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CONTENTS

A. Brief Notes

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G.RÖBBELEN

A. BRIEF NOTES

Arabidopsis thaliana (L.) HEYNH. in the western hemisphere (Compiled from the floristic literature)

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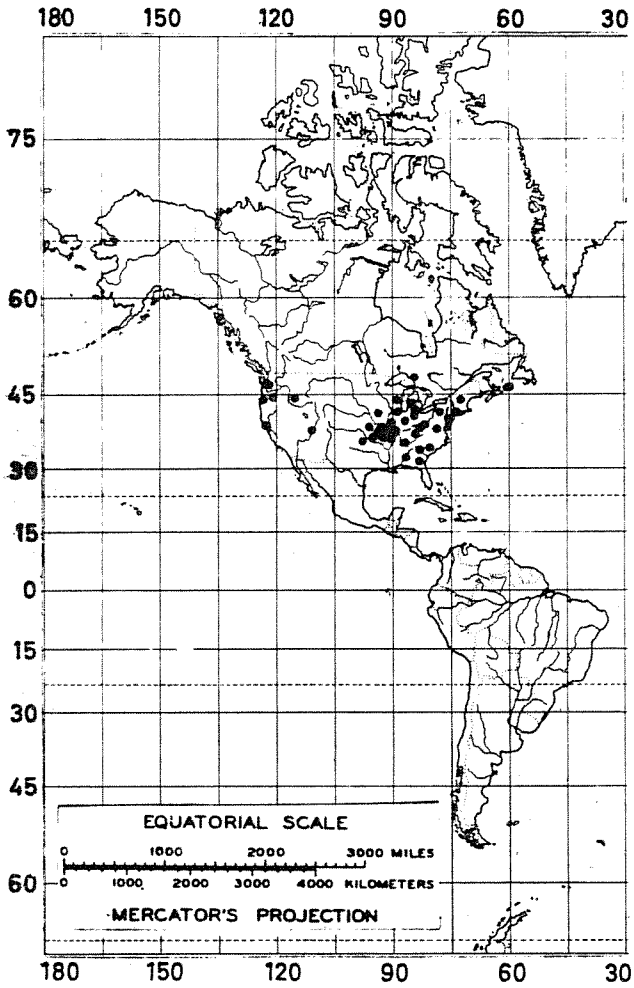
In the Western Hemisphere, A. thaliana has been reported from relatively limited areas. The plant seems to be most common in the Northeast and Midwest regions of the United States (BECK, 1833; CHAPMAN, 1883; GRAY, 1895-97; SMALL, 1913; BRITTON and BROWN, 1913; RYDBERG, 1932; RICKETT, 1967). Most taxonomists agree that it was introduced originally from Europe, while MACOUN (1883) in view of the abundance of the species in the region of the Great Lakes believed that A. thaliana is indigenous to America. Floras of PURSH (1814) amd NUTTALL (1818) describe Arabidopsis in the area extending from New England to the Virginias. The early botany of Vermont (GROUT, 1898) did not mention A. thaliana, while according to DOLE, WEATHERBY, CARPENTER and KITTREDGE (1937), it could be found along the Wells River in that State. BIGELOW (1824) did not mention it in the Florula Bostoniensis. According to GLEASON (1962) it can be found in the vicinity of the New York City. Several reports are available on the occurence of the plant in different locations in Pennsylvania (TWINING, 1917; BRENNER, 1932; WAGNER, 1943). In Maryland (REDMOND, 1932) and in the Carolinas (BATESON, 1964; MATZINGER, 1963 personal communication), Arabidopsis was not uncommon. I did not find any report on this plant from the parts of the states of Florida, Mississippi, Louisiana and Texas bordering on the Gulf of Mexico. It was widely scattered over the state of Alabama (MOHR, 1901). Several authors mentioned A. thaliana in Georgia (CHAPMAN, 1883; RADFORD, AHLES and BELL, 1964). No specific locations were mentioned in Ohio where A. thaliana occurs. It is apparently frequent in most parts of West Virginia, Virginia, Kentucky and Tennessee (CORE, McNEILL, BARNETT, HALE, AMMONS and BEAR, 1960; REDFORD, AHLES and BELL, 1964).



In the Midwest Arabidopsis could be found in several localities in Illinois (PEPOON, 1927; JONES, 1945; MOHLENBROCK and VOIGT, 1959), in Indiana (DEAM, 1940) and in Iowa (GREENE, 1907). In the state of Missouri the numeros locations of the habitats of Arabidopsis are excellently mapped (DANIELS, 1907; PALMER and STEYERMARK, 1935; STEYERMARK, 1963; HENDERSON, 1967 personal communication). The "Columbia wild type" of our laboratory was not extracted from wild populations of the area of Columbia, Missouri. It was selected from the Landsberg ecotype, provided to me by Professor LAIBACH. Within the city limits of Columbia, Missouri, wild populations of Arabidopsis have not yet been found and the escapees from our cultures rarely survived the second year outside the greenhouse.

Arkansas (DEAM, 1940) and Oklahoma (WATERFALL, 1952) apparently had A. thaliana growing while Central Texas was apparently free of the plant (COULTER, 1891-94; SHINNERS, 1958; IRWIN, 1961).

In the West, Kansas (GATES, 1940) and Utah (RYDBERG, 1932) were A. thaliana habitats but from Colorado (RYDBERG, 1906; HARRINGTON, 1954; WEBER, 1961), Arizona (KEARNEY, PEEBLES, HOWELL and McCLINTOCK, 1960), New Mexico (WOOTON and STANDLEY, 1915) no reports were available on the plant. In California there was apparently no A. thaliana in the south or in the desert areas (COVILLE, 1893; DAVIDSON and MOXLEY, 1923; THOMAS, 1961) or in the Sierra Nevada foothills (MUNZ, 1961). An old Flora of the San Francisco Bay area (GREENE, 1894) did not list this plant; the newer Flora of MUNZ and KECK (1959) indicated its presence in the vicinity of the Bay. Oregon (PECK, 1941), Washington (ABRAMS, 1944), and Idaho (DAVIS, 1952) had this plant growing only sparsely.

In North Central United States, it has been reported in the area of the Great Lakes in Michigan (HANES and HANES, 1947), Wisconsin (RICKETT, 1967), and Minnesota (MacMILLIAN, 1892; BRITTON and BROWN, 1913; SMALL, 1913). The recent handbook of the North Dakota plants by STEVENS (1963) did not list A. thaliana however.

Seemingly A. thaliana did not spread northward from the Great Lakes into Canada; it was not listed in the Botanical Survey of the Lawrence Seaway area (DORE and GILLETT, 1955). The several Alaskan Floras consulted failed to provide any evidence on the occurrence of A. thaliana in these northern areas, continental or islandic, though numerous crucifers and even A. mollis (A. hookeri) were reported there (ANDERSON, 1959; HULTEN, 1960; WIGGINS and THOMAS, 1962).

There was no evidence for the occurrence of A. thaliana from Mexico (LONGWORTH, 1942), from the Panama Canal Zone (STANDLEY, 1928), from the Bahamas (GRISEBACH, 1864; BRITTON and MILLSPAUCH, 1962), from the Antilles (BOLDINGH, 1909), or from the Barbados (GOODING and LOVELESS, 1965).

The Floras of Ecuador (DIELS, 1937), Chile (MUNOZ, 1959) the old monumental work on Brazil by MARTIUS and EICHLER (1841-1872) or CABRERA's Flora on Argentina did not indicate the presence of A. thaliana in Middle or South America.

The cited references are listed in the publication of G.P. RÉDEI in Bibliographia Genet. 21, 1-151 (1969).

Genetic analysis of lines derived of natural populations of Arabidopsis thaliana (L.) HEYNH.

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The genetic determination of flowering time in Arabidopsis has been studied in several papers (HÄRER, 1951; NAPP-ZINN, 1957; DIERKS, 1958; SEYFERT, 1964; Van der VEEN, 1965) which discovered some corresponding features (e.g., dominant alleles for late flowering and presence of gene interactions). But none of these investigations led to general conclusions neither about the number of factors controlling this character nor about the way of their activity. Each author choose only relatively few forms and the possibility of comparison is lacking. For this reason, it is yet unknown whether there are few or many different genes for early vs. late flowering.

In our Department the genetic variability of this character is studied in natural populations (CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1969). Besides of breeding experiments with natural forms it is necessary to include corresponding analytical crossings. Three years ago, we carried out a model experiment with the aim to indicate a suitable system of genetic analyses making possible the inclusion of a large number of genotypes. This system is based (1) on crosses of many genotypes with a common tester line and (2) on diallel crosses in smaller groups according to the results of the forementioned crosses.

In this paper, some conclusions of experiment (1) are given. I studied early and medium late lines isolated in 1963 from three different natural populations and autogamized from three to five generations. From these, 16 apparently homozygous lines were chosen and crossed with the line S 96 (an early segregant from the cross Dijon x Li-2 obtained by Van der VEEN, 1965). This line is highly uniform, it reaches the physiological limit of earlines and it seems not to contain any known dominant gene for late flowering. The experiment was carried out under controlled experimental conditions securing an expressive delay of flowering in forms with vernalization requirement. (For technical details see CETL, HELICHOVÁ-DOBROVOLNÁ, and KRSKOVÁ, 1970.) Mean number of days to appearance of flower primordia ($\bar{x}$), mean number of rosette leaves ($\bar{y}$), and mean combined score ($\bar{x}+\bar{y}$) with corresponding distributions were studied in both parents, in the F_1 and the F_2. The studied lines were taken as mothers, the line S 96 as father.

The 16 lines showed different behaviour as to dominance. For example, in the number of days to appearance of flower primordia 3 lines showed dominance, 2 partial dominance and 1 recessivity of the later flowering while in 9 lines the F_1 means were significantly higher than those of the maternal line. One line was not different from S 96.

In the F_2, in 11 lines very late flowering plants appeared, the majority of which was found to be segregating for late and early in the F_3.

We initially expected that it should be possible to study the segregation in the F_2 by analyzing the positions of individual peaks on the distribution curves and to search in this way for corresponding segregation ratios. But in most of the cases, the distributions were multimodal, often with larger number of undefinable peaks.

The detailed study of all these phenomena is underway.

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The changes of width of cotyledons with increasing vernalization rate in Arabidopsis thaliana (L.) HEYNH.

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In cereals, the size of the coleoptile and of the few first leaves diminishes with increasing vernalization rate (PURVIS, 1934; ČAJLACHJAN and ŽDANOVA, 1938; THIMANN and LANE, 1938; HÄNSEL, 1953; CETL, 1962).

In this preliminary experiment, it was searched for similar phenomena in Arabidopsis. As has been found in former experiments, the width of the cotyledons is a well definable quantity while the length is less advantageous as it cannot be precisely bordered against the petiole. At the same time it was stated that both dimensions increase during the early stages of growth till the first two true leaves reach approximately the size of the cotyledons. After this stage, the dimensions of the cotyledons change no more (unpublished data).

For this reason, the changes of width of cotyledons with increasing vernalization rate were studied in a late line Je 31-5-3-4. The seeds were vernalized for 0 (control), 10, 20, 31, 49, and 60 days. When the third true leaf appeared the width of the cotyledons was measured in single plants and expressed as a mean ($\bar{w}$) for each variant. Later the mean number of days to appearance of flower primordia ($\bar{x}$) and of rosette leaves ($\bar{y}$) were similarly determined. (For technical procedures and conditions of cultivation see CETL, RELICHOVÁ-DOBROVOLNÁ, and KRSKOVÁ, 1970.)

The results are given in the Table. As can be seen, all three quantities studied decreased with increasing vernalization rate. The decrease till the 20th or 30th day of vernalization was very fast while afterwards it was relatively slow. This means

Table: Changes in the mean number of days to appearance of flower primordia ($\bar{x}$), in the mean number of rosette leaves ($\bar{y}$), and in the mean width of cotyledons in mm ($\bar{w}$) with increasing vernalization rate

Days of vern.	n	$\bar{x} \pm s_{\bar{x}}$	$\bar{y} \pm s_{\bar{y}}$	n	$\bar{w} \pm s_{\bar{w}}$
0	82	35,97 ± 0,92 (57)*	15,94 ± 0,86 (28)*	92	2,36 ± 0,04
10	82	33,36 ± 0,73 (38)*	16,48 ± 0,64 (17)*	94	2,11 ± 0,02
20	104	18,91 ± 0,23	10,15 ± 0,08	106	2,01 ± 0,02
31	87	17,29 ± 0,26	8,52 ± 0,09	95	1,76 ± 0,02
40	86	15,79 ± 0,10	7,86 ± 0,06	92	1,68 ± 0,03
49	100	14,78 ± 0,14	6,92 ± 0,19	102	1,64 ± 0,02
60	83	12,55 ± 0,26	5,39 ± 0,14	88	1,59 ± 0,03

*Corrected in respect to the portion of vegetative plants

that not only the two characters, directly connected with the development, but also the width of cotyledons diminished in the same way as a consequence of vernalization.

Highly significant positive correlation coefficients were found between the three characters: Between the $\bar{x}$'s and $\bar{y}$'s: $r = 0{,}9952$, $P < 0{,}01$; between the $\bar{x}$'s and $\bar{w}$'s: $r = 0{,}9267$, $P < 0{,}01$; between the $\bar{y}$'s and $\bar{w}$'s: $r = 0{,}9406$, $P < 0{,}01$. Therefore, the

decrease in all three quantities during vernalization was directly proportional to each other. From the physiological point of view it might be of interest to study the corresponding causal relations.

In any case, the changes of the width of the cotyledons with increasing vernalization rate in Arabidopsis represent a phenomenon of the same expressivity as similar changes in the first leaves of cereals.

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The quantitative determination of the vernalization requirement in two late flowering mutants of Arabidopsis

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There are data that late flowering mutants can be produced from early forms. In Arabidopsis, HUSSEIN (1968) determined the genetic constitution of seven such mutants. At the same time, he studied their vernalization response after 4 weeks of seed vernalization and he found that all mutants responded well to vernalization with some differences in the amount of response.

Methods of quantitative determination of the vernalization requirement were applied to independent late mutants obtained from M_2 populations of the race Dijon G after treatment of dry seeds by N-nitroso-N-methylurea. So far, experiments with two mutants (L5 and L6) are completed. Seeds in soil culture (cf. CETL, RELICHOVÁ-DOBROVOLNÁ, and KRSKOVÁ, 1970) were vernalized for 0 (control) to 40 days at 2 ± 1°C in a dark room. Then, the cultures were kept at 25 ± 3°C under continuous illumination (1250 lux) for 42 days. The mean number of days from germination to appearance of flower primordia ($\bar{x}$) and the mean number of rosette leaves ($\bar{y}$) were determined. The results are given in the Table.

Table: Changes in the mean number of days to appearance of flower primordia ($\bar{x}$) and in the mean number of rosette leaves ($\bar{y}$) with increasing vernalization rate

Days of vern.	n	$\bar{x} \pm s_{\bar{x}}$	$\bar{y} \pm s_{\bar{y}}$	n	$\bar{x} \pm s_{\bar{x}}$	$\bar{y} \pm s_{\bar{y}}$
	Dijon G			L5		
0	33	10,30 ± 0,13	4,00 ± 0,00	39	16,85 ± 0,31	7,59 ± 0,16
5	34	9,82 ± 0,05	4,00 ± 0,00	38	14,74 ± 0,30	7,18 ± 0,17
9	46	10,17 ± 0,08	4,00 ± 0,00	44	14,61 ± 0,29	6,73 ± 0,09
25	43	9,67 ± 0,11	4,00 ± 0,00	35	13,46 ± 0,13	6,17 ± 0,07
30	47	9,96 ± 0,04	4,00 ± 0,00	39	13,54 ± 0,25	6,39 ± 0,09
40	46	10,02 ± 0,08	4,00 ± 0,00	50	12,32 ± 0,15	6,02 ± 0,05
	Dijon G			L6		
0	28	10,07 ± 0,13	4,00 ± 0,00	18	32,17 ± 0,87	14,22 ± 0,58
5	44	9,05 ± 0,10	4,00 ± 0,00	46	25,67 ± 0,36	9,61 ± 0,27
10	51	10,24 ± 0,11	4,00 ± 0,00	46	25,96 ± 0,82	9,02 ± 0,29
20	45	10,11 ± 0,10	4,00 ± 0,00	40	16,60 ± 0,20	7,40 ± 0,10
30	49	10,08 ± 0,10	4,00 ± 0,00	23	17,00 ± 1,11	6,83 ± 0,14
40	38	10,84 ± 0,16	4,00 ± 0,00	32	15,88 ± 0,26	6,25 ± 0,09

While the initial race Dijon G did not react on vernalization both late mutants showed a clear response, the amount of which could be expressed (1) as the $\bar{x}_C/\bar{x}_{40}$ and $\bar{y}_C/\bar{y}_{40}$ ratios (cf. HUSSEIN, 1968); (2) as the percentage of generative plants in the controls in a 42 days' experiment; (3) as the minimum vernalization rate necessary for to have all plants generative.

As to the criterion (1), the ratios $\bar{x}_C/\bar{x}_{40}$ in the initial race Dijon G were about 1,00 (in two experiments 0,93, and 1,03, respectively), but 1,37 in the mutant L5, and 2,03 in the mutant L6. The $\bar{y}_C/\bar{y}_{40}$ ratios were similarly 1,00 in Dijon G, 1,26 in the mutant L5, and 2,28 in the mutant L6. These values show that the race Dijon G does not react on vernalization while in the less late mutant L5 the response was weak in comparison with that of the more late mutant L6.

The comparison according to criteria (2) and (3) furnished similar conclusions, but the behaviour of the less late mutant L5 was undiscernible from that of the race Dijon G: In both genotypes the percentage of generative plants in the controls was 100, while in the mutant L6 only 78,22% of the plants showed flower primordia after 42 days. The minimum vernalization rate leading to the generative development of all plants was zero days in Dijon G and L5, but five days in L6. Apparently, the other late mutants also show different amounts of vernalization requirement. At any rate, in both late mutants studied as yet vernalization reaction was induced and at the same time, quite different amounts of vernalization requirement were found.

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CETL, I., J. RELICHOVÁ-DOBROVOLNÁ, and M. KRSKOVÁ: Arabid.Inf.Serv. 7, 35-36 (1970)
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The phenotypic stability of leaf characters in mutants of Arabidopsis thaliana (L.) HEYNH.

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In the race Dijon G and in its eight monogenic recessive mutants (see CETL, DOBROVOLNÁ, and NESRSTA, 1969) 15 physiological and morphological characters were studied in three environments differing widely in daylength and light intensity. The effects of genotypes, environments, replications and corresponding interactions were studied by means of a factor analysis of variance. Complete results will be published elsewhere.

Table: "Macro-environmental" (a) and "micro-environmental" (b) variability coefficients in the race Dijon G and its mutants

Genotype	Component	Whole leaf length	Petiole length	Blade width	Blade length	Blade length /width	Petiole /leaf length
Di G	a	12,33	18,63	2,16	21,06	3,03	2,87
	b	9,42	11,20	6,91	7,78	3,00	1,39
aut	a	29,22	29,90	24,48	27,28	4,71	3,03
	b	10,92	11,46	12,29	12,98	2,60	1,24
da	a	22,86	20,80	21,84	25,23	6,78	3,64
	b	8,48	7,64	6,13	7,12	3,56	0,74
dl	a	28,20	32,91	22,35	25,47	11,31	3,48
	b	16,26	18,49	10,67	14,25	3,42	1,16
gr	a	13,14	10,05	14,73	14,70	4,28	5,56
	b	2,63	2,18	2,31	8,05	0,88	0,81
lc	a	24,45	23,90	21,29	25,87	6,39	3,97
	b	6,37	2,29	7,82	7,96	1,34	3,08
ln	a	30,45	35,44	28,31	29,61	9,41	8,31
	b	7,85	6,18	7,88	8,97	1,35	1,41
qn	a	37,33	40,34	28,21	34,16	16,19	2,72
	b	5,37	2,88	6,29	5,05	1,14	1,27
rf	a	18,32	15,16	18,18	22,53	5,13	3,42
	b	6,22	5,65	4,07	7,25	2,79	0,75

In 6 leaf characteristics (length of whole leaf; length of petiole; width of leaf blade; length of leaf blade; length/width of leaf blade; length of petiole/length of whole leaf) the variances due to environments and to replications estimated in all nine genotypes were transferred to variability coefficients so that comparable amounts of both "macro-environmental" and "micro-environmental" variation, could be measured.

As it could be expected the "macro-environmental" variation was considerably higher than the "micro-environmental" one. It seems that there is no clear correlation between the quantities of these two components. For the mutant qn, e.g., very high values of the "macro-environmental" variability were characteristic, while as to the amount of the "micro-environmental" one this mutant appeared to be less variable.

Among the characteristics studied both indexes (length/width of leaf blade, length of petiole/length of whole leaf) showed low values of both components. This means that

the indexes represent relatively stable quantities. As the mutants studied differ mostly in the leaf shape (e.g., the mutants gr and rf have roundish leaves, while aut, dl, and ln have oblong ones), just the most typical character was found to be one of the least variable.

On the other hand, there were conspicuous differences also among the genotypes. One of the most stable genotypes was the initial race Dijon G. Also the mutant gr showed very low "macro-environmental" and "micro-environmental" variability. The other mutants appeared to be phenotypically far less stable in almost all characters studied.

The larger phenotypical stability of the initial race Dijon G as compared to the majority of the mutants studied, suggests that one of the typical consequences of mutation is the decrease of the developmental homeostasis.

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CETL, I., J. DOBROVOLNÁ, and M. NESRSTA: Arabid.Inf.Serv. 6, 31 (1969)

Gibberellin effects in dwarf mutants of Arabidopsis thaliana

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During the last years we have undertaken a number of experiments on the influence of gibberellins on several developmental phenomena (flower induction, apical dominance, growth of the main axis) in numerous Arabidopsis races and mutants. A publication of SUGE and MURAKAMI (1968) induces us to anticipate some results concerning two darf mutants which had been obtained by McKELVIE (1962) with a summer annual strain.

Dwarf forms of Arabidopsis thaliana have already been known as early as 1877 (VIVIAND-MOREL, 1877-78). One of the types we have used (le) is characterized by a very reduced stalk (one or two internodes of some millimetres length, besides the inflorescence); lateral shoots are poorly developed. The other one (ca) has a short stalk, too, a still more "compressed" inflorescence, longer siliques, and a more bushy growth habit. Upon a treatment with several gibberellins, both dwarf mutants reacted in a different way: With the ca-plants, most gibberellins led to earlier flowering without changing the growth habit noticeably. On the other hand, the gibberellins did not influence significantly the age of flowering of the le-plants (which, in any case, flowered earlier than the ca-plants); their stalks, however, became longer which is reflected, among others, by the inclusion of a greater number of internodes into the stalk, while the number of rosette internodes was reduced and the total internode number remained practically the same (see Table).

Table: Influence of several gibberellins upon flowering and stalk formation in the dwarf mutants ca and le of Arabidopsis thaliana. Plants treated 10 times at half-weekly intervals (from the 3rd to the 7th week of growth) with 0,04-0,05 ml of a 20 ppm solution (Tween 80 added), put upon the rosette leaves; grown at 20°C and continuous fluorescent plus incandescent light (4.500 lx). Numbers of plants per group between parentheses.

	Gibberellins							Control
	A_1	A_3	A_4	A_5	A_7	A_8	A_9	
ca days to flowering	46,4*(8)	43,6*(8)	43,6*(7)	45,0*(5)	43,2*(6)	49,0*(6)	50,8 (6)	54,6 (9)
le days to flowering	30,6 (5)	30,4 (5)	30,0 (5)	31,5 (4)	29,8 (5)	31,8 (5)	31,8 (5)	31,6 (5)
le total internode number	13,8	13,2	13,4	14,0	13,4	13,6	13,8	13,2
thereof stalk internodes absolute number	3,2	3,2	3,6	3,0	3,4	2,4	3,4	1,8
in per cent	23,2*	24,2*	26,9*	21,4	25,4*	17,7	24,6*	13,6

* Difference significant at the 0,27% level, as compared with controls

Thus, the influence of gibberellins upon shoot growth and upon flowering may be easily separated with these dwarf mutants of Arabidopsis. With regard to growth habit, the behaviour of the mutant ca corresponds to that of certain dwarf mutants of Zea mays and of Oryza sativa which do not react upon a gibberellin treatment by increased stalk growth, or the gibberellin contents of which are not lower than those of comparable normal types (cf., SUGE and MURAKAMI, 1968; PHINNEY, 1956); nevertheless, flowering is clearly influenced by gibberellins in this case. Only as far as the mutant le is concerned, dwarfism is overcome by gibberellins, as it is known from many other dwarf forms.

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Linear order of flowering time mutation

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HUSSEIN (1968, 1969) could identify three linkage groups for flowering time in Arabidopsis thaliana. One of these is the linkage group (e_3/ e_4/ e_7/ e_8) for genes having large magnitude of effect towards late flowering. As the genes (mutations) were in repulsion phase, selection experiments were carried out to isolate the double and triple recessives. Selfing experiments are in progress in order to ensure homozygosity of the recessive selections.

A programme of two and three point experiments was designed to study the linear order and the map distances among the four loci involved in the above linkage group.

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A quantitative evaluation of cell temperature resistance in a wild population of Arabidopsis thaliana (L.) HEYNH. and a selection line thereof

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Cell temperature resistance has been shown to be a conservative character of the species in plants as well as in animals (for details see ALEXANDROV, 1964; USHAKOV, 1964). It is strongly influenced by changes in ploidy (ASHRAF, 1967, 1970) as well as mutation (SUKHANOVA, 1968). This led us to investigate the quantitative variability of this character in a wild population of A. thaliana collected from the suburban of Peterhof in the vicinity of Leningrad and to compare it with the same characteristic in a selection line P-8 derived from a population of the same locality. The selection line was isolated by Dr. KVITKO, Department of Genetics of the Leningrad State University, who kindly provided verified seeds of this line. His criteria for selection did not include considerations of cell temperature resistance or resistance to any other unfavourable factor.

The cell temperature resistance was measured according to the method developed by ALEXANDROV (for details and review see ALEXANDROV, 1964). Leaf pices of the plant were infiltrated with water with the help of a medical syringe. They were then subjected to the high temperature in a water thermostate for five minutes. Under a microscope with water immersion objective (90x) and eye-pices (7x) the temperature was determined which stops the protoplasmic streaming after 5 min.

As can be seen from the Figure the wild population and the pure line markedly differ in their cell temperature resistance. The differences are not only true for the mean values (40,8° and 43,2°C for the pure line and the wild population, respectively), but also hold for the distribution curve with a clear-cut decrease of the variability in the pure line. The variability coefficient was found to be 0,87 for the selection line and 0,99 for the wild population. It is interesting to note that the cell temperature resistance was affected even though the material had never been subjected to any selection in this direction. While the curve for the selection line realized the requirements of a normal distribution curve, the curve for the wild population lacked a single peak because here inspite of large sampling (75 plants) the mode spread over several classes (from 42,8° to 44,4°C).

So far very little is known on the population dispersion of this character. USHAKOV et al. (1963) studied cell temperature resistance in a population of frog tadpoles; they succeeded to increase it by high temperature treatments of the spermatozoids eliminating the less resistant ones. AMOSOVA et al. (1967) studied cell temperature resistance on the basis of thermal suppression of muscle cell excitability in Calliphora erythrocephela. They tried both, positive and negative selection for cell temperature resistance. The attempts to increase the resistance through selection during many generations failed in the end though temperary increases were recorded. In the negative direction, however, the selection was successfull already within three generations. The curve continued to go down and the ultimate results were highly significant. Finally ALTUKHOV et al. (1968) studied cell temperature resistance in hermaphroditic and parthenogenetic selection lines of the silk worm. They found that hermaphroditic lines had a higher coefficient of variability than the parthenogenetic lines and that different selection lines differed significantly from each other in their cell temperature resistance.

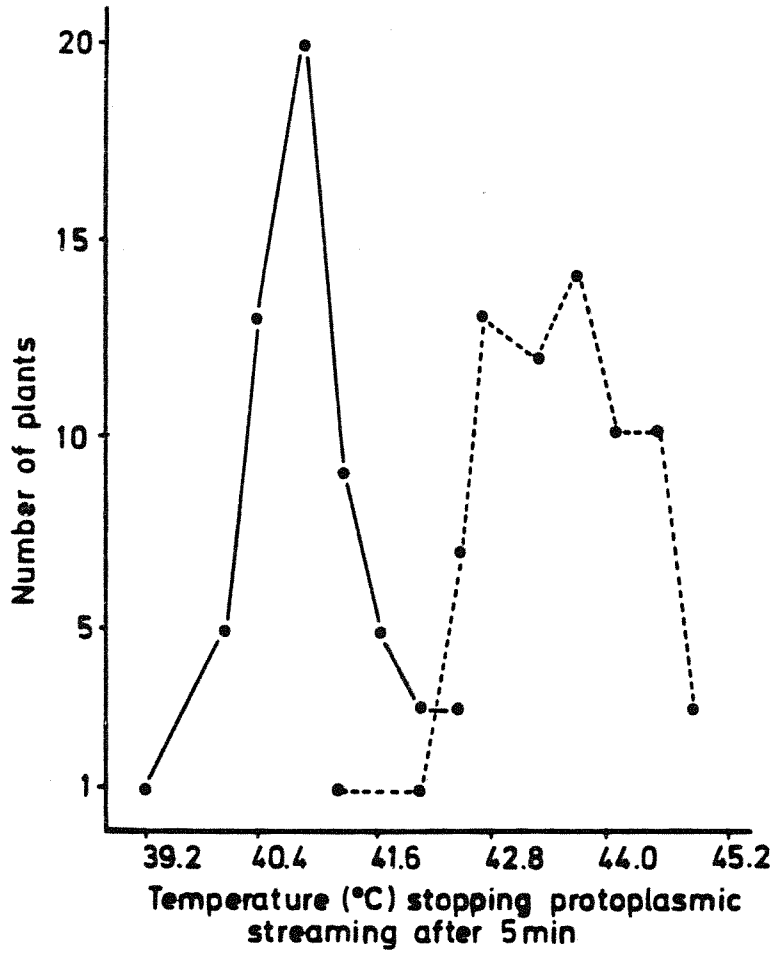

Figure: Population distribution curve of cell temperature resistance in Arabidopsis thaliana. Plants grown in soil culture under artificial light.
—— Pure line selection A-8 from Peterhof.
---- Wild population collected from the same locality in Peterhof.

Our experimental data point to the possibility that cell temperature resistance might be linked up with characters other than those of cell physiology and consequently demonstrate the possibility that fruitful work can be done in searching links between resistance, morphological characters and growth factors.

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Variability of cell temperature resistance in ecological races of Arabidopsis thaliana (L.) HEYNH.

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Our studies on the variability of cell temperature resistance in populations reported earlier in this issue led us to investigate different ecological races and to study the genetics of this type of resistance. For this purpose ecological races from the collection of Prof. LANGRIDGE and Prof. RÖBBELEN were used, the seeds of which were provided kindly by the two authors. ALEXANDROV's criteria of a stop of the protoplasmic streaming in the cell (1964) was taken as a sign of thermal damage. By using this method we determined the temperatures that stop the protoplasmic streaming by their action over different durations. The results are presented in the Figure.

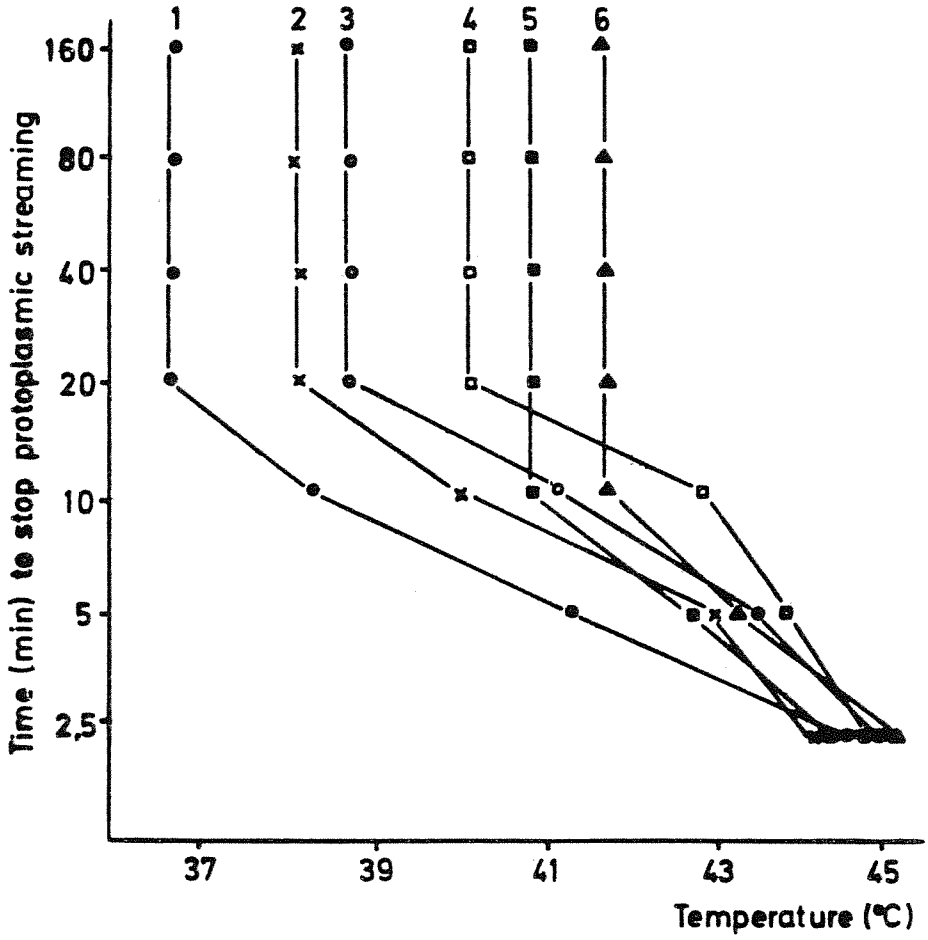

Figure: Cell temperature resistance in ecological races of Arabidopsis thaliana. Plants grown in soil culture at room temperatur with 3000 lux light intensity. 1) Estland, 2) Enkheim, 3) Kopenhagen, 4) Blanz, 5) A-8, 6) Kazbek

It can clearly be seen that different races form a continuous series. However, at the relatively high temperatures of short duration, like 2,5 or 5 minutes, the different races show different points of break in the temperature curve, i.e., 10 minutes and 20 minutes for races A-8 and Estland.

ALEXANDROV (1964, 1965, 1967, 1969) who further developed NOSONOV and ALEXANDROV's protein theory of cell excitation and damage (1940) showed that the two parts of the temperature curve before and after the breaking point correspond to two types of cell temperature resistance. In the first part of high temperatures and short duration the straight line correlation indicates that the cause of the temperature damage is thermal denaturation of protoplasmic proteins leading to a stop of the protoplasmic streaming. In the second part of relatively milder temperatures of a longer duration, which gives an almost vertical line, the observed damage is a resultant of two opposite forces: the damaging action of the temperature and the active resistance offered by the cell through repair of the damage already going on during the temperature treatment. The graph suggests that different races can show differences in both the active (second) and the passive (first) mode of temperature reaction.

LANGRIDGE (1959) showed that ecological races in Arabidopsis thaliana differ in their response to temperature treatment when grown under the same conditions throughout the experimental period; he argued that in some cases this difference can be attributed to single biochemical components, the synthesis of which is blocked in the susceptable race. Our work tries to trace this resistance problem back to the cellular level. Since the straight line before the break in the temperature curve is expected to characterise the protein denaturation, it is quite possible that the differences in this region may ultimately be traced to some cell protein components.

The available literature (ALEXANDROV, 1964, 1969; USHAKOV, 1964, 1967) shows that the cell temperature resistance is a character which is species specific. It may be changed by mutation or selection, but remains unchanged while the organism (except lower plants and animals who show a "tuning up" of their cell resistance according to the temperature of the surroundings) is grown under different temperature conditions. It can be changed, however, by pretreatment with temperatures which harden the cell (ALEXANDROV, 1964, 1967).

Thus, while confirming the general observations so far reported in the cytophysiological literature, our work shows that this criteria can be fruitfully employed for a characterization of temperature resistance within races of a species.

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On the participation of phytochrome in the photoperiodic reaction of Arabidopsis thaliana (L.) HEYNH.

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To elucidate the question whether phytochrome participates in the photoperiodic reaction of Arabidopsis, plants were irradiated with far-red light (FR) for 15 min at the beginning of the darkness at different photoperiods.

For the experiments the race Estland 2075/4 was chosen for its early growth and small dimensions. The plants grew in sterile flasks on agar medium with the half concentration of a Knop solution prepared according to VELEMÍNSKÝ and GICHNER (1964). Luminescent tubes LB-40 giving 20 wt/m^2 at the level of the plant rosettes were used as a light source. The duration of the diurnal illumination varied from 4 to 24 hrs with two hrs intervals. FR was obtained from reflecting incandescent lamps (300 wt) by filters consisting of 2 cm water, a dark-blue glass filter as well as 3 red and 1 dark-blue cellofan film. The maximum of radiation corresponded to 730 nm; the intensity of the radiation at the rosette level was 17 wt/m^2.

The figure shows the typical quantitative dependence of flowering on the duration of the diurnal illumination, with a local maximum at 10-12 hour's day length (curve I). FR given immediately at the beginning of the dark period markedly accelerated the development of the plants (curve II). This acceleration was faster with the shorter days than with the longer ones. FREDERIQ and De GREEF (1966) observed similar effects in Marchantia, where the active form of phytochrome (Pfr) controlled the orthotropic growth of the thallus.

As FR extracts Pfr out of the system, it is reasonable to assume that the absence of Pfr hinders the reproduction, and the lowering of its level below a certain limit promotes it. At the other hand, in the experiments without FR radiation the bud formation of the plants is faster with the longer days or with the continuous illumination (CI), whereas the amount of Pfr is sufficient during the whole cycle. To overcome this contradiction, one may suppose that during the day the level or the activity of Pfr is low; but on turning the light out this level of activity increases and remains high during the greater part of the night. In this case it should be expected that a short day is at least not less favourable for the development than C.I.

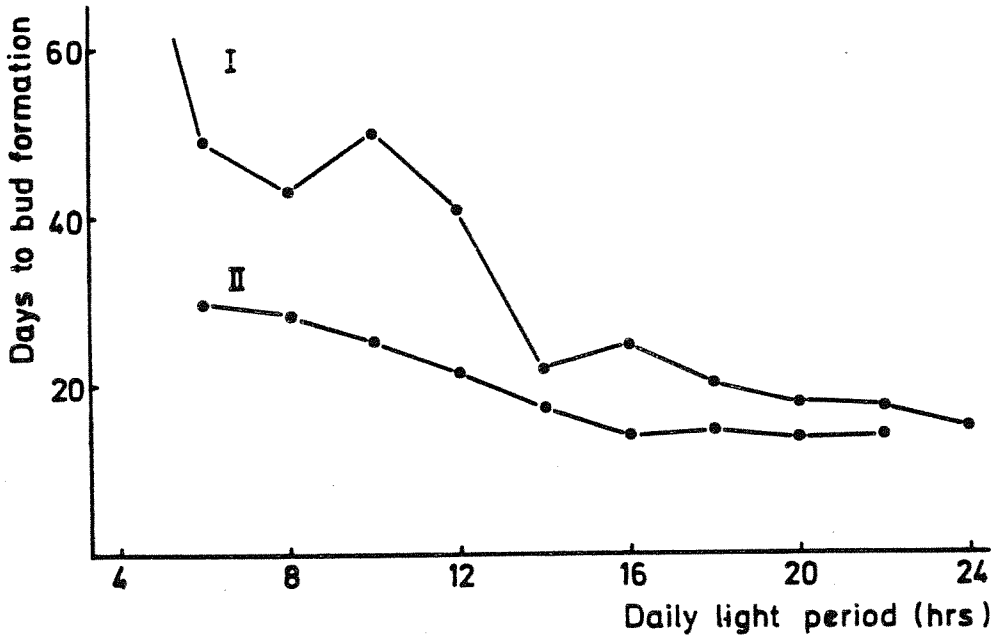

Figure: Influence of day length on the mean number of days before bud formation in Arabidopsis thaliana (L.) HEYNH., race Estland 2075/4. I = control plants; II = plants receiving 15 min far-red irradiation at the very beginning of dark period

However, this is not true. This non-coincidence of the observed dates with the expected ones may partly be explained by the delay of the morphological manifestation rate of the reaction. In fact, in other experiments the addition to the medium of 3% sucrose considerably accelerated the bud formation and under these conditions plants irradiated by FR after turning out the light formed buds under a 4-hour's dayly illumination at the same day as those under C.I.

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The effect of gibberellin on D_2O damage of seed germination

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In our earlier studies (BHANDARKAR and GAUR, 1967) we have reported delayed germination and retarded growth of Arabidopsis thaliana seedlings in heavy water (D_2O). It has been frequently observed that seed germination time is reduced by gibberellin treatment (STOWE and YAMAKI, 1957). KUCERA (1966) and REINHOLZ (1967) have shown that the inhibition of germination in Arabidopsis seeds by ionizing radiation could be nullified by post-radiation application with gibberellic acid (GA). In the present study it was aimed to examine whether GA is effective in overcoming the D_2O-induced delay in germination of Arabidopsis seeds.

The material and culturing techniques were the same as those employed in our previous experiment (BHANDARKAR and GAUR, 1967) with minor modification viz., the medium was devoid of sucrose. While the growth medium was autoclaved, the GA was sterilized by passing the solution through a millipore filter (22 mμ pore size). D_2O and GA concentrations varied over 25 to 100% and 2.5×10^{-6} to 2.5×10^{-2}M range, respectively. The time taken to attain 100% germination (radical appearance) was used as criterion of the treatment effect.

Compared to the water control, a delay of about 144 hours in germination was brought about by 50% D_2O (Table 1). While GA concentrations up to 2.5×10^{-3}M stimulated germination both in water and D_2O, 2.5×10^{-2}M proved completely inhibitory. This stimulation represented a 2-phase effect, characteristic of the action of hormone concentration. GA was found optimum in a concentration of 2.5×10^{-5} M hastening germination by about 26 and 96 hours in H_2O and 50% D_2O, respectively.

Table 1: Time (hours) taken to obtain 100% germination of seeds sown in H_2O and 50% D_2O and treated with gibberellic acid (GA)

Treatment	GA Concentrations (M)					
	0	2.5×10^{-6}	2.5×10^{-5}	2.5×10^{-4}	2.5×10^{-3}	2.5×10^{-2}
H_2O	96	78	70	88	88	No germination
50% D_2O	240	222	144	198	216	No germination

Table 2: Time (hours) taken to obtain 100% seed germination in the presence and absence of 2.5×10^{-5} M gibberellic acid (GA) and of various D_2O concentrations

Treatment	H_2O	D_2O Concentrations (%)			
		25	50	75	100
- GA	96	144	240	No germination	
+ GA	72	120	140	52% germination at 240 hrs	No germination

In Table 2 a concentration dependent delay in germination can be seen induced by D_2O. While the percent of germination was unaffected up to 50% D_2O, higher concentrations completely prevented germination, at least up to 10 days. On the other hand, the presence of 2.5×10^{-5}M GA not only hastened the germination up to 50% D_2O; but also its relative effect increased with increasing D_2O concentrations. Further, GA brought about as much as 52% germination in the presence of 75% D_2O. There was, however, no germination in 100% D_2O even with application of GA.

The current investigation shows that the seeds sown for a prolonged period in D_2O, followed a slackened rate of germination. The reports on D_2O effect on some of the germination factors viz., seed hydration and seed respiration (BHATTACHARYA et al., 1969), RNA synthesis (GROSS and HARDING, 1961; GROSS et al., 1964), and cell division (GROSS and SPINDEL, 1960) reveal the inhibitory action of the deuterium. The GA application to seeds treated with D_2O might have acted collectively on any one or more of these processes responsible for germination. It is known that GA improves the hydration level in H_2O (STOWE and YAMAKI, 1957) as well as in D_2O soaked seeds (BHANDARKAR et al., 1970). Also it promotes RNA and protein synthesis (BROUGHTTON, 1969) and cell elongation (SACHS, 1965). This may explain the overcoming of the D_2O- induced delay in germination by GA.

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Effect of the growth retardants potassium nitrate, thiourea, and gibberellin on seed germination of Arabidopsis thaliana

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Like gibberellic acid (GA), potassium nitrate and thiourea are also well known as effective promotors of germination in positively photoblastic seeds (EVENARI, 1965). In contrast, growth retardants as well as coumarin act as inhibitors of seed germination (CATHEY, 1964; KNYPL, 1967). However, exogenously applied GA can effectively overcome the inhibitory effect of these chemicals in several plant systems including seed germination (CATHEY, 1964; RENNERT and KNYPL, 1967; KNYPL, 1967). The apparent similarity of the effects of GA, KNO_3, and thiourea encouraged us to plan some experiments to explore, whether the latter chemicals, like GA, also counteract the inhibitory effect of growth retardants or not. The results of this study will be reported in this communication.

It is clear that when the seeds were germinated at 25°C under a light intensity of about 1000 lux as detailed earlier (SANKHLA and SANKHLA, 1968) all the three growth retardants tested (Table) inhibited the germination of seeds to a very great extent,

Table: A comparative study of the effects of the growth retardants potassium nitrate, thiourea, and GA on germination of Arabidopsis

Treatment	Percentage of germination after hrs 48	72	96
Water	65	95	100
100 ppm CCC	35	45	48
1.000 ppm CCC	10	15	20
100 ppm B-nine	25	35	40
1.000 ppm B-nine	10	10	15
10 ppm Phosfon	50	80	80
50 ppm Phosfon	8	10	10
100 ppm KNO_3	85	92	100
1.000 ppm KNO_3	90	95	100
100 ppm Thiourea	60	88	98
1.000 ppm Thiourea	65	95	100
10 ppm GA	75	98	100
50 ppm GA	80	98	100
100 ppm CCC + 100 ppm KNO_3	45	60	80
100 ppm CCC + 1.000 ppm KNO_3	55	75	90
100 ppm CCC + 100 ppm Thio.	40	50	60
100 ppm CCC + 1.000 ppm Thio.	40	55	75
100 ppm CCC + 10 ppm GA	60	65	80
100 ppm CCC + 50 ppm GA	70	85	95

although phosfon indicated some toxic effects. GA and KNO_3, on the other hand, appeared to enhance the rate of seed germination. Thiourea, when alone, did not ellicit any positive effect on the rate of seed germination. Furthermore, it was observed that in appropriate concentrations KNO_3, GA, as well as thiourea greatly reversed the effect of CCC on seed germination.

Earlier we observed that in lettuce, strain 'Attraktion', CCC inhibited the germination of seeds only slightly while in strain 'Rapide' it proved completly ineffective in inhibiting the germination. Not only this, in strain 'Attraktion' thiourea when applied simultaneously with CCC reversed the inhibition of root growth caused by the growth retardant. In strain 'Rapide', on the other hand, thiourea did not indicate any positive response on seedling growth either alone or in the presence of CCC (SANKHLA, 1970). In some mutants of Arabidopsis also neither thiourea nor KNO_3 could reverse the inhibition of seed germination caused by CCC (our unpublished data). These results indicate differential behaviour and specificity of the response ellicited by two strains of the same species. A similar behaviour of specificity of response to added growth retardants has also been reported earlier for different cultivars of other species (CATHEY, 1964). Our present knowledge indicates that growth retardants (in particular CCC) affect auxin-, gibberellin-, cytokinin-, and choline metabolism of the treated plants (CATHEY, 1964; RENNERT and KNYPL, 1967; TUNG and RAGHAVAN, 1968). Thiourea, on the other hand, is known to change the activity of hydrolytic enzymes, to affect respiration, alter the nature and amount of naturally occuring endogenous growth regulators and affect nucleic acid metabolism (POLJAKOFF-MAYBER and MAYER, 1960). Recent evidences suggest that in reversing the effect of growth retardants KNO_3 as well as KCl, like GA, might also act through their effect on DNA, RNA and protein synthesis (SANKHLA, 1969; KNYPL, 1969). Therefore, it might be possible that KNO_3, thiourea, and gibberellin reverse the inhibition of seed germination caused by CCC, by stimulating the metabolism in a balanced way.

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Effect of IAA, GA, and ABA on senescence of leaf discs of Arabidopsis thaliana

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The visible symptoms associated with leaf senescence are an irreversible yellowing and loss of chlorophyll. The changes are in turn closely associated with a progressive decline in the efficiency of the leaf tissue to synthesize new RNA and protein (OSBORNE, 1965, 1967). It has been shown that, although the action is species specific, the functional life span of leaf discs of various plants can be experimentally extended by a variety of growth substances including auxins, gibberellins, kinins and growth retardants (OSBORNE, 1965; HARADA, 1966; KNYPL, 1967). This hormonal retardation of leaf senescence is mediated through maintenance of DNA as a functional template for DNA - dependant RNA synthesis (OSBORNE, 1967). Earlier we demonstrated cytokinin regulation of senescence in Arabidopsis; the present report indicates that the senescence of leaf discs of this plant could also be prevented by gibberellic acid (GA). Not only this, gibberellic acid also reversed the senescence of leaf discs caused by a naturally occuring, abcission accelerating, senescence promoting, and dormancy inducing substance viz., abscisic acid (ABA) (ADDICOTT and LYON, 1969).

Since often leaves of different age group ellicit different response, the present experiments were performed with leaf discs punched from mature leaves (mostly obtained from 30-33 days old plants). Chlorophyll was determined by extracting the pigment in 80% acetone and measuring the optical density of this extract at 665 nm, while the proteins were estimated by a modified biuret method. The results were expressed as the net decrease during the experimental period in the percentage of chlorophyll or protein from that initially present. During the experimental period the leaf discs, floating on water, IAA, ABA and on a combination of IAA and ABA, became yellow while those floating on GA solution as well as these on GA and ABA in combination retained their green colour. It was found that in the control the chlorophyll content decreased to as much as 75%, on IAA to 85% and on ABA to 90% (Figure a). In contrast the leaf discs on GA lost less than 20% of their total chlorophyll during this time. It is significant to note that the chlorophyll content was also maintained to a very great extent in the discs floated on solutions of ABA and GA in combination. The results also clearly indicated that GA delays the degradation of the proteins (Figure b) alone as well as in combination with ABA. In other words, in Arabidopsis, GA not only itself acted as regulator of senescence in leaf discs but also appreciably reversed the senescence promoting action of ABA. Auxins did not retard senescence, and appeared to enhance the process.

Retardation of the senescence of excised leaf discs by GA has been noted in comparatively few species (Tropeolum, Taraxacum, Rumex and a few crop plants) only (FLETCHER and OSBORNE, 1965; BEEVERS, 1966; WHYTE and LUCKWILL, 1966; GOLDTHWAITE and LAETSCH, 1968; MISHRA and MISHRA, 1968). Thus Arabidopsis should also be added to the list of the species in which senescence is regulated by GA. Also since in this plant cytokinins (SANKHLA and SANKHLA, 1968a, b) successfully retard the senescence of leaf discs, this effect is not restricted to GA alone. In Taraxacum, GA maintained the DNA as a functional template for DNA dependent synthesis of RNA (FLETCHER and OSBORNE, 1965). In Tropeolum, on the other hand, RNA content declined in the GA treated leaves without the onset of any visible symptoms of senescence, thereby suggesting that only certain types of RNA are essential for the maintenance of function, and synthesis of this component may be sustained in the presence of GA (BEEVERS, 1966). The manner in which GA treatment delayed senescence in Arabidopsis remains to be explored. However, in the presence of GA protein degradation was considerably checked. Recently, OSBORNE (1967)

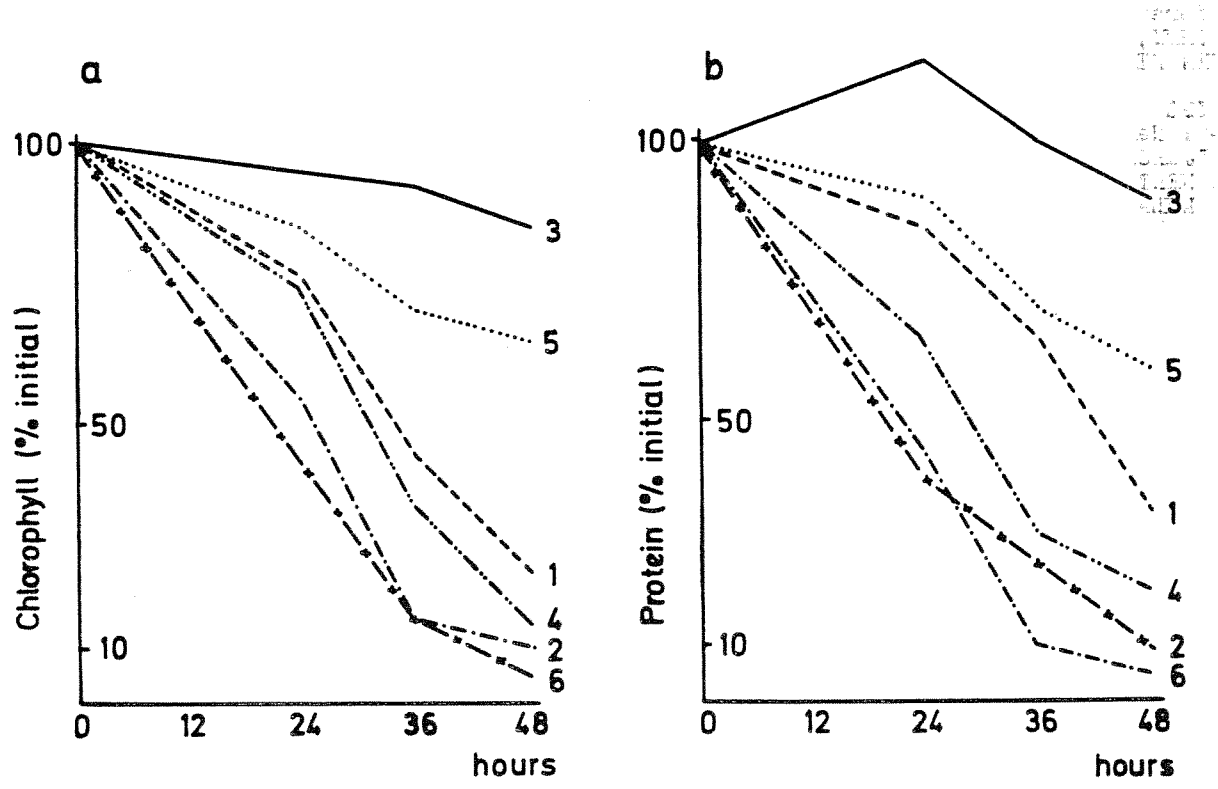

Figure: Chlorphyll (a) and protein (b) in leaf discs of the control (1) as well as in those floated on 2 ppm ABA (2), 10 ppm GA (3), 10 ppm IAA (4), 2 ppm ABA + 10 ppm GA (5) and 2 ppm ABA + 10 ppm IAA (6)

observed that pretreatment of leaf discs with ABA reduced the subsequent incorporation of ^{14}C-leucine into protein. We have observed that ABA also inhibits incorporation in RNA (SANKHLA, 1970). Thus, in view of the above results, it would appear possible that GA probably reverses the senescence accelerating effect of ABA by exerting its influence on protein and RNA synthesis.

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Further observations on reversal of abscisic acid induced senescence by kinetin

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Abscisic acid (ABA) - a naturally occuring plant hormone - now ranks in importance with auxins, gibberellins, and cytokinins as a plant growth controlling factor (ADDICOTT and LYON, 1969). Its role in (a) acceleration of abscission in fruits and leaves, (b) induction and prolongation of dormancy in shoots of deciduous trees and in tubers, (c) inhibition of germination, (d) inhibition of flowering of long day plants held under short days, as also in the senescence of leaves, now appear to be almost established (EL ENTABLY et al., 1967; SANKHLA and SANKHLA, 1968a, b, c, d; ADDICOTT and LYON, 1969). We observed that ABA greatly enhanced the chlorophyll degradation of excised leaf discs of Arabidopsis, and this effect was reversed if cytokinins were added simultaneously (SANKHLA and SANKHLA, 1968a). The present report summarizes our observations relating to the effect of kinetin and ABA, alone and in combination, on degradation of proteins and incorporation of leucine and uracil in the senescing leaf discs of Arabidopsis.

The leaf discs were prepared for experimentation in the same way as described earlier (SANKHLA, 1970); the protein content was measured periodically by a modified biuret method. Radioactivity was estimated with the help of a Packard's liquid scintillation spectrophotometer after appropriate incubation of the leaf discs.

Treatment	Residual protein after hours (% of initial) 24	36	48	^{14}C-Leucine cpm/mg protein	^{3}H-Uracil cpm/mg RNA
Water	85	65	35	965	254
2 ppm ABA	45	10	5	543	96
5 ppm Kinetin	105	90	75	1238	398
2 ppm ABA + 5 ppm K	85	75	60	1052	338

The results in the table clearly indicate that ABA drastically affected the maintenance of protein content; as a result ABA treated leaf discs lost a much as 95% of their protein after 48 hrs. In contrast, kinetin greatly delayed the disorganization of the proteins. Kinetin also checked the disintegration of protein resulting from ABA action. These findings were found to be supported by incorporation studies. Kinetin stimulated incorporation of ^{14}C-leucine into proteins, while ABA appreciably reduced it. When applied with ABA, kinetin appreciably restored the incorporation of leucine into proteins. ABA also decreased the incorporation of ^{3}H-uracil into RNA, but kinetin slightly enhanced it. Thus it is clear that kinetin not only delayed the decrease in RNA content, but also maintained the protein content in the leaf discs. In this respect the response of Arabidopsis leaf discs to kinetin treatment appears to be similar to that exhibited by other species to auxins, gibberellin, and kinetin (OSBORNE, 1967).

ABA has been reported to block the synthesis of specific DNA's (VAN OVERBEEK et al., 1967). Although this report yet remains to be confirmed, several investigators believe that ABA certainly inhibits RNA synthesis. Treatment of leaf discs with ABA led to a marked reduction of RNA, the reduction of polyribosomes fraction was particularly rapid (WAREING, GOOD and MANUEL, 1969). In leaf discs of Xanthium, ABA prevented the incorporation of ^{14}C-leucine into protein (OSBORNE, 1967). In embryos of Fraxinus, ABA inhibited the incorporation of ^{3}H-uridine and ^{3}H-thymidine but not the incorporation of ^{3}H-leucine. It was concluded that ABA maintains dormancy by inhibiting the production of specific types of mRNA and, therefore, the formation of specific proteins (VILLIERS, 1968). In Arabidopsis, ABA induced inhibition of chlorophyll, proteins and RNA (SANKHLA and SANKHLA, 1968a; SANKHLA, 1970) could be restored by kinetin. Also gibberellin reversed the senescence promoting effect of ABA (SANKHLA, 1970). Since many of the effects of ABA are in opposition to the growth promoting hormones, auxins, GA and cytokinins, it would appear possible that ABA-like substances have an important role in the homeostasis of the plants (ADDICOTT and LYON, 1969).

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Effect of germanium and gibberellin on the development of Arabidopsis plants

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A discussion of the role of mineral nutrition in the maintenance of the physical organization and activities of living cells, as well as in the regulation of metabolic patterns has recently acquired ample attention (EVANS and SORGER, 1966; BOLLARD and BUTLER, 1966; HEWITT, 1963). In these studies ARNON's (1950) concept of the 'essentiality of nutrients' has been recognised as too rigid and instead a concept of 'functional nutrients' has been proposed. According to NICHOLAS (1961) the term 'functional or metabolism nutrient' includes any mineral element that might function in some precise way in plant metabolism, irrespective of whether or not its action is completely specific or indispensable. Germanium (Ge) is the next higher analogue of silicon in the main sub-group of Group IV of the Periodic table of elements. In diatoms and certain plants germanium acts as a competitive inhibitor of silicon (LEWIN, 1966; WERNER, 1966, 1967 a). The latter author demonstrated that growth of Sinapis, Lemna, Wolffia, Nicotiana, Tradescantia, Zinnia and Secale was depressed by germanium and that the inhibition could be reversed by silicon addition. SANKHLA and SANKHLA (1967) observed that in several plants treatment with germanium greatly inhibited germination, retarded the elongation of the hypocotyl, checked the growth of the main root, inhibited the formation of the laterals and affected the pigmentation of the cotyledons. Preliminary studies indicated that in Arabidopsis, on the other hand, this element did not affect germination and early seedling growth. Therefore, additional experiments were planned to study the effect of germanium on the development of Arabidopsis; the results are being succintly summarized hereunder.

Plants of Arabidopsis were raised from seeds in pots at 20°-25°C under a light bank consisting of white and coloured cool fluorescent lamps which, at the level of the plants, supplied a light intensity of about 8.000-10.000 lux. The treated plants were sprayed with appropriate concentrations of germanium solution and data were regularly recorded. The results presented in the table clearly indicate that although this chemical failed to influence seed germination, it did affect the subsequent development. With an increase in concentration a progressive decrease in extention growth (Figure), fresh weight, and dry weight could be clearly observed (Table). Treatment with germanium also delayed flowering, reduced the number of fruits, and affected their size adversely. Since gibberellin is now universally recognised as a hormone which affects the elongation, growth experiments were also planned to study the effect of germanium and gibberellin in combination. It was extremely interesting to observe that gibberellin successfully reversed the inhibition of elongation growth caused by germanium, and the treated plants appeared very similar to those reared in gibberellin alone.

Table: (1) Germination percentage 8 days after sowing, (2) length (mm), (3) fresh weight (mg), and (4) dry weight after 4 weeks, (5) flowering after days, (6) number of fruits and (7) size of fruits (mm) after 35 days

Treatment	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Water	100	270	768	140	17-20	140-160	10-13
10 ppm Ge	100	270	765	138	17-21	140-160	10-12
50 ppm Ge	100	250	535	100	21-24	80-100	7-10
100 ppm Ge	100	200	525	85	25-28	70- 90	6- 8
500 ppm Ge	100	100	370	62	26-28	55- 75	5- 7
50 ppm GA	100	280	485	75	16-19	90-120	8-14
500 ppm Ge + 50 ppm GA	100	240	425	68	17-20	100-120	8-12

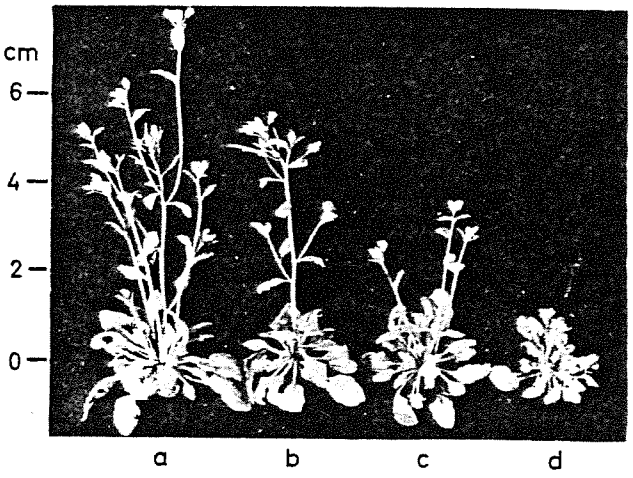

Figure:
Plants (from left to right) sprayed with (a) water, (b) 50 ppm, (c) 100 ppm, and (d) 500 ppm germanium, respectively

Earlier it was shown that germanium specifically and retrogressively inhibited growth of Cyclotella, but not that of Chlorella and Anacystis (WERNER, 1966). In Sinapis it did not affect early growth but inhibited the subsequent growth. Therefore it is assumed that apparently in certain plants silicon is not an essential growth factor in early developmental stages, whereas in later development the same plants need silicon (LEWIN and REIMANN, 1969).

It is worthwhile to note that germanium did not inhibit uptake of silicic acid, but interfered with further reactions of silicic acid within the cell. In the treated plants both chlorophyll synthesis and protein synthesis were drastically inhibited. However, germanium did not directly influence respiration, photosynthetic oxygen evolution, and synthesis of carbohydrates, although it prevented the breakdown of carbohydrates in the dark (LEWIN, 1966; WERNER, 1967 b). The reversal of germanium induced inhibition of growth by gibberellin in the present investigation clearly indicates interesting involvement of gibberellins in the regulation of some specific reactions which are possibly antagonised by germanium.

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A comparative study of the effect of cycocel (CCC), morphactin and gibberellin on growth and development of Arabidopsis thaliana

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Growth retardants (CCC, phosfon, B-nine, AMO-1618 etc.) apparently resemble morphactins in their overall growth retarding property (SCHNEIDER, 1964; CATHEY, 1964). However, morphactins, unlike growth retardants (NINNEMAN et al., 1964) do not affect GA biosynthesis (TOGNONI et al., 1967) and are known to disturb phototropism and geotropism of plant organs (KHAN, 1967; SANKHLA and SANKHLA, 1968). Recently, we have observed that morphactin induces formation of polypetalous flowers in snapdragon, reverses the original compound leaf to simple leaf in Ipomea, selectively inhibits formation of vessels in Petunia, inhibits tendril formation and alters leaf morphology in Momordica and modifies the flower sex in Nicotiana in a manner similar to auxin and growth retardants (SANKHLA, 1969 and unpublished data). The present report describes our results relating to the effect of CCC, morphactin (M), and gibberellin (GA) on growth and development of Arabidopsis.

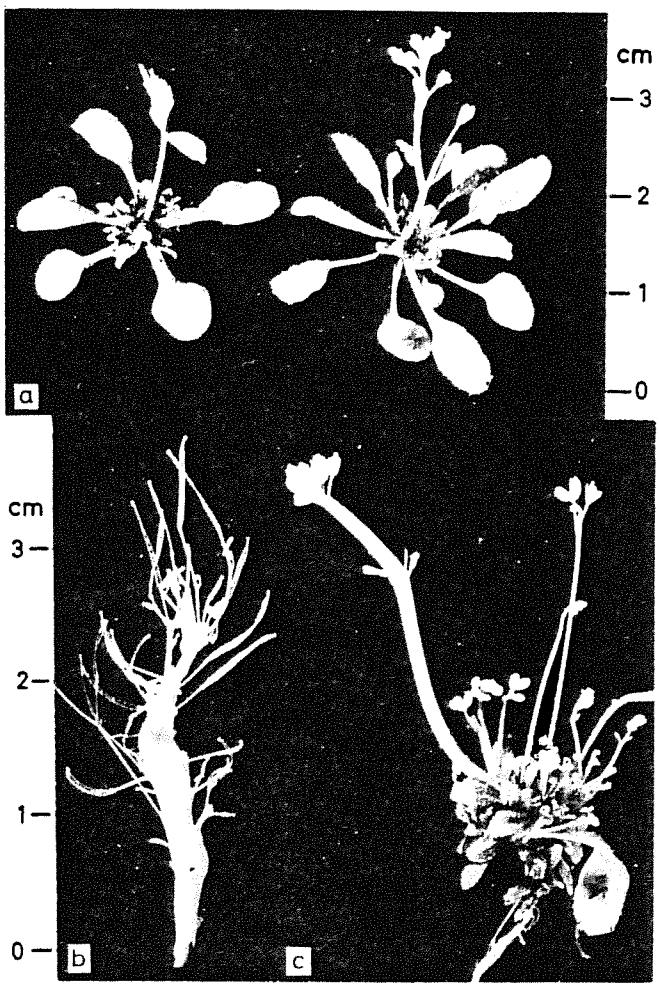
Plants of A. thaliana, race En-2, were raised in the green house and treated with the desired concentrations (Table) of CCC-, M-, and GA solutions. From the results

Table: (1) Lenght of plant (mm), (2) fresh weight and (3) dry weight (mg) after 4 weeks, (4) number of lateral branches, (5) number of 'secondary rosette' leaves, (6) flowering after days, (7) number and (8) size of fruits after 35 days

Treatment	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Water	270	768	140	3- 4	---	17-20	140-160	10-13
10 ppm M	200	576	91	5- 6	9-15	17-21	120-130	8-10
100 ppm M	120	377	59	14-20	28-40	25-27	40- 50	6- 8
500 ppm M	70	215	56	10-15	32-35	30-32	---	5- 6
50 ppm GA	280	485	75	2- 3	---	16-19	90-120	8-12
100 ppm M + 50 ppm GA	200	705	108	7- 8	22-25	17-20	70- 80	5- 8
3 000 ppm CCC	160	654	86	6- 8	---	20-22	140-160	9-11
6 000 ppm CCC	140	789	106	4- 6	---	22-24	130-150	7- 9
6 000 ppm CCC + 50 ppm GA	210	583	98	3- 4	---	18-20	120-140	9-10

presented in the table it appears that both CCC and M have profound effects on growth and development of Arabidopsis plants. With an increase in the concentration of M a decrease was observed in the extention growth, fresh weight and dry weight of the treated plants. Also till the effect of the chemical persisted, the flowering was delayed considerably and the number and size of fruits was greatly reduced. However, later on because of the production of a large number of lateral branches, the treated plants produced an enormous number of fruits, and also their senescence was greatly checked. A unique response to added morphactin was reflected in the appearance of a

large number of lateral branches and production of many short, hairy, upturned spatula-like 'secondary rosette'-leaves which frequently developed enormous amounts of an anthocyanin-like pigment on their morphological lower side. Although GA when added simultaneously tended to reverse the effect of morphactin it always failed in reversing the effect of the latter chemical on 'secondary rosette' leaves (Figure a and c). In plants receiving higher morphactin concentration a few lateral branches invariably indicated various degrees of fasciations (Figure b) which also could not be prevented by a simultaneous addition of gibberellin. It was interesting to note that even after an extreme degree of fasciation the branches produced normal fruits containing viable seeds (Figure b). On a few plants CCC and morphactins were also sprayed simultaneously (data not shown). These plants also indicated effects of both CCC and morphactin, thereby suggesting independent effects of the two chemicals on growth and development. The exact site of the action of morphactins is unknown. Several evidences suggest that these chemicals disturb the polar transport of auxin (KRELLE and LIBBERT, 1967) and possibly also affect the endogenous levels of the known growth regulators (ZIEGLER, 1968). Therefore, it appears likely that these chemicals ellicit the characteristic morphogenetic effects by affecting the overall modulation of endogenous growth regulators in such a way that the smooth running of an 'endogenous growth regulator gradient' is prevented (BOPP, 1968).



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Toxicity of 5-bromodeoxy-uridine to Arabidopsis thaliana in agar medium

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HIRONO and SMITH (1969) have reported that 5-bromodeoxyuridine at the rate of 1×10^{-5} M in the medium caused single gene mutations in Arabidopsis. This rate seems very low when compared to the one used in phage work. The writer had obtained conditional lethal mutants in P22 phage with the same mutagenic agent at the rate of 100 micrograms per cc (CORCOS and ORIAS, 1963). Therefore, he wanted to check if 5-BDU at higher concentrations would be more effective and concentrations of 100 micrograms per cc, 1×10^{-4} M, 1×10^{-5} M of 5-BDU were added to the Velemínský and Gichner medium (VELEMÍNSKÝ and GICHNER, 1964). The medium was then autoclaved for 15 minutes at 15 pounds pressure.

At the concentration of 100 micrograms per cc, no seeds of Arabidopsis germinated. At the concentration of 1×10^{-4} many of the plants germinated, but died 2 or 3 days later. At the concentration of 1×10^{-5}, the one used by HIRONO and SMITH, germination was not impaired, some plants died after a few days, but 84 of 128 survived and those surviving are now progeny-tested for possible mutant lines.

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Colchicine-induced chimerism in Arabidopsis

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Ploidy mutations induced by colchicine were scored by measuring pollen diameters. Mutated plants or plant-sectors give larger pollen. Some flowers in the M_1 yield normal as well as larger pollen, and are therefore chimeric. Studies of the pollen of flowers on the main inflorescences (the first 15 flowers) and on inflorescences of side shoots of different orders gave the following results:

(1) The main inflorescence is often chimeric especially at the base, loosing chimerism towards the top. (2) Side shoots of higher order show progressively less chimerism. (3) Within the chimeric parts of the inflorescence the flowers also are often chimeric (36% on the main inflorescence in our experiment). This is in contrast to what is usually accepted after EMS treatment, viz. little within-flower chimerism. We are now testing the same for EMS by studying the M_3 to detect heterozygotes in the M_2 that may occur in fruits otherwise scored as normal. (4) Mutated sectors are not parallel to the vascular bundles, but slightly twisted in a direction opposite to the short leaf spiral.

This is part of a study on chimerism; more detailed results will be published later.

Studies on the action of 8-azaguanine in Arabidopsis thaliana

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The purine analogue 8-azaguanine (AG) is known to be incorporated into the RNA of organisms like Bacillus cereus; it affects the synthesis of proteins at the m-RNA level (ZIMMERMAN et al., 1967). Apart from this, AG can also induce other harmful effects, e.g., endopolyploidy in the cells of pea root meristems (NUTI-RONCHI et al., 1965). The experiments reported below were conducted to study the action of AG on the biosynthesis of RNA by measuring the rate of incorporation of uridine into the acido-insoluble (AIS) fractions.

Labelled uridine was adjoined in the mineral medium of the culture in all these experiments. Seeds/plants were subjected to photoperiods of 24 hrs with a light intensity of 9000 lux; temperature was $24 \pm 1.5^{\circ}C$ and relative humidity at a level above 70%.

E x p e r i m e n t No. 1: Lots of 100 seeds were immersed in the following media: (1) Mineral medium (MM)+^{3}H-uridine (25 µC/ml) and (2) MM+AG (2.5×10^{-5}M)+^{3}H-uridine (25 µC/ml). Seeds were recovered at different intervals and were thoroughly washed with an equimolar solution of cold uridine and then with distilled water. The AIS fractions were extracted by a modified method of JACOBS (1969). The radioactivity

Table 1:

Time im hrs	Cpm for 100 seeds in the AIS fraction	
	MM+^{3}H-Uridine	MM+AG+^{3}H-Uridine
18	41	25
24	60	40
28	82	50
32	196	121
36	907	425
40	1402	803
44	1428	978
48	1352	1073

Table 2:

Time in hrs	Cpm for 10 plants in the AIS fraction	
	MM+^{3}H-Uridine	MM+AG+^{3}H-Uridine
2	602	441
4	1756	1228
5	2404	1839
6	2852	2031
7	3806	2895
8	3727	3016

Table 3:

Medium	Cpm for 5 plants in the AIS fraction
MM+^{3}H-Uridine (U)	899
MM+AG (1×10^{-5}M)+^{3}H-U	754
MM+AG (2.5×10^{-5}M)+^{3}H-U	664
MM+AG (5×10^{-5}M)+^{3}H-U	601
MM+AG (7.5×10^{-5}M)+^{3}H-U	528
MM+AG (1×10^{-4}M)+^{3}H-U	501

Time of incubation: 4 hrs

(Cpm = counts per min) was measured in a Tri-Carb liquid scintillation spectrometer in a BRAY's medium. Results are presented in Table 1.

E x p e r i m e n t No. 2: Seeds were sown in MM and after eight days lots of ten seedlings (C+2) were incubated in the same media as in the previous experiment. Seedlings of uniform size were selected and only the root system was immersed in the solutions. They were recovered at different intervals of time and the AIS fractions were extracted by the Millipore technique (DRIESSCHE and BONOTTO, 1969). Data are given in Table 2.

E x p e r i m e n t No. 3: In this experiment eight day old seedlings grown in MM were transplanted into media having different concentrations of AG, the specific activity of the labelled metabolite being the same in all cases (25 µC/ml). The seedlings were recovered after four hours and the rate of incorporation of ^{3}H-uridine was studied as in experiment No. 2. The readings are presented in Table 3.

From the results of these experiments it is clear that AG has an inhibitory effect on the rate of the uridine incorporation into the RNA of this cruciferous plant. The exact nature of this inhibition is being analysed. Results of similar experiments with ^{3}H-leucine instead of ^{3}H-uridine show that AG has an even more serious inhibitory effect on the rate of incorporation of this amino acid into proteins.

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A chlorate resistant mutant of Arabidopsis thaliana

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After nitrosoguanidine treatment of seeds of A. thaliana we isolated in the M_2 a mutant, showing resistance to elevated concentrations of chlorate. If wildtype seedlings of about 6 days old are exposed to chlorate in a concentration of 250 mg/l, the plants loose their green colour and die within 6 days. Toxicity of chlorate, however, depends on the nitrogen source of the seedlings. We found that plants, grown on a solution with ammonium as the only nitrogen source (solution F), are more sensitive than plants grown on ammonium nitrate (A). Plants grown on nitrate (D) are least sensitive to chlorate, but as mentioned, do die within 6 days.

Our chlorate-resistant mutant grown on solution D shows very little damage from 250 mg chlorate/l. The relative nitrate reductase activities of extracts of the wildtype (extract preparation and enzyme-essay following SANDERSON and COCKING, 1964) for seedlings grown on F, A, and D, respectively, are: 1, 6, and 8. On solution D our chlorate resistant mutant turned out to have a nitrate reductase activity of about 1.5 - 2 times higher than the wildtype.

The results suggest a positive correlation between the resistance to chlorate and the level of nitrate reductase activity. This is quite different from the data of LILJESTRÖM and ÄBERG (1966) obtained with young wheat plants. Their experiments strongly support the hypothesis that chlorate resistance in wheat plants is negatively correlated to the level of nitrate reductase activity, suggesting that damage is caused by the chlorite generated from chlorate by the nitrate reductase.

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Species homologous methylation of nucleic acids from Arabidopsis

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In three preceding articles (ABEELS, 1968; ABEELS and DIGNEFFE, 1969; ABEELS and DUBOIS, 1969) the existence of DNA- and tRNA-methylases was shown in Arabidopsis thaliana. For each enzyme the assay was performed with a heterologous source of nucleic acid, because it was so far accepted at least for bacteria that a homologous methylation is impossible except for a specific mutant of E. coli (58/161 RC^{rel} met^-) and, more recently, for a yeast mutant with a similar genetic modification (KJELLIN-STRABY and BOMAN, 1968).

The activity of tRNA-methylases was well proven to vary quantitatively in the insect Tenebrio molitor and in virus infected bacteria, while a qualitative variation was found in tumourous cells (BALIGA et al., 1965; WAIFAN et al., 1965; MAGEE et al., 1966). More recently KALOUSEK and MORRIS (1969) demonstrated that also a homologous methylation of DNA from pea seedlings may occur. Results of our own experiments, based on methylation of both, DNA and tRNA, also point towards a homologous methylation during different developmental periods of Arabidopsis thaliana. The DNA-methylases of Arabidopsis, just as those of pea seedlings or tissues, are bound to insoluble material; but tRNA-methylases, similar to those of bacteria, are soluble and further purification of these enzymes may therefore be more readily achieved. Whereas KALOUSEK and MORRIS believe homologous methylation to be just unusual, our hypothesis is that specific variations of the methylation of both, DNA and tRNA, occur during the development of the plant, thus varying the genetic information.

E x p e r i m e n t a l p r o c e d u r e . The same technics were used as those reported in No. 5 and 6 of this newsletter. The DNA-methylases were available only in crude protein extracts; but the tRNA-methylases were purified by precipitation with ammonium sulfate (30-50%) and by DEAE-cellulose chromatography. Nucleases were inhibited in the incubation mixture and during the extraction by bentonite or polyvinyl sulfate (BONOTTO and JACOBS, 1968; ABEELS, 1970).

R e s u l t s . The DNA methylases are bound to insoluble material after a centrifugation at 30.000 x g.

DNA	Enzymes	Incorporation
E.coli starved	Arabidopsis precipitate	2420 cpm
no	Arabidopsis precipitate	1390
E.coli starved	Arabidopsis supernatant	107
no	Arabidopsis supernatant	17

This experiment also proves the existence of a species homologous methylation in Arabidopsis. Another homologous methylation results from a crude extract of Arabidopsis mixed in the incorporation mixture with

DNase = 334 cpm	DNase+RNase = 51 cpm
RNase = 1238 cpm	nothing else = 1423 cpm

It is also possible to supply different crude extracts from the same plant with purified tRNA from Arabidopsis.

tRNA	Enzymes	Incorporation
no	Arabidopsis flowering	374 cpm
Arabidopsis rosette	Arabidopsis flowering	636
no	Arabidopsis in fruit	275
Arabidopsis rosette	Arabidopsis in fruit	563
no	Arabidopsis rosette	229
Arabidopsis rosette	Arabidopsis rosette	259

C o n c l u s i o n . We have shown that a species homologous methylation can occur for both, DNA and tRNA. We believe that methylation plays an important role in cell differentiation.

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Estimation of pH in the seed of Arabidopsis

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The aim of the following measurements was to estimate the pH inside the Arabidopsis seed after soaking in water and to determine whether buffers at different pH can influence the pH inside the seed.

For the pH measurements the range indicator method (R.I.M.) of SMALL (1956) was used. Seeds of Arabidopsis, soaked for 24 hrs in Britton-Robinson I buffer of pH 3 or pH 11 or in distilled water were washed briefly in neutral water; then the seed-coat was removed.

Indicator	Presoaked in: water		buffer pH 3		buffer pH 11	
	colour of embryo	pH	colour of embryo	pH	colour of embryo	pH
Phenol red	yellow	<6.8	yellow	<6.8	yellow	< 6.8
Bromo-thymol blue	yellow	<6.2	yellow	<6.2	yellow	< 6.2
Bromo-cresol purple	yellow	<5.9	yellow	<5.9	yellow	< 5.9
Methyl red	red	<5.2	red	<5.2	red	< 5.2
Bromo-cresol green	blue	>4.4	blue	>4.4	blue	> 4.4
Bromo-phenol blue	blue	>4.0	blue	>4.0	blue	> 4.0
Resulting range of pH	<5.2 > 4.4		<5.2 > 4.4		< 5.2 > 4.4	

The naked embryos were put into a drop of the indicator. After approximately 30 min the embryos were rinsed briefly in neutral water and the colour of the embryos was evaluated under a microscope. The indicators, given in the Table, were dissolved in 20% ethanol, only methyl red was dissolved in 60% ethanol. As demonstrated in the Table, the pH of the seed embryos was in the range of pH 4.4 to 5.2, regardless if the seeds were soaked for 24 hrs in water or in buffers adjusted to pH 3 or pH 11. This proves a high buffering capacity of Arabidopsis seeds.

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The influence of pH on the mutagenic effectiveness of nitroso compounds in Arabidopsis

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The pH of the solution during the treatment of Arabidopsis seeds influenced the mutagenic effectiveness of 7 nitrosoamides: N-methyl-N-nitrosourea, N-ethyl-N-nitrosourea, N,N'-dimethyl-N-nitrosourea, N,N',N'-trimethyl-N-nitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine, N-ethyl-N'-nitro-N-nitrosoguanidine and N-methyl-N-nitroso-p-toluenesulfonamide. When the half-life of decomposition of a tested nitrosoamide was shorter than the duration of treatment, a considerable decrease in the mutagenic effectiveness was observed. This was mostly at alkaline pH.

The pH inside the Arabidopsis seeds (pH 4.4 to 5.2) was not influenced by the buffers applied, even at extreme pH values. The decrease in mutagenic effectiveness can be explained, therefore, by decrease in the nitrosoamide concentration, due to its decomposition outside the seeds.

The nitrosoamines ethylvinylnitrosoamine and methylbenzylnitrosoamine, although very stable at all pH levels, decreased in mutagenic effectiveness at pH 11 or 12. Explanations for this phenomenon, e.g., a decreased rate of incorporation, are discussed.

No evidence that the nitrosoamides at low pH induce mutations in Arabidopsis via a "nitrous acid mechanism" could be demonstrated. Nitrous acid, however, can participate in the lethal action of some nitrosoamides at low pH.

Summary of a paper which will appear in Mutation Research

Comparison of the mutagenic activity of N-methyl-N-nitrosourea, N,N'-dimethyl-N-nitrosourea, and N,N',N'-trimethylnitrosourea

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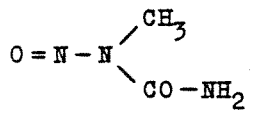
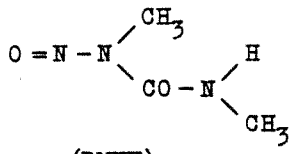
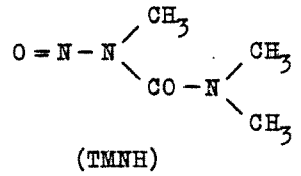
In the paper of GICHNER and VELEMÍNSKÝ (1967) the mutagenic activity of different N-alkylnitrosoureas was compared. The tested compounds differed in their alkyl groups (methyl, ethyl etc.) whereas their acyl group was the same. An increasing number of C-atoms in the alkyl group caused - with certain exceptions - a decrease in the mutagenic effectiveness, whereas the mutagenic efficiency was approximately the same. It seemed, therefore, to be interesting to compare the mutagenic effectiveness and the efficiency of nitroso compounds, having the same alkylnitroso group, but differing in the group bound on the acyl moiety. For this purpose 3 compounds were chosen: N-methyl-N-nitrosourea (MNH), N,N'-dimethyl-N-nitrosourea (DMNH) and N,N',N'-trimethyl-nitrosourea (TMNH).



(MNH)



(DMNH)



(TMNH)

All three compounds are strong cancerogens in rats (DRUCKREY et al., 1967). The chosen nitrosoureas differ in their hydrolysis rates, especially in neutral and in alkaline solutions. MNH is the most unstable compound, TMNH the most stable one.

All three compounds are strong mutagens for Arabidopsis thaliana. The mutagenic effectiveness, expressed by the concentration in mM, needed to induce 50% segregating M_1-

Table: Mutagenic efficiency and mutagenic effectiveness of MNH, DMNH and TMNH

Compound	mM $m_{b\ 50}$	mM S_{50}	S $m_{b\ 50}$
MNH	0.21 ± 0.004	0.31 ± 0.012	21 ± 5.3
DMNH	5.10 ± 1.1	8.25 ± 0.15	21 ± 7.0
TMNH	16.5 ± 1.5	35.8 ± 2.6	18 ± 6.8

mM $m_{b\ 50}$ = concentration in mM inducing 50% segregating M_1 siliquae

mM S_{50} = concentration in mM inducing sterility degree = 50

S $m_{b\ 50}$ = sterility degree at 50% segregating M_1 siliquae

95% confidence interval

siliquae differed significantly (Table). With the increasing number of methyls, bound to the NH_2 group, the effectiveness decreased. Of several possible explanations for these differences, the different stabilities of the tested compound seems to be the most plausible. The mutagenic efficiency of the tested compounds, expressed by the degree of sterility, accompanying 50% segregating M_1 siliquae, did not significantly differ (Table).

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Abstracted from a paper published in Arzneimittel-Forschung 19, 1053-1055 (1969)

Complex complementation map of 10 recessive chlorina mutants

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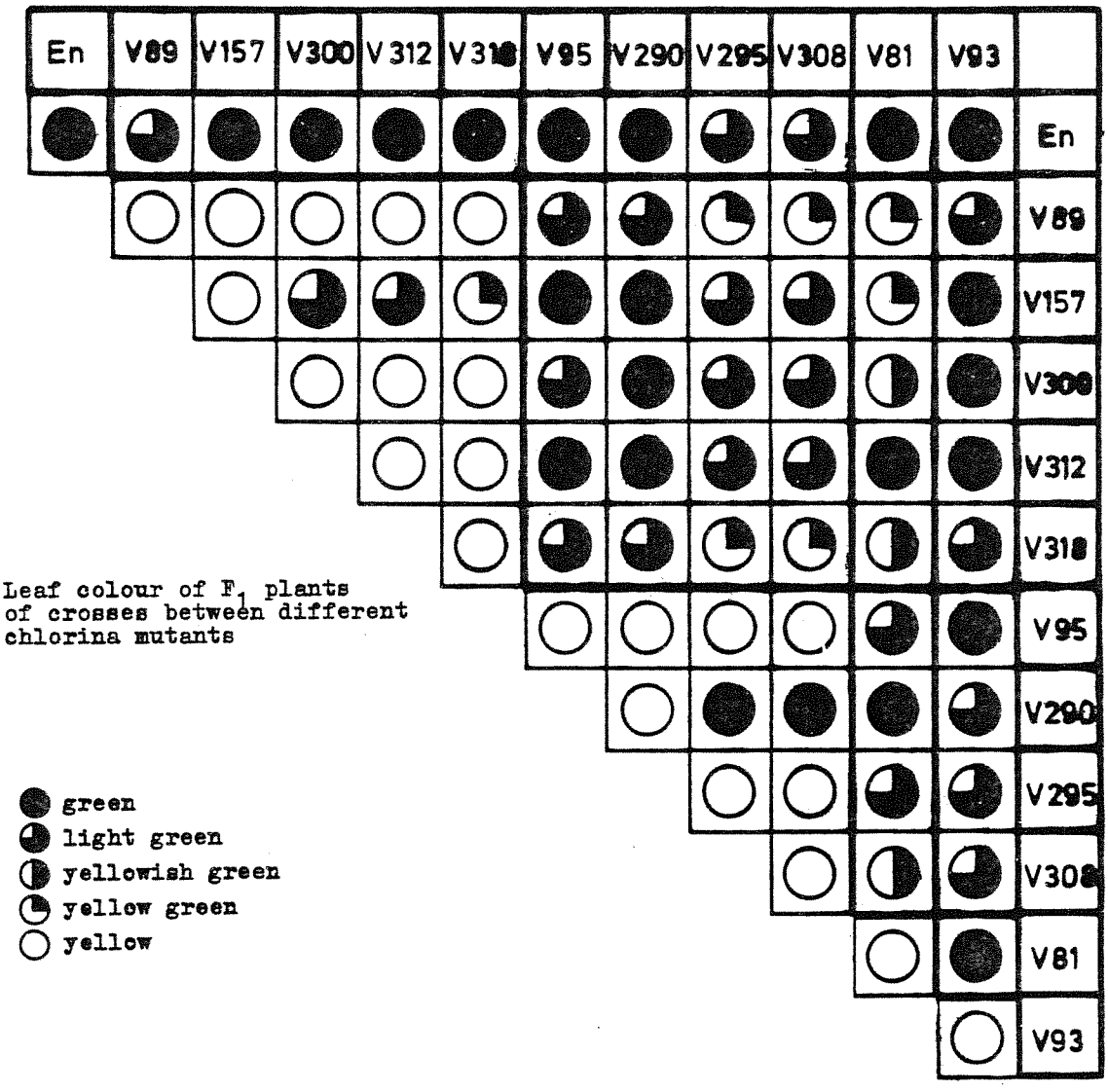
The reversion tests on which already reported in the last issue of this newsletter (RÖBBELEN, 1969) were performed with different chlorina mutants, all of which were characterized by plain yellow(-green) rosette leaves and a more or less normal plant morphology and growth. During the investigations, crosses were also made between these



original mutants in a diallelic fashion. When the F_1-plants were grown, it turned out that the leaf colour of the hybrids followed an unexpectedly complex pattern which is tabulated in the Figure. Notice for example that the F_1 between V300 x V318 is yellow, indicating allelism between these two mutants; accordingly 252 plants which were raised as F_2 generation from this cross, were all of the mutant type. On the other hand, the

green F_1 plants of the cross between V290 x V300 demonstrate non-allelism. Other F_1 combinations, however, show intermediate colouring, e.g., in V81 x V89 yellow-green rosettes. Still more complicated is the situation if the interactions of more than two mutant genes are compared; e.g., the two apparently allelic mutants V300 and V318 clearly differ in combinations with chlorina mutants like V93, V157, V290, V295, V308. In addition it is interesting to note that 3 mutant allels (V89, V295, V308) are partially dominating the + allel of the wild typ (race Enkheim). Finally 9 of the tested 11 mutants can be classified into 2 groups, within which at least one F_1 combination is yellow; just so is the rest of F_1's within each group more yellow than the F_1 combinations with members of the other group.

Also the F_2 segregation data of this diallel test are so far rather confusing. Among the 25 F_2's grown up to now, simple monogenic ratios were only recovered from all the (5) combinations with yellow F_1 plants, where the two parent types could be distinguished from one another. The whole rest of F_2's was more or less distorted. From green F_1 plants, e.g., in the cross V157 x V290 from a total of 1049 individuals 37.9% were alike V290, 16.1% V157, and 45.9% wild type, giving a significant surplus of mutant phenotypes compared to a recessive dihybrid pattern. Especially in F_2's from light green to yellow-green F_1 plants 4-6 classes of leaf colours could be clearly distinguished with various frequencies, of which detailed analyses are just underway.

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Interallele Wechselwirkung und Pleiotropie des ch-Locus von Arabidopsis

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Im Zusammenhang mit der Aufklärung der Biosynthese des Chlorophylls b und seiner Rolle in den Lichtreaktionen der Photosynthese sind die quantitativen Funktionen des ch-Locus von besonderem Interesse (KRANZ, 1968). Bekanntlich haben HIRONO und RÉDEI (1963) nach Röntgenbestrahlung folgende Mutanten erhalten und papierchromatographisch

Tabelle 1: Kreuzungsanalyse mit phänotypischer und genotypischer Klassifizierung der F_1- und F_2-Pflanzen sowie der F_3-Linien

		$ch^1 \times ch^+$	$ch^2 \times ch^+$	$ch^1 \times ch^2$
F_1	Blattfarben-bonitur	14 24 9 6 6 } = ch^+	51 35 28 9 10 } = ch^+	23 8 1 21 11
F_2	Papierchromat. Test	ch^+ = { 30: 9 20: 8 21: 7 25: 8 22:12 } = ch^1	ch^+ = { 22: 6 32:14 7: 3 6: 6 9: 4 } = ch^2	ch^2 = { 12: 9 29:13 22:20 16:20 17:10 } = ch^1
		$ch^+/ch^\cdot$ ch^1/ch^1	$ch^+/ch^\cdot$ ch^2/ch^2	ch^2/ch^2 ch^2/ch^1 ch^1/ch^1
F_3	Quantitative Pigmentmessung	15:5 8	16:5 8	5 : 16 : 5
		$ch^+/ch^\cdot$ ch^1/ch^1	$ch^+/ch^\cdot$ ch^2/ch^2	ch^2/ch^2 ch^2/ch^1 ch^1/ch^1

Tabelle 2: Quantitative Verteilung der Blattpigmente zur Blütezeit bei den in F_3 untersuchten Genotypen (Mittelwert ± Streuung) und den 3 Elternlinien als Vergleich

Kreuzung	Genotyp F_3	Pigment mg/g Trockengewicht Ca	Cb	Cc
ch^1 x ch^+	ch^+/ch^+	3.29 ± 0.30	1.11 ± 0.11	1.02 ± 0.11
	ch^+/ch^1	2.13 ± 0.60	0.84 ± 0.22	0.69 ± 0.21
	ch^1/ch^1	1.23 ± 0.43	0.11 ± 0.05	0.36 ± 0.21
ch^2 x ch^+	ch^+/ch^+	3.84 ± 0.26	1.32 ± 0.08	1.26 ± 0.09
	ch^+/ch^2	2.47 ± 0.72	0.92 ± 0.22	0.78 ± 0.26
	ch^2/ch^2	1.19 ± 0.27	0.22 ± 0.05	0.37 ± 0.09
ch^1 x ch^2	ch^2/ch^2	1.37 ± 0.42	0.12 ± 0.04	0.42 ± 0.17
	ch^1/ch^2	1.22 ± 0.39	0.17 ± 0.06	0.36 ± 0.14
	ch^1/ch^1	1.33 ± 0.36	0.06 ± 0.03	0.43 ± 0.11
Eltern	ch^+/ch^+	4.47 ± 0.04	1.48 ± 0.03	1.66 ± 0.05
	ch^1/ch^1	1.60 ± 0.18	-0.02 ±<0.01*	0.51 ± 0.05
	ch^2/ch^2	1.41 ± 0.15	0.11 ±<0.01	0.43 ± 0.07

* Noch innerhalb der methodischen Fehlergrenzen von ± 3 %

bestimmt: ch^1 = völlig Chlorophyll b (Cb)-frei, ch^2 = stark reduzierter Cb-Gehalt. Eine dritte Mutante (ch_3) besitzt etwas weniger Cb als die Wildform (ch^+). Ihre Plastidendifferenzierung und Pigmentbildung wurden von VELEMÍNSKÝ und RÖBBELEN (1966) eingehend untersucht und keine wesentlichen Thylakoiddestruktionen festgestellt. Die bisher veröffentlichten Genanalysen ergaben eine monogen rezessive Grundlage der Mutationen, wobei ch^1/ch^1 und ch^2/ch^2 einer Allelenreihe des ch-Locus der Kopplungsgruppe 4 angehören (LEE-CHEN and STEINITZ-SEARS, 1967). Dagegen ist die von RÖBBELEN (briefl.Mitteilung) induzierte ch_3 Mutante (=V81) nicht mit diesen allelisch, da die F_1-Pflanzen aus Kreuzungen mit ch^1/ch^1 und ch^2/ch^2 normal grün sind. Bisher ungeklärt sind die quantitativen Wirkungen der genannten Allele und ihre molekulargenetische Bedeutung.

Die hier angewandte Pigmentanalytik und die Anzuchtbedingungen in einer programmgesteuerten Klimakammer wurden bereits beschrieben (KRANZ, 1966, 1968). Die Mutanten wurden wie üblich (KRIBBEN, 1964) mit der Wildform (En-2) diallel gekreuzt. Zunächst zeigte es sich, daß uns eine sichere phänotypische Klassifizierung der F_1-, F_2- und F_3-Pflanzen mit der von RÉDEI (1968) vorgeschlagenen papierchromatographischen Schnellmethode allein nicht gelang. Daher wandten wir ein kombiniertes Verfahren an, bestehend aus einer visuellen Blattfarbenbonitur der F_1-, papier- bzw. dünnschichtchromatographischer Pigmenttrennung der F_2-Pflanzen und einer quantitativen Farbstoffmessung der F_3-Linien (siehe Tabelle 1). Dabei wurde aus den Kreuzungen mit dem Wildtyp erhalten: In der F_1-Generation der normalgrüne Phänotyp der Wildform, in F_2 eine Aufspaltung im 3 : 1-Verhältnis und in F_3 eine erneute Spaltung der Heterozygoten sowie eine Konstanz der F_3-Nachkommen aller mutantengleichen F_2-Genotypen (ch^1/ch^1 und ch^2/ch^2 (χ^2 ist stets mit $P \approx 0{,}2$ bis 0,8 signifikant). Schwieriger war die phänotypische Beurteilung der Nachkommen aus der Kreuzung ch^1 x ch^2. Die Blattfarbenbonitur der F_1-Pflanzen ließ hier keine sichere Differenzierung zu, und auch mit den chromatographischen Tests waren die Cb-freien Phänotypen nicht sicher zu erfassen. Erst nach Herstellung größerer Proben aus den F_3-Linien gelang es, die erwartete Spaltung der Genotypen im Verhältnis 1 ch^2/ch^2 : 2 ch^2/ch^1 : 1 ch^1/ch^1 quantitativ nachzuweisen ($\chi^2 = 1{,}385$, $P = 0{,}50$).

Bei den hier publizierten Pigmentanalysen von F_3-Linien erhielten wir ferner eindeutig pleiotrope quantitative Genwirkungen, die stets alle drei Blattpigmente, Chlorophyll a (Ca), Chlorophyll b (Cb) und die Carotinoide (Cc) betrafen. Die F_3-Genotypen besaßen nämlich zur Blütezeit stets niedrigere Farbstoffmengen als ihre Kreuzungseltern

(siehe Kontrolle in Tabelle 2), und der meßbare Cb-Anteil lag bei den mutantengleichen F_3-Nachkommen etwas höher. Die Heterozygoten zeigten intermediäre Werte mit stärkerer Expressivität des Wildallels; allein die Mutantenkreuzung ch^1 x ch^2 lieferte in der F_3 Heterozygote mit signifikant heterotischem Cb-Gehalt (siehe den unterstrichenen Wert in Tabelle 2), womit Superdominanz des ch^2- gegenüber dem ch^1-Allel angezeigt wird.

Damit ergaben die Untersuchungen bisher, daß der ch-Locus offenbar auf mehrere Stellen der Blattpigmentbiosynthese einwirkt, und komplementative Effekte in den Heterozygoten und Rekombinanten die Blockade der Cb-Bildung teilweise aufheben. Das könnte auch bedeuten, daß die Cb-Synthese auf wenigstens zwei gengesteuerten Reaktionsschritten beruht. Ähnliche Superdominanzreaktionen wurden bereits früher bei Arabidopsis von WRICKE (1955) für die Chlorophyllbildung und zuletzt von LI und RÉDEI (1969) für den Thiaminstoffwechsel beschrieben. Versuche zur genaueren molekularbiologischen Charakterisierung der Genwirkungen des ch-Locus von Arabidopsis wurden inzwischen mit weiterführenden Methoden begonnen.

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Mit Unterstützung durch eine Sachbeihilfe der Deutschen Forschungsgemeinschaft

Umsatzraten einiger Photoreaktionen in isolierten Chloroplasten von Arabidopsis thaliana

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Chloroplasten aus einer Wildform von Arabidopsis thaliana (En) und einer Chlorophyll-b-freien Mutante (ch^1) wurden - im Rahmen einer größeren vergleichenden Arbeit - auf ihre photosynthetische Leistungsfähigkeit untersucht. Dabei ergab sich unter anderem die Frage, ob in den Chloroplasten der Mutante mit dem Chlorophylldefekt auch Änderungen der bekannten Photoreaktionen einhergehen. Chloroplasten aus Spinacia oleracea dienten als Vergleichsstandard. Über methodische Einzelheiten wird an anderer Stelle ausführlich berichtet werden.

Zunächst mußte die Präparationstechnik dem Objekt angepaßt werden. Wir isolierten in Tris-HCl-Puffer, pH = 7,2, mit 0,33 M Sorbitol und den notwendigen Cofaktoren (vgl. JENSEN und BASSHAM, 1966; SANTARIUS und ERNST, 1967). Der Erhaltungszustand der Chloroplasten wurde im Phasenkontrastmikroskop und durch analytische Daten überprüft (HEBER, 1960; JACOBI, 1963). Aus den ermittelten Relationen von Chloroplasten- zu Blattprotein oder Chlorophyll- zu Proteingehalt, aus der Ausbeute an Chloroplasten und anderem konnten wir auf einen guten, den Spinatchloroplasten vergleichbaren Erhaltungszustand schließen. Demnach hätte man erwarten können, daß in den Chloroplasten der Wildform von Arabidopsis die primären Photosyntheseschritte zumindest mit ähnlichen Umsatzraten wie in Spinatchloroplasten ablaufen. Die in Tabelle 1 zusammengestellten Ergebnisse zeigen jedoch, daß das nicht der Fall war. Die Sauerstoffentwicklung und die HILL-Reaktion mit Fe^{+++} als Elektronenakzeptor lagen beträchtlich niedriger als bei Spinatchloroplasten. Aus den errechneten stöchiometrischen Verhältnissen geht hervor, daß die entscheidenden, vom Elektronentransport abhängigen Photoreaktionen, nämlich die NADPH- und die ATP-Bildung, offenbar noch stärker gehemmt waren.

Tabelle 1

Pro mg Chlorophyll x h gemessen bzw. berechnet	Chloroplasten Spinacia	Arabidopsis
µMole O_2 entwickelt	52	11
µMole Fe^{+++} reduziert	160	69
NADP-red/O	1,85	0,24
NADP-red/ATP	1,27	4,25
ATP/O	1,47	0,06

Eine mögliche Hemmwirkung des Elektronentransportes durch zelleigene Glucosinolate (0,6% im Blattfrischgewicht, bestimmt nach LEIN und SCHÖN, 1969) oder andere Inhaltsstoffe konnte durch Versuche an Spinatchloroplasten mit angereicherten, partikelfreien Extrakten aus Arabidopsis-Blättern und -Chloroplasten ausgeschlossen werden.

Dagegen gelang eine teilweise Stimulierung des Elektronentransports und der offenkettigen Phosphorylierung auf zwei Wegen:
a. durch Zugabe des synthetischen Elektronendonatorsystems DCPIP/Ascorbat und
b. durch Hinzufügen der für die NADP-Reduktion notwendigen Fd-NADP-Reduktase (erhalten aus Spinacia bei der DEAE-Säulenchromatographie zur Gewinnung von Fd).
Die Tabelle 2 enthält einige Daten zu diesen Stimulierungsversuchen.

Tabelle 2

Arabidopsis-Chloroplasten	µMole NADP-reduziert	µMole ATP gebildet
Standardansatz	1,1	0,27
+ DCPIP/Ascorbat	5,3	3,1
+ Fd-NADP-Reduktase	4,59	-

Diese Befunde lassen die folgenden hypothetischen Schlüsse zu:
1. Die Elektronentransportkette ist in isolierten Chloroplasten von Arabidopsis gehemmt. Ihre Stimulierung durch Einschleusen von Elektronen (vgl. a) zwischen Photosystem I und II (AVRON and NEUMANN, 1968) erlaubt, diese Hemmung zwischen Plastochinon und Cytochrom b zu vermuten.
2. Arabidopsis-Chloroplasten unterscheiden sich von Spinatchloroplasten auch darin, daß sie bei der Isolation neben Fd auch Fd-NADP-Reduktase verlieren. Trotz der erzielten Stimulierungseffekte lagen die stöchiometrischen Verhältnisse zum entwickelten Sauerstoff noch zu niedrig. Aus diesem Grunde darf man aus der erhöhten Umsatzrate nicht auf die natürliche, physiologische Leistungsgrenze der Arabidopsis-Chloroplasten schließen.

Mit Chloroplasten aus der Chlorophyll-b-freien Mutante ch^1 wurden analoge Ergebnisse erzielt. Allerdings lagen die Sauerstoffentwicklung, die HILL-Reaktion und die Umsatzrate des Elektronentransports etwa 50% unter den Werten der Wildform. Da jedoch kein grundsätzlicher Unterschied zur Wildform zu finden war und sich die Mutante ähnlich wie diese entwickelt, dürfte auch in Arabidopsis als Funktion des Chlorophyll b nur eine zusätzliche Lichtabsorption und -übertragung infrage kommen. Zu gleichartigen Schlußfolgerungen kamen BOARDMAN und HIGHKIN (1966) und BOARDMAN und THORNE (1968) bei Untersuchungen an einer Chlorophyll-b-freien Gerstenmutante.

Folgende Abkürzungen wurden benutzt:
ATP: Adenosin-5'-triphosphat; Cyt.b: Cytochrom b; DCPIP/Asc: Dichlorphenolindophenol/Ascorbat; DEAE: Diäthylaminäthyl-Zellulose; Fd: Ferredoxin; NADP: Nikotinamid-adenindinukleotidphosphat; O: Atome Sauerstoff,

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Mit Unterstützung der Deutschen Forschungsgemeinschaft

Leucine - requiring biochemical mutants of Arabidopsis thaliana

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It was shown earlier by LANGRIDGE (1955), RÉDEI (1960) and JACOBS (1965) that Arabidopsis thaliana is a suitable organism for the biochemical genetics of higher plants. In particular chlorophyll mutants which revert phenotypically on supplemented media are a proper tool for such investigations. In our experiments 35 chlorophyll mutants were screened for reversions on nutrient media supplemented with single amino acids. Only one mutant - viridoalbina 40/3 - reverted phenotypically to the wild type on a medium with leucine. Previously it was reported that this mutant is allelic to the mutation "Weiss ausbleichend", V-155/2 (KASYANENKO, 1969). Indeed, both alleles responded similarly to the addition of leucine, though the reaction was more clearly expressed in the viridoalbina 40/3. The addition to the nutrient medium of 200 mg/per liter DL-leucine increased the contents of the photosynthetic pigments and especially carotene; it restored the morphology and ultrastructure of chloroplasts and noticeably rose the intensity of photosynthesis. The leaves of the plants, grown on the leucine were more resistant to high intensity of light. Leucine also increased the rate of development and the dry weight of the plants.

Table: The influence of DL-leucine on the allelic mutants viridoalbina 40/3 and "Weiss ausbleichend" V-155/2

Genotype	Nutrient medium	Pigment/g fresh weight				
		chlorophyll a	chlorophyll b	carotene	lutein	violaxanthine
Enkheim	mineral	910	290	52	89	39
	mineral+ DL-leucine	580	240	50	76	34
Viridoalbina 40/3	mineral	220	127	20	28	20
	mineral+ DL-leucine	534	258	49	73	48
Weiss ausbleichend V-155/2	mineral	269	133	10	19	13
	mineral+ DL-leucine	506	211	42	38	40

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A spontaneous mutant of Arabidopsis thaliana with an altered type of inflorescence

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In summer 1968 on the experimental site of our highland research station Siya Kuh (2500 m above sea level), a spontaneous mutant was discovered among plants of race Enkheim showing retarded leaves and an unusual type of inflorescence. In most cases, three siliques as well as some single ones were formed. If in the first type one fruit failed to appear, a double silique resulted (Figure). The branching of the stem was also altered. When grown in tubes, the mutant started fruiting on the 28-th day after sowing, i.e. 3 days later than the control. Crossing with the wild type revealed a recessive pattern of inheritance. The absolute penetrance and high fertility make this mutant a useful marker for the genetic analysis.

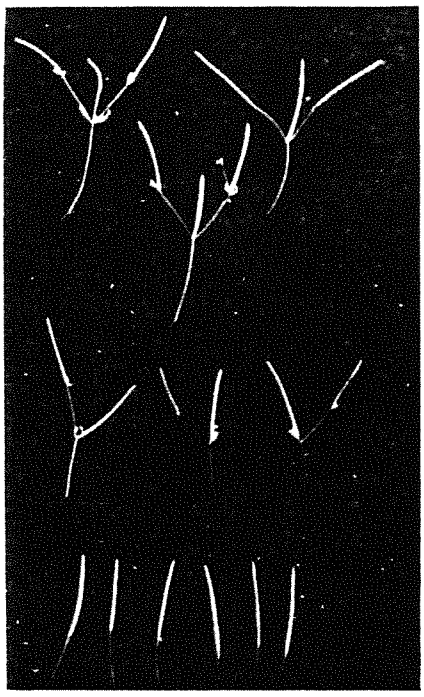

Figure:
(from above to below) Triplex, double and normal siliques of a mutant plant

Some observations on McKELVIEs axillaris mutant

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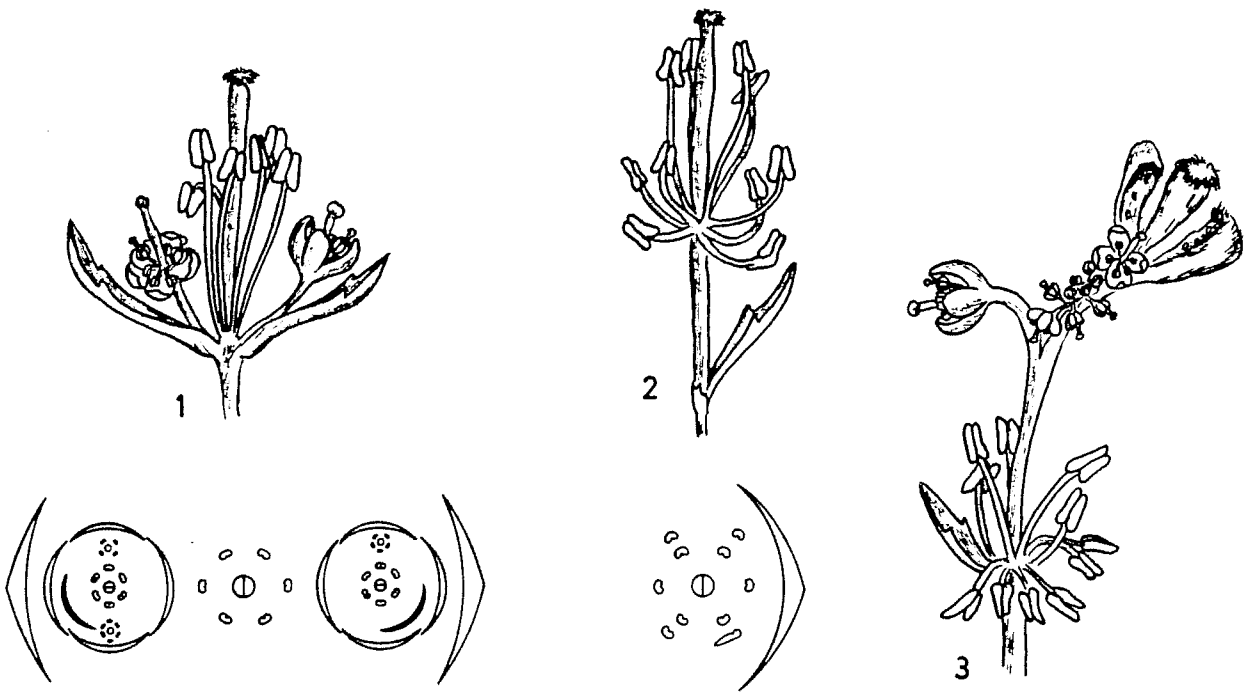
Already in 1962 McKELVIE described a mutant which he named axillaris (ax) "with two flowers growing from the base of each 'pod'". There is almost no doubt that this mutant is at least phenotypically identical with the spontaneous variant mentioned above by USMANOV.



Throughout extensive mutational studies we have not paid much attention in our laboratory to those genetic changes which are expressed not earlier than in the generative phase. Nevertheless, the axillaris-mutant was found three times. According to our crossing results these three types are allelic, though they differ in the expressivity of the critical characteristic. The mutant ax_3 which showed up (as M 45/15) after 14 kR of X-irradiation on seeds presoaked for 24 hrs, exhibits an abnormal branching in almost every flower. Mutant ax_4 was induced (as M 41/61) by 4 hrs treatment of presoaked seeds with 0.1% ethylene imine; it is phenotypically alike ax_3 except that in starts flowering 2-3 days later. The expressivity of ax_5, however, is much lower, many flowers showing normal white petals and no or only a single extra bud. The above description of USMANOV as well as the Figure 7 given by McKELVIE (1962) fits best to the phenotype of this allele ax_5 which was induced by the same chemical treatment as ax_4.

During the whole vegetative growth period our three axillaris-mutants are entirely of wild-type appearance. At flowering stage ratios of segregation in F_2's were 24,1% ax_3-types among 838 and 26,1% ax_4-mutants among 829 individuals. In Figure 1 a typical aspect of the ax_3-mutant is shown. It is apparent that the original 'first order' flower forms no calyx nor petals. But two stem leaves protrude from its pedicle. The diagram to Figure 1 and the microscopical section in Figure 4 indicate that the two additional flower buds mentioned above originate from the axis of these pedicle leaves. In general these 'second order' flowers carry a calyx with 4 sepals and may even have single petals. From the axis of two opposite sepals 'third order' flower buds may arise in which - like in the 'first order' bud - only up to 6 anthers and a pistil are present.

In agreement with the statement of USMANOV, this prototype of the axillaris-mutant can vary to quite an extent. Not only may the 'second order' flowers eventually be single or missing. But also more drastic deformations occur, of which the Figures 2 and 3 illustrate two examples. In Figure 2 the 'first order' flower shows additional 4 anthers instead of 4 sepals (or petals). Whenever this happens, any further development of secondary buds seems to be inhibited. Curious enough, this flower type exclusively produces one larger pedicle leaf only, the other being reduced to a small scale. The flower in Figure 3 apparently demonstrates a further step in the malformation compared to the former flower. In this case the base of the pistil is elongated and set with a mass of buds of different size and developmental stage ranging from almost normal flowers to those consisting merely of a pistil and anthers.

These morphological features as well as the simple genetic determination leads us to assume that the genetic defect in the axillaris-locus causes a relatively simple change within the array of the various growth hormones which are known to be active during flower morphogenesis.

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Physiological peculiarities of species and ecotypes of the genus Arabidopsis from the Pamir-Alay region

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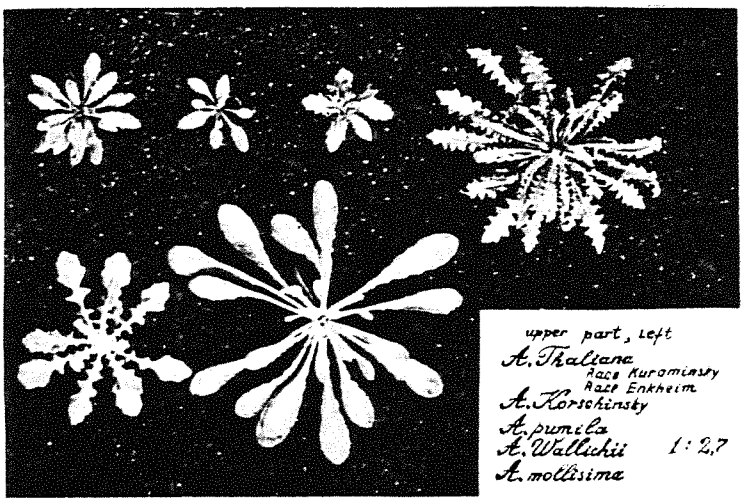
It was reported earlier that at least 5 species of the genus Arabidopsis are growing in Tadjikistan: A.Thaliana (L.) HEYNH., A.pumila (STEPH.) N.BUSCH, A.Korschinskyi BOTSCH., A.mollissima (CAM) N.BUSCH and A.Wallichii (HOOK et THOMS) (Figure). All the species are represented by various ecological forms. The ecotypes of Arabidopsis Thaliana used in the following investigations were collected from different parts of Tadjikistan. Several of them were characterized by specific morphological peculiarities, others only by physiological characteristics, some of which are indicated in the table. The vegetation period of the different species differed widely but ecotypes of A.Thaliana, under the same conditions, were characterized by a comparatively identical velocity of development. With a saturative concentration of CO_2 (1%) the photosynthetic activity of species and ecotypes was approximately similar. But the optimal intensity of light and the optimal temperatures for photosynthesis were dependend on the altitude of the habitat. Species and ecotypes from the highlands were characterized by the higher optimal light intensity and by the lower temperature for photosynthesis.



Species and ecotypes	Altitude of the habitat above sea lev. (in m)	Vegetation period in days (fruiting)	Maximal potential intensity of photosynthesis (mg CO_2 per gr dry weight per hour)	Saturation of light for photosynthesis (10^3 lux)	Optimal temperature for photosynthesis C°
Arabidopsis pumila	350-2100	103	235	40	24-28
A.Thaliana	-	56	200	50	20-24
A.Wallichii	850-4000	219	225	55	18-22
A.Korschinskyi	3600-4000	84	230	60	15-17
Ecotypes of A.Thaliana					
Renger - Tau	1100	67	210	35	22-26
Harangon	1900	55	205	40	21-25
Shugnan	2700	63	200	50	19-22
Turkestan	3600	63	200	55	16-18

Reference:

B. TECHNIQUES

A simple technique of uniform soil culture for the study of developmental physiology and genetics in Arabidopsis thaliana (L.) HEYNH.

I. CETL, Jirina RELICHOVÁ-DOBROVOLNÁ, and Marta KRSKOVÁ

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Some years ago, a series of experiments in developmental physiology and genetics was started in our Department. As so far the technique of cultivation and scoring was not yet published in detail it might be of interest to give some more important aspects here.

The main objective of the following procedures is to achieve the highest possible uniformity of germination and of further development and, thereby, to reduce all possible sources of non-genetic variation in determining the flowering time and other developmental characters in both, unvernalized and vernalized conditions. At the same time, the simplest method is preferred avoiding laborious operations (pre-sowing preparation of seeds, transplantation of seedlings etc.). In short, all exacting requirements (cf. Van der VEEN, 1965, 1967) should be fulfilled with relatively non-exacting means.

The vessels from pressed material are 60 cm wide, 30 cm deep and 6 cm high with 12 holes in the bottom. They are furnished with a paraffin layer on the internal walls and filled with a 2 cm high layer of coarse sand and with a 4 cm high layer of a soil mixture (3 parts of garden soil, 3 parts of compost soil and 1 part of sand, all fractions approximately 2 mm in diameter). The filled vessels are irrigated and allowed to stand for 24 hours. The seeds are sown in 16 to 20 rows (parallel with the shorter side of the vessel) with 16 to 20 pits each 0,8 cm deep so that 256 to 400 plants fit in. The density of the sowing is dependent on the aim of the experiment and on the expected size of plants. (If small plants of early forms are grown the density can be higher, and vice versa.)

Uniform germination is secured (1) by the choice of the suitable period after harvest when the dormancy of the seeds is already broken (a preliminary trial on wet filter paper is often necessary), or (2) by a simple procedure with cooling the seeds after sowing. The vessels covered by glass plates 60 x 30 cm padded with wooden laths 30 x 1 x 0,5 cm are placed for 24 hours in a dark cooling box at +1 to +3°C. This period is sufficient to achieve for the seeds a full and regular imbibition with water without starting the germination process (unpublished data). Then, the covered vessels are placed in the growing room with constant conditions of temperature and light: 25+3°C and continuous illumination given by incandescent bulbs, 1250 lux. (The reasons for the choice of the given regime see CETL, DOBROVOLNÁ, and EFFMERTOVÁ, 1969.) After 3 to 4 days, in the average, very uniform germination sets in. The germination of each seed is scored individually during the regular daily inspection practised between 10 and 11 hours a.m. not only throughout the period of germination but during the entire course of the experiment.

Only if the young cotyledons are stretched into the horizontal position the seed is taken for germinated to the given date. In simple experiments, the date of germination is determined comprehensively for the whole specimen according to the date when the majority of the seeds germinated. In more exact experiments, the plants germinating each day are marked with signs (3 x 0,5 cm) placed left from the plant. All evaluations are then related to this date. In both cases, the plants germinating three days or later after the date of maximum germination are not taken into account. If it concerns to single (usually weak) plants (up to 3 per cent) they are removed; if the portion of such plants is higher the experiment is to be repeated in the next convenient term.

In vernalization experiments the vessels with the sown seeds, covered with the glass plates, are placed in the cooling room for 24 hours as above; but this time they are first kept at 25 ± 3°C and in continuous illumination (1250 lux) for the next 24 hours. The reason for this treatment is the same as that given by Van der VEEN (1965). Only after this procedure, the vessels are placed in the dark with the vernalization temperature (+2 ± 1°C). During the vernalization, the vessels are not irrigated so that the hypocotyl growth is inhibited at higher vernalization rates, where the seeds already start to germinate. In experiments with gradually increasing vernalization rates (up to 80 days), the seeds are sown successively so that the vernalization of all variants can be finished at the same date. Then, the vessels are transferred into the growing room. As the plants of variants, where the seeds already germinated, are very susceptible the vessels are transferred gradually, whereby both temperature and intensity of illumination are increasing. Only after 10 to 12 hours from the end of the vernalization, the vessels can be placed on the definitive place in the growing room. The determination and scoring of

germination is not different from the experiments without vernalization. In seeds already germinating during the vernalization, the date of germination corresponds to the date of the end of vernalization.

F u r t h e r c a r e o f t h e p l a n t s. After full germination the glass plates and wooden laths are removed. When the cotyledons are approximately 3 mm above the soil surface the pits are filled with the above mentioned soil mixture. The cultures are irrigated both between rows and plants two times daily: before the inspection and in the afternoon.

The determination of the n u m b e r o f d a y s to appearance of the flower primordia. As the appearance of the flower primordia in the middle of the leaf rosette is the first well defined and well observable sign that the flower formation is accomplished, we take this date for the determination of earliness. The later stages of the flower morphogenesis appear to be less advantageous, in so far as they lenghten the course of experiments and can be influenced by factors only indirectly connected with the developmental processes. To the time of the daily inspection, we carefully roll off the highest rosette leaf and search with naked eye for the presence of the first flower primordium which can be well distinguished from a leaf primordium by its roundish shape and paler colour. This stage is not necessarily connected with the elongation of the first stem internodium. Only in early forms, the flower primordia appear simultaneously with this elongation.

The determination of the n u m b e r o f r o s e t t e l e a v e s. The number of rosette leaves can often be counted at the same time as the flower primordium appears. In doubtful cases, the plants are marked with signs as above and the accurate number is determined the next day. The two cotyledons are included. In late forms, the lower rosette leaves die away before counting. In such cases, it is necessary to count carefully the rests of the petioles or the traces of them.

In some experiments, plants can be removed after scoring. If it is necessary to obtain ripe seeds the plants are marked with signs placed right from the plant and furnished with corresponding data.

Valuable and well comparable data result from the fulfilment of all given procedures so that in homozygous specimens the results as to the number of days to appearance of the flower primordia and the number of rosette leaves show minimum variability.

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Aseptic culture of Arabidopsis thaliana (L.) HEYNH.

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For aseptic cultures of Arabidopsis on agar medium 100 ml volume flasks are used covered with a transparent polyethylen film of 60 µ thickness which is fixed on the glass by means of a ring rubber. The film is sufficiently permeable for air, but impermeable for water vapour, thus protecting the nutrient medium against drying for many months. Under these conditions the plants are equally open for the upper and lateral light. The specific advantage of this technique is the standardized gas exchange, which is more easily reproducible with the film than with corks or layings of cotton. The film may be sterilized by placing it in alcohol for several days.

For mass culture of Arabidopsis a device is used for accelerating and simplifying the sowing of seeds. This represents a plastic cylinder with small openings drilled along its axis in a definite order. At the top of the cylinder there is a flange which may rest on the borders of the glass. On the upper butt-end of the cylinder there are some ring hollows which are filled with seeds before sowing. The cylinder is inserted into the glass with the agar medium, until the flange rests upon the borders of the glass; then by means of a sterile needle the seeds are successively thrown into the holes. To maintain the given arrangement of the seeds the height of the cylinder should provide for a minimum distance between the surface of the medium and the lower butt-end of the cylinder. The device is sterilized by alcohol. 19 seeds are sown in each 100 ml flask. The method can, of course, be applied to glasses of any volume and to any quantity of seeds.

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D. ANNOUNCEMENTS

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