ARABIDOPSIS INFORMATION SERVICE

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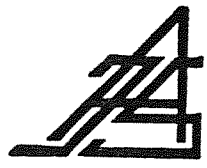

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A R A B I D O P S I S

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

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G. RÖBBELEN

A. BRIEF NOTES

Application of multivariate statistical methods for the investigation of quantitative characters of natural populations of Arabidopsis thaliana (L.) HEYNH.

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Multivariate statistical methods allow the quantitative description of populations. One of the important statistical parameters is the correlation coefficient r, which estimates the degree of conjugation for variations of population characters. In evolution, particular organism-environment interrelations create the prerequisite for the formation of correlation galaxies, i.e. the presence of correlations within complexes of characters in the absence of correlations between characters of different complexes. Correlation galaxies exist at different levels: ontogenetical, specific, biocenological, etc. Ecological reasons for the formation of correlation galaxies during the natural selection process and the general principle of the occurrence of independence for individual character is formulated by R.L. BERG (1964).

Suppose a population of individuals is described by n-characters: $x_1, x_2, \ldots, x_n$. Let the new character, presenting a linear combination of initial characters

$$w_1 = b_1x_1 + b_2x_2 + \ldots + b_nx_n$$

be the first factor. The coefficients $b_1, b_2, \ldots, b_n$ are to be found from the condition of maximum correlation of w_1 with all the initial characters, i.e. we obtain the maximum

$$[r(w_1,x_1)]^2 + [r(w_1,x_2)]^2 + \ldots + [r(w_1,x_n)]^2$$

Evidently, $b_1, b_2, \ldots, b_n$ are the contributions of each of the initial characters in w_1. Let the second factor w_2 be a new character, introduced in the same way as w_1, but with the additional condition $r(w_1,w_2) = 0$. Let the third factor w_3 be another character, introduced similar to w_1 and w_2 but with the additional condition: $r(w_1,w_2) = 0$ and $r(w_2,w_3) = 0$. As the factors absorb the maximum information of the initial characters, the procedure allows to choose the most essential characters and groups of characters and aids in interpreting the biometrical investigation of populations.

Our investigations were based on a natural population of Arabidopsis thaliana (L.) HEYNH., race Enkheim. The plants were grown in testtubes on mineral medium (IVANOV et al., 1966). The testtubes were placed in randomised blocks under light installation. The studied quantitative characters and their principal statistical parameters are given in Table 1. All the statistical parameters have been calculated with high accuracy ($P < 0.01$).

Table 1: Principal statistical parameters of quantitative characters of a natural population of Arabidopsis thaliana

Characters	Parameters M ±	S_M	σ^2	C%	Correlation matrix 2	3	4	5	6	7	8
1. Germination (days)	2.6 ±	0.08	0.52	27.3	0.66	0.54	0.55	0.2	0	0	0
2. Appearance of rosette (days)	6.8 ±	0.14	1.61	18.6		0.64	0.62	0	0	0	0
3. Budding (days)	11.8 ±	0.14	1.72	11.1			0.69	0	0	0	0
4. Beginning of flowering (days)	18.2 ±	0.12	1.30	6.3				0	0	0	0
5. Height to the 1st silique (cm)	9.3 ±	0.16	2.11	15.5					0.2	0	0.3
6. Number of rosette leaves	7.5 ±	0.16	2.13	19.6						0	0
7. Number of siliques	8.8 ±	0.20	3.52	21.4							0
8. Number of side shoots	3.4 ±	0.11	1.08	30.8							

The total of the quantitative characters, describing the main development phases of the plants, forms a correlation galaxy of ontogenetical level. It can be assumed that close correlation is caused by the control of the coupled genetical factors over the characters.

Table 2 presents the data on factor analysis of quantitative characters for the investigated natural population.

Table 2: The data on factor analysis

Factors	Characters 1	2	3	4	5	6	7	8	Weight of factors (%)	Dispersion
w_1	0.81	0.87	0.85	0.82	-0.25	0	0	-0.20	36	2,9
w_2	0	0	0	0.26	0.74	0.62	0.29	0.62	18.6	1.5
w_3	0	0	0	0	-0.15	0.23	0.85	-0.47	12.9	1.0

In w_1 the contributions of the characters 1 to 4, describing the main development phases for Arabidopsis thaliana, are significantly higher than the contributions of the characters 5 to 8. Opposite to that, in w_2 the contributions of the vegetative characters 5,6 and 8 are large, and in w_3 the contribution of the character 7, typical for fertility, is large as well. This allows to give a new complex characteristics of Arabidopsis populations, and to interpret w_1 as "the development phase factor", w_2 as "the vegetation factor" and w_3 as "the fertility factor". These three factors absorb 68% of the information on the population.

References:
BERG, R.L.: Application of mathematical methods in biology. Leningrad University 2, 23-60 (1964)
IVANOV, V.I., A.G. KASYANENKO, A.V. SANINA, and N.A. TIMOFEEFF-RESSOVSKYA: Genetics 8, 57-70 (1966)

Estimations of heritability in an uniform natural population of Arabidopsis thaliana (L.) HEYNH.

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In our studies on the variability of some developmental characters of natural populations of Arabidopsis thaliana, a large majority of 134 population samples was found to be highly variable and only two of them were found to be uniform, showing narrow, continuous, unimodal and symmetric empirical distributions (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1969).

As it was stated, the corresponding heritability coefficients in typical variable populations were relatively high and significantly different from zero (DOBROVOLNÁ, 1968; 1969a,b). Thus, it appeared to be interesting to study similar relations in the rare uniform populations.

The experiment was carried out with the population Cha where the mean number of days to appearance of the flower primordia was 13.1, the standard deviation was ± 0.65 days, and the coefficient of variability 4.9 per cent. Progenies of 42 mother plants were studied on heritability in the same way as in our cited papers. The h^2 values were calculated (1) as a regression coefficient of individual progeny data on repeated maternal ones (DOBROVOLNÁ, 1968) and (2) as the intraclass correlation coefficient, W (DOBROVOLNÁ, 1969). For cultivation conditions see CETL, RELICHOVA-DOBROVOLNÁ, and KRSKOVÁ, 1970.

In the Table, results obtained in the uniform population Cha are given together with the earlier results obtained in a variable population Stř.

Table: The estimates of heritability in the number of days to appearance of the flower primordia in two natural populations, Cha and Stř

Population	Estimates of h^2 as (1) $b_{OP} \pm s_b$	(2) $W \pm s_W$
Cha	0.74 ± 0.06	0.49 ± 0.02
Stř	0.46 ± 0.02	0.57 ± 0.01

The values of b coefficients in both populations are high and significantly different from zero at $P < 0.001$. But in the uniform population Cha, this value was even significantly higher than in the highly variable population Stř. On the other hand, the W values are similar in both populations. This result suggests that also in an uniform population at least a half or more of the phenotypic differences between individuals are of genetic nature.

According to DOBROVOLNÁ (1969b), calculated b and W values correspond to estimates of h^2 in narrower sense so that the role of genes in determining this character may be still higher. Correspondingly, underestimation must be the larger, the more heterozygous plants with dominance or interactions are present in the population. For this reason, the high b value found in the uniform population Cha is comprehensible.

It seems that in uniform populations of Arabidopsis relatively the same amounts of genetic variability can be present as in variable ones.

References:
CETL, I., J. DOBROVOLNÁ, and E. EFFMERTOVÁ: Folia Fac.Sci.Nat.Univ.Purkynianae Brunensis, Biologia 22, 10 (3), 1-42 (1969)
- , J.RELICHOVÁ-DOBROVOLNÁ, and M. KRSKOVÁ: Arabid.Inf.Serv. 7, 35-36 (1970)
DOBROVOLNÁ, Jiřina: Arabid.Inf.Serv. 5, 15-16 (1968)
- : Arabid.Inf.Serv. 6, 20 (1969a)
- : Biologia Plantarum 11 (4), 310-318 (1969b)

The genetic behaviour of families derived from one natural population

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Our earlier studies with natural populations of Arabidopsis thaliana (L.) HEYNH. were based on mixed samples of seeds from 25 to 100 plants growing in one locality (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1969). In the present paper another method of sampling was used in a relatively early flowering population Mo-4: the seeds from individual plants at the same locality were harvested individually (cf. NAPP-ZINN, 1964; JONES, 1968). In 25 families sampled in this way the number of days to appearance of the flower primordia and other developmental characters were studied in successive generations under laboratory conditions (CETL, RELICHOVÁ-DOBROVOLNÁ, and KRSKOVÁ, 1970).

Three characteristic examples of results obtained with these families and their individual subfamilies (4 to 10 plants of each family were taken for the study of one subfamily) are given in the Figure. The families Nos. 4 and 8 were uniform

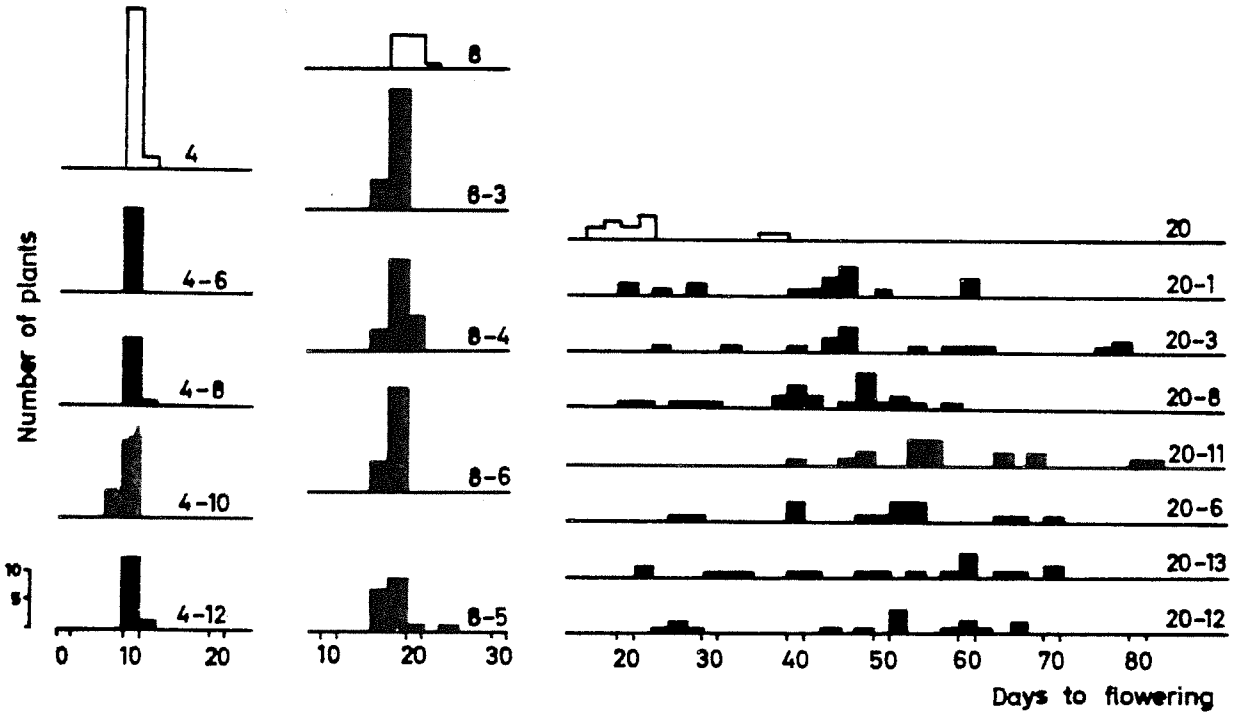

Figure: Distribution of the number of days to appearance of the flower primordia in three families (□) and in their corresponding subfamilies (■)

and this uniformity also recurred in their subfamilies. On the opposite, the family No. 20 can serve as an example of a relatively non-uniform family with clearly variable subfamilies. Among all 25 families studied, about a quarter was found to be of this non-uniform type. Few transitional cases were also present between the two extreme types of families. In this way, it seems to be possible to determine the proportion of uniform (= homozygous?) and of non-uniform (= heterozygous?) plants present in a natural population. At present, later generations of this material are studied, and corresponding analyses of variance are underway.

References:

CETL, I., J. DOBROVOLNÁ, and E. EFFMERTOVÁ: Folia Fac.Sci.Univ.Purkynianae Brunensis, Biologia 22, 10 (3), 1-42 (1969)

- , J. RELICHOVÁ-DOBROVOLNÁ, and M. KRSKOVÁ: Arabid.Inf.Serv. 7, 35-36 (1970)

JONES, M.E.: Arabid.Inf.Serv. 5, 11-13 (1968)

NAPP-ZINN, K.: Beiträge zur Phytologie, Festschr. H. WALTER. Pp. 39-49, 1964

The breeding system of Arabidopsis thaliana

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A number of more recent investigations of variation in natural progenies of Arabidopsis have cast some doubt on the long-held belief that the species is strictly autogamous (NAPP-ZINN, 1964; DOBROVOLNÁ, 1966; JONES, 1968, 1971; KARBE and RÖBBELEN, 1968). Since all of these investigations concern metrical characters, however, none provide an unambiguous test for the presence of heterozygotes in open-pollinated progenies. The present experiment was designed to provide such a test.

Each of 10 plants of the Wilna-2 inbred line was closely (6 cms) and completely surrounded by a ring of 6 plants of the LANGRIDGE line, making ten groups of seven plants in all. Five of these groups were placed, outdoors, in an open situation (block 1) and the remainder in a more sheltered place between a pair of glasshouses (block 2), the groups in both blocks being not less than 2 m apart. The Wilna-2 line breeds true for the recessive trait, glabrous leaves, while plants of the LANGRIDGE line exhibit the dominant, wild-type character, hairy leaves. The plants of both lines came into flower in the open at approximately the same time and were allowed to continue with the minimum of attendance until they died naturally. Plants of the LANGRIDGE line were then removed carefully and ripe siliquae were harvested from the Wilna-2 plants over a three-week period on up to four separate occasions at the end of the experiment. At this time, the flowering stems of both lines had grown into one another, the plants being approximately 30 cm tall.

The seed so obtained was sown individually in boxes of soil in the glasshouse, 400 seeds being sown, where supplies allowed, from each harvesting occasion - plant combination. Germination was good with a rate of 96.4% and a total of 10,395 seedlings were scored three weeks after the seed was sown.

Now if each of the ten Wilna-2 plants under test had set seed autogamously, none of their progeny, mutation apart, would be expected to bear hairy leaves. In fact, a total of 180 seedlings did so, a proportion which yields an overall estimate of the frequency of outcrossing in these plants of 1.73 ± 0.12%. There were, furthermore, significant differences between both plants and blocks with respect to their frequency of outcrossing, as the following table makes clear:

Plant no. (block 1)	1	2	3	4	5
% outcrossing	4.71	1.27	1.97	1.58	1.56
Plant no. (block 2)	6	7	8	9	10
% outcrossing	0.47	1.31	0.43	2.78	0.38

Thus the average frequency of outcrossing in the first block, occupying the more open situation, is 2.15%, while that in the relatively more sheltered second block is only 1.19%. There are also significant differences between harvesting occasions within plants. In block 2, the frequency of outcrossing is highest among the progeny of the first harvest. In block 1, on the other hand, though the progenies are heterogeneous in this respect, no consistent trend emerges.

There can be no doubt from these results that outcrossing can occur in this species; its cause, however, is much less clear. The test was carried out under a high plant density in the belief that contact between adjacent inflorescences caused by wind movement might be the causal mechanism of cross-pollination, a belief which receives some support from these results in that the frequency of outcrossing is higher in block 1 than in the more sheltered block 2. On the other hand, the frequency of outcrossing on this view, is expected to increase with age in that later flowers on well-developed side shoots can more easily make contact than those on the main one.The results from the second block, however, suggest that the frequency of outcrossing is highest for these early flowers. Cross-pollination by contact therefore appears to be ruled out. Though visits by insects to flowers of this species are not common in the urban environment in which this experiment was performed, hover flies (family Syrphidae) have been observed occasionally to visit plants in the glasshouse. Indeed, casual, infrequent visits by insects of this type would be expected to cause the rather erratic frequency of outcrossing observed. In a more rural environment, on the other hand, the frequency of insect visitation to open flowers of Arabidopsis may well be higher because these visitors are more common. Thus our estimate of the frequency of outcrossing may well be lower than that which is obtained in natural populations of the species.

We should welcome further information concerning both the frequency of outcrossing in the species, as well as the causal agent of its cross-pollination.

References:

DOBROVOLNÁ, J.: Arabid.Inf.Serv. 4, 6-7 (1967)
JONES, M.E.: Arabid.Inf.Serv. 5, 11-13 (1968)
- : Heredity (in press) 1971
KARBE, C., and G. RÖBBELEN: Arabid.Inf.Serv. 5, 13-15 (1968)
NAPP-ZINN, K.: Beiträge zur Phytologie, Festschr. H. WALTER, pp. 39-49, 1964

Further data on the extent of outcrossing in Arabidopsis

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Earlier experiments on the extent of outcrossing in natural populations of A. thaliana (cf. KARBE and RÖBBELEN, 1968; BLISS and RÖBBELEN, 1969) did not yield conclusive estimates. Therefore, in 1969 another test was sheduled. This experiment, as it appears only now, was very similar to the investigation described above by LAWRENCE and SNAPE, in that not racial characteristics but a monogenic marker was used for detection of the hybrids.

From the wild type "En" and a hairless mutant thereof with yellow seeds, designated as F1, each 30 mg of seeds were mixed and sown in an outdoor underglass hotbed. Thus on an area of about 6 m^2 1450 En-plants and 1750 F1 mutants were grown in a dense mixed stand. Both genotypes showed no major differences in vigour, flowering time, pollen vitality and seed fertility. Only seeds from the glabrous mutant were harvested. These were bulked and again chequed for wild type admixtures by eliminating even the few erraneously included brown seeds. Two subsequent sowings were performed from this seed bulk giving

1)	5,256	hairless and	57	hairy and	
2)	15,192	" "	171	"	plants,
i.e., a total of	20,448	" "	228	"	individuals.

From the latter group 210 hairy plants could be harvested and progeny tested. The result was, that according to the expectation 199 progenies segregated 3:1 [with a total of 12,603 wild type : 3,168 hairless phenotypes (= 25.2%)], while by non-segregation the rest 11 proved to be wild type admixtures.

From these data the calculation of the outcrossing percentage in the original mixed population runs as follows:

199 hairy phenotypes in the "F_1" generation demonstrated to be hybrids;
+ 18 hairy phenotypes not progeny tested, therefore possibly including 11/210 ≈ 5% wild type admixtures =
17 not tested hybrids, giving a total of
216 hybrids within the grown "F_1" of 20,448 + 216 = 20,664 plants.

The outcrossing percentage thus amounts to 1.05%, a value very close to that found in the preceding paper by LAWRENCE and SNAPE (1971). On the basis of other sporadic observations we also agree with these authors that in Arabidopsis the outcrossing percentage may well vary considerably with the actual environment.

References:
BLISS, F.A., and G. RÖBBELEN: Arabid.Inf.Serv. 6, 4 (1969)
KARBE, Christine, and G. RÖBBELEN: Arabid.Inf.Serv. 5, 13-15 (1968)
LAWRENCE, M.J., and J.W. SNAPE: Arabid.Inf.Serv. 8, 6 (1971)

Facilitation of crossing by the use of appropriate parental stocks

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Crossing of Arabidopsis may cause some difficulties to the inexperienced experimenter because of the small size of the flowers and the relative paucity of pollen grains in the anthers. Various methods of cross-pollination have been described (MÜLLER, 1961; FEENSTRA, 1965). In our laboratory we have used successfully for years sharp tweezers under a 2 inch focal distance 5X watchmaker's magnifier held on the head or under a dissecting microscope equipped with zoom-lens. With this technique the most time-consuming part of crossing is the careful emasculation. If the plants are very vigorous one person may produce daily several thousands of hybrid seed by such a procedure. Accidental selfing can be easily detected if the female parent is homozygous for a recessive seedling marker or the pollen donor is labelled with a dominant mutation.

Recently we observed that mutant as (asymmetric rosette leaves) (linkage group 2; RÉDEI, 1965) allelic to REINHOLZ's (1947) mutation magnifica, generally develops in their flowers a protruded style carrying a stigma receptive several days before the anthers dehisce.

Two series of experiments by pollinating non-emasculated as^1 homozygotes with two pollen donor stocks yielded the following results:

	Pollen donor	No. of seeds tested	Percentage of hybrid seedlings
Group I	gl[1], re	507	38.3
Group II	co, er	183	93.4

The population of single fruits in group I varied from almost all maternal to 100% hybrid progenies, depending on the age and physiological stage of the stigma. In group II the percentage of hybrid seedlings was much larger among the offspring obtained by pollination carried out at two different dates. This higher success was due partly to the early pollination and partly to the fact that mutant er is a very good pollen donor.

Mutants similar to as have been repeatedly described in the tomato (RICK and ROBINSON, 1951). While the ex (exerted) mutants of the tomato set only 7-15% seed by selfing depending on the degree of extrusion of the stigma the as[1] mutant of Arabidopsis is as fertile as the wild type even when artificial pollination is not used. The shape of the rosette leaves of as is characteristic; thus the selfed individuals can be easily identified at a very early developmental stage. The use of as females increases the efficiency of crossing several fold and it is comparable to that of cytoplasmic male sterility in other plant species, a condition not yet available in Arabidopsis. Actually the employment of as stocks shows some advantages above a cytoplasmic material in as much as no restorer is needed to produce normal fertility and yield upon selfing, if so desired for maintenance.

References:
FEENSTRA, W.J.: Arabid.Inf.Serv. 2, 34 (1965)
MÜLLER, A.: Kulturpflanze 9, 364-393 (1961)
RÉDEI, G.P.: Genetics 51, 857-872 (1965)
REINHOLZ, E.: Fiat Rep. 1006, 1-70 (1947)
RICK, C.M., and J. ROBINSON: Amer.J.Bot. 38, 639-652 (1951)

Androgenesis in Arabidopsis thaliana (L.) HEYNH.

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Three plants of A.thaliana were found which originated evidently by androgenesis. They appeared in the F_1 generation from the cross C.arenosa x A.thaliana. The tetraploid (2n = 32) C.arenosa from the locality Libčice near Praha (cf. population Nr. P 106; MĚSÍČEK, 1970) was pollinated by a mixture of pollen grains taken successively from 4 winter-annual individuals of A.thaliana, race "Libčice". These individuals were treated with colchicine in October (apical meristems of rosette

Table: The F_1 generation of the crossing between C.arenosa (2n = 32 , petals pinks) and A.thaliana (mixture of pollen, pet. white)

Seeds				Plants				
Embryo	Radicle	Size mm	Number	Number	2n	Petal colour	Fertility	Habit and morphological characters
well developed	± incumbent or accumbent	large, 1.2 x 0.7	1	1	32	pink	reduced	C.arenosa
			1	1	32	pink	very low	(gynogenesis)
	incumbent	middle 0.7 x 0.5	1	1	24	pink	low	new
			1	1		white	low	new
			1	1	26	white	rather	ad Hylandra
			1	1		white	high	suecica
	incumbent	small, 0.5 x 0.35	3	3	10	white	full	A.thaliana (androgenesis)
abnormally or poorly devel.	often straight or irreg. curved	0.7-0.8 x 0.5-0.6	20	6	26	white or pink	rather high	ad Hylandra suecica
				3	21	pink	very low	new
underdevel.	-	diverse	170	-	-	-	-	-

plants), and used for the pollination the next spring. Thus, the following microspores were expected to be present in the used pollen mixture: a) haploid m. with n = 5 produced by diploid sectors or inflorescences, b) polyhaploid m. with n = 10 arising from tetraploid sectors, c) aneuhaploid m. and probably also some high polyploid m. and d) sterile microspores.

200 seeds were obtained from 42 pollinated flowers. Embryos were well developed, however, in only 9 seeds; while embryos at different (deficient) stages of development were found in 20 seeds, and underdeveloped or dwarfish ones in ca. 170 seeds. The seeds of the first category were further differentiated in 3 groups with respect to their size and structure (cf. Table).

Three small seeds (embryos with incumbent radicles) were identical with those of the diploid Arabidopsis thaliana, and this identity was also confirmed in the mature plants: no differences were found between the normal diploid Arabidopsis and the plants grown from these small seeds. The meiosis in PMC's of these individuals was quite regular (5_{II}).

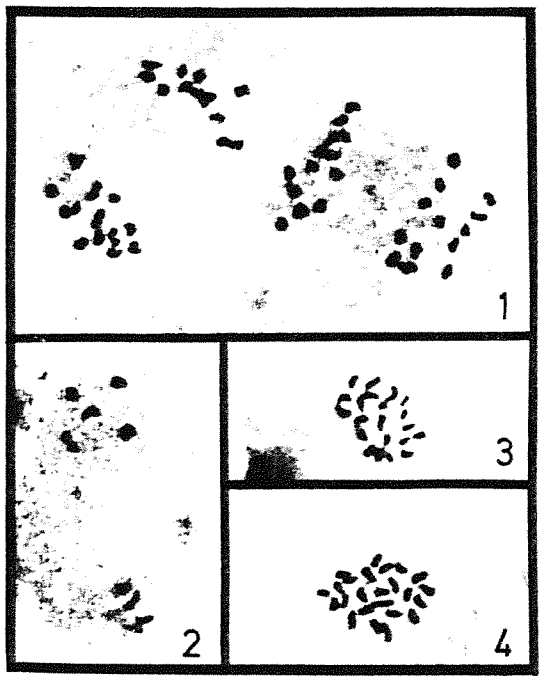

Figure:
Meioses and mitoses in F_1 plants from the crossing C.arenosa x A.thaliana.
1: anaphase II (n = 13) in an F_1 individual with 2n = 26;
2: metaphase II (n = 5) in the androgenetic Arabidopsis;
3: 2n = 21
4: 2n = 24

From the data mentioned the conclusion may be drawn that the 3 small seeds must have developed by androgenesis from the male nuclei of diploid Arabidopsis microspores (n = 10). Even though remarkable meiotic disturbances and irregular chromosome pairing were often observed in the tetraploid PMC's of the male parents treated by colchicine, in the given case the number n = 10 must represent the complete diploid chromosome set of Arabidopsis. This assumption is obvious because of the regular meiosis in the PMC's and the high fertility in the three F_1 plants. Therefore these plants may be characterized as pseudohaploids, i.e. autopolyhaploids.

Reference:
MĚSÍČEK, J.: Preslia (Praha) 42, 225-248 (1970)

Frequency of androgenesis

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ARNOLD (1964) and ARNOLD and CRUSE (1968) reported on experiments of induced androgenesis in Arabidopsis. Quantitative information has not been published yet, however. The experimental study of apomixis is of considerable interest in plants both from pure biological viewpoint and also for applied purposes in crop plants.

The actual frequency of gynogenetic apomixis is extremely difficult to determine because a large number of the maternal embryos are diploidized at an early stage. Thus accidental selfing can be ruled out only in the haploid maternal

offspring. Androgenetic plants can be conveniently produced in some species by pollen cultures (NITSCH, 1969) but to some other species this method cannot be applied yet.

The frequency of androgenesis undoubtedly depends on genetic factor(s) in the same species (KERMICLE, 1969). By the use of appropriate genetic techniques the recognition of offspring containing only paternal chromosomes in maternal cytoplasm can be facilitated and contaminations of various origin can be identified with certainty.

As female parent we used plants homozygous for the recessive gene *gl*^{1} (hairless) and their cytoplasm was marked with a plastom syndrome (RÉDEI, 1971) induced by a nuclear chloroplast-mutator (*chm*) gene. The pollen donor plants were homozygous for *hy* (long hypocotyl) and either for *as* (asymmetric rosette leaves) or for *re* (reticulate leaf blades) recessive genes, respectively. All three were located in linkage group 3 (RÉDEI, 1965). The male parents were homozygous dominant for other factors including *chm*$^{+}$ and *gl*$^{+}$.

Accordingly all normal diploid biparental offspring was expected to carry the cytoplasmic syndrome and to be wild type for all the nuclear markers employed. Androgenetic progeny was supposed to be variegated, hairy, with elongated hypocotyl and to display reticulate or asymmetric rosette leaves, respectively.

The genetic markers used were very convenient for the studies because all could be identified generally within two weeks after germination.

Attempts were made to detect the possible influence of extrinsic factors on the frequency of androgenesis but no significant difference was detectable under conditions of treatments:

Treatment	Size of the population	No. of androgenetic offspring
1. Pollination followed emasculation within 48 hours	11,800	1
2. Pollination was delayed by 4–8 days	4,100	0
3. After pollination like in treatment No. 1 the plants were transferred from the greenhouse to cold room (15–18° C)	700	1
4. After pollination the cold room (15–18°C) grown plants were transferred to the greenhouse	300	0
5. Pollen irradiated with 7,200 r	1,750	1
6. *Cardaminopsis arenosa* or *Hylandra suecica* pollen was used for *Arabidopsis* females	450	0
7. Diploid or triploid pollen was employed to haploid eggs	600	0
	19,700	3

Two of the androgenetic plants appeared diploid and one was possibly a haploid. It appears that androgenesis is well detectable in *Arabidopsis* but its frequency is low, approximately 1.5×10^{-4}. In normal maize the frequency of androgenetic progeny is approximately 8×10^{-5}; in the presence of the genetic factor *ig* a frequency of $2\text{-}3 \times 10^{-2}$ was observed (KERMICLE, 1969).

References:
ARNOLD, C.G.: Arabid.Inf.Serv. 1, 16-17 (1964)
- , and D. CRUSE: Arabid.Inf.Serv. 5, 39 (1968)
KERMICLE, J.L.: Science 166, 1422-1424 (1969)
NITSCH, J.P., and C. NITSCH: Science 163, 85-87 (1969)
RÉDEI, G.P.: Genetics 51, 857-872 (1965)
- : Arabid.Inf.Serv. 8, 28 (1971)

Variability of cell resistance to damage by high hydrostatic pressure in ecological races of Arabidopsis thaliana (L.) HEYNH.

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As was reported earlier (ASHRAF, 1970a,b), the stopage of protoplasmic streaming can be used to study the damaging effects of various agents in vivo. The experiments showed a wide range of variability that is met with in ecological races as well as within a single selection line and its parental race under conditions when selection had n o t taken this point into consideration. Using the same criterium we attempted to uncover the variability that is present in ecological races of Arabidopsis in respect to cell resistance to damage caused by high hydrostatic pressure (HHP). It should, however, be emphasized that HHP is not a factor that is d i r e c t l y affected by selection either in nature or in the laboratory.

For the investigations we used the same material that was described earlier (ASHRAF, 1970). Stopage of protoplasmic streaming was performed in a hydrolic press according to ALEXANDROV et al. (1959). Since ALEXANDROV et al. (1971) assumed that the concentration of the osmotically active substances in the cell is the mechanism by which the cell resistance to HHP varies, we determined the concentration of these osmotically active substances in the cell by means of grenzplasmolysis in a KNO_3 solution during a 10 minute exposure. The following table sums up the obtained data:

Race	HHP in atm, 5 minutes action of which stops protoplasmic streaming	Concentration of KNO_3, 10 minutes exposure of which causes grenzplasmolysis
Estland	900	0.26
Enkheim	1000	0.23
A-8	1070	0.20
Kazbek	1000	0.33
Copenhagen	1070	0.35
Blanz	1100	0.30

Thus our data show that the level of cell resistance to HHP and the concentration of the osmotically active substances in the cell are not correlated in our material. Earlier, ASHRAF (1971) and ALEXANDROV et al. (1971) had shown that this correlationship is valid for changes in HHP resistance of the cell that follow changes in ploidy or seasons, respectively. It is also of interest to note that one and the same criterium (stopage of protoplasmic streaming) can show differences in the direction of the changes that result from the different agents (HHP and high temperature). These resistances, therefore, seem not to be related to each other.

References:
ALEXANDROV, V.Ya., et al.: Tsitol. 1, 672-691 (1959)
- : Tsitol. 13, in press (1971)
ASHRAF, J.: Arabid.Inf.Serv. 7, 11-12 (1970a)
- : Arabid.Inf.Serv. 7, 9-10 (1970b)
- : Tsitol. 13, in press (1971)

The range of reversibility of temperature damage in ecological races of Arabidopsis thaliana (L.) HEYNH.

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The resistance to damage caused by high temperature varies between ecological races or even within a single selection line of Arabidopsis (ASHRAF, 1970a,b). It has also been shown that this reaction is affected by genetical changes (ASHRAF, 1967, 1971). The following paper presents data on the capacity of ecological races to recover after a high temperature treatment. For this purpose the very races were used that were used in the earlier experiments (ASHRAF, 1970b). The temperature resistance of the cell and its reversibility was determined according to ALEXANDROV et al. (1963). The following table summarises the obtained data.

In case of the races Enkheim and A-8 the range of reversibility was investigated with vernalized and unvernalized plants; but we failed to find any difference.

Table: The range of reversibility of temperature damage in various ecological races of Arabidopsis thaliana

Race	Temperature in °C, 5 minutes action of which stops protoplasmic streaming	Range of damage reversibility in °C	Temperature in °C, 80 minutes action of which stops protoplasmic streaming	Range of reversibility
Estland	41.1	2	36.5	2
Enkheim	42.3	3	38.2	3
Peterhof (A-8)	42.4	3	40.8	3
Kazbek	42.8	3	41.2	3
Copenhagen	43.0	3	38.7	2
Blanz	43.6	3	39.8	3

Furthermore, ALEXANDROV et al. (1963) had shown that the range of reversibility of the temperature damage significantly narrows at an increase of the treatment time with a correspondingly lower temperature. This effect is also true for Arabidopsis if diploid and polyploid plants are compared (ASHRAF, 1967). But in the cases presented here this shortening could not be demonstrated. From a total of 6 races only one (Copenhagen) revealed a difference between the reversibility ranges after 5 minutes and 80 minutes of heating. The rest showed identical reversibility inspite of a 16 fold difference in the duration of the temperature exposure. This identical reaction includes races showing significantly different levels of temperature resistance (e.g., Blanz as compared to Enkheim). Thus there seems to be no tight correlationship between the level of thermal resistance and the recovery from this damage.

References:
ALEXANDROV, V.Ya. et al.: Tsitol. 5, 159-168 (1963)
ASHRAF, J.: Tsitol. 9, 804-810 (1967)
- : Arabid.Inf.Serv. 7, 11-12 (1970a)
- : Arabid.Inf.Serv. 7, 9-10 (1970b)
- : Tsitol. 13, in press (1971)

Does Arabidopsis follow the sun?

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Arabidopsis plants of the race Li_2 were grown in the greenhouse in September 1970. The rosette leaves were radially arranged till they came into contact with neighbouring pots or plants, and started to be twisted (see photo). They were all twisted in the same direction, independent of the phyllotaxis, as if the stem had been twisted in a clockwise direction leaving the leaf tips behind. Illustrations of twisted mutants (REINHOLZ, 1947) also show a clockwise twist. This may be related to the movement of the sun. In that case plants in the southern hemisphere should twist anticlockwise.

Reference:
REINHOLZ, E.: Naturwiss. 34, 26-28 (1947)

Der Anteil der einzelnen Organe an der photosynthetischen Produktivität der Gesamtpflanze bei Arabidopsis thaliana (L.) HEYNH.

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Bei der Beurteilung der photosynthetischen Produktivität einer Pflanze darf insbesondere bei krautigen Objekten der Anteil der grünen Achsenteile nicht vernachlässigt werden. Um diesen Betrag quantitativ zu erfassen, wurden von unter Laborbedingungen angezogenen Arabidopsis-Pflanzen, Rasse En-1, (HOFFMANN, 1965, 1968) im Blühstadium die Rosettenblätter (B), die Stengelblätter (C) bzw. alle Blätter (D, auch die nachwachsenden) entfernt und nach 3 1/2 Wochen wesentliche Kenngrößen ihrer stoffwechselphysiologischen Leistungsfähigkeit im Vergleich mit den unbehandelten Kontrollpflanzen (A) ermittelt (HOFFMANN, 1968). Die Ergebnisse in Abb. 1 zeigen, daß auch die Pflanzen ohne Blätter noch zu einer beträchtlichen Stoffbildung befähigt sind: gegenüber dem Versuchsbeginn (Blühstadium) nimmt ihr Frischgewicht bis zum Schotenstadium um 615% (von 17 auf 122 mg/Pfl.), ihr Trockengewicht um 400% (von 3 auf 15 mg/Pfl.) und der Chlorophyllgehalt um 1600% (von 6 auf 104 µg/Pfl.) zu (vgl. auch GREENWOOD und TITMANIS, 1968). Bei den Kontrollpflanzen liegen die Zuwachsraten für diese Größen bei 230%, 220% bzw. 190%.

Auf die Rosettenblätter entfallen zu Versuchsbeginn vom Chlorophyllgehalt sowie von der photosynthetischen CO_2-Aufnahme etwa 60% des jeweiligen Wertes für die Gesamtpflanze. Die Entfernung der Rosettenblätter wirkt sich jedoch auf die Dauer in weit geringerem Maße auf die Stoffproduktion aus. Pflanzen, denen nur die Stengelblätter entfernt wurden, sind kaum beeinträchtigt, in der Substanzproduktion sogar dominierend.

Die Untersuchungen zeigen somit, daß die Photosyntheseleistung der Sproßachse von Arabidopsis thaliana durchaus ausreicht, um nicht nur das System zu erhalten, sondern auch noch zu einem beträchtlichen Stoffgewinn und zur Ausbildung reifer Schoten zu führen. Der große Assimilatbedarf (Sog) wirkt sich also fördernd auf die Produktivität der verbleibenden grünen Gewebe aus (vgl. auch MAGGS, 1965; KING und Mitarb., 1967; TROBISCH, 1967; LUCAS und ASCANA, 1968; WAREING und Mitarb., 1968; MEIDNER, 1969) und kompensiert durch die Ausschöpfung der vorhandenen beträchtlichen Reserven im Photosynthese-Apparat bis zu einem gewissen Grade den partiellen oder totalen Verlust der Assimilationsorgane. Dieses spiegelt sich auch in dem gegenüber der Kontrolle erhöhten Chlorophyllgehalt der Sproßachse wider (Tabelle 1).

Tabelle 1: Chlorophyllgehalt verschiedener Organe von Arabidopsis-Pflanzen (in mg Chlor. a+b je g Frischgewicht)

	Behandlg. (s.Text)	Rosetten-blätter	Stengel-blätter	Sproß-achse	Schoten
Versuchsbeginn (Blühstadium)		0.87	3,0	0,35	-
Versuchsende (Schotenstadium)	A	0,57	1,32	0,36	0,8
	B	-	1,44	0,48	0,93
	C	0,52	-	0,39	0,73
	D	-	-	0,57	0,81

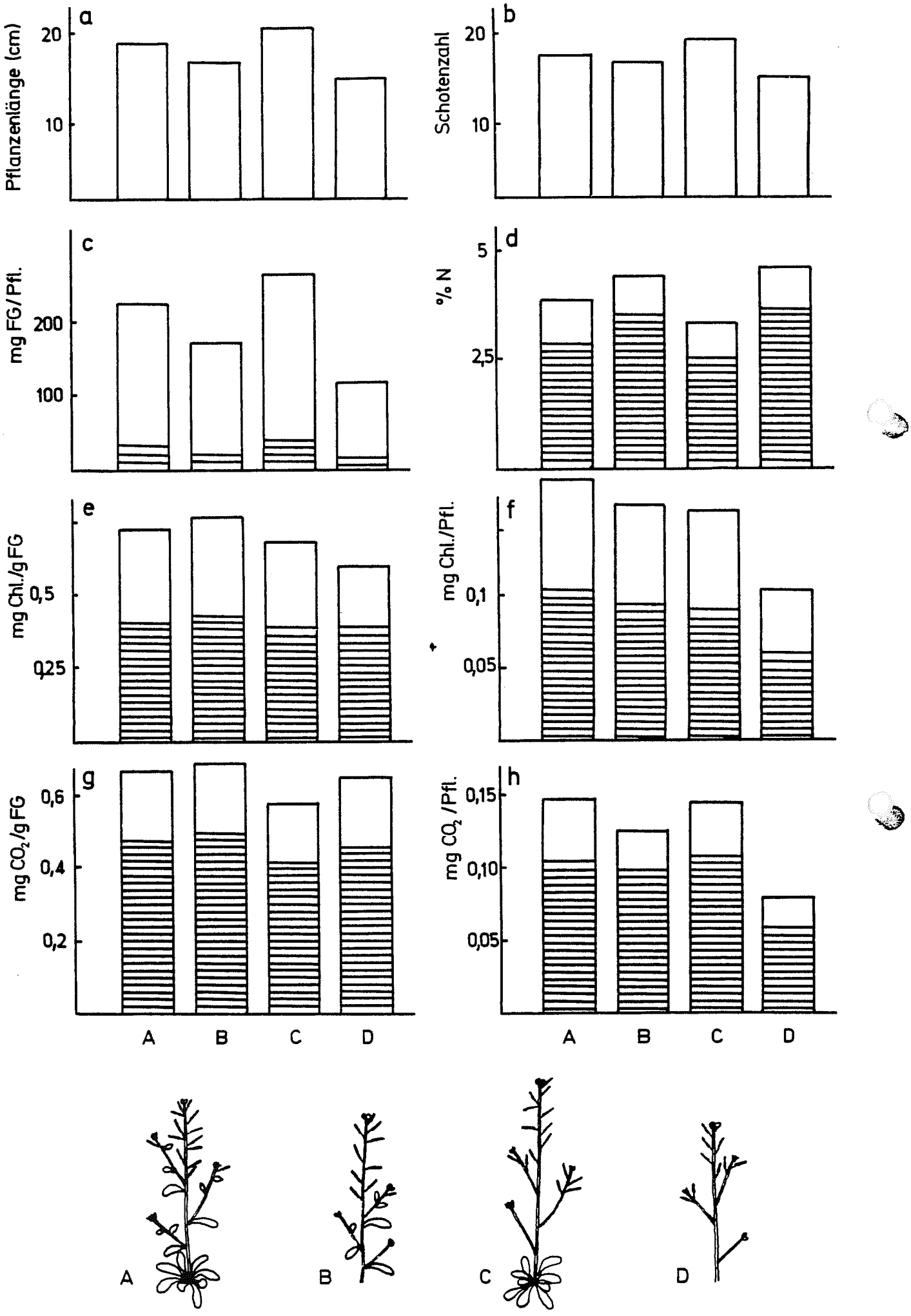
Andererseits zeigen Untersuchungen an Mutanten von Arabidopsis (VELEMÍNSKÝ und Mitarb., 1969), daß auch der Pigmentgehalt die Photosyntheserate nicht immer begrenzt.

Eine besondere Bedeutung kommt der photosynthetischen CO_2-Fixierung der sich entwickelnden Schoten selbst zu (Tabelle 2) (vgl. auch Van der MEER und WASSINK, 1962; TROBISCH, 1967; KRIEDEMANN, 1968). Sie beträgt bei grünen Schoten im Licht-Warburg-Apparat (0,1 Vol.% CO_2) etwa 50% der Blattphotosynthese (pro Frischgewicht) bzw. 200% derjenigen des Achsengewebes.

Tabelle 2: Gaswechsel verschiedener Organe von Arabidopsis-Pflanzen (im Licht-Warburg-Apparat)

	Blätter		Sproßachse		Schoten	
	µl O_2/g Frischgew.	µl O_2/mg Chloroph.	µl O_2/g Frischgew.	µl O_2/mg Chloroph.	µl O_2/g Frischgew.	µl O_2/mg Chloroph.
Blühstadium						
Photosynthese	+ 814	+ 784	+ 391	+ 1150	-	-
Atmung	- 128	-	- 155	-	-	-
Schotenstadium						
Photosynthese	+1153	+1095	+ 279	+ 706	+ 592	+ 712
Atmung	- 93	-	- 102	-	- 120	-

Eine ausführliche Diskussion dieser komplexen Problematik der Bedeutung von Assimilatsog und -stau, Wurzelstoffwechsel und Assimilatverteilung auf die Produktivität der einzelnen photosynthetisierenden Organe (vgl. auch TROBISCH, 1967; NEALES und INGOLL, 1968) soll an anderer Stelle erfolgen.


a
Pflanzenlänge (cm)
20
10
b
Schotenzahl
20
10
c
mg FG/Pfl.
200
100
d
% N
5
2,5
e
mg Chl./g FG
0,5
0,25
f
mg Chl./Pfl.
0,1
0,05
g
mg CO2/g FG
0,6
0,4
0,2
h
mg CO2 /Pfl.
0,15
0,10
0,05
A
B
C
D
A
B
C
D

Summary: Under our experimental conditions Arabidopsis plants in the flowering stage reach a height of 7 cm and produce 9 mg dry matter per plant. When the stem leaves, the rosette-leaves or both are removed at this stage of development, the weight of the plants in the silique-stage is reduced. The water-content, the chlorophyll content, the fresh weight as well as the photosynthetic activity and the relativ amount of different N-fractions in partially defoliated plants are not markedly changed. Although the assimilatory area of these plants is limited to the green tissue of the stem only, the plants partly compensate for this loss and produce about 85% of the ripe siliques as compared to the control plants by increasing the photosynthetic productivity.

Literatur:
GREENWOOD, E.A.N., and Z.V. TITMANIS: Austr.J.agric.Res. 19, 9-14 (1968)
HOFFMANN, G.: Habilitationsschrift Berlin 1970 (Eberswalde 1969)
HOFFMANN, P.: Arabid.Inf.Serv. 1, 128-131 (1965)
– : Botan.Studien 18, 1-151 (1968)
KING, R.W., I.F. WARDLAW, and L.T. EVANS: Planta 77, 261-276 (1967)
KRIEDEMANN, P.E.: Austr.J.biol.Sci. 21, 907-916 (1968)
LUCAS, D., and R.D. ASCANA: Physiol.Plantarum 21, 1217-1223 (1968)
MAGGS, D.H.: J.exp.Bot. 16, 387-404 (1965)
MEER, Q.P.van der, and E.C. WASSINK: Meded.Landbouwhogeschool (Wageningen) 62, 1-9 (1962)
MEIDNER, H.: Nature 222, 876-877 (1969)
NEALES, T.F., and I.D. INOLL: Bot.Review 34, 107-125 (1968)
TROBISCH, S.: Habilitationsschrift Jena 1967
VELEMÍNSKÝ, J., J. SVACHULOVÁ, and W. STARZECKI: Photosynthetica 3, 97-103 (1969)
WAREING, P.F., M.M. KHALIFA, and K.J. TREHARNE: Nature 220, 453-457 (1968)

Fixation of $^{14}CO_2$ activity of carboxydimutase and the content of free sugars in two chlorophyll mutants of Arabidopsis thaliana (L.) HEYNH.

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The rate of $^{14}CO_2$ fixation was followed in lethal chlorophyll mutants, strain 1467 of the race Dijon and strain 42 of the race St 56, cultivated on agar with sucrose. Both mutants fixated less $^{14}CO_2$ than the normal plants.

The mutant 1467 with a chlorophyll content of 30-50% of the normal plants and with a normal ratio of chlorophyll a : b fixated less $^{14}CO_2$ than the mutant 42 with a chlorophyll content of 10-20% of the normal plants and with an extremely low content of chlorophyll b.

The distribution of ^{14}C in the alcohol-soluble and alcohol-insoluble fraction in leaves was the same for both, the mutants and the normal plants. There were no differences in the activity of carboxydismutase. Similarly the 5 free sugars: raffinose, maltose, saccharose, glucose and fructose, determined chromatographically, were detected in the same quantitative relation in mutants and normal plants.

Abstract of a paper which will appear in Photosynthetica.

Abbildung 1 (vgl. links, S. 14): Kenngrößen der unten schematisch wiedergegebenen Arabidopsis-Pflanzen, die seit dem Blühstadium für 3 1/2 Wochen ohne Rosettenblätter (B), ohne Stengelblätter (C) bzw. ganz ohne Blätter (D) wuchsen. A = Kontrollpflanzen.
a) Pflanzenlänge (cm); b) Schotenanzahl; c) Frischgewicht (mg) je Pflanze, schraffierter Teil der Säule = Trockengewicht; d) Stickstoffgehalt (%) bezogen auf Trockengewicht und aufgetrennt in löslichen Stickstoff (oberer) und Proteinstickstoff (unterer Teil der Säule); e,f) Chlorophyllgehalt, aufgetrennt in Chlorophyll a (unten) und b (oben) je Frischgewicht bzw. Pflanze; g,h) Gaswechsel-(URAS-)Messungen, unterteilt in Nettoassimilation (unten) und Atmung (oben) je Frischgewicht bzw. Pflanze

Quantitative modifications of the phytochrome trigger mechanism in seed dormancy of homoallelic genotypes

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The wildtype En-2 and two homoallelic chlorina mutants, ch^1 and ch^2, of Arabidopsis thaliana (L.) HEYNH., which are quantitatively deficient in chlorophyll b, were cultivated in a growth chamber during six consecutive generations under continuous yellow light (λ>550 nm, ~ 4.10 μ watts·cm^{-2}.10 nm^{-1}). The seed dormancy of the wild type ch^+/ch^+ decreased significantly from the I_1 to I_6 generation, but in the seeds of the ch^1/ch^1- and ch^2/ch^2-types significant dormancy was never observed (KRANZ, 1970). Therefore experiments were started to analyse this different germination behaviour.

AMEN (1968) proposed a model that embodies a hormonal regulation of four phases of dormancy: an inductive, a maintenance, a trigger and a germination mechanism. The available experimental evidence indicates that the "triggering agent" is a photochemical reaction and / or a thermochemical one, while the other mechanisms are under hormonal control exclusively. For the photochemical reaction the phytochrome mechanism appears to be mainly responsible (MANCINELLI, BORTHWICK and HENDRICKS, 1966). Hence, the first part of our experiments was to investigate the phytochrome system within the seed material pretreated with yellow light.

Applied methods: seed germination in one-way-petri-dishes on wet filter paper after cold pretreatment at 5°C for 24 hours, subsequent radiation treatment under constant climate (25 ± 2°C, 60 ± 8% rel. humid.) with the plexiglass filters: far red (FR) RÖHM and HAAS 700 (λ> 750 nm, ~ 1.02 μ watts·cm^{-2}.40 nm^{-1}) and red (R) RÖHM and HAAS 501 (λ> 650 nm, ~ 4.40 μ watts·cm^{-2}.20 nm^{-1}).

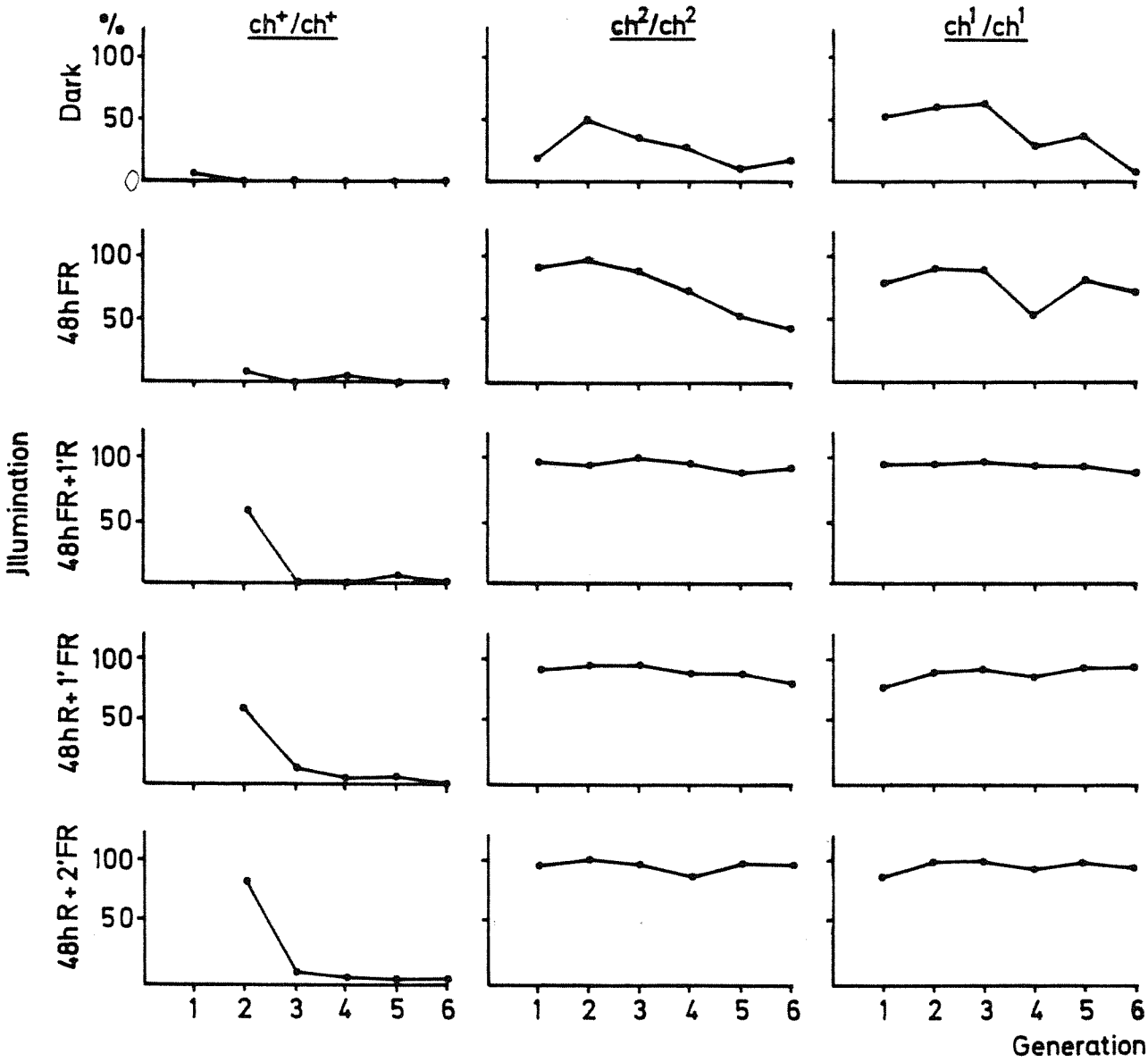

Figure 1: Relative values of seed germination depending on different R/FR-treatments examined during six consecutive generations of three homoallelic genotypes

The results are summarized in Figure 1 giving the relative values of seed germination seven days after sowing for six consecutive generations. While the seeds of the I_0 still developed under normal light, those of I_1 to I_5 grew under the yellow light. The germination of the mutants was significantly better than that of the wildtype. For seeds which ripened under yellow light, the germination time decreased from I_2 to I_6 in the dark control and under the far red filter. This means that the quantitative modifications of the seed dormancy induced by yellow light, can be broken up by the red/far red radiation. Subsequent treatment of FR (2 minutes) after R (48 hours) probably yields the best results. But there are large differences between the wildtype and the mutants. The mutants differ significantly after FR treatment and in the dark control. The ch^2/ch^2 mutant containing small amounts of chlorophyll b stands intermediate between the normal ch^+/ch^+ and the b-less ch^1/ch^1.

Evidently, the phytochrome dependent trigger mechanism is influenced by the chromosomal gene ch which mainly affects the contents of chlorophyll b in the plants. We are now starting experiments to analyse the effect of plant hormones (GA_3, ABA, Kinetin), which may interact with the phytochrome system and these genotypes. Hence, new information can be expected (CHEN and OSBORNE, 1970) on the hormone and/or gene dependent regulation system using m-RNA which is stored and/or synthesized de novo in the embryo.

References:
AMEN, R.D.: Bot.Rev. 34, 1-31 (1968)
CHEN, D., and D.J. OSBORNE: Nature (Lond.) 226, 1157-1160 (1970)
KRANZ, A.R.: Theor.Appl.Genet. in press (1970)
MANCINELLI, A.L., H.A. BORTHWICK, and S.B. HENDRICKS: Bot.Gaz. 127, 1-5 (1966)

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The fate of bromodeoxyuridine in Arabidopsis tissues

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The basic mechanism of flower induction remains obscure in spite of the extensive efforts devoted to this problem over a long period of time. Numerous attempts have been made to identify the chemical basis of flower differentiation (cf. KOLLI, 1969). No single substance could be identified so far with proven specificity concerning flower induction. Recently HODSON and HAMNER (1970) reported the extraction of an unidentified compound capable of promoting flowering in two short day plants, Xanthium and Lemna.

Arabidopsis, a very sensitive long-day plant appears to be a useful test organism for investigations on the mechanism of flowering because of (1) the great facility by which it can be cultured under well controlled conditions, (2) the homogeneity and stability of its genetic background, and (3) the availability of several monogenic mutants qualitatively different from the wild type in their photoperiodic response (RÉDEI, 1962).

The flower promoting effect of the antimetabolites, 8-azaadenine (HIRONO and RÉDEI, 1966a) and halogenated deoxynucleosides (BROWN, 1962; HIRONO and RÉDEI, 1966b) is well demonstrated in this plant. On the basis of the autoradiographic studies conducted in the laboratories of BESNARD-WIBAUT (1966, 1968) and BROWN (1968), BROWN has suggested that bromodeoxyuridine promotes flower induction by temporary incorporation of the analog into the nuclei of the flank meristem of the vegetative apex and the transient inhibition of the metabolism of those critical cells. The lowered activity of the flank meristem would induce precociously the cells of the axial region and thereby conditions similar to those of natural flower determination would be created. It was hypothesized that after the activation of the axial meristematic region the analog is removed from the DNA of the flank meristem cells and normal flower development would proceed. According to this hypothesis the flower promoting activity of bromodeoxyuridine would be exerted through its incorporation into DNA. This interpretation seems to be in agreement with the observations made on plants to which the analog is applied only through the apex and only for a limited duration. It is difficult to visualize however, how the selective uptake and elimination of the analog can take place under the conditions of continuous supply of bromodeoxyuridine through the roots in the aseptic test tube cultures.

In order to clarify these inconsistencies we followed a different approach for tracing the metabolic fate of 5-bromodeoxyuridine, which was provided to the plants in agar cultures. Our recent experiments are in perfect agreement with previous

observations that this analog is a very potent inducer of flowering in mutant gi[2]. We also cultured plants on ^{3}H-bromodeoxyuridine (1.25 microcurie per ml mineral-sucrose agar medium) with the appropriate controls. From 15 to 20 days after germination we harvested individual plants, removed the roots and washed the leaves under running tap water for approximately ten minutes to remove surface contamination. In order to detect whether the plants would have some adsorbed radioactivity we dipped some control plants (grown on "cold" nutrient solution) into isotope containing agar medium. After washing no traces of radioactivity were found in the tissues of such plants. The washed plants were then homogenized thoroughly in cold (-20°C) absolute acetone to remove all visible leaf pigments, and the extraction was repeated. The pellet was then transferred to pH 5 acetate buffer (0.01 M) containing EDTA and sodium dodecyl sulfate and sometimes also bentonite. To this suspension we added an equal volume of water-saturated freshly distilled phenol. This extraction procedure was essentially similar to that of KUO and RÉDEI (1971) but the repeated phenol extraction were omitted. After centrifugation the buffer-phenol extract was collected with a Pasteur pipette in four fractions. The top layer was treated with double volume 95% ethanol containing NaCl and left overnight at -20°C. After centrifugation at -20°C, for 1-3 hours in a swing-out Sorvall HB rotor, we obtained a small whitish pellet and a completely clear supernatant. The scheme of fractionation is illustrated in Figure 1.

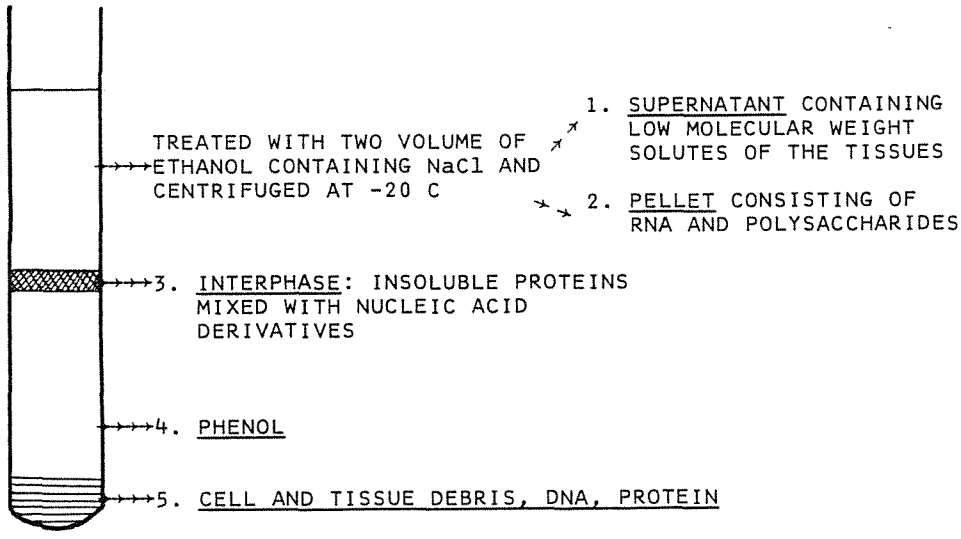

Figure 1: Fractionation of plant tissues into major cellular components

Fraction No. 2 was taken up in a small volume of buffer, the others were used without any further treatment for measurement of their radioactivity. This was carried out by adding 0.2 ml of each fraction to ten ml scintillation liquid of the following composition:

Dioxane 2 l, naphthalene 240 g, PPO 16 g, dimethyl POPOP 0.8 g, methanol 400 ml, diethylene glycol monomethyl ether 80 ml brought to a volume of 4 l, with dioxane.

This mixture was analyzed in an automatic Packard liquid scintillation counter and the following results were obtained:

Fraction number (See Figure 1 for description)	Relative total radioactivity (%) Experiment No. 1	2	Average
1	72	60	66.0
2	3	5	4.0
3	14	14	14.0
4	7	12	8.5
5	4	9	6.5
	100.0	100.0	100.0

The total detectable radioactivity of single plants varied between 4,000 to 6,000 cpm.

It should be noted that this separation does not produce absolutely clean fractions and only the radioactivity of fractions 1 and 3 is significant. Fraction 3 being the phenol-water interphase is certainly contaminated with fraction 1, thus only fraction 1 can be considered to contain appreciable amount of tritium. None of the comparable fractions obtained from the plants grown on non-labelled medium contained any detectable radioactivity.

These data indicate that the flower promoting activity of bromodeoxyuridine does not involve incorporation of significant amounts of the analog into macromolecules. The radioactive fraction was further analyzed by ascending paper chromatography (Whatman No. 1 paper, n-butanol (water-saturated) 100 part, 15 n ammonium hydroxyde 1 part) and no radioactivity was associated with bromodeoxyuridine, bromodeoxyuridine monophosphate, or bromodeoxycytidine. All the radioactivity remained at the origin.

Our observations do not rule out the possibility that small amount of the analog was actually incorported into DNA but the bulk of the radioactivity was present in other molecules of the cell. The report of BONOTTO et al. (1968) indicates only weak incorporation of BDU into DNA. This observation is supported by low mutagenicity of halogenated nucleosides in Arabidopsis (HIRONO and SMITH, 1969).

It was surprising to us that no one looked for the presence of BDU in non-macromolecular components of the cells of Arabidopsis.

References:

BESNARD-WIBAUT, C.: C.R.Acad.Sci. (Paris) Ser.D. 263, 1582-1585 (1966)
- : In "Cellular and Molecular Aspects of Floral Induction" (G. BERNIER, Ed.). Longmans, Green and Co. - London 1968, Pp. 7-9
BONOTTO, S., M. JACOBS, M.C. LONI, S. van PUYMBROCK, and R. KIRCHMANN: Arabid.Inf.Serv. 5, 33 (1968)
BROWN, J.A.M.: Nature (Lond.) 196, 51-53 (1962)
- : In "Cellular and Molecular Aspects of Floral Induction" (G. BERNIER, Ed.). Longmans, Green and Co. - London 1968, Pp. 117-138
HIRONO, Y., and G.P. RÉDEI: Planta 68, 88-93 (1966a)
- , - : Planta 71, 107-112 (1966b)
- , and H.H. SMITH: Genetics 61, 191-199 (1969)
HODSON, H.K., and K.C. HAMNER: Science 167, 384-385 (1970)
KOLLI, S.: Bot.Rev. 35, 195-200 (1969)
KUO, S.C., and G.P. RÉDEI: Arabid.Inf.Serv. 8, 25 (1971)

Further studies on the effect of gibberellic acid on the dwarf mutant ca of Arabidopsis thaliana

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In a previous communication (NAPP-ZINN and BONZI, 1970) it was reported that two dwarf mutants of Arabidopsis thaliana react differently to gibberellins. The age of flowering was reduced in the case of ca plants, without having any appreciable effect on their growth habit.

As these observations were based on treatments given at very low concentrations of different gibberellins, further studies on ca plants were carried out with higher concentrations (upto 250 ppm). The seeds were sown in the greenhouse at the 12th of February. The treatment was started when the plants were 3 weeks old. 0.04-0.05 ml gibberellic acid solutions were given every third day until the flower primordia appeared. The following Table summarizes the observations.

The present observations further confirm that gibberellic acid has decreased the number of days to flowering in ca plants. It has also influenced its vegetative growth. For example, the number of stalk-leaves and the length of the stalk have increased. But gibberellic acid has not stimulated the growth of the inflorescence to the same extent. Upto 100 ppm concentration of gibberellic acid, the plants show no significant response, but with further rise in concentration (250 ppm) significant differences appear. Therefore one can anticipate, that doses higher than 250 ppm of gibberellic acid can certainly promote the growth of the inflorescence.

It may be concluded from the obtained results, that exogenous treatment of plants with gibberellic acid reduces the number of days to flowering and also stimulates the vegetative growth. Thus if in the experiment of NAPP-ZINN and BONZI ca plants failed to overcome their dwarfism, this was due to the low

Table: The effect of gibberellic acid on flowering and growth habit of the dwarf mutant ca of Arabidopsis thaliana

The days to flowering were calculated from the actual day of the calendar, on which the first stigma became visible. Counting of the stalk-leaves, and measurement of the "length of the stalk" and "length of the inflorescence" was done after six weeks of the flowering of the control plants

Gibberellic acid conc. (ppm)	Days to flowering (from sowing)	No. of stalk leaves	Length of the stalk (mm, upto the first flower)	Length of the inflorescence (mm)
250	67.3 +++	16.5 +++	26.0 +++	20.0 ++
100	68.3 +++	16.3 +++	17.2 +++	14.5 +
50	69.4 +++	15.1 +++	16.4 +++	14.5 o
20	70.4 +++	11.5 +++	14.5 +++	12.7 o
10	71.5 +++	11.7 +++	8.8 o	11.4 o
5	75.1 +++	10.1 +++	9.3 o	11.1 o
1	73.5 +++	7.5 o	10.8 o	9.2 o
Dist. water	84.9 o	9.4 o	6.6 o	10.9 o
Control (untreated)	85.0	6.8	9.5	10.28

Significance of difference as compared with controls (after PÄTAU, 1943):

+++ $P < 0.0027$
++ $P < 0.01$
+ $P < 0.05$
o $P > 0.05$

concentration of gibberellin used by these authors. In the present experiment, even the plants treated with 20 ppm concentration of gibberellic acid are showing clear cut results with regard to growth habit. This is probably due to the entirely different experimental conditions prevailing in both experiments; the previous one was carried out in growth chambers with continuous illumination, but the present one under greenhouse conditions.

References:
NAPP-ZINN, K., and G. BONZI: Arabid.Inf.Serv. 7, 8 (1970)
PÄTAU, K.: Biol.Zbl. 63, 152 (1943)

Thanks are due to Prof. K. NAPP-ZINN for his valuable guidance and help.

Isoenzyme variability in Arabidopsis thaliana

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With the purpose to initiate developmental genetic studies with Arabidopsis mutants, we have prospected for enzyme polymorphism by means of electrophoretic techniques. Since isozymes provide a unique opportunity to look at gene activity throughout the development of a plant and as, to date, genetic studies of enzymes in higher plants have been almost confined to maize, it seems worthwhile to report some preliminary results on isozymes in Arabidopsis.

This report deals with two enzymes, acid phosphatase (AP) and leucine amino-peptidase (LAP), which are made visible after electrophoresis on starch gel (SMITHIES, 1955) by specific histochemical stains. The gels were immersed in a solution containing for AP, 15 ml of 0.2 M acetate buffer, pH 4.7; Fast Garnet GBC 150 mg; α-naphtyl acid phosphate 150 mg and distilled water to 150 ml; for LAP, 1-leucyl-β-naphtylamide 43 mg, 15 ml of 0.2 M acetate buffer, pH 6.0; Fast Garnet GBC 150 mg and distilled water to 150 ml.

In this study, plant material was homogenized weight per volume in 0.1 M Hepes buffer, pH 7.4, including 5.10^{-4} M dithiothreitol and centrifuged at 17,000 g, 15 min to remove cell debris. For electrophoretic analyses of individual plants, the crude extract of 2-4 rosette leaves obtained by squashing them with a drop of buffer, was directly adsorbed onto the filter paper sections to be inserted in the starch gels. By the last method, it is possible to determine the genotype of a plant and still to keep the latter for further experiments. All operations were performed at 4°C.

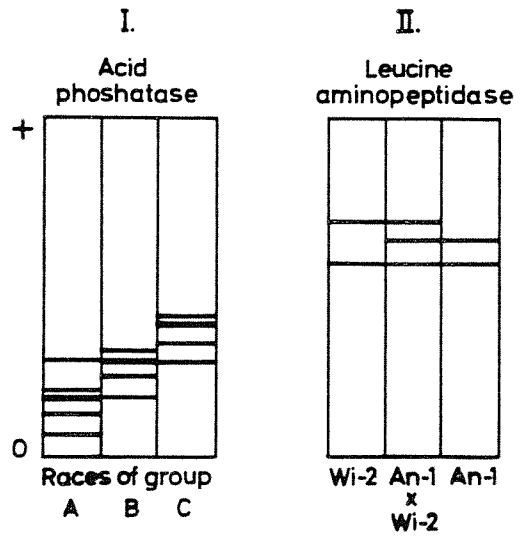

Figure 1:
Schematic zymogram from rosette leaves of various geographical races of Arabidopsis. I Electrophoretic patterns of acid phosphatase.
Races type A: Zü-0, 232.
Races type B: Wi-2, St-0, Tsu-0, Pa-1, Stw-0, Ost-0, Bl-1, Mr-0, Ei-5, Te-0, Je-0, Pi-0, Ct-1.
Races type C: Chi-0, Wa-1, Rschw-0, Kn-0, In-0, Gie-0, Rou-0, Hi-0.
II Electrophoretic patterns of leucin aminopeptidase with the 1' variant in An-1 and the heterozygote with Wi-2 which represents the standard type. Note the absence of hybrid enzyme in the heterozygote.
0 denotes the origin, + the anode

The results obtained for AP are shown diagrammatically in Figure 1,I. Three main types of electrophoretic pattern have been observed. Other more anodal faint bands are not taken into account here. The various geographical races of Arabidopsis are more or less equally distributed among type B and type C, but type A is only represented by two populations, Zü-0 and 232 (originated from an original St-0 stock). The genetic control of the multiple band pattern is now studied. F_1 data give a summation of the parental bands. As for developmental aspects, we have noted in the case of Wi-2, the absence of the two first cathodal bands in roots and the appearance of new cathodal bands in extracts of dry seeds.

Leucine aminopeptidase shows two main zones of activity, represented in Figure 1,II. Among thirty-one races tested, only one variant has been detected in the race An-1. It is characterized by a slower migration of the more anodal band. The three parental bands are present in F_1 plants, without formation of a hybrid enzyme between 1 and 1' forms. Absence of interaction between subunits produced by different alleles has been reported for LAP in Drosophila (BECKMAN and JOHNSON, 1964) and Zea mays (BECKMAN et al., 1964). The hypothesis "one gene with two codominant alleles" is now under experimentation. LAP main bands were found to have a relatively homogenous distribution in the major organs of the plant. Seeds were however characterized by a supplementary, more anodal, band. This search for enzyme polymorphism is being extended to include esterases, peroxidases and various dehydrogenases. It is interesting to note that among populations of plants grown from the original Laibach collection of races, no intravariation has been so far observed.

References:
BECKMAN, L., and F.M. JOHNSON: Hereditas 51, 212-220 (1964)
- , J.G. SCANDALIOS, and J.L. BREWBAKER: Genetics 50, 899-904 (1964)
SMITHIES, O.: Biochem.J. 61, 629-641 (1955)

Part of the research was carried out at the Genetics Institute, State University of Groningen, Haren, The Netherlands.

Chlorate resistant mutants of Arabidopsis thaliana

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Recently we reported (OOSTINDIER, 1969) a chlorate resistant mutant of A. thaliana. Further investigations showed that the resistance is due to one recessive gene (chl-1). Two more chlorate resistant mutants were isolated. Here too resistance proved to be recessive and monogenic (genes tentatively named chl-2 and chl-3). Crosses between the mutants showed complementation between chl-2 and chl-1, resp. chl-3, and no complementation between chl-1 and chl-3.

We also reported that the extracts of chl-1 chl-1 seedlings, grown on solution A (ammoniumnitrate as nitrogen source) or D (nitrate as only nitrogen source) have a rather high level of nitrate reductase activity compared with wildtype. The same is the case with extracts of chl-3 chl-3 seedlings, but chl-2 has a quite different effect: extracts of chl-2 chl-2 seedlings have a much lower level of nitrate reductase activity than wildtype. Furthermore, it was found that chl-2 chl-2 seedlings stop growing on solution D at the stage of two small rosette-leaves, while wildtype, chl-1 chl-1, and chl-3 chl-3 go on growing on D very well. This supports the idea that it is possible to isolate mutants with a low nitrate reductase content by means of selection for chlorate resistance, as has been done with microorganisms (AZOULAY et al., 1969; GUEST, 1969; STOUTHAMER, 1967a,b).

Results of experiments on chlorate uptake by chl-1 chl-1 plants suggest that the resistance to chlorate here depends on a lower uptake of chlorate ions. The production of active nitrate reductase in wildtype plants is induced by nitrate. Further experiments with chl-2 chl-2 plants will have to reveal whether the low nitrate reductase activity in these plants is due to low uptake of nitrate, a change in the regulatory system, or a mutation in a structural gene for nitrate reductase.

References:
AZOULAY, E., J. PUIG, and P. COUCHOUD-BEAUMONT: Biochim.Biophys.Acta 171, 238-252 (1969)
GUEST, J.R.: Molec.Gen.Genet. 105, 285-297 (1969)
OOSTINDIER-BRAAKSMA, Fietje J.: Arabid.Inf.Serv. 7, 24 (1970)
STOUTHAMER, A.H.: Arch.Microbiol. 56, 68-80 (1967a)
- : Arch.Microbiol. 58, 228-247 (1967b)

Separation and identification of ribonucleotides of Arabidopsis

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Though higher plants are relatively rich in RNA, the quantitative determination of the nucleic acid content poses several problems, and no ideal methods can be found in the literature (cf. INGLE, 1963). The results vary not only from organism to organism but also from tissue to tissue within the same organism (HOLDGATE and GOODWIN, 1965).

In our laboratory we found, in agreement with the literature, that crude digests obtained with the SCHMIDT and THANNHAUSER (1945) procedure were not satisfactory if fresh or freeze-dried rosettes of Arabidopsis were used. Considerable improvement can be attained if the extract is further purified by Dowex 1 x 8,200 mesh resin in chloride or formate form. A sharp maximum can be observed in the acid eluate at 260 mμ but the O.D. 260/234 ratio is generally 2.10-2.20 indicating the presence of considerable amount of non-nucleic acid derivatives. Very high degree of purity can be obtained by chromatographic separation of individual nucleotides on Dowex 1-8, 200 mesh, formate form with a procedure similar to that of MARKHAM (1955).

The essential steps of the preparation (adapted from MILLIKAN and PICKETT, 1964; HOLDGATE and GOODWIN, 1965) are the following:

1. Lyophilized rosettes 1.5 g, ground in mortar with sand and extracted 10 x or more with a mixture of methanol-acetone-water 95:95:10 (MAW), -10°C. In between extractions centrifuged at 8,000 rpm (-10°C), 10 min. — Supernatants discarded

2. Pellet extracted 3 x with 10 ml ice cold 10% trichloroacetic acid, centrifuged — Supernatants discarded

3. Pellet extracted 3x with 20 ml ethanol:0.1 M $KOOCH_3$ (4:1), centrifuged at 8,000 rpm — Supernatants discarded

4. Pellet extracted with MAW 2x, centrifuged at 8,000 rpm — Supernatants discarded

5. Pellet extracted with 20 ml ethanol, centrifuged at 8,000 rpm — Supernatants discarded

6. Pellet extracted 2 x with 15 ml ethanol:ether (2:1) for 30 min at 50°C on water bath, centrifuged at 8,000 rpm — Supernatants discarded

7. Pellet digested with 0.33 N KOH for 7 hours, centrifuged at 5,000 rpm — Supernatant saved

8. Pellet discarded after washing 2x with NH_3 water, pH 8 — Supernatants combined

9. Supernatants brought to pH 3 with concentrated perchlorid acid, a few drops of 1 M $MgCl_2$ and 1 volume ethanol added and stored in icebath for at least 2 hours, centrifuged at 10,000 rpm for 20 min

Pellet discarded

10. Supernatant brought to pH 7.0 with KOH and centrifuged

Perchlorate sediment discarded

11. Supernatant concentrated to a few ml and pH adjusted to 8.3 with NH_4OH

12. This material is applied to the top of 15 cm long column of Dowex 1-8, 200 mesh, formate form prepared in 0.6 cm diameter tube (purification according to MARKHAM, 1955), washed with 50 ml glass distilled water and eluted sequentially by

275 ml	0.01 M HCOOH
250 ml	0.15 M HCOOH
225 ml	0.01 M HCOOH containing 0.05 M NaOOCH
250 ml	0.10 M HCOOH containing 0.15 M NaOOCH

The volume of each eluant may need adjustment as monitoring the absorbance indicates. 5 ml fractions were collected and optical density determined at the appropriate wavelengths of the UV spectrum (Fig. 1).

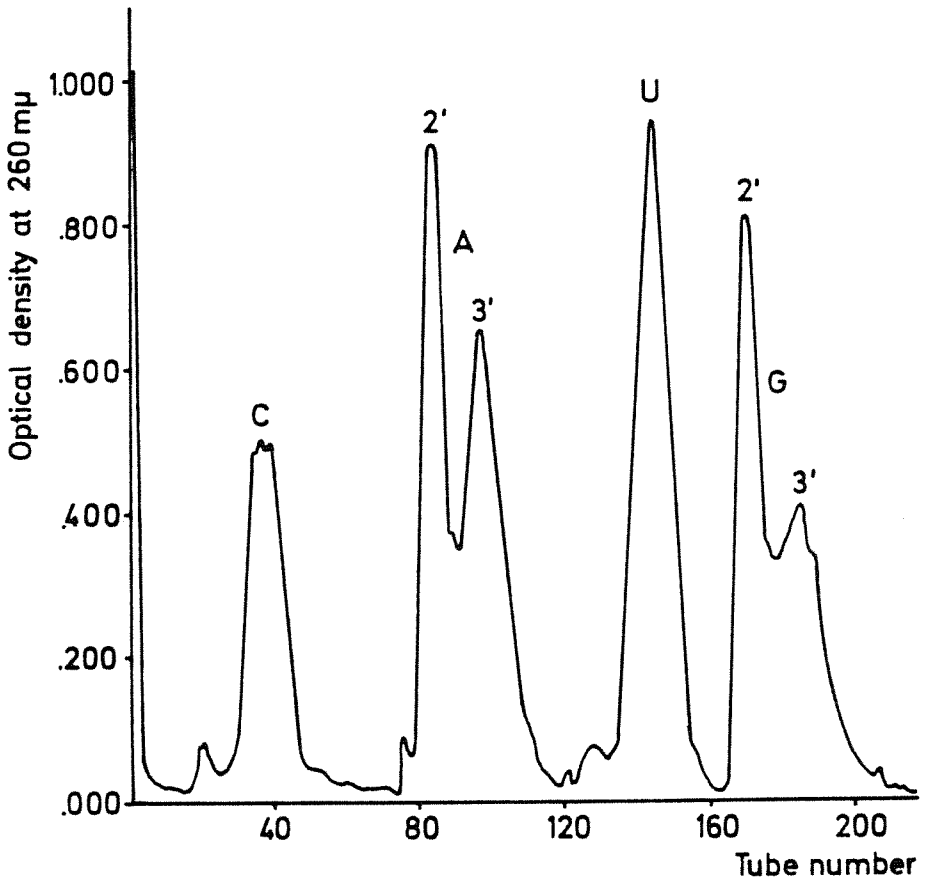

Figure 1: Dowex eluate of Arabidopsis RNA hydrolysate. C denotes cytidylic acid (sometimes the 2' and 3' isomers are separated), A designates adenylic acid with the 2' and 3' components, U marks uridylic acid and G stands for the 2' and 3' isomers of guanylic acid. The first two peaks contain relatively small quantities of bases and nucleosides

The purity of individual nucleotides appeared remarkably good (Table 1).

Table 1: Absorbance ratios of ribonucleotides eluted from the Dowex column and adjusted to pH 2.0. Expected values from DAWSON et al. (1959)

	Observed			Expected		
	250/260	280/260	290/260	250/260	280/260	290/260
Cytidylic acid	0.54	1.76	1.27	0.42-0.48	1.83-2.14	1.22-1.61
Adenylic acid	0.86	0.30	0.012	0.85	0.23	0.038
Uridylic acid	0.83	0.36	0.01	0.80	0.28	0.030
Guanylic acid	0.98	0.70	0.44	0.90	0.68	0.480

References:
DAWSON, R.M.C., D.C. ELLIOTT, W.H. ELLIOTT, and K.M. JONES: Data for Biochemical Research. Clarendon Press, Oxford 1959
HOLDGATE, D.P., and T.W. GOODWIN: Phytochemistry 4, 831-843 (1965)
INGLE, J.: Phytochemistry 2, 353-370 (1963)
MARKHAM, R.: In "Moderne Methoden der Pflanzenanalyse". (K.PAECH and M.V. TRACEY, Eds.). Vol. 4. Springer - Berlin 1955. Pp. 246-304
MILLIKAN, D.F., and E.E. PICKETT: Phytochemistry 3, 667-670 (1964)
SCHMIDT, G., and S.J. THANNHAUSER: J.Biol.Chem. 161, 83-89 (1945)

Extraction and fractionation of high molecular weight RNA of Arabidopsis

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Methods for the extraction and partition of high molecular weight RNA were reported by BONOTTO and JACOBS (1967, 1968). Since the separation according to their procedure requires the use of ultracentrifuge another simpler technique is outlined below, as adapted from the methods of GIRARD (1967) and OSAWA and SIBATANI (1967):

1. Homogenize 2 grams lyophilized tissue in 22.5 ml cold acetate EDTA buffer + 1.5 ml 10% SDS (Sodium duodecyl-sulfate) + 6 ml bentonite (50 mg/ml).
2. Add 30 ml 88% phenol (purified according to DRAPER and POLLARD, 1949), shake vigorously in 60°C water bath for 3 min, cool in ice bath and centrifuge at 1,000 x g for 3-5 min, discard the phenol (lower) phase.
3. Add again 20 ml phenol and shake for 2 min at 60°C, cool and centrifuge at 1,000 x g for 3-5 min, discard the phenol phase.
4. Repeat with 14 ml phenol, carefully collect upper phase.
5. Add 0.1 volume of 2 M NaCl and 2 volume of 95% ethanol (-20°C), mix and transfer to centrifuge tubes, keep it at -20°C for at least 2 hours, collect the precipitate by centrifugation at 10,000 rpm for 1/2 to 1 hour, -10°C.
6. Repeat alcohol precipitation twice.
7. Suspend the pellet in a small volume (5 ml) of 0.2 M acetate buffer, dialyze against 0.01 M acetate buffer for 24 hours.
8. Fractionate on MAK column (prepared by the method of OSAWA and SIBATANI, 1967), wash column with 150 ml 0.1 saline buffer then elute with 400 ml 0.4 M + 400 ml 1.6 M saline buffer (gradient elution), collect 5 ml fractions of the eluate and measure optical density at 260 mμ.

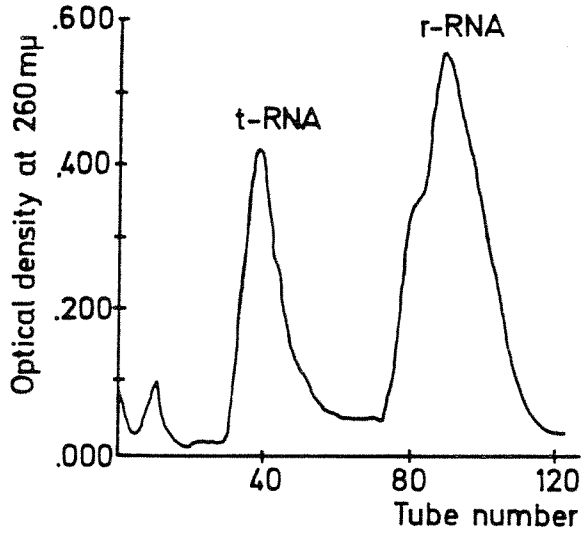

Figure 1:
Profile of RNA fractions of Arabidopsis obtained from MAK column. The first two small peaks contain bases, nucleosides and nucleotide contaminations. The first major peak is transfer RNA, the second major peak contains ribosomal RNA

References:
BONOTTO, S., and M. JACOBS: Arabid.Inf.Serv. 4, 65-67 (1967)
- , - : Arabid.Inf.Serv. 5, 59-60 (1968)
DRAPER, O.J., and A.L. POLLARD: Science 109, 448-449 (1949)
GIRARD, M.: In "Methods of Enzymology". (S.P. COLOWICK and N.O. KAPLAN, Eds.). Vol. XIIA. Acad.Press - New York 1967. Pp. 581-596
OSAWA, S., and A. SIBATANI: In "Methods of Enzymology". (S.P. COLOWICK and N.O. KAPLAN, Eds.). Vol. XIIA. Acad.Press - New York 1967. Pp. 678-686

An approach to estimate genetic redundancy in Arabidopsis

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Earlier surveys of our laboratory (RÉDEI, 1965; LI, RÉDEI and GOWANS, 1967) indicated that the spectra of biochemical mutation in green plants (Arabidopsis, Physcomitrella, Chlamydomonas) is different from those of bacteria and fungi. In the latter groups of species auxotrophic mutants concerned with the biosynthesis of amino acids, vitamins, purines and pyrimidines can be readily induced. In green plants mutants absolutely depending on external supply of the above mentioned compounds are rare, and restricted to substances synthesized through relatively few pathways.

Recently CARLSON (1970) obtained seven auxotrophic cultures of callus in tobacco and all of them were leaky. Essentially similar was the observation of WALLES (1963) on the amino acid requiring mutants of barley.

The difficulty of obtaining obligate auxotrophs in higher plants may be attributed to several causes. BRITTEN and KOHNE (1968) postulated genetic redundancy on the basis of the observation of a high multiplicity of repeated sequences in the DNA. The ribosomal RNA sites of the chromosome of Drosophila indicated well over hundred-fold redundancy on the basis of nucleic acid hybridization studies (RITOSSA, ATWOOD, and SPIEGELMAN, 1966). The widespread occurrence of isozymes (cf. SHANNON, 1968; SCANDALIOS, 1969) can also be considered as evidence for genetic redundancy. Recently ANDERSON and ADVANI (1970) distinguished by isoelectric focusing three photosynthetic enzymes from chloroplast and non-particulate cytoplasm, respectively, in Pisum.

We have isolated in an aqueous medium by differential centrifugation chloroplasts (2,000 x g, 10 min) and non-particulate cytoplasm (110,000 x g, 2 hrs) and the ribonucleolytic activity of the two fractions displayed different pH optima. The organelle contained RNase digested yeast RNA best at pH 7.5 and exhibited a sharp optimum, the RNase of the non-particulate cytoplasm was most active around pH 6 with no clear optimum (RÉDEI, 1967). Recently we isolated chloroplasts from fresh plant material in pH 5.0 (0.1 M) citrate buffer containing 0.5 M sucrose and we purified it through density gradient centrifugation (Sorvall HB 4 roter) (400 x g, 10 min) by layering it 1.5 M sucrose in citrate buffer (pH 5, 0.1 M). Intact chloroplasts were obtained in a sharp band in the middle of the tube. Above the sharp green band broken organelles were scattered and below some clumped chloroplasts and tissue debris could be distinguished.

The "intact" chloroplasts were washed three times with sucrose buffer, then suspended in distilled water, lyophilized, extracted with many changes of acetone, dried, dialyzed against several changes of distilled water for 24 hours and after freeze drying a slightly colored powder was obtained. Water extracts of this thoroughly ground material failed to show any detectable phosphomonoesterase activity. Incorporation of this particulate material into an incubation mixture containing p-nitrophenyl phosphate displayed high phosphatase activity. Some enzyme activity was present also in 0.1 M KCl extracts of this chloroplast powder. The pH optimum of the bound enzyme could not be clearly determined. Some preparations exhibited maximal activity at pH 6.0 in others the activity was about identical between pH 5.0 and pH 6.0.

The non-particulate cytoplasm contains readily water-soluble acidic phosphatase(s) with a pH optimum at 5.6. The relative activity of this enzyme shows a sharper decline in the alkaline range than the chloroplast bound protein.

Thus it appears that Arabidopsis cells compartmentalize at least two clearly distinguishable enzymes which seem to contain smaller variants. This observation lends additional support to the genetic redundancy postulated on the basis of the restricted spectrum of obligate auxotrophic mutations.

References:

ANDERSON, L.E., and V.R. ADVANI: Plant Physiol. 45, 583-585 (1970)
BRITTEN, R.J., and D.E. KOHNE: Science 161, 529-540 (1968)
CARLSON, P.S.: Science 168, 487-489 (1970)
LI, S.L., G.P. RÉDEI, and C.S. GOWANS: Molec.Gen.Genet. 100, 77-83 (1967)
RÉDEI, G.P.: Amer.J.Bot. 52, 834-841 (1965)
- : Genetics 56, 431-443 (1967)
RITOSSA, F.M., K.C. ATWOOD, and S. SPIEGELMAN: Genetics 54, 663-676 (1966)
SCANDALIOS, J.G.: Biochem.Genet. 3, 37-79 (1969)
SHANNON, L.M.: Ann.Rev.Plant Physiol. 19, 187-209 (1968)
WALLES, B.: Hereditas 50, 317-344 (1963)

A novel type of nutritional mutant

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At the present time three main types of "nutritional" mutants are available in our laboratory. Type 1 require normal metabolites for growth, type 2 cannot be normalized with natural cellular products but respond favourably to antimetabolites and type 3 is extremely sensitive to metabolites supplied in the nutrient solution and becomes lethal on media favorable to the growth of the wild type.

In our laboratory the absolute organoauxotrophic mutants reported so far all required thiamine or some precursors of this compound (RÉDEI, 1965a; LI and RÉDEI, 1968).

Some of the late flowering mutants were inducible to flower early with 8-azaadenine and halogenated deoxyribopyrimidines (HIRONO and RÉDEI, 1966a,b). Similarly another antimetabolite, 6-azauracil, was found to normalize mutant phenotype by promoting chloroplast differentiation in a variegated mutant (RÉDEI, 1965b, 1967a,b). The same mutant displayed an improved phenotype also upon irradiation with high doses of X-rays (RÉDEI, 1967c). Actually mutants at as many gene loci were found to respond favourably to antimetabolites as many responded to normal metabolites.

This year another unusual type was isolated. This mutant, though easily distinguishable from the wild type in soil cultures or on mineral-sucrose-agar media, produces fair amounts of seed under these conditions. On our complete medium, containing supplements (mgs/l) of yeast extract (10), yeast hydrolysate (10), casein hydrolysate (50) and coconut milk (20 ml), plants of the same genotype fail to grow beyond the four leaf stages and bleach and die within a period of 3-4 weeks

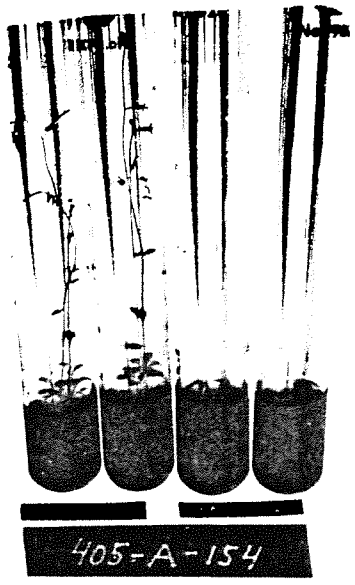

Figure 1:
Mutant 405-A-154 grown on minimal (left) and "complete" media (right) respectively. The print was made from a positive color film, therefore the plants appear dark on the complete medium though in reality they were white.

(Fig. 1). On this same nutrient solution the wild type and several other genotypes, with defective photosynthetic apparatus grow very well. The identification of the physiological mechanism of mutant No. 405-A-154 is underway.

References:

HIRONO, Y., and G.P. RÉDEI: Planta 68, 88-93 (1966a)
- , - : Planta 71, 107-112 (1966b)
LI, S.L., and G.P. RÉDEI: Biochem.Genet. 3, 163-170 (1968)
RÉDEI, G.P.: Amer.J.Bot. 52, 834-841 (1965a)
- : In "Arabidopsis Research". (G.RÖBBELEN, Ed.) Göttingen 1965b. Pp. 119-127
- : Genetics 56, 431-443 (1967a)
- : J.Hered. 58, 229-235 (1967b)
- : Radiation Botany 7, 401-407 (1967c)

New cytoplasmic mutations

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The frequency of hereditary cytoplasmic alterations is only a small fraction of that of nuclear gene mutations in the majority of organisms. In maize several hundred chromosomal genes have been analyzed but only about half dozen plastom factors were discovered. In Arabidopsis RÖBBELEN (1962) reported 23 cases of maternally inherited plastid variations verified either by reciprocal crosses or by the microscopic detection of cells with "mixed" plastids. ARNOLD and CRUSE (1966) confirmed RÖBBELEN's discovery that in Arabidopsis - contrary to Oenothera - plastid variation can be induced by X-rays.

Similar variations have been frequently observed also in our laboratory, but these plants could not be maintained. There is no information in the literature how many of the reported cases of the plastom mutations of Arabidopsis are maintained in other laboratories.

A particular type of plastom alteration was obtained and analyzed in detail by RÖBBELEN (1966). A nuclear gene (am) when homozygous induced structural alterations in the plastid and the newly arisen defect displayed maternal transmission independent from the genome. Thus am behaved as a specific plastom mutator. This unusual condition, similar to the ij (RHOADES, 1943) or cm (STROUP, 1970) systems in maize can be maintained easily through several generations.

A mutation designated as chm (chloroplast mutator) showing similarities to am were obtained in our laboratory in an EMS-treated gl[1] population (LI and RÉDEI, 1967, unpublished). The affected plants display white or yellow sectors often of considerable size; sometimes however only small discolored dots appear on the normal green background. About 10% of the selfed progeny show no appreciable number of green plastids in the cotyledons, approximately one fourth of the young seedlings are sectorial after emergence. Frequently all selfed plants become variegated before maturity, some other times there is a strong selection against the variegated tissues. When chm is used as female the majority of the F_1 plants are variegated, the reciprocal F_1 plants are not sectorial, however. In the F_2 of the first type of cross the extent of variegation is similar to that of the preceeding generation. In the reciprocal F_2 - contrary to the F_1 - variegation is observed in 25% or in somewhat lower percent of the plants. Thus the chm factor of Arabidopsis behaves differently from cm of maize where the frequency of the variegated plants in both types of F_2 generations is about the same, approximately 5%. It seems that chm is capable of inducing hereditary alterations in the plastids at at least three different "sites". RÖBBELEN (1966) did not mention that am would induce more than one type of plastid alteration. Data of a limited extent indicate that chm belongs to linkage group 3. Linkage information on am has not been reported yet. Since the phenotype of chm plants resembles to that of homozygotes for am they may be allelic, and in this case the use of different gene symbols is not permissible. Just as genes with complete dominance cannot be tested directly for allelism, the conventional allelism test is not applicable for plastid mutators, because the autonomous plastom is maternally inherited, and both types of F_1 plants are expected to be similar even in case of non-allelism (contrary to the description of STROUP, 1970). The difficulties can be overcome, however, by a simple genetic manipulation. If an individual heterozygous for am (am^+/am) and possessing normal cytoplasm (derived from an am^+/am^+ female) is produced, this plant is expected to be of non-variegating type. Using such a genotype as pistillate parent and crossed with a pollen donor of chm/chm constitution the immediate progeny may be of two alternative types. If am and chm are non-allelic all the immediate offspring is expected to be non-variegating. In case of allelism theoretically half (practically, a significant percent) of the population is supposed to be sectorial. In case of non-allelism, the bulked second generation of the cross should segregate 3 normal - 2 variegating (if penetrance and expressivity are good). In case of allelism the selfed second generation, under ideal conditions, is expected to be 3 normal and 5 variegating. Localization of a gene in a different linkage group does not absolutely rule out allelism because "new mutations" at the same gene locus may arise by transposition.

Recently another viable plastom mutant - non identical with the chm class - has been obtained. Characterization is underway.

References:
ARNOLD, C.G., and D. CRUSE: Arabid.Inf.Serv. 3, 17 (1966)
LI, S.L., and G.P. RÉDEI: 1967 unpublished
RHOADES, M.M.: Proc.Nat.Acad.Sci.(U.S.) 29, 327-329 (1943)
RÖBBELEN, G.: Z.Vererb.-Lehre 93, 25-34 (1962)
- : Z.Pflanzenphys. 55, 387-403 (1966)
STROUP, D.J.: Heredity 61, 139-141 (1970)

RBE of fast neutrons in inducing somatic and genetic effects in Arabidopsis

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In the present note general results are summarized of two independent series of experiments, one using D/Be neutrons about 5.6 MeV (work carried out in collaboration with Mrs. G. HABERER and coworkers from the Institut für Biophysik der Deutschen Akademie der Wissenschaften zu Berlin), and the other using reactor neutrons about 2 MeV. In both cases air-dry dormant seeds of A.thaliana (L.) HEYNH., race En-1, were irradiated and dose-response curves were determined for ten end-points tabulated below. For the estimation of the RBE values of neutrons the averaged dose-response data were used obtained in numerous experiments with 0.1 MeV X- and ^{60}Co gamma-rays. For each of the end-points the RBE values were calculated as mean ratios of isoeffective doses of X+gamma versus the correspondent neutron irradiation throughout the available range of response levels. The obtained RBE-values are presented in the first two columns of the following Table.

End-point	RBE of fast neutrons		
	2 MeV	5.6 MeV	Mean value
Seedling survival	10.8	10.8	10.8
Overall plant survival	8.4	9.3	8.9
Root growth	13.1	9.9	11.5
Rate of vegetative development	11.1	11.0	11.1
Arrest of plant development in the vegetative phase	8.4	8.7	8.5
Shoot growth	8.7	7.7	8.2
Rate of reproductive development	8.2	7.3	7.7
Seed setting	12.2	11.1	11.6
Sterility grade	12.1	10.8	11.4
Mutation rate (recessive embryonic lethals + cotyledon colour mutations)	8.8	5.7	7.2

The analysis of the data, presented in the table, reveals a close agreement between the RBE values of neutrons determined in the two completely independent series of experiments ($r^S = 0.70$; $P = 0.01 - 0.05$), thus indicating the non-random nature of variation in the RBE-values between the ten tested end-points. This inference is still strengthened by the absence of significant differences within the correspondent pairs of neutron dose-response curves for any of the end-points. Therefore, in the last column of the Table mean values of RBE of fast neutrons are calculated.

At least two sources of differences in RBE of fast neutrons in inducing diverse somatic and genetic effects may be suggested: (1) quantitative and qualitative differences in the primary mechanisms, responsible for the initiation of diverse end-responses, and (2) pre-differentiation of the embryonic meristematic cell population into vegetative and reproductive compartments, differing in their responses to irradiation.

A detailed description and discussion of the results will be published elsewhere.

Leaf colour mutants in Arabidopsis induced by gamma-irradiation of dormant seeds

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In this brief note some data are presented on the pattern of segregation of cotyledon colour (chlorophyll-) mutants in M_2- and M_3-progenies of A.thaliana (L.) HEYNH., race En-1, after irradiation of dormant seeds with ^{60}Co gamma-rays (dose rate about 3.7 kr/min, doses = 0, 10, 20, 40, 70, and 110 kr, respectively). M_1 to M_3 seedlings and plants were grown on mineral agar medium either on Petri dishes or in test tubes.

The majority of the cotyledon colour mutants of Arabidopsis are early recessive lethals. Therefore the procedure of detection of induced mutants includes three consecutive steps: (1) testing M_2-families for segregation, (2) raising phenotypically normal sibs from segregating M_2-families to seed setting, and (3) checking the latter in M_3 for heterozygosity. According to LANGRIDGE (1958) and LI and REDEI (1969) the following segregation ratios are expected in Arabidopsis at these steps: 7 to 1 of normals to mutants in segregating M_2-families, 5:2 of normal homozygotes to heterozygotes among phenotypically normal sibs in segregating M_2-families, and 3:1 of normals to mutants in M_3-families obtained from M_2-heterozygotes.

In the present study the following mutant phenotypes were distinguished: white (alb), pale yellow to yellow (xa), yellow-green (ch), and pale green (vi). All the alb and xa M_2-seedlings were proven by M_3 data to be true mutations, while about a half of ch and vi seedlings was comprised by a dose-independent admixture of non-heritable phenocopies. The M_2 and M_3 segregation data for proven mutations are presented in Tables 1 and 2.

Table 1: Segregation ratios in M_2 after different doses of ^{60}Co gamma-irradiation

Dose (kr)	0	10	20	40	70	110	Total
Normals	57	488	556	301	326	63	1791
Mutants	6	46	79	29	62	7	229
Ratio	9.5:1	10.6:1	7:1	10.4:1	5.3:1	9:1	7.8:1

The overall M_2 segregation ratio of normals to mutants (Table 1) was found to be in good agreement with a 7:1 expectation (P = 0.10 - 0.25) and showed no dose-dependent trend, in spite of excessive variance between the different dose-levels (P = 0.005 - 0.010), the latter being apparently due to the limited material available. On the other hand, distinct differences were observed (Table 2) between M_2 segregation ratios for the four types of mutations scored (P = 0.001 - 0.005), and a significant correlation was found between segregation ratio and germination rate in segregating the M_2-families studied (r^S = 0.43; P = 0.01 - 0.05).

Table 2: Segregation ratios for different chlorophyll mutants in M_2 and M_3 after ^{60}Co gamma-irradiation

		alb	xa	ch	vi	Total
M_2:	Normals	383	382	657	369	1791
	Mutants	47	71	79	32	229
	Ratio	8.1:1	5.4:1	8.3:1	11.5:1	7.8:1
M_3:	Normals	1076	1097	1733	1692	5598
	Mutants	277	275	343	327	1222
	Ratio	3.9:1	4:1	5.1:1	5.2:1	4.6:1

The overall M_2 segregation ratio of normal homozygotes to heterozygotes was found to be 240:83 or 5.8:2, which is in agreement with a 5:2 expectation (P = 0.25). Due to limited material available a detailed analysis of these data by dose-levels or mutation-types seems to be unreasonable.

The overall M_3 segregation ratio of normals to mutants (Table 2) was found to disagree with the 3:1 expectation (P less than 0.001) with distinct differences between mutations (P less than 0.001). The observed mutant deficit may be caused (at least, partially) by a poorer germination in M_3 as compared to M_2 (85 and 97%, respectively). However, no correlation was found between segregation and germination in M_3 (r^S = 0.15; P more than 0.05). As a reason for the absence of such correlation (contrary to M_2) a relatively high contribution of "physiological" disturbancies in M_3 germination (mascing the genetical effects on the latter) may be suggested. It therefore appears that to obtain reliable segregation data an excellent germinability of the seeds is required.

References:

LANGRIDGE, J.: Australian J.Biol.Sci. 11, 58-68 (1958)
Li, S.L., and G.P. RÉDEI: Radiation Botany 9, 125-131 (1969)

Factors limiting the increase of mutation rate after treatment of Arabidopsis seeds with nitrosamides and nitrosamines

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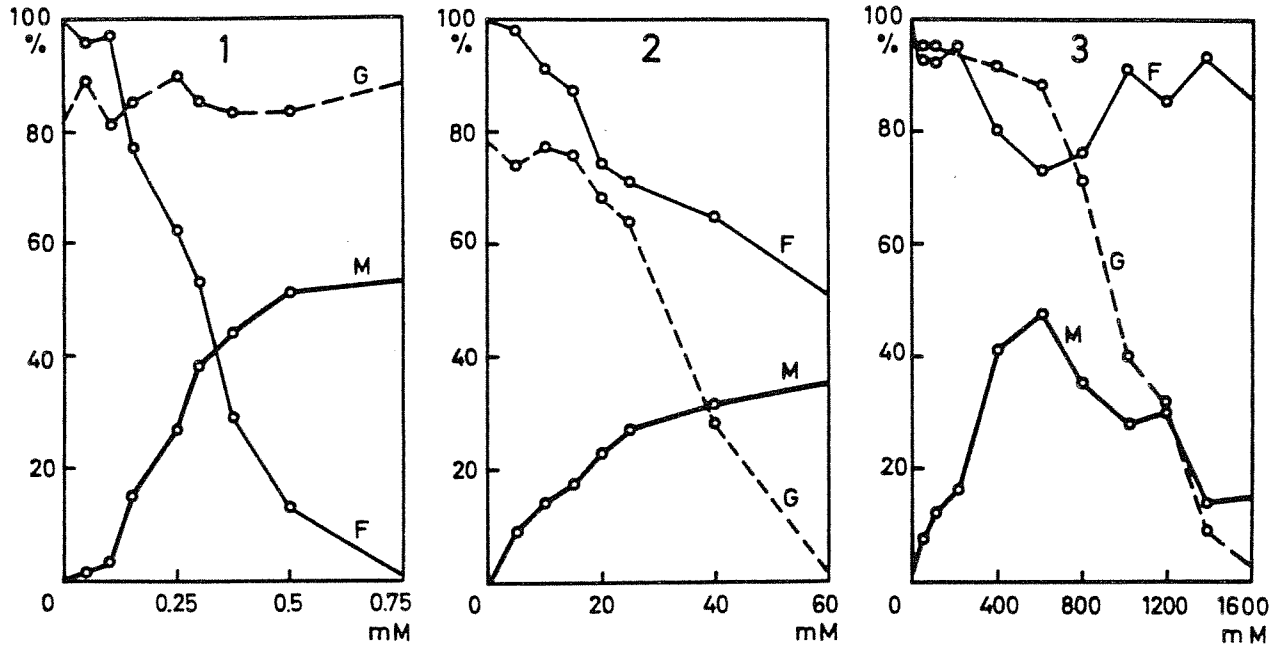
Paralell with the increasing mutation rate after mutagenic treatments, there is an increase of some types of biological damage, such as inhibition of germination, sterility etc. From a certain level this biological damage prevents the application of higher doses of mutagens and thus limits the increase of the mutation rate. The type of damage, which hinders the application of higher doses of mutagens, depends on the character of the mutagen, on the treated material etc.

In Arabidopsis according to the dose-response curves three types of limiting factors for mutagenic nitrosocompounds can be demonstrated:



Figures 1-3: Dose-response curves in mutation experiments with (1) N-methyl-N-nitrosourea, (2) ethylvinylnitrosamine and (3) dimethylnitrosamine for germination of the treated seeds (G), fertility of the M_1-plants (F) and mutation rate in the M_2 (M)

The first type is illustrated by the N-methyl-N-nitrosourea dose-response curve (Figure 1). With the increasing concentration of the mutagenic compound, the mutation rate and sterility increases, whereas the germination remains unchanged. This type of dose-response curve is typical of nitrosamides (MÜLLER and GICHNER, 1964; MÜLLER, 1964; GICHNER and VELEMÍNSKÝ, 1967).

The second type is illustrated by the ethylvinylnitrosamine curve (Figure 2). The mutation curve is characterized by a similar shoulder shape as in the case of nitrosamides; the germination, however, declines in dependence on the increased concentration. A similar dose-response curve was observed following methylbenzylnitrosamine treatment (VELEMÍNSKÝ and GICHNER, 1971).

The third type is illustrated by the dimethylnitrosamine dose-response curve (Figure 3). The shape of the mutation and sterility curve is roughly parabolic, characterized by an increase at lower concentrations and by a decline nearly to the spontaneous rate at the highest concentrations. Germination decreases steadily with the increased concentration. The same curve shape is typical of butylmethylnitrosamine (VELEMÍNSKÝ and GICHNER, 1971).

From these dose-response curves follows:

a) The high sterility is the factor limiting the increase of mutation rate after treatments with nitrosamides.
b) The toxic action on the seed-germination is the factor limiting the increase of the mutation rate following treatment with ethylvinylnitrosamine or similar compounds.
c) The limiting factor for the increase of the mutation rate following dimethylnitrosamine treatment is neither the decreased germination nor the sterility, but the extremely high concentration itself. This high concentration may bring about the inhibition of enzymes or enzyme-like system, which hydroxylate nitrosamines in vivo and thus enable the production of a mutagenic alkylating intermediate (VELEMÍNSKÝ and GICHNER, 1971).

References:

GICHNER, T., and J. VELEMÍNSKÝ: Mutation Res. 4, 207-212 (1967)
MÜLLER, A.: Züchter 34, 102-120 (1964)
- , and T. GICHNER: Nature 201, 1149 (1964)
VELEMÍNSKÝ, J., and T. GICHNER: Mutation Res., in the press (1971)

The relation of the toxicity of dialkylnitrosamines to the number of C-atoms in their molecules

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Regardless of their mutagenic ability, dialkylnitrosamines inhibit the germination of Arabidopsis seeds in a dose dependent relation (VELEMÍNSKÝ and GICHNER, 1971). The ratio between this biological damage and the concentration of the dialkylnitrosamines can be applied as a measure of their toxicity. When a logarithm of the concentration of the dialkylnitrosamines (in mM), inducing 50% inhibition of seed germination in a 24 hr treatment at 25°C, was plotted against the number of C-atoms, a straight line could be drawn for saturated non-branched derivatives, i.e. dimethyl-(2C), ethylmethyl-(3C), diethyl-(4C), n-butylmethyl-(5C) and amylmethylnitrosamines (6C) (cf. in the Figure). With the increasing number of C-atoms, the concentration needed to induce the same germination inhibition decreases, i.e. the toxicity of the compounds increases.

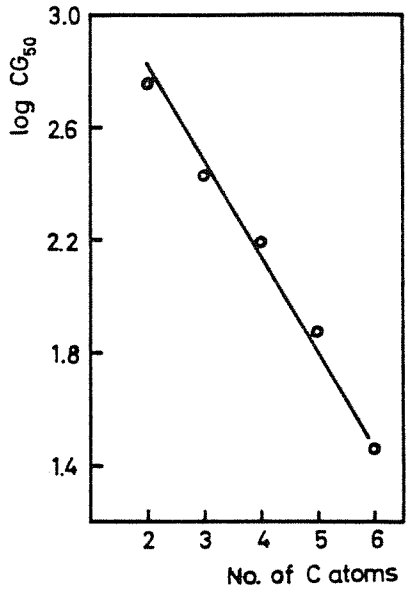

Figure:
The relation of concentration (mM) inducing 50% inhibition of seed germination (CG_{50}) to the number of C-atoms in the alkylgroups of saturated nonbranched dialkylnitrosamines. The points represent mean values established in several independent experiments.

DRUCKREY et al. (1967) established for the same dialkylnitrosamines a linear relationship of the number of C-atoms to the log. of their distribution coefficients (hexan/buffer) and to the log. of their solubility in water (g/100 ml). With the increasing number of C-atoms the distribution coefficient (lipophilic character) increased and the solubility in water decreased. These findings could perhaps explain the relation of the toxicity of dialkylnitrosamines to the number of C-atoms in their molecules. Especially the relation of toxicity to the lipophilic character can be of biological importance, since the lipophility influences the penetration of agents through membranes and their intracellular compartmentation into various structures (cf. SCHWAIER et al., 1966).

In contrast to the toxicity, no clear relation of the number of C-atoms to the mutagenic effectiveness (i.e. frequency of mutations / concentration of mutagen) of mutagenic dimethyl-, ethyl-, methyl-, n-butylmethyl- and amylmethylnitrosamines could be demonstrated. This supports the conclusion based on other facts (VELEMÍNSKÝ and GICHNER, 1971) that the toxic action of nitrosamines and their mutagenic ability are governed by two, more or less independent mechanisms.

References:

DRUCKREY, H., R. PREUSSMANN, S. IVANKOVIC, and D. SCHMÄHL: Z.Krebsforsch. 69, 103-201 (1967)

SCHWAIER, R., F.K. ZIMMERMANN, and R. PREUSSMANN: Z.Vererbungsl. 98, 309-319 (1966)

VELEMÍNSKÝ, J., and T. GICHNER: Mutation Res., in the press (1971)

Mutation induction by alkylating agents: Post-treatment storage of soaked seeds

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Arabidopsis seeds treated with solutions of chemical mutagens can be stored in water if germination is inhibited by anaerobiosis. It has been shown previously (MÜLLER, 1965, 1966, 1967) that such post-treatment "water storage" for 2 days does not alter significantly the frequency of mutations induced by monofunctional alkylating agents. These results suggest that mutation induction is the consequence of alkylation of non-replicating DNA and that mutation fixation is not detectably influenced by delaying the start of DNA replication for 2 days. Later we found that it is possible to apply much longer periods of "water storage". There are at least two storage conditions under which the germinability of soaked seeds is preserved for several weeks (at 24°C): (1) Strong anaerobiosis created by submersion of seeds in N_2 water (oxygen replaced by nitrogen), or (2) holding the seeds in the dark. (The obligate light requirement for germination of strain "Dijon G" is fulfilled by a short impulse of light. If light is avoided from the beginning of soaking, seeds do not germinate under aerobic conditions.)

Seeds of strain "Dijon G" were presoaked for 12 hrs in N_2 water and treated for 3 hrs at 24°C with 60 mM ethyl methanesulfonate (EMS), 30 mM methyl methanesulfonate (MMS), or 0.3 mM N-methyl-N-nitrosourea (MNH). After 5 min of washing, the seeds were either germinated immediately or stored for periods of 2 to 20 days in N_2 water in the dark. Germination was induced by spreading the seeds on wet filter paper and exposing them to light. Germinated seeds were transferred to soil and grown in the greenhouse.

In all variants (with the exception of those treated with MMS) the percentage of germinated seeds was 97-100% and the survival of M_1 plants ranged from 85 to 95%. (No significant difference as compared with the control!) After MMS treatment there was a storage-dependent decrease of germination rate from 98% (no storage) to 68% (20 days storage). The frequencies of embryonic lethal and chlorophyll mutations were determined by the embryo test, using one silique per M_1 plant.

Table:

Mutagenic treatment	Duration of storage (days)	No. of M_1 plants scored	Segregating silique progenies (%)	
			embryonic lethals	chlorophyll mutations
60 mM EMS	0	200	82.5	23.0
	2	200	79.5	21.5
	5	200	81.0	20.5
	12	200	78.0	23.0
	20	250	71.6	22.4
30 mM MMS	0	200	83.0	21.5
	5	200	80.5	22.5
	12	200	79.3	20.5
	20	250	81.2	20.0
0.3 mM MNH	0	300	84.3	25.0
	2	200	77.0	23.0
	5	200	75.5	23.5
	12	200	70.0	17.5
	20	250	68.0	15.2
Control	0	300	2.7	0
	20	200	1.5	0.5

The results (see Table) of the EMS and MMS experiments show that the frequencies of both types of mutations are not significantly influenced by storage. As anaerobiosis should not inhibit hydrolytic processes (as it does with enzymatic processes), this constancy of mutation frequency indicates that hydrolytic degradation of alkylated DNA is not involved in the formation of the mutations observed. It is well known from in vitro studies that methylation and ethylation of DNA lead to depurination and backbone breakage (see RHAESE and FREESE, 1969). These spontaneous changes of alkylated DNA are often discussed as possible causes of mutations. Our results suggest furthermore that non-germinating seeds are unable to repair premutational lesions in alkylated DNA, - a conclusion which is also supported by the results of experiments with fractionated doses (MÜLLER, 1969).

In an additional experiment, EMS treated seeds were stored aerobically on wet filter paper for 20 days (no germination induction by light!). Also under these conditions the mutation frequency remained constant during storage.

In the experiment with MNH we found a decrease of mutation frequency during storage (see Table). Although we cannot explain the difference between MNH and the methanesulfonic esters as to the response to storage, it should be mentioned that a mutation frequency decline is not necessarily an indication of repair of lesions in the DNA.

References:

MÜLLER, A.J.: Mutation Res. 2, 426-437 (1965)
- : Züchter 36, 201-220 (1966)
- : Arabid.Inf.Serv. 4, 43 (1967)
- : Arabid.Inf.Serv. 6, 28 (1969)
RHAESE, H.J., and E. FREESE: Biochim.Biophys.Acta 190,418-433 (1969)

B. TECHNIQUES

Submerged aseptic culture of intact plants in liquid medium

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Arabidopsis can be cultured by a wide variety of aseptic techniques using whole plants, isolated organs or tissues (cf. RÉDEI, 1970).

In aseptic media the plants are generally supported by agar or perlite. NEALES (1968) grew isolated roots in liquid media, however. Methods for growing intact higher plants under aseptic conditions in liquid submerged culture do not seem to be available (cf. KLEIN, 1970).

In the past years we successfully grew Arabidopsis in liquid culture. The basic mineral solution is the same as generally used (RÉDEI, 1965) and with 2% glucose added this constitutes the "minimal" nutrient medium. From this liquid 80 ml is distributed to 250 ml Erlenmeyers, then plugged and autoclaved. After cooling 30 seeds (disinfected by treatment with 5% calcium hypochlorite for 8 min and washed with 4-5 changes of sterile distilled water) are dropped into each flask under aseptic conditions. The Erlenmeyers are placed on a slow moving reciprocating shaker to insure sufficient aeration. Under such conditions the plants grow more rapidly than on agar. The improved growth can be attributed to the fact that a large surface of the plants is exposed to the nutrients and apparently the uptake through the leaves is very efficient. The growth of the roots is remarkably good in such submerged cultures. This technique seems very promising for investigations on nutrition through the leaves of intact plants, a problem of interest for plant physiologists and agronomists (cf. BOYTON, 1954; WITTWER and TAUBNER, 1959; SARGENT, 1965; FRANKE, 1967; EPSTEIN and JEFFERIES, 1964), but difficult to study on plants of large size. The generally used spray techniques are hard to quantitate, besides leaf burns often occur because of the evaporation of the solvents.

This type of culture also facilitates the economical, aseptic production of fair quantities of plant material for biochemical analyses.

References:
BOYNTON, D.: Ann.Rev.Plant Physiol. 5, 31-54 (1954)
EPSTEIN, E., and R.L. JEFFERIES: Ann.Rev.Plant Physiol. 15, 169-184 (1964)
FRANKE, W.: Ann.Rev.Plant Physiol. 18, 281-300 (1967)
KLEIN, R.M., and D.T. KLEIN: "Research Methods in Plant Science". Natural History Press - Garden City, N.Y. 1970
NEALES, T.F.: New Phytol. 67, 159-165 (1968)
RÉDEI, G.P.: Amer.J.Bot. 52, 834-841 (1965)
SARGENT, J.A.: Ann.Rev.Plant Physiol. 16, 1-12 (1965)
WITTWER, S.H., and F.G. TAUBNER: Ann.Rev.Plant Physiol. 10, 13-27 (1959)

Continuous culture of excised roots of lethal fusca mutants

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Most of the recessive fusca mutants of Arabidopsis thaliana (MÜLLER and HEIDECKER, 1968) are unable to grow on mineral medium or on medium supplemented with sugars, amino acids, vitamins or extracts from wildtype plants. These lethal fusca mutants can be roughly divided into two groups: (1) mutants with an early lethal phase which die immediately after germination, and (2) mutants with a somewhat later lethal phase developing a short root and some very small rosette leaves. In order to get some information about organ-specific expression of the genes involved, we tried to culture excised roots of lethal fusca mutants. Whereas the early lethals failed to grow, all the mutants with residual growth of the seedlings developed excisable roots which were continuously propagated for over 9 month. The root cultures of three mutants (fusA344, fusD262, fusD122) differed insignificantly in growth rate from the wildtype "Dijon G". Excised roots of the other mutants (fusD120, fusD136, fusD123, fusD307, fusD282, fusD109) are characterized by lower growth rates, but can be cultivated continuously by repeated cloning and transfer to fresh medium.

We used the m e t h o d s described by NEALES (1968) for culture of excised roots of A.thaliana with some modifications: The seeds were surface sterilized in 0.01% $HgCl_2$ for 10 min, three times washed with sterile distilled water and set to germinate in petri dishes on agar medium (24°C, light). After 8-12 days the seedlings were transferred to 100 ml Erlenmeyer flasks containing 50 ml of liquid medium. The cultures were then incubated at 27°C in the dark for 2 weeks, during which the main root of the wild type reached 5-7 cm and many lateral roots emerged. The shoot bleached out and died. Only at this stage the roots were excised and cut into 4 or 5 pieces which were then singly transferred to new flasks. Late excision proved advantageous for cultivation of the mutants.

The medium used was that of WHITE modified according to STREET and McGREGOR (1952) which contains inorganic salts, sucrose and vitamins. Iron was supplied as Fe-EDTA. The pH was adjusted to 5.0 before autoclaving.

Table: Effect of temperature on growth over 34 days of excised roots of strain "Dijon G". Final weight is given by the mean values of 5 cultures. The inocula weighed 20-30 mg (fresh) and 2-3 mg (dry)

Temperature (°C)	Growth of roots		
	Fresh weight (mg)	Dry weight (mg)	Dry weight (rel.%)
19	33.2	3.3	14.6
23	101.8	9.1	40.3
27	233.5	22.6	100.0
31	27.9	4.2	18.6

The effect of temperature on growth of excised roots of the wildtype "Dijon G" is given in the Table. The optimum temperature was about 27°C. A comparison of our results with the results obtained by NEALES (1968) shows that excised roots of "Dijon G" are much more sensitive to deviations from optimum temperature than roots of strain "Estland".

References:
MÜLLER, A.J., und U. HEIDECKER: Arabid.Inf.Serv. 5, 54 (1968)
NEALES, T.F.: New Phytol. 67, 159-165 (1968)
STREET, H.E., and S.M. McGREGOR: Ann.Bot. 16, 185-205 (1952)

Preliminary report on callus formation and growth in vegetable-juice and WHITE's liquid media

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Wildtype root pieces were inoculated into liquid vegetable-juice medium and into modified WHITE medium. Callus appeared after one month and grew into balls around 1 mm in diameter after three months.

The vegetable juice medium was HELLER's (WHITE, 1963) to which had been added 2% dextrose and 10% "V8" juice. V8 juice is a blend of tomatoes, carrots, celery, beets, parsley, lettuce, watercress, and spinach. The modified WHITE medium is the one used for growing tobacco cells (FILNER, 1965).

In other experiments, roots were blenderized 15 seconds and used to inoculate the following three media:

A. Heller minerals + 2% dextrose + 3% coconut milk
B. Heller minerals + 2% dextrose + 6% V8 juice
C. Heller minerals + 2% dextrose

Callus formation was obtained in both media A and B, but subsequent growth of the callus is extremely slow. See Table 1 for growth results.

Table 1: Growth of Arabidopsis root tissue in three media. The results are in mg of dry weight

	Medium		
	A	B	C
Inoculum	4.6	4.6	4.6
Week 3	10.0	26.0	3.2
Week 6	10.4	18.0	10.0
Week 8	8.0	36.6	4.4

When whole plants, 2'' tall, were used as inoculum in modified WHITE medium, callus nodules appeared in a month. However, when placed in V8 juice medium, the plants usually grew into irregular green balls 11 to 15 mm in diameter with numerous roots, stems, and leaves. Parts of these balls, when transfered to fresh medium, grew into balls in about two weeks. Hence fresh material can be kept by transferring into fresh medium every two weeks.

Following the work of ZIEBUR (1965) who has subcultured tissue from a piece of hypocotyl, we have transferred the callus culture obtained from modified WHITE medium into agar culture containing 2,4-D at the concentration of 3 mg/liter and 20% coconut milk and into another agar culture where the coconut milk was replaced by 10% corn liquid. In both media growth is very rapid and results are very promising.

References:
FILNER, P.: Exp.Cell Research 39, 33-39 (1965)
WHITE, P.: "The Cultivation of Animal and Plant Cells". 1963. P. 61
ZIEBUR, N.: Arabid.Inf.Serv. 2, 34-35 (1965)

C. MATERIAL

Die morphologischen Mutanten des Göttinger Arabidopsis-Sortiments, einschließlich der Mutanten mit abweichender Samenfarbe

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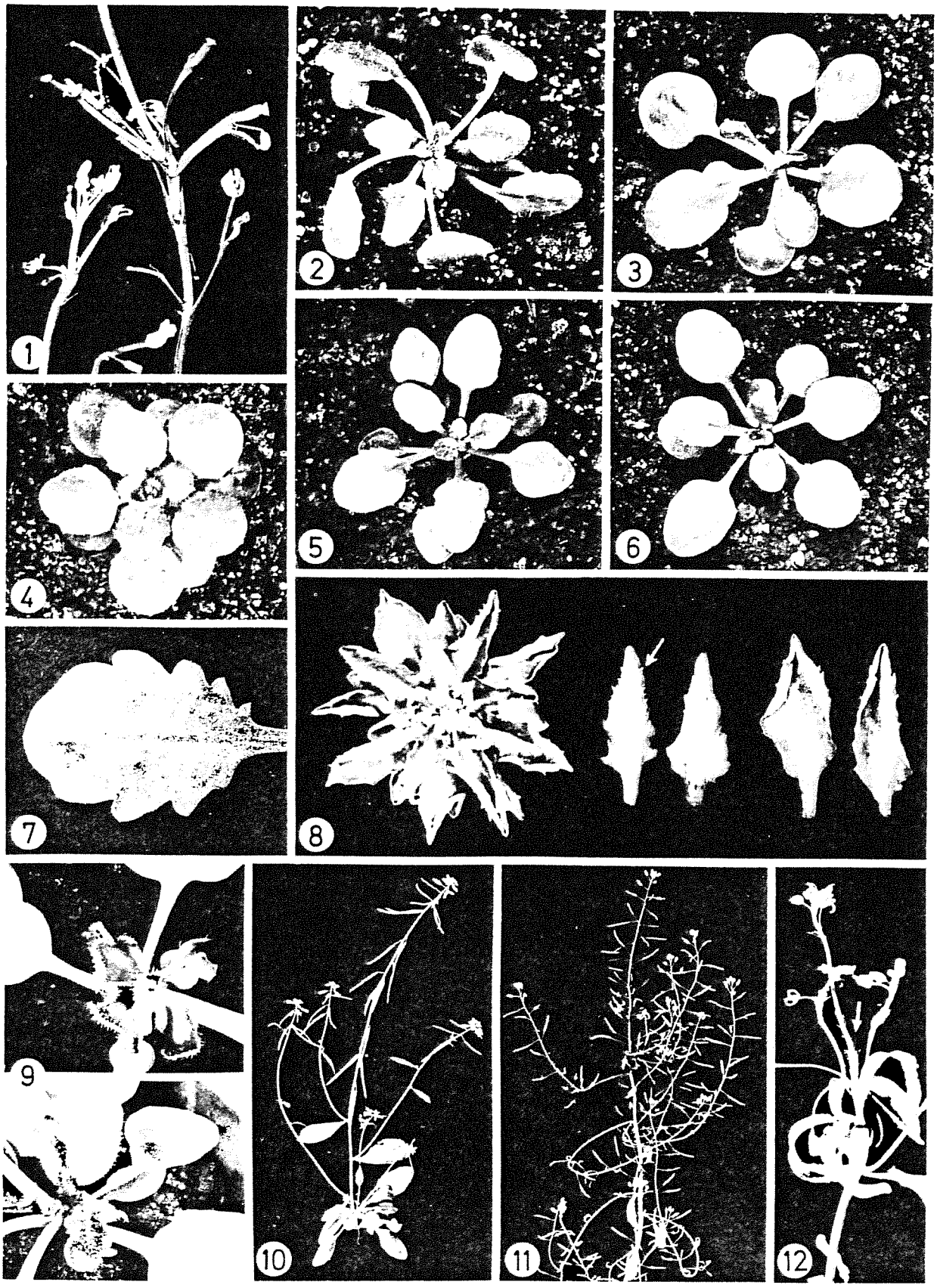
Seit vielen Jahren werden in Göttingen zahlreiche, zumeist durch Chemikalien induzierte Mutanten aus der Arabidopsis-Rasse "En" (Enkheim) und zum Teil auch aus der Rasse "An" (Antwerpen) zu vorwiegend genetischen Untersuchungen verwendet. Aus verschiedenen Gründen muß ein Teil dieser Arbeiten z.Zt. zu einem vorläufigen Abschluß gebracht werden. Es erscheint daher zweckmäßig, eine Reihe von Mutanten, die für Kreuzungsuntersuchungen oder andersartige, z.B. entwicklungsphysiologische Studien von Interesse erscheinen, im folgenden kurz zu skizzieren. Dabei wurde bewußt auf eine Benennung der verschiedenen Typen verzichtet, da zwar die Konstanz der veränderten Merkmale nachgewiesen, jedoch nicht in allen Fällen weitere genetische Analysen angeschlossen werden konnten.

Wesentliches Merkmal der Mutantengruppe	Nr. der Mutante	Zusätzliche Merkmale der Mutante
1) Veränderungen an der Keimpflanze		
Kotyledonen verändert	F 37 (Abb. 1)	Kotyledonen becherförmig (Erhaltung nur über Heterozygote)
	F 61	Kotyledonen klein glänzend, Rosette winzig, aber Blätter lang gestielt (Erhaltung nur über Heterozygote)
	F 62	Kotyledonen grau, Rosetten verwachsen
	F 63	Kotyledonen klumpig verwachsen (Erhaltung nur über Heterozygote)
	F 38 } F 39 }	1 Keimblatt (unvollständige Penetranz), Rosette klein, subletal
Hypokotyl verlängert (ähnlich hy)	M 42/250	weiße Samen. Kreuzung mit hy ergibt in F_1 Wildform, offenbar sind beide Faktoren verschieden
	M 41/100	Hypokotyl nur etwas verlängert, Blätter schmaler, heller grün
	V 317	keimt schlecht, Rosette hellgrün, groß, leicht verbändert



Morphologische Mutanten aus dem Göttinger Sortiment

Abb. 1: F 37
" 2: F 104
" 3: F 130
" 4: F 91

Abb. 5: F 44
" 6: F 41
" 7: V 315
" 8: F 98

Abb. 9: R 81/23
" 10: M 41/72
" 11: M 42/244
" 12: M 42/300

Wesentliches Merkmal der Mutantengruppe	Nr. der Mutante	Zusätzliche Merkmale der Mutante
2) Veränderungen an der Rosette		
Zwerge	F 64	Blätter keulenförmig kurz, gelblich grün
	F 65	Kotyledonen gelb, Primärblätter dunkelgrün, Rosette moosartig, subletal (Erhaltung durch Heterozygote)
	F 66	Kotyledonen klein, graugrün, z.T. weißlich gefleckt, Primärblätter heller, fädig
	V 105 V 125	gelbgrüne, kurzgestielte Rosettenblätter, sehr spät
Dichte Rosette durch kurz gestielte Rosettenblätter (wie rosula)	F 132	Samen dunkelbraun
	F 127	Rosette etwas lockerer, Samen dunkelbraun
	F 30	sehr klein
	F 31	unbehaart, gelbsamig
	F 4 u. F 83	sowie 21 weitere F-Mutanten und 4 M42-Mutanten mit unterschiedlicher Ausprägung des Mutantenmerkmals
Blätter mit den Rändern nach unten gewölbt, kurz gestielt, aus den runden, kurzen Knospen ragen die Narben hervor, Blütenblätter oft mehr oder weniger verkümmert (ähnlich as)	F 34 M 42/52 M 42/234	Bei Kreuzungen mit as und untereinander sieht die F_1 wie as aus, also scheinen alle drei Mutanten mit as allelisch zu sein
	M 42/302	breitere Blätter und längere Blattstiele als as, heller grün
	M 42/244	Seitenäste der Infloreszenz schlingenartig tordiert
	7 weitere Mutanten aus M 42	
Schraubige Blattstellung entgegen dem Uhrzeiger (schon an den Koyledonen kenntlich)	F 104 (Abb. 2)	gekoppelt mit vc_2, Gruppe 6
Blätter runder, fast glattrandig, Blattstiele und Infloreszenz verkürzt (ähnlich er)	3 Mutanten aus F-Sortiment und 7 Mutanten aus M 42	
3) Veränderungen der Blattgestalt		
Blattrand nach oben eingerollt, Blätter z.T. heller grün, kleiner; frühblühend, Blütenblätter meist etwas verkümmert, Kelchblätter persistierend	F 21	klein, stark eingerollte Rosettenblätter
	F 68	Blätter leicht eingerollt, Blütenblätter reduziert bis fehlend
	F 150	Blätter schwach grünadrig
	F 72	Blätter schwach gerollt, variable Manifestation
	4 weitere Nummern aus dem F- und 2 aus dem V-Sortiment	
	Bei Kreuzungen von F 21, F 72 und F 150 untereinander ist die F_1 Mutantentyp; alle drei Mutanten sind somit allelisch	
Unebene Blattspreite	F 44 (Abb. 5)	Blätter rhombisch; frühblühend
	M 41/19	insbesondere die inneren Blätter wie verknüllt, etwa normal groß; frühblühend, fertil

Wesentliches Merkmal der Mutantengruppe	Nr. der Mutante	Zusätzliche Merkmale der Mutante
Löffelartige Blätter	F 130 (Abb. 3)	
	F 98 (Abb. 8a,b)	kurz gestielt (ähnlich rosula), unbehaart, warzenähnliche Auswächse an Blattunterseite und Kelchblättern, Knospen relativ kurz, meist frühzeitig offen
	F 75	und weitere 3 Mutanten mit mehr oder weniger stark ausgeprägter Löffelblättrigkeit
Schmale Blätter (wie an)	M 41/88 M 42/189	Bei Kreuzungen mit an oder untereinander ist die F_1 schmalblättrig, alle 3 Mutanten sind somit allelisch
	M 42/278	
	M 42/226	kleine Blätter, lange Blattstiele
Breite Blätter	F 26	rel. stark behaarte Blätter, kurze Blattstiele
	F 91 (Abb. 4)	Rosette hellgrün, leicht gelbherzig
	F 100	Same heller und größer als normal (4 n ?)
	Weitere 9 Mutanten meistens aus dem F-Sortiment	
Grob gezähnte Blätter	F 101	Blätter breit, vor allem im basalen Teil stark gezähnt, lange Blattstiele, sehr spät (4 n ?)
	F 125	Blätter lang und schmal
	V 315 (Abb. 7)	hellgrüne Rosette
	4 weitere Mutanten	
Abweichend behaarte Blätter	M 42/383	hellgrüne, schmalere Blätter
wenig behaart (meist kürzere und weniger gegabelte Haare)	M 45/78	Pflanze niedrig, Infloreszenz buschig
	F 153	
	F 154	Blätter oval, fast glattrandig; frühblühend
unbehaart	F 1	Samen gelb
	M 42/200	
	M 42/209	allelisch mit gl und Wil-2
	F 31	kurz gestielte Rosettenblätter, Samen gelb
	F 41 (Abb. 6)	nicht allelisch mit gl und F 1
	F 136	Samen gelb
	F 155	gelbherzige Rosette (nicht allelisch mit gl)
	V 211	grünadrige Blätter (nicht allelisch mit gl)
	V 360	Rosette je nach Belichtung grün bis fast weiß, Samen gelb
	Weitere 15 Mutanten meist aus M 42 und M 45	
4) Veränderungen im Bereich der Infloreszenz		
Infloreszenz niedrig und buschig	F 146	Rosette graugrün, rötlich; frühblühend
	F 74	Rosette dunkel, leicht rötlich
	F 92	Rosette gedrungen, rötlich; spät
	M 41/100	Pflanze bis 3 cm hoch

Wesentliches Merkmal der Mutantengruppe	Nr. der Mutante	Zusätzliche Merkmale der Mutante
	M 41/103	gut fertil
	M 41/110	längeres Hypokotyl; fertil
	M 41/126	ähnlich acaulis
	M 42/141	Blätter rundlich, Stiele lang, viele Knospen in Blattachseln; fertil
	M 42/411	Blätter etwas heller grün; frühblühend
	M 45/73	
	M 45/85	gut fertil
	F 2	normal gestalteter Zwerg, frühblühend
	Weitere ca. 20 Mutanten meist aus M 42 und M 45	
Schoten abwärts gerichtet	M 41/71	
	M 41/72 (Abb. 10)	
	M 42/164	Schoten nur leicht abwärts, Blätter hellgrün, Pflanze niedrig, Seitenäste sperrig, z.T. steril
Abnorme Verzweigung	M 45/15	(vgl. AIS 7, 33, 1970)
	M 41/61	
	M 41/92	Verzweigungsanomalie nur schwach ausgeprägt. Alle 3 Mutanten sind allelisch
Verbänderte Infloreszenz	F 40	Verbänderung variabel
	M 45/19	Seitenzweige weitgehend fehlend, Schoten breit (mit 4 Karpellen)
	M 45/17	Infloreszenz kurz, Schoten breit
	F 15	Samen gelb, nachdunkelnd
	6 weitere Mutanten mehr oder weniger stark verbändert und 15 Mutanten mit mehr oder weniger breiten Schoten, z.T. verbändert	
Sperriger Wuchs	V 313	Rosette hellgrün, leicht grünherzig, Blätter etwas schmaler und stärker gezähnt
	M 42/244 (Abb. 11)	Rosette anfänglich ähnlich as, Seitenäste schlingenförmig tordiert
	M 42/139	Blätter graugrün, stärker gezähnt, Schoten waagerecht abstehend; spät blühend
	M 42/164	Blätter hellgrün, Infloreszenz niedrig, Schoten leicht abwärts gerichtet, z.T. steril, spät
Glänzender Stengel (wie $\underline{vc}_2$)	M 45/33	nicht allelisch mit $\underline{vc}_2$
	M 45/42	Rosette gelblich grün, leicht grünherzig
	M 45/55	nicht allelisch mit $\underline{vc}_2$
	17 weitere Mutanten aus M 42 und M 45	
Sonstige Abweichungen	F 151	Infloreszenzen enden häufig blind, Blätter hellgrün, groß, oval, fast glattrandig; relativ frühblühend
	R 81/23 (Abb. 9a,b)	Trichterblätter, Infloreszenzen oft blind endend, weitgehend steril
	V 191	Teile der Infloreszenz sterben nekrotisch ab (Ausprägung je nach Jahreszeit verschieden stark)

Wesentliches Merkmal der Mutantengruppe	Nr. der Mutante	Zusätzliche Merkmale der Mutante
5) Veränderungen an den Blüten		
Blütenorgane umgebildet oder verkümmert	M 41/118	fast keine Blütenblätter, Infloreszenz zierlich, schlank, fast steril
	M 42/300 (Abb. 12)	kaum Blütenblätter, Blüte oft kümmerlich (sehr wenig Samen), kleine pelzartige Ringe an den Infloreszenzen (offenbar aus Blütenanlagen hervorgehend)
	R 81/3	Antheren zu Samenanlagen umgebildet, dadurch ♂-steril, hellgrün
6) Veränderte Samenfarbe		
Gelb	F 1	Blätter unbehaart
	F 97	Blätter unbehaart, kurz gestielte Rosettenblätter, im
	F 98	Blätter unbehaart, kurz gestielte Rosettenblätter mit warzenförmigen Anhängen
	F 136	Blätter unbehaart, mehr graugrüne Rosette
	M 42/1 bis 8	Blätter unbehaart
	M 42/465 bis 477	Blätter unbehaart, Same etwas dunkler gelb
	M 45/1	Blätter unbehaart
	M 45/6 bis 8	Blätter unbehaart
	F 18	Rosette dunkelgrün, klein, verschieden groß
	F 107	Same fast weißlich
	F 142	Same fast weißlich, Rosette ungleich groß, heller grün
	F 17	Rosette etwas verwachsen
	M 42/9	Samen etwas dunkler, Blätter spitz, Infloreszenz ziemlich niedrig, buschig
	M 45/2 bis 5	
	M 45/9 bis 13	Unterschiede in Blattfarbe und Blühzeit
	M 42/459 bis 464	
	M 42/250	Same fast weißlich, langes Hypokotyl
	V 220	Same dunkelgelb
Gelb, nachdunkelnd	F 15	verbänderte Infloreszenz
	F 19	
	V 205	Rosettenblätter gescheckt
	V 208	Rosette gelbgrün, gelbherzig; spät blühend
Hellgrau-braun	F 100	anfangs hellgrün, Samen groß (4 n ?)
	M 42/114	Rosette gelb
	M 42/168	Blätter leicht grünadrig
Grünlich-graubraun	F 36	Pflanze kleiner, heller grün
	F 52	Blätter rund
	F 131	
	F 135	Blätter rund, dunkler grün
	M 45/44	
	M 42/455	Schoten hell rötlich
	V 124	gescheckte Rosettenblätter

Wesentliches Merkmal der Mutantengruppe	Nr. der Mutante	Zusätzliche Merkmale der Mutante
Dunkelbraun	F 127	kurz gestielte Rosettenblätter
	F 132	kurz gestielte Rosettenblätter
	V 119	ähnlich lu (lutescens)
	V 127	ähnlich lu

A four-leaf stage lethal mutant in Arabidopsis thaliana

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Lethal-4 (l_4): Seedlings (l_4l_4) grow normal and differentiation continues up to the four-leaf stage. Cessation of development starts with a degradation of chlorophyll at this stage. The four rosette leaves turn yellowish and dry within 2-3 days.

Origin: "Landsberg-erecta", line 5I, after 9 mM EMS. Heterozygous plants (L_4l_4) normal, segregate l_4l_4-seedlings with a frequency 25% in M_3 to M_6, indicating complete recessivity of this mutation.

The material is expected to be suitable for studies on developmental processes in A.thaliana.

D. BIBLIOGRAPHY

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

DALY, K.R.: Effect of temperature on survival of γ-irradiated seeds of Arabidopsis thaliana. Experientia 27, 81 (191)

GICHNER, T., and J. VELEMÍNSKÝ: The mutagenic activity of nitrosamides and nitrosamines on a plant - Arabidopsis thaliana. Environmental Mutagenic Society, Newsletter 3 (1970)

GICHNER, T., J. VELEMÍNSKÝ, and V. POKORNÝ: Comparison of the mutagenic activity of N-methylnitrosourea, N,N',-dimethylnitrosourea and N,N',N',-trimethylnitrosourea. Arzneimittelforschung 19, 1053-1055 (1969)

HIRONO, Y., H.H. SMITH, J.T. LYMAN, K.H. THOMPSON, and J.W. BAUM: Relative biological effectiveness of heavy ions in producing mutations, tumors, and growth inhibition in the Crucifer plant, Arabidopsis. Radiat.Res. 44, 204-223 (1970)

KASYANENKO, A.G., and Yu.S. NASYROV: The effect of genetic factors on the photosynthetic apparatus of Arabidopsis thaliana (L.) HEYNH. Fiziologiya Rastenii 15, 422-429 (1968)

MATAGNE, R.: Toxicité et mutations induites chez Arabidopsis thaliana (L.) HEYNH. par les isomères du diépoxybutane. Bull.Soc.roy Bot. Belgique 102, 239-248 (1969)

- : Induction of chromosomal aberrations and mutations with isomeric forms of L-threitol-1,4-bismethanesulfonate in plant materials. Mutation Res. 7, 241-247 (1969)

McCULLOUGH, J.M., and W. SHROPSHIRE, jr.: Physiological predetermination of germination responses in Arabidopsis thaliana (L.) HEYNH. Plant and Cell Physiol. 11, 139-148 (1970)

MĚSÍČEK, J.: Chromosome counts in Cardaminopsis arenosa agg. Preslia (Praha) 42, 225-248 (1970)

RÉDEI, G.P.: Gene function and phenotypic repair. In "Induced Mutations in Plants". International Atomic Energy Agency, Vienna 1969. Pp. 51-60

SEARS, Lotti M.S., and Suzanne LEE-CHEN: Cytogenetic studies in Arabidopsis thaliana. Can.J.Genet.Cytol. 12, 217-223 (1970)

SHIRALIPOUR, A., and D.S. ANTHONY: The effect of supraoptimal temperatures on the contents of KREBS' cycle acids in Arabidopsis thaliana. Phytochemistry 8, 1179-1183 (1969)
- , - : Chemical prevention of growth reduction caused by supraoptimal temperatures in Arabidopsis thaliana. Phytochemistry 9, 463-469 (1970)
SMITH, H.H., D.C. JOSHUA, N.C. COMBATTI, and K.H. THOMPSON: Relative effects of fission neutron vs. X-irradiation of seeds over a wide range of genotypes, doses and moisture levels. In "Symposium on Neutrons in Radiobiology". USAEC Conf.-691106 (TID-4500), Oak Ridge, Tenn. 1970. Pp. 173-191
USMANOV, P.D., and A.J. MÜLLER: Use of the embryo test for the analysis of embryonic lethals induced by irradiation of pollen of Arabidopsis thaliana (L.) HEYNH. (In Russian) Genetika (Moscou) 6, No. 7, 50-60 (1970)
VELEMÍNSKÝ, J., and T. GICHNER: The influence of pH on the mutagenic effectiveness of nitroso compounds in Arabidopsis. Mutation Res. 10, 43-52 (1970)
VELEMÍNSKÝ, J., S. OSTERMAN-GOLKAR, and L. EHRENBERG: Reaction rates and biological action of N-methyl and N-ethyl-N-nitrosourea. Mutation Res. 10, 169-174 (1970)
VELEMÍNSKÝ, J., J. ŠVACHULOVÁ, and W. STARZECKI: Photosynthesis of two chlorophyll mutants of Arabidopsis thaliana growing on a sucrose medium. Photosynthetica 3, 97-103 (1969)

(In the press)

JONES, M.E.: The population genetics of Arabidopsis thaliana. (I) The breeding system. Heredity
- : The population genetics of Arabidopsis thaliana. (II) Population structure. Heredity
- : The population genetics of Arabidopsis thaliana. (III) The effect of vernalisation. Heredity
KRANZ, A.R.: Genphysiologie quantitativer Merkmale bei Arabidopsis thaliana (L.) HEYNH. Teil 1. Spaltungsanalyse und Pigmentbiosynthese in quantitativen Chlorophyll b-Mangelmutanten. Theor.Appl.Genet. (Berl.) 40, 1970
- : Genphysiologie quantitativer Merkmale bei Arabidopsis thaliana (L.) HEYNH. Teil 2. Modifikation primärer und sekundärer Genwirkungen durch langwellige Strahlung bei monogenen Chlorophyll b-Defektmutanten. Theor.Appl.Genet.(Berl.) 40, 1970
- : Genphysiologie quantitativer Merkmale bei Arabidopsis thaliana (L.) HEYNH. Teil 3. Dauermodifikationen in der Pleiotropie homoalleler Genwirkungen. Theor.Appl.Genet. in preparation (1971)
ŠVACHULOVÁ, J.: Fixation of $^{14}CO_2$ activity of carboxydimutase and the content of free sugars in two chlorophyll mutants of Arabidopsis thaliana (L.) HEYNH. Photosynthetica
VELEMÍNSKÝ, J., and T. GICHNER: Two types of dose response curves in Arabidopsis thaliana after the action of nitrosamines. Mutation Res.
WESTERMAN, J.M.: Genotype-environment interaction and developmental regulation in Arabidopsis thaliana. (II) Inbred lines; analysis. Heredity
- : Genotype-environment interaction and developmental regulation in Arabidopsis thaliana. (III) Inbred lines; analysis of response to photoperiod. Heredity
- : Genotype-environment interaction and developmental regulation in Arabidopsis thaliana. (IV) Wild material; analysis. Heredity
- , and M.J. LAWRENCE: Genotype-environment interaction and developmental regulation in Arabidopsis thaliana. (I) Inbred lines; analysis. Heredity

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