	130%

Apparently the B25 mutation (nitrate reductase-less) in combination with the allele chl-1$^+$ induces an enhanced uptake of nitrate and chlorate under the experimental conditions.

Experiments on the kinetics of nitrate uptake are being carried out. Preliminary results indicate the excistance of at least two phases, with different K_m and V_{max}. Experiments on the uptake of nitrate, both by wild type plants and by mutants are being continued. The next step will be to complete the study of the kinetics of the uptake, and to establish the influence of external conditions like pH, counter ions and temperature.

ACKNOWLEDGMENTS.
We wish to thank Miss Dieuwke de Heer for her initiative and cooperation in the development of the method for growing plants in water culture.

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Nitrate reduction in Arabidopsis thaliana

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In order to isolate nitrate reductase-less mutants M_2 offsprings of 18,000 M_1 plants treated with N-methyl-N'nitro-N-nitrosoguanidine (NG) and 17,000 M_1 plants treated with ethylmethanesulphonate (EMS) were screened for chlorate-resistant mutants. The NG treatment yielded 1 mutant, exhibiting a lowered level of nitrate reductase activity (chl-2) and 14 mutants of type chl-1, with a lower uptake of chlorate (reported previously, Van der LAAN e.a. 1971; OOSTINDIER-BRAAKSMA and FEENSTRA 1973a). The EMS treatment yielded 1 mutant, B25, without nitrate reductase activity (OOSTINDIER-BRAAKSMA and FEENSTRA 1973b and 1974), 7 mutants with a lowered level of nitrate reductase activity (isolation numbers B29, B31-1, B31-2, B33, B35, B36 and B40), and 37 mutants of type chl-1. EMS appears to be a more suitable mutagen than NG for the type of mutations we are looking for.

Complementation tests showed that the mutants B29, B31-2, B33 and B35 belong to the same complementation group. All newly isolated mutants showed complementation with the previously isolated mutants chl-2 and B25.
Linkage data obtained thusfar suggest that B40 may be closely linked with chl-2; B31-1 and B36 may be linked with chl-2, but not closely; the mutants B29, B31-2, B33 and B35 are not linked with chl-2. All mutants with a low level of nitrate reductase activity showed independent segregation with B25. Localisation experiments with marker lines are presently being pursued. The first results show that B25 is probably very closely linked with the marker an, even so close that it appears to be difficult to isolate a double mutant line. Since an is in chromosome 1 (RÉDEI and HIRONO, 1964; RÉDEI, 1965) this suggests that the B25 mutation and chl-2 are in different chromosomes, since chl-2 was located in chromosome 2 (OOSTINDIER-BRAAKSMA and FEENSTRA 1973a and 1973b).

When mutants with a low nitrate reductase activity (5-10 times lower than in the wildtype) are grown on media containing nitrate, extracts of most of them show, when compared to wildtype, an enhanced nitrate content and a 2-4 times higher nitrite reductase activity. The mutant B25, exhibiting no nitrate reductase activity, shows a high nitrate content and an extremely high level of nitrite reductase activity. Enzyme induction experiments were carried out with wildtype and B25 rosettes, which, after having grown on perlite substrate with ammonium as the N-source, were cut off and transfered to a liquid medium, to which, after 16 hours of N-starvation, nitrate (6 mM) was added as the only N-source. Increase of nitrite reductase activity was more rapid and to a higher level in B25 than in wildtype. These results suggest that synthesis of nitrite reductase is induced by nitrate rather than by nitrite. More support for this hypothesis comes from the finding that plants of wildtype and B25, when grown on nitrite medium, show a nitrite reductase activity about equal to that found in plants grown on ammonium medium, and thus considerably lower than in plants grown on nitrate.

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Revertants of the nitrate reductase-less mutant B25

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The nitrate reductase-less mutant B25 (OOSTINDIER-BRAAKSMA and W.J. FEENSTRA, 1974) grows very poorly on nitrate as the only N-source. Selection for revertants can therefore be carried out simply by looking for well growing plants on a nitrate medium. After EMS treatment about 30,000 M_1 plants were screened, but no revertants were found. Among M_2's of 1000 M_1plants 7 independently arisen revertants could be isolated (isolation numbers B25R1, B25R2 etc.). Some data about the first 4 revertants will be given here.

All revertants grow well on media with nitrate as the sole N-source, and are chlorate sensitive like the wildtype. All revertants regained the ability to synthesize nitrate reductase, though on ammoniumnitrate medium B25R1 exhibits a low level of nitrate reductase activity, about equal to the level on ammonium medium.

F_1's of revertant x B25 showed chlorate resistance (B25 phenotype) and all except the F_1 of B25R1 x B25 showed poor growth on a nitrate medium (also B25 phenotype). Thus for B25R1 dominance relationship depends on the criterium used, whereas for the other revertants the restoration of nitrate reductase activity is recessive.

Results from F_2-analysis of the cross revertant x wildtype suggest for all revertants a mutation in a gene segregating independently of B25.

Results of Km studies of nitrate reductase from wildtype and revertants do not suggest that the properties of the enzyme are different.

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A block in the electron's cyclic transport in mutant 58/15 Arabidopsis thaliana (L.)HEYNH.

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It is known that chlorophyll mutants are very good for the research in questions concerning genetical control of the photosynthetic process (VOSKRESENSKAYA et al., 1968; JAKUBOVA, 1972a). The task we had in mind was to study photochemical activity of the photosynthetic apparatus in monohybrid foliage chlorophyll mutant 58/15 (viridis), induced by 1% ethylmethansulfonate. The chlorophyll content in mutant 58/15 is twofold less than in the initial form (JAKUBOVA et.al., 1972b).

The following parameters have been considered: delayed light emission, absorbance changes in the 520 nm ares and photoinduced changes of absorption in the 554 nm area in intact leaves; the detailed characteristic of these parameters is given in the publications of STRELER (1951) and RUBIN (1973).

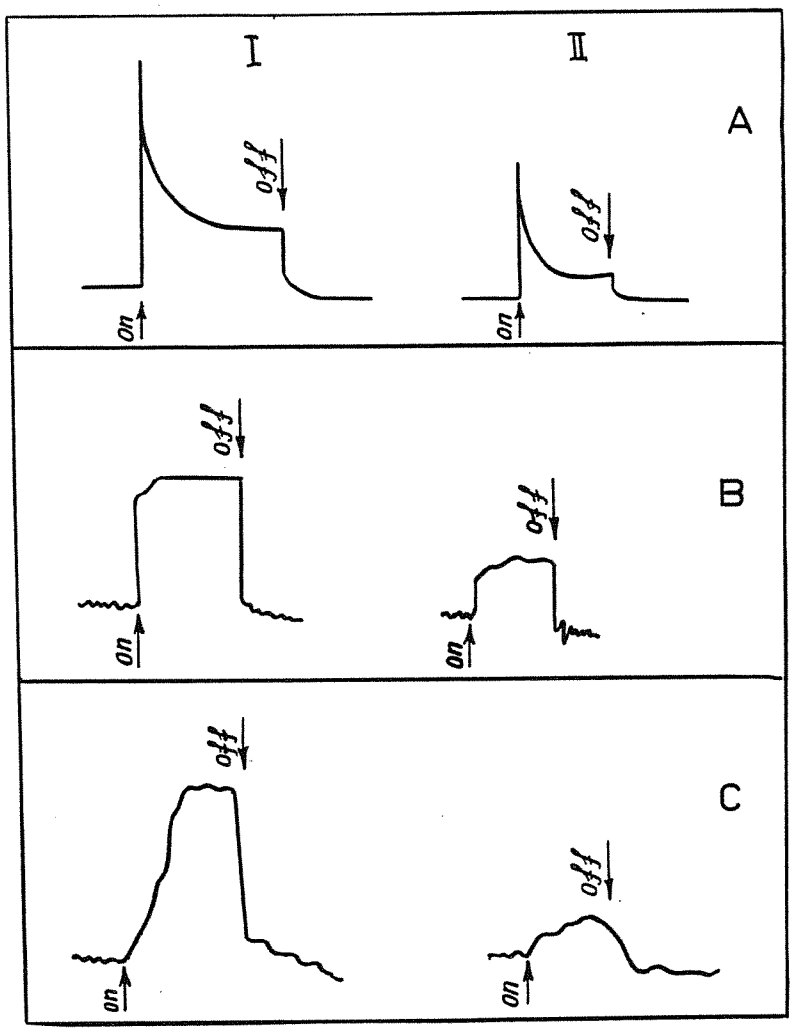

Figure A - Induced curvex of delayed light emission

Figure B - absorbance changes in the 520 nm area

Figure C - photoinduced changes of absorption of cytochrome in the 554 nm area.

I - initial form

II - mutant 58/15

As figure 1 A shows, A.thaliana leaves have a normal induction curve showing the presence of two components of delayed light emission. The induction curve of 58/15 is characterized by the reduced delayed light emission as compared with the control.

These data answer well the present ideas on the relationship of the delayed light emission intensity with the chlorophyll content and the differentiation of the photosynthetic apparatus. Mutant 58/15 does not manifest the violation but the slowdown in the process of transport of electrones and the membranes'energy supply as compared to the initial form, which is indicated by the course of the induction curves of the delayed light emission.

The data of figure 1 B prove that the kinetic of the absorbance changes in the 520 nm area of the normal type and of the mutant of A.thaliana is one-phase as opposed to the leaves of peas, tobacco and cotton that show two-phase curves. When excited by the light signal the amplitudes in the initial form are higher than in the mutant. These data also indicate the lower energy supply of the membranes in connection with the transport of the electrones in 58/15.

The results of the photoinduced changes of cytochrome absorption in the 554 nm area prove the absence of the effect in 58/15 (fig.1 C). These data suggest that the membranes' energy reduction in connection with the transport of the electrones is associated with the absence of the indicated effect in the mutant.

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Characterization of Arabidopsis thaliana ecotypes on the basis of genetic variation at ten isozyme loci

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Isozymes, or multiple molecular forms of enzymes, as resolved by electrophoresis in gel matrices, have added a new dimension to genetic studies. Arabidopsis thaliana provides a very suitable material for genetic analyses in higher plants. However, not many reports are available in literature concerning the inheritance of electrophoretic variants in this plant, except those by GROVER and BYRNE (1972) and JACOBS and SCHWIND (1972). Further to our previous report, we have been able to study the genetics of isozyme variation at ten loci as observed in 17 of the geographic races. These races or ecotypes, form a part of the collection maintained by the division of Plant Industry, CSIRO at Canberra (see LANGRIDGE and GRIFFING, 1959).

The details of the growth conditions and electrophoretic assays, will be described elsewhere. Briefly, the leucine aminopeptidases (Lap), glutamate oxalacetate transaminases (Got), acid phosphatases (AcPh) and esterases (Est) were extracted from rosette leaves of 13-day old plants growing in agar medium, whereas the peroxidases (Per) were derived from their roots. Also, for reasons not yet clear, it was found that Lap, Got and AcPh isozymes are best resolved in starch gels, while polyacrylamide gels only gave satisfactory separation of esterases and peroxidases.

Figure 1: Electrophoretic variants for isozyme loci in Arabidopsis thaliana. The electrophoretic mobilities relative to the buffer front are given for each band.

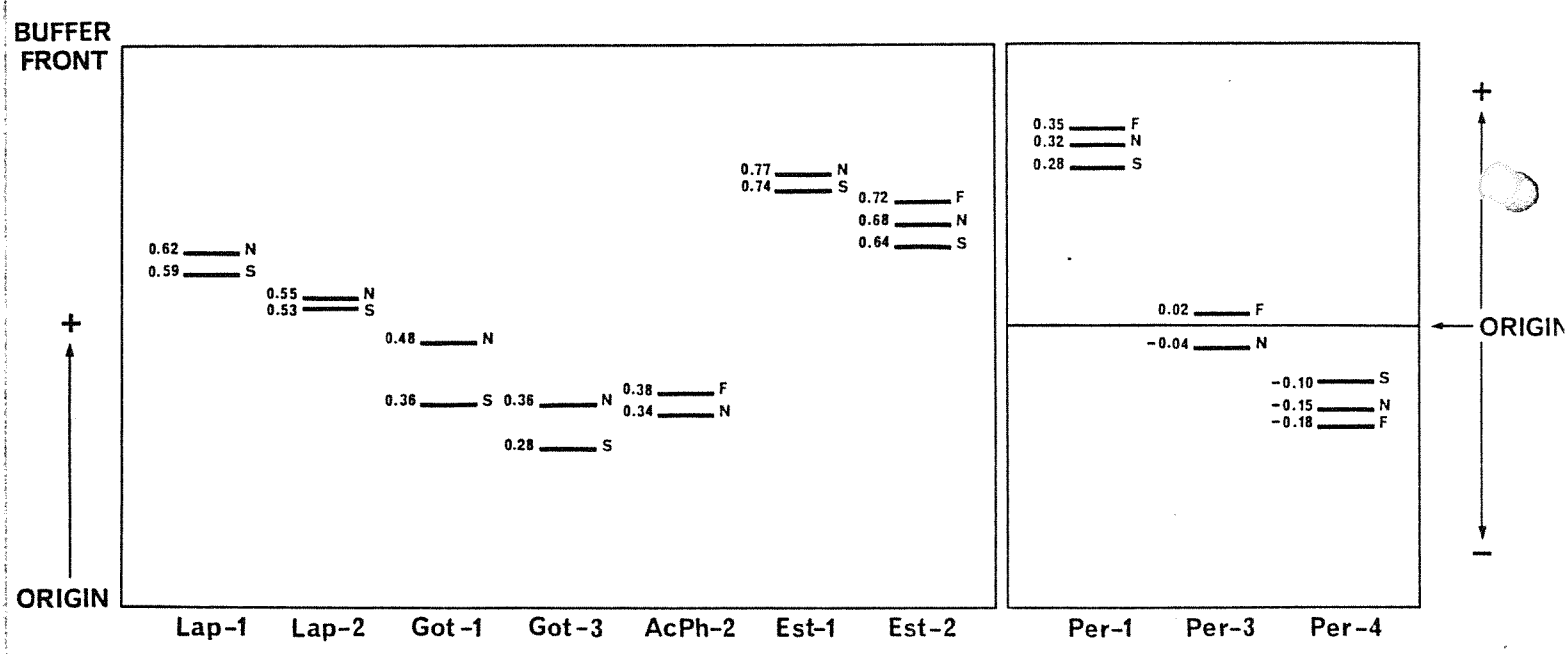

Fig.1 shows the electrophoretic variants for different enzyme systems that we have studied so far. The relative electrophoretic mobilities of these bands are also given. For any one enzyme system, the isozyme variant which is the most common among the 17 geographic races, has been called the normal (N) allele. Other allelic variants (all codominant) as determined by genetic analyses, are designated S (slow) or F (fast) depending on their migration relative to the N band. On the basis of these analyses, it has been possible to characterize each race at ten different isozyme loci, and these results are summarised in Table 1. This information, combined with further analyses on variation at other loci, would be useful for the purposes of constructing detailed linkage maps.

A word of caution should be given here. The races or ecotypes, used in the present study, have been maintained as single plant progenies for a number of generations. Thus, any one race named after the place of collection may have little resemblance with the population from which it is sampled and, as also pointed out by RÉDEI (1970), the designation of a race may have different meanings in different laboratories. Therefore, the results reported here are good only for our collection of races, and other research workers will be well advised to compare their results with ours before designing any further experiments.

Locus / Race	Lap-1	Lap-2	Got-3	Got-1	AcPh-2	Per-3	Per-4	Est-2	Est-1	Per-1
GR	S	N	N	N	N	N	S	F	S	N
RLD	N	N	N	N	F	N	F	S	N	N
SA	N	S	N	N	N	N	N	S	S	N
ANT	N	N	N	N	N	N	N	F	S	N
BD	N	N	N	N	N	N	N	N	S	N
BE	N	N	S	N	N	F	F	S	S	N
BR	S	N	N	N	N	N	F	N	N	S
DA	N	N	N	N	N	N	F	N	S	N
ER	N	S	N	N	N	N	N	N	N	F
EST	S	N	N	N	N	N	S	F	S	N
FR	S	N	N	N	N	N	N	N	N	S
HI	N	N	N	N	N	N	F	S	S	N
KOL	S	N	S	N	N	N	N	N	N	N
KS	N	N	N	S	N	N	N	N	N	N
MARB	N	N	S	N	N	N	N	N	N	N
MT	N	N	N	N	N	N	S	S	S	N
TA	N	N	N	N	N	N	N	S	N	N

Table 1. Allelic constitution of 17 geographic races of Arabidopsis thaliana homozygous with respect to ten isozyme loci. N, S and F refer to normal, slow and fast alleles respectively, at each locus.

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Arabidopsis thaliana (L.)HEYNH.as an object for studying population structure of predominantly self-pollinating plant species.

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In an extensive series of papers starting in 1960 (JAIN and ALLARD, 1960), a group of workers of University of California, Davis, have subjected to a penetrating analysis not only the theoretical principles governing the population structure of predominantly self-pollinating plant species but also the statics and dynamics of artificial and natural populations of those species as, f.i., Hordeum vulgare, Triticum aestivum, Phaseolus lunatus, Avena fatua, Festuca microstachys, and others. The most interesting conclusion was that the empirical genotypic frequencies corresponded to a common effect of a low proportion of random cross-fertilization and of definite selection pressures favouring heterozygotes.

Some natural populations were found to be in equilibrium at high frequencies of heterozygotes and thus in the condition of balanced polymorphism. In this way, it was possible to demonstrate that the genetic evolutional potential of predominantly self-pollinating plant species compared with the cross-fertilizing ones is controlled by qualitatively same mechanisms.

Three hypotheses are connected with this model: (1) the low proportion of random cross-fertilization; (2) the selection pressures against homozygotes; (3) the equilibrium condition at $H > 0$ in some natural populations.

In our experiments with more than 200 samples of natural populations of Arabidopsis from MORAVIA carried out since 1963 it was shown that there exists in the genetic determination of the developmental rate not only a large geographical variability among populations but also a high degree of variability both between and within lines derived of them. The geographical variability is clinal the late flowering genotypes being bound to the lowland with relatively high temperatures. On these localities, selection pressures exist against early flowering genotypes, the latter being, on the contrary, bound to the highland localities with lower temperatures. An extensive transitional zone of "mixed" populations appears in this geographical-climatical gradient. The "mixed" populations show an extremely high degree of variability in the flowering time both between and within lines (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1969). The genetic character of this variability can be demonstrated by high values of coefficients of heritability (DOBROVOLNÁ, 1969). Alleles for late flowering are mostly dominant over those for early flowering (EFFMERTOVÁ and CETL, 1968). By means of comparison with homozygous lines it can be shown that often up to 70 per cent lines are segregating for the flowering time and thus a very high proportion of heterozygotes is suggested (DOBROVOLNÁ, 1967).

Unfortunately, the number of loci and alleles responsible for the differences in the flowering time is unknown (KARLOVSKÁ, 1974), and more simple model situations must be chosen to analyze the population structure of Arabidopsis.

If two recessive mutants were sown densely (9 cm^2 per plant) in alternating rows, and complementing standard individuals were counted in the following generation, the proportion of cross-fertilization was found to be 1.96 ± 1.24 per cent. Under conditions favouring cross-fertilization (artificial motion of flowering plants), the proportion of random crossing rose to 4.24 ± 3.34 per cent. Thus, in consent with previous studies, Arabidopsis thaliana goes with other self-fertilizing plant species with a low frequency of spontaneous random crossing.

In the experiments carried out to determine the possible selection advantage of heterozygotes, recessive chlorophyll mutations lethal in the seedling stage were used. Eight artificial populations carrying different mutant alleles each were started with seeds harvested from heterozygotes, $\underline{ch^+ch}$, so that the initial frequency of heterozygotes was $H_o = 1$. The embryo test (MÜLLER, 1963) made possible to distinguish between dominant homozygotes and heterozygotes and to determine the frequencies of both surviving genotypes. In each following generation, a random sample of seeds of previous generation was sown.

Frequencies of heterozygotes expected in each successive generation under absence of any selection were compared with those actually observed. At the same time, relative viabilities (v_1) and adaptive values (w_1) the latter being calculated as a product of relative viability and relative fertility ($w_1 = v_1 \cdot f_1$) were estimated in the dominant homozygote $\underline{ch^+ch^+}$ when all these values in the heterozygote were put equal 1. Table 1 shows that in seven of eight populations studied, selection favouring heterozygotes was present. Only in the population carrying the ch 42 allele selection against the homozygote $\underline{ch^+ch^+}$ was not proved. In some populations (f.i.,with ch 2411), the v_1 and w_2 values were stable while in others (f.i., with ch 4062), they diminished from generation to generation. As the frequency of heterozygotes decreased with each following generation this downward tendency of v_1 and w_1 values might be explained by means of the frequency-dependent selection (JAIN and JAIN, 1970).

Alleles	Generations			
	1	2	3	4
	H_1 v_1; w_1	H_2 v_1; w_1	H_3 v_1; w_1	H_4 v_1; w_1
	Expected			
	0.6667 1.00; 1.00	0.4 1.00; 1.00	0.2222 1.00; 1.00	0.1176 1.00; 1.00
	Observed			
ch 42	0.6029 1.32; 1.40	0.3919 1.03; 1.08	-	-
ch 2411	0.7054 0.84; 0.79	0.4661 0.76; 0.71	0.2634 0.80; 0.71	-
ch 2040	0.6629 1.02; 1.11	0.5351 0.57; 0.62	0.4144 0.40; 0.44	0.2680 0.36; 0.39
ch 4062	0.6667 1.00; 0.93	0.4755 0.74; 0.69	0.5461 0.24; 0.22	0.3704 0.23; 0.21
ch 1467	0.7353 0.72; 0.54	0.5359 0.58; 0.44	-	-
X 21-4	0.8031 0.49; 0.49	0.5176 0.62; 0.62	-	-
X 21-53	0.7544 0.65; 0.46	0.4419 0.84; 0.59	-	-
X 28-19	0.8607 0.32; 0.26	0.5333 0.58; 0.46	-	-

Table 1.

Expected and observed frequencies of heterozygotes $\underline{ch^+ch}$, H_1 to H_4, with relative viabilities, v_1, and adaptive values, w_1, of dominant homozygotes $\underline{ch^+ch^+}$.

It is yet difficult to predict whether balanced polymorphism might arise in some populations. It is known (CETL, 1974) that w_1 values lower than 0.5 are necessary to attain equilibrium at $H > 0$ and to avoid fixation of the ch^+ allele. Our data suggest that this possibility is not excluded in the case of populations with ch 2040, ch 4062, ch 1467, and X 28-19.

A numerous group exists among "mixed" populations whose frequency of early homozygous recessive genotypes amounts from few tenths per cent to about 20 per cent. The members of this group occupy a narrow range of localities on the outmost S-E extremities of the mountains called ČESKOMORAVSKÁ VRCHOVINA. They are scarcely affected by anthropic influences, and complete selection against early genotypes take place in them. One of these populations, HV-3, was found to be in equilibrium (EFFMERTOVÁ and CETL, 1968). As the frequency of heterozygotes was also known it was easy, assuming complete self-fertilization, to estimate the adaptive value of the dominant homozygote, $w_1 = 0.46$. Under the assumption that also other members of this group are in equilibrium, we attempted to determine the w_1 values in them, using several ways of estimation. Similar values were found. But with the increasing proportion of early genotypes the estimated w_1 values decreased and rapidly came to zero just at 25p.c.early genotypes, i.e., at the upper limit of this group. The estimated values for this entire group are thus always lower than 0.5. In this way, the deciding condition is fulfilled to attain equilibrium at $H > 0$ and pass to the condition of balanced polymorphism. As the estimated w_1 values approach rather 0.5 than 0, the equilibrium is attained very slowly and thus these populations must be of long standing.

Our results confirm that Arabidopsis thaliana proved competent to be used in testing all three above hypotheses.

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Further analysis of heterosis and its expression for the rosette diameter length in Arabidopsis thaliana (L.)HEYNH.

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This paper continues the first publication in AIS (EL ASMI, 1974). It describes experiences conducted as incomplete diallel crosses (GRIFFING, 1956, methode 2: direct crosses and parents only used) which has been realized for five inbred lines of Arabidopsis thaliana including A_5 (Dijon, France), A_9 (Eastland, Baltique), A_{64} (Edinburgh, Scotland), A_{51} (Bologna, Italia), A_{80} (Turin, Italia).

We must summarize briefly, that this experiment has been conducted as a complete randomized block design under artificial light of 8000 lux during 16 hours a day. Some of those crosses had shown a very important heterosis, examined on the rosette diameter length, 19 days after sowing (EL ASMI, 1974).

In the present paper, we intend to give some further results which are very important in relation to heterosis expression in the following stages of growth in those plants observed.

For this purpose, the rosette diameter length (cm) has been measured each two days. This operation has been realized regularly from sowing to the stage at which diameter length is stopped in its growth (about 50 days after sowing). Observations had been made in the hybrids as well as in the parents, with in each case about 16 repetitions (blocks). Those results yield, in each case, a good statistic mean for the study of the expression of the diameter length at this period.

Figure 1 shows the growth of the hybrid (A_9 x A_{51}) comparatively to their parents. Values of the graph are the mean of the repeated measures obtained in each case. Figure 2 shows the growth in the cross (A_9 x A_{80}). We have choosen these two crosses as an example and particulary because of their difference in spite of using the same parent A_9. The two graphs show with great evidence that in the case of the hybrid A_9 x A_{80} as well as in the case of A_9 x A_{51}, growth rate is always superior to that of the parents in each case and this difference remains in the same manner from the beginning of the growth to the stabilization of the diameter length at the final observation (about 50 days after sowing).

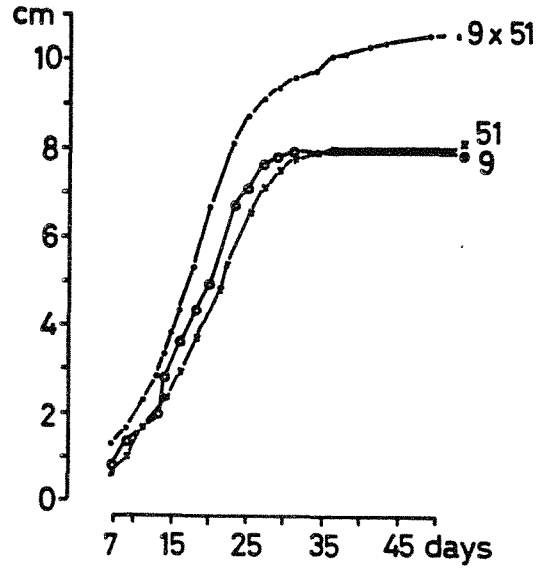

Figure 1: growth of the rosette diameter length hybrid $A_9 \times A_{51}$

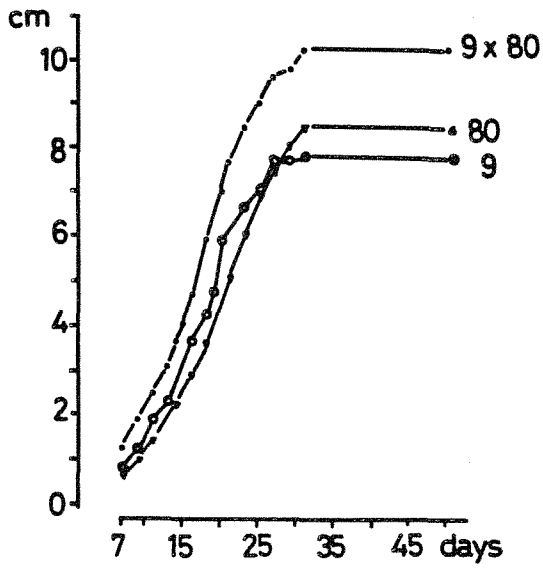

Figure 2: growth of the rosette diameter length hybrid $A_9 \times A_{80}$

Careful quantitative examination of differences between the hybrids and their parents for the rosette diameter length at the final stage of growth gives many important results as follows. Table 1 shows the means of the rosette diameter length, according to the incomplete diallel model described.

	A_5	A_9	A_{51}	A_{64}	A_{80}
A_5	7.43	8.13	10.48	7.34	6.06
A_9		7.64	10.53	8.81	9.71
A_{51}			7.76	9.24	9.18
A_{64}				6.30	8.78
A_{80}					8.38

Table 1: Rosette diameter length (cm) (mean value, 50 days after sowing)

As it has been studied for the rosette diameter length at an early stage (EL ASMI, 1974) we have estimated the quantity of heterosis expressed on the phenotype according to FALCONER (1960)

$$H_{F_1} = m_{F_1} = \frac{1}{2}(m_1 + m_2)$$

These results and their analysis are summarized in table 2. Comparisons of the means according to a t-test of Student has been executed between the hybrid mean and the mid parent in each case as follows:

The significance of the t-test is also indicated:

t_{50} : comparison for the results quoted above

t_{19} : comparison for the results exposed in A.I.S.no.11.

hybrids	$A_5 \times A_{80}$	$A_5 \times A_{64}$	$A_5 \times A_9$	$A_{51} \times A_{80}$	$A_{64} \times A_{80}$	$A_9 \times A_{80}$	$A_9 \times A_{64}$	$A_{51} \times A_{64}$	$A_9 \times A_{51}$	$A_5 \times A_{51}$
H_{F_1}	-1.850	+0.469	+0.590	+1.122	+1.443	+1.697	+1.834	2.219	2.831	2.884
%	-23.4	6.828	7.824	13.91	19.665	21.189	26.305	31.531	36.766	37.962
t_{50}		N S	N S	T S	H S	H S	H S	H S	H S	H S
t_{19}	N S	S	N S	T S	H S	H S	S	H S	H S	H S

Table 2: Analysis of the quantity of heterosis

(N S :not significant, S : significant at 5%, T S : at 1% and H S : at 1‰)

The data of table 2 show for each cross the superiority expressed in 1% of the hybrid mean relatively to its parent's mean value with the exception of $A_5 \times A_{80}$. From these results we can see easily that the same effect of heterosis is significant for the two stages. There is no difference if we compare with the significance at 19 days. According to these results we must conclude that the heterosis expressed by the hybrid genotype has a clear advantage relatively to the parents (i.e.it is maintained as such) from the beginning until the final stages of growth. It doesn't disappear during growth and on the contrary, differences between the hybrid and parent's mean value increase positively.

Furthermore there is an important positive correlation between the rosette diameter length at 19 days and the final diameter for the ten hybrids presented as well as for the quantity of heterosis expressed by H_{F_1} as it is shown in Figure 3.

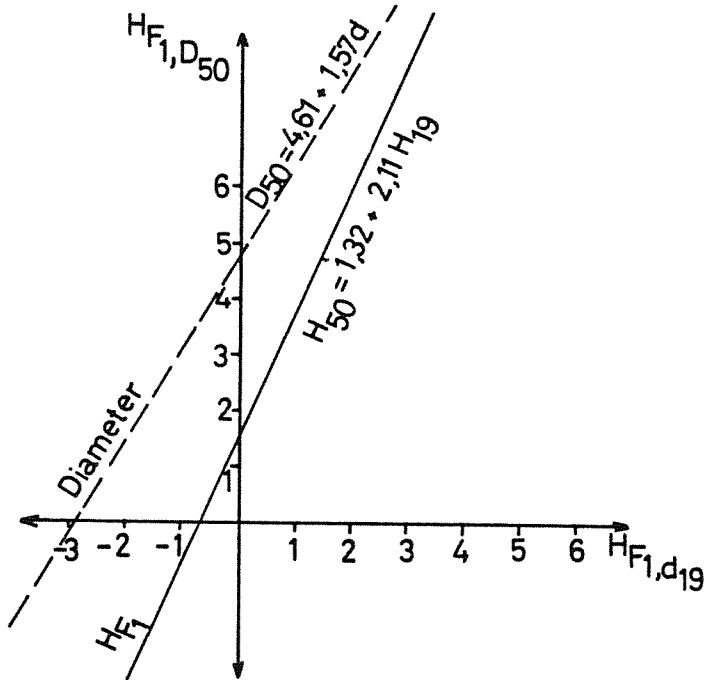

Figure 3: Final stage linear regressions for H_{F_1} and diameter length on 19 days measurements.

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B. TECHNIQUES

"In vitro" culture of Arabidopsis thaliana

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To use Arabidopsis as a model system in somatic cell genetics of higher plants some prerequisites must be fulfilled:

- establishment of calli and cell cultures;
- possibility to regenerate a plant from a tissue culture;
- isolation of haploid tissue by means of anther culture;
- isolation and culture of protoplasts which should allow fusion between somatic cells.

Some results in this direction have already been mentioned in Arabid.Inf.Serv.(ANAND, 1966; LOEWENBERG, 1967; HOWELL, 1969; CORCOS and MÜLLER, 1972; CORCOS, PIPER and LEWIS, 1973; GRESSHOFF and DOY, 1973).

We report here some complementary data.

1. Callus induction and culture experiments were performed on GAMBORG's B5 medium (GAMBORG and EVELEIGH, 1968). The amount and the growth of the callus depend on the cultured organ (stem and leaf pieces, seeds, anthers), hormone composition and ratio (2,4D, NAA, IAA and kinetin) and the race used in the experiment.

In leaf, but mainly in seeds there is a poor callus formation (up to 47% and 22% respectively) at the following low 2,4D concentrations: 0.05 and 0.5 mg/l. In stems the production of calli is above 90% at all 2,4D concentrations tested, i.e. from 0.05 to 2 mg/l, but it is only above 1 mg/l 2,4D that the callus looks completely undifferentiated. Leaves require a high 2,4D concentration, 2 mg/l, for good callus induction.

The induction time is shorter for stems (9 days) than for seeds (up to 18 days).

With seeds better growth was obtained on B5 medium including 2% glucose instead of 2% sucrose as carbon source, in the presence of 2,4D with or without kinetin.

On GRESSHOFF and DOY medium, using more than 10 various hormonal combinations (NAA, IAA, 2,4D and kinetin), we induced callus from seeds and stems with abnormal appearance and structure unsuitable for growth in suspension culture. Among the races we have tested, Columbia, Coimbra, Estland and Chisdra gave the best callus induction, i.e. 100% germinated seeds lead to the development of calli.

In anther culture, performed according to the method described by GRESSHOFF and DOY (1972), 65%, 56% and 54% callus induction was obtained for Martuba, Estland and Wassilewskije respectively. No haploid lines were isolated until now.

2. Suspension culture. In a series of experiments we used heavy liquid inocula in a ratio of 1:1 to the fresh medium*, initiating several growing cycles suspension cultures. For practical reasons growth was measured using cell packed volume (CPV)** and the number of viable cells/ml. The subcultures were done by replacing 45 to 60 per cent of the suspension "supernatant" CPV with fresh medium to the final volume of 40 ml. This subculture system enable us to exploit the suspension cultures as sources of actively growing single cell suspensions (see table 1).

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* a modified B_5, containing 2% glucose, two times concentrated GRESSHOFF and DOY MS_1 solution, and 0.05 mg/l kinetin

** using an 0.35 CPV factor (i.e. 35 g tissue are contained in 100 ml CPV) we express volumetric measurements in grams

Table 1. Growth rates in established cell suspensions, subcultured every 8 to 10 days in a modified B_5 medium.

Cycle of growth	Growth rates			Generation time (days)
	as CPV in ml/day/40ml susp.culture	in g/1/day (factor 0.35)	as CPV, during the exponential phase	
1	1.00	8.75	1.50	2.60
2	1.10	9.62	1.40	7.30
3	0.55	4.80	1.45	18.00

In spite of the fact that growth rates vary with the growing cycle these rates remain rather constant for the log portion of the curve. It is precisely the duration of the log phase which is varying in inverse ratio to the biomass.

When growth is favoured by optimum inocula rather short generation times can be obtained.

At higher biomasses (30 per cent and more CPV of the whole culture volume) dissociation is favoured in suspension cultures. In such established cell suspensions there is a balance between cell division and cell expansion, i.e.between growth and dispersion, the number of viable cells/ml reaching values as high as 7.5×10^5 cells/ml. Meanwhile, cell densities can reach values of 5×10^6 cells/ml. Cell separation and dissociation of cell aggregates are variable characters among different lines used in the experiments.

Depending on the number of viable cells/ml, amount of tissues in culture and increase in biomass, the duration of a growing cycle is regulated from case to case.

3. Karyology of callus and cell suspension. A classical FEULGEN staining method was used for chromosome countings. In our conditions the tested callus cells showed heteroploidy, but 2,4D concentrations (0.05 to 2 mg/l) did not influence the range of the chromosome number variation. The age of the callus seems to have an influence on the ploidy level. In 3 weeks old seed induced callus an average of 65% diploid cells was found, while after three months of subculture diploidy dropped to a mean of 20%.

When different plant organs were used, leaf and especially stem pieces showed a high degree of polyploidy after only 3 to 4 weeks in culture (44% diploid cells and 23.9% respectively).

Media were then supplied with different auxins as 2,4D (NAA and IAA respectively). It seems now that natural auxins in the presence of kinetin at very low concentration can produce a reasonable percentage of diploid tissue; for ex., with 8 mg/l NAA + 0.01 mg/l kinetin there were as much as 70% of diploid primary calluses, while with 2 mg/L 2,4D + 0.01 mg/L kinetin only 20% of the checked primary calluses showed the diploid chromosome number. However, the properties of the calluses obtained in these conditions are not so favourable to initiate suspension cultures then on a modified B_5 medium. At high kinetin concentrations (1.5 mg/l) high ploidy levels were registered.

4. Protoplasts have been obtained from mesophyll of full-grown leaves, callus tissue and cells in suspension culture. Enzymes used were mixtures of meicelase 2% and macerozyme 0.3% in case of leaf material, driselase 1.5% or cellulase R10 2% and macerozyme R10 1% in case of cells from callus and suspension cultures. In the last case the necessary incubation time and the amount of released protoplasts appeared to depend on age of cells and culture conditions.

Young, actively dividing cells in callus and cell suspension give the best release of protoplasts within the shortest incubation time. Incubation took place on a slow moving gyrotory shaker (30-50t/min), during 3-6 hours in case of the cellulase R10-macerozyme R-10 mixture; 1/5 - 4 hours in case of the riselase-macerozyme R10 mixture, and, not shaken, up to 16 hours for the leaf protoplasts. Although driselase seemed to digest the cell walls within a shorter time period, the released protoplasts were not very stable and showed less resistance in the next steps of centrifugation. A washing and culture solution mainly B_5 medium was used to which was added sucrose 1.5%, sorbitol 6%, mannitol 6%, $CaCl_2$ 4.3 mM, 2,4D 1mg/l, at pH 5.8.

The protoplasts stay alive for some weeks, however up to now no cell divisions have been observed. The osmotic agents sorbitol and mannitol have shown a strong inhibition effect on growth in cell suspension culture. Other agents are being investigated.

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Cultures of Arabidopsis thaliana cuttings

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Successful cloning defined as a multiplication of an identical genotype to an indefinite number of fully developed copies has been achieved so far by REINHOLZ, E. (1972). She obtained vegetative reproduction by shifting plants from the generative to the vegetative phase applying short day conditions. Flower buds thus developed a small leaf rosette. When these rosettes were planted in soil and exposed to long day conditions, they gave rise to normal inflorescences. Since we did not succeed in repeating REINHOLZ result with genotypes at our disposal another approach, starting from Arabidopsis cuttings, was attempted.

Arabidopsis thaliana (L.) Heyhn race "Go"-o" were grown under long day conditions (19 h artificial light per day) at 24°C ± 1°C and a relative humidity between 90% and 70%. The minimal medium is basically that of HOAGLAND and ARNON (JACOBS 1964) supplemented with 0,5% glucose, 2,5 ppm fungicide (carbendal) and solidified with 0,8 % agar. Shoot cuttings were collected at the time the petals were distinctly visible or fully open and taken just above the rosette leaves, while the shoot leaves were facultatively eliminated.

In our experiments a synthetic auxine, PRB-8 (ORTHONIL or α-chloro-β- (3-chloro-o-tolyl)-propionitrile) was used since its assimilation is considerably higher compaired to other growth regulators (DIERICKX, P.J., VENDRIG, J.C., 1973, 1974).

Cuttings held under the above mentioned conditions and continously exposed to 5 ppm PRB-8 showed callus and root development respectively after two and three weeks.
When the cuttings were held in a double strength PRB-8 solution for a 24 hour period, root development was observed without prior production of callus. Although rooting was not dependent on the presence of leaves, a direct influence on the rate of growth became evident.

With respect to reproduction, the further development of the inflorescence was seen to be dependant upon the phase of florescence at cutting. In case the petals became visible at the time of cutting further growth of the main shoot was completely inhibited, followed by an abundant proliferation of the lateral shoots. A different pattern was observed when the cuttings were taken at a later phase of florescence. Although the main and lateral shoots showed a further development, their fructification had been altered. Flower buds present at the time of cutting grew out without bearing fruits, while the fructification process appears to be normal through the newly formed flower buds.

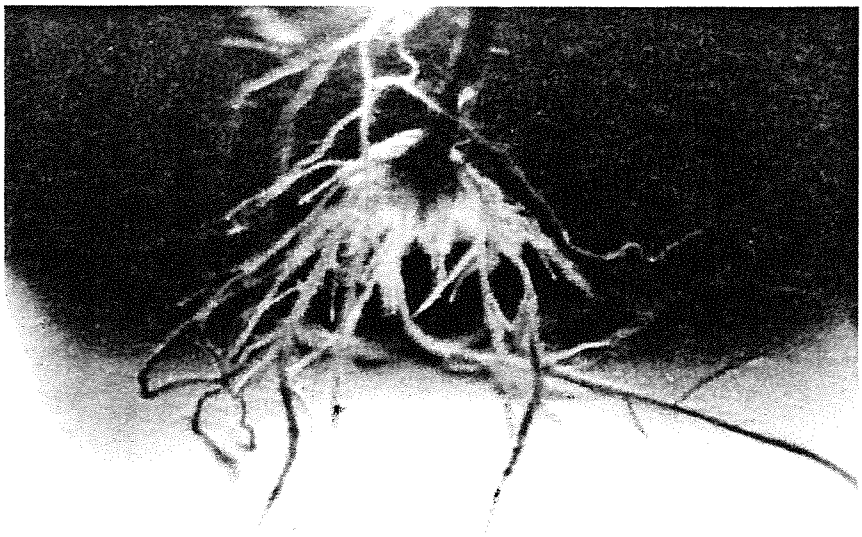

Figure 1: Root development in a four week old culture of a cutting treated with FAB-8 (10 ppm) for 24 hours.

A similar pattern was observed on a lateral shoot cutting. Consequently, this method may be considered as useful for vegetatitve reproduction of Arabidopsis thaliana.

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Investigations on Arabidopsis in the Apollo-Soyuz Test Program

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The common US-Soviet Apollo-Soyuz Test Program starts on July 15, 1975. As at the last two Apollo flights (Apollo 16 and 17) the Biostack experiment will be included. The objective of the Biostack experiment is to study the biological effects of individual heavy particles (HZE-particles) of cosmic radiation. Several kinds of biological subjects were selected e.g.: Bacillus subtilis spores, Artemia salina eggs, Tribolium castaneum eggs and as plant material seeds of Arabidopsis thaliana En-2.

The Biostack consists of a series of monolayers of biological subjects with each layer sandwiched between several different physical track detectors. The stack is stored in a hermetically sealed cylindrical aluminium container, which is placed in the Apollo-command module. The biological material is embedded in water soluble foils of polyvinyl-alcohol (PVA) before placement into the Biostack. REINHOLZ proposed Arabidopsis thaliana for this experiment. The embedding of Arabidopsis seeds in PVA has no influence on germination and growth even after storage up to 18 months (REINHOLZ, 1972). The biological layers are either in contact with nuclear emulsions or cellulose nitrate (CN)-sheets. The nuclear emulsions which were attached to a biological layer, carry an optical photo of the distribution pattern in the biological layer and of coordinate grid in

addition to the tracks of the penetrated particles. Microscopical analysis indicate the hit biological objects. In case of CN-sheets the PVA, in which the Arabidopsis seeds are embedded is directly poured on the detector as carrier. The fixed contact is maintained during the procedures of processing and scanning of the detector. The correlation of hit seeds and tracks can be done microscopically without changing this physical relationship considering some geometrical properties.

In the Biostack experiments I and II (Apollo 16 and 17) layers of Arabidopsis seeds were flown in contact with nuclear emulsions. Hit, unhit flown seeds and ground controls were separately examined by REINHOLZ (BÜCKER et al., 1973). The germination of the hit seeds seems to be delayed. Further studies on more seeds are necessary to ensure this effect statistically. Moreover multicaulity was observed by the hit seeds and occasionally by the unhit flown seeds. Growth, flowering time and fruiting time were not significantly influenced.

For the Apollo-Soyuz flight 1975 the following improvals were made:

I. A computer-program was established to determine the hit regions within the seed. This was done by approximating the shape of the seeds as rotational ellipsoids.

II. A new embedding procedure was developed to prevent the conglobation of the seeds during drying of the PVA-layer.

1. A lifting plate with a petri dish cover spread with Parafilm will be moved against a stretched fine nylon net attached on statives. The width of one square of the net and the thickness of the net is in agreement with the size of the seed.
2. Seeds are distributed on the net with a piece of spatula sized paper.
3. The lifting plate will be lowered. Now the individual seeds are lined up uniformely. Because of their rough surface they adhere in the Parafilm.
4. A CN-sheet covered with PVA (10%, thickness 200-300 u)which is not hard yet, has to be rolled on the Parafilm with the seeds. The seeds are sticking now in the PVA. After drying of the PVA the seeds were embedded with PVA (15%).

By this method a layer with a diameter from 7 cm is covered with 2000 - 2500 seeds.

After the flight, the hit Arabidopsis seeds will be devided into three groups comprising seeds which are hit either in the radicula, or in the cotyledons, or in the shoot apex. Development of the M_1- plants will be measured periodically. Twenty descendants of each M_1- plant will be observed in regard to morphological changes during their growth and development. In order to state whether the observed changes are due to mutations the progeny of the morphologically changed plants will be examined again. Biochemical mutations will be detected during growth in synthetic nutrient medium.

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C. INFORMATION

IMPORTANT EDITORIAL INFORMATIONS

In order to save in the future costs and labour the following reminder is necessary:

i) no further requests for manuscripts and orders of AIS will be sent out annually, As indicated on the inside of the cover page of each volume your contribution will be expected not later than February 15, each year.

ii) the editor expects that the contributors will follow in detail informations given on that inside cover page. Costs and labour are substantially reduced, if you are typing your manuscript analogous to the size (DIN A 3) of the specimen mailed with this volume.

ERRATA
of "Arabidopsis Information Service" No.11
The following text should be added:
page 21, line 26:
R e s u l t e s

Very similar results had been obtained in barley cells (BOUNIAS, 1972a) and figure (1c) shows radioactive sites into isolated chloroplasts, after incorporation of ^{14}C-leucine. Then, on figure 1d it appears that the alkaline phosphatase activity (E.C. 3.1.3.1.) revealed by the GOMORI technique is also located in peripheric and intra chloroplastic grana; the main part of the enzyme activity being concentrated in the sole chloroplasts (BOUNIAS, 1972a).

D i s c u s s i o n

Previous experiments gave evidence that L-leucine is an effector for the regulation of alkaline phosphatase activity and photosynthetic apparatus (BOUNIAS, 1972a, BOUNIAS and PACHECO, 1972 and 1974). Thus, it appears that such a role of L-leucine is possible because of the same localization of the amino acid, enzyme and photosynthetic pigments into chloroplastic sites.

BACK VOLUMES OF AIS

The supply of AIS No.1, 2, 3, 4, 6 and copies of "Arabidopsis Research" (Rep.Intern.Symp., Göttingen 1965) are entirely out of order. Only a few copies of the other AIS-volumes are still available. This will be distributed at cost price by the editor.

BIBLIOGRAPHY

In order to continue the list of articles pertaining to Arabidopsis the editor expects, that the contributors will send

i) special printings of their available publications, and
ii) a continued list of their publications.

References should be of the form as indicated under D. Bibliography of that inside page 34.

A SECOND INTERNATIONAL SYMPOSIUM ON ARABIDOPSIS RESEARCH FOR SUMMER 1976

The First International Symposium on Arabidopsis Research was organized in 1965. It was felt at the time that a forum for contact in Arabidopsis research was needed and so was created - Arabidopsis Information Service. Since ten years have elapsed, it may be useful to all Arabidopsis research workers and to those interested in the progress of AIS to make personal contacts during a second international meeting. Though the preparations for this second symposium will come at a period of world-wide economic problems we should not be disconraged.

Since the secretariat of AIS is stationed at Frankfurt/Main, the Editor and an executive board has planned to organize this meeting in that city. Frankfurt/Main is highly suitable for international meetings because of its central geographic location in Europe and its overall convenient connections by trains, cars, and planes. Furthermore, the location is highly appropriate since it was there that Arabidopsis research started 40 years ago.

An excellent reason for a second meeting on Arabidopsis research is that important national and international organizations, in particular the International Union of Biological Sciences (IUBS)have decided the following:

1. "To make a thorough study of, and to take active steps to render appropriate assistance to, on-going and new international activities in the following fields:
   a. The preservation of genetic diversity in selected natural areas;
   b. Culture collection of micro-organisms, cells, tissues, etc.;
   c. Living collections (including seed banks) in botanical and zoological gardens, agricultural institutes, etc.;
   d. Efforts to survey and catalogue the diversity in organisms.
   e. Dissemination and their availability for use for human benefit.
2. To assure the free movement of biologists and free circulation of scientists in order to serve the objective of biology."

Arabidopsis gives an excellent example of these activities. IUBS is convinced of this and will support the meeting by a grant. Other institutions have been contacted to get additional support.

Attractive topics of interest could be the following:

1. Experimentation (gene-enzyme systems, somatic cell genetics, mutagenesis, cosmobiology, quantitative genetics, photomorphogenesis and growth regulation, photosynthesis and productivity, etc.)
2. Exploration (population studies, cytogenetic relationships, ecological adaptations, plant community studies, etc.)
3. Conservation (preservation and documentation of collections, organization of seed banks for mutants and population samples, etc.)
4. Information (Arabidopsis as a subject in courses, computerized bibliography, new techniques with Arabidopsis)

Please, let me know as soon as possible your main field of interest and of probable participation.

The exact time period of the symposium has not been scheduled as yet. The best time of the year at Frankfurt/Main is spring and early summer.

A.R.Kranz

D. BIBLIOGRAPHY

(Seventh addition to the list compiled in A.I.S. No.5)

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E. ANNOUNCEMENT

Meeting of Scientists interested in Arabidopsis

During the XIIth International Botanical Congress, held in Leningrad (USSR), during July 3 - 10, 1975 an informal meeting is scheduled in order to give the possibility for discussion on present Arabidopsis research activities. This is to inform you that

1) the report of Arabidopsis included in the programme of the symposium "Origin of the cultivated plants" will take place on July 5, 10 a.m.in Tavrid Palace audit.119, and

2) the report-demonstration will take place on the same day and place at 6 p.m., audit.114.

For details, please, pay attention to the announcement at the boards of the Congress Registration Office or contact Drs.D.V.TER AVANESIAN or KRANZ.