ARABIDOPSIS INFORMATION SERVICE

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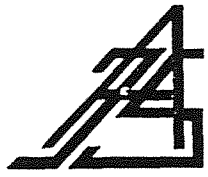

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Arabidopsis Information Service

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

At present one number is issued annually in March. The original articles should preferably be written in English, but also German or French (with english summary) will be accepted. Contributions should reach the Editor not later than January 15, each year. For preparation of the manuscript see inside the back-cover. It is understood, that the contents of the contributions are the sole responsibility of the authors.

The price of this number is DM 4,- domestic and DM 4,50 or US $ 1,25 foreign, all postage free. Payment can be done by remittance to "Arabidopsis Information Service", Konto-No. 2069, Städtische Sparkasse, Hauptstelle, 34 Göttingen, Germany or by personal cheque to the Editor.

All business correspondence including orders, change of address, manuscripts, and communications concerning the newsletter should be addressed to the Editor: Gerhard RÖBBELEN, Institut für Pflanzenbau und Pflanzenzüchtung der Universität, von-Siebold-Str.8, 34 Göttingen, Germany

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Acknowledgement

The cost of the present publication has been defrayed partly by a grant in aid from the Universitätsbund Göttingen e.V. to which we wish to convey our sincere thanks.

We should also like to express our gratitude for many favorable comments and valuable contributions. Increased support for further issues would be appreciated.

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A. BRIEF NOTES

Distribution of spring and winter types in the local populations of Arabidopsis thaliana (L.) HEYNH. from various localities in Western Moravia

I.CETL, J.DOBROVOLNÁ and E.EFFMERTOVÁ

(Department of Plant Physiology and Genetics, Purkyně-University, Brno, Czechoslowakia)

In the last two years seed of more than 60 local populations of Arabidopsis thaliana have been collected from various localities. The plants from post-dormancy seed were grown at 25±3°C in continuous illumination (1250 lux), i.e., in the conditions which induce flowering in spring types only and inhibite it in winter ones. The experiments were finished after 42 days from full germination. The percentage of generative (=spring) plants was determined at the same time.

The distribution of spring types has been found in the first part of the whole material (32 populations) as follows:

% of spring plants	5	15	25	35	45	55	65	75	85	95
Number of populations	6	0	3	1	2	0	1	0	4	15

Thus, the majority of the populations (78%) was of pronouncedly spring or winter type with a small portion of individuals of the opposite type, while only 22% populations were mixed.

A confrontation with the geographical-climatic conditions shows that the boundary between the localities with spring and winter types corresponds to the limit of the range of the xerotherm vegetation: in the more warm and dry lowland we find the winter populations, in the more cold and wet highland the spring ones. The mixed populations occur in the boundary localities.

Racial differences in the number of days to appearance of the flower primordia, in the number of rosette leaves, and in the number of rosette leaves per day in Arabidopsis thaliana (L.) HEYNH.

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The variability of the number of days to appearance of the flower primordia (x), of the number of rosette leaves (y), and of the number of rosette leaves per day (y/x) was studied in 19 different races without or with only a quantitative vernalization requirement (4 samples from the exchange service of the botanical gardens, 6 lines A_1 from the west-moravian populations, 8 races from the collection of Prof.F.LAIBACH, Limburg, and 1 race from the collection of Dr.M.SOSNA, Praha). Plants from unvernalized and vernalized seed (2 weeks at +2 ±1° C) were grown at 25 ±3° C

in continuous illumination (1250 lux) for 84 days. The quantitative vernalization requirement ascertained in another experiment in some races was fully covered by the used vernalization.

Vernalization (weeks)	Variability	N	x		y		y/x	
			V	F	V	F	V	F
0	between races	18	1714,48	48,72*	133,13	12,41*	0,0433	15,46*
	within races	327	35,19		10,73		0,0028	
2	between races	18	381,43	83,06*	32,12	36,09*	0,0323	17,94*
	within races	277	4,59		0,89		0,0018	
V_0/V_2	between races			4,49*		4,15*		1,34
	within races			7,66*		12,06*		1,56

*Exceeds the 1 per cent point

There exist significant racial differences in control as well as in vernalized plants in all three characteristics. In the vernalized plants, the variability between and within races decreases significantly only in the number of days (x) and rosette leaves (y), while in the rosette leaves per day (y/x) this decrease is not significant. Thus, the y/x ratio is not changing by vernalization and represents an important racial constant.

On the given basis, it is possible to search for suitable races in which the negative correlation between the earliness and the fertility is expressed in a minimal degree. For genetic experiments in artificial conditions early forms with high y/x ration appear to be ideal; in our material, e.g., the line BP 5 with $\bar{x}_o \pm s = 13,4 \pm 2,99$, and $\bar{y}_o/\bar{x}_o \pm s = 0,49 \pm 0,065$.

Changes of earliness in the M_2-generation of Arabidopsis thaliana (L.) HEYNH. as caused by application of methylnitrosourea

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About 1.100 seeds of three races (early race BP 4-1, middle-early race ST 56, and late race Zb 25) were treated by the 0,2 mM solution of methylnitrosourea at 25°C during 24 hours. The control was analogously made in distilled water. The M_1-populations were cultivated in the green-house at 12 - 20°C. The M_2-populations were sown by 20 seeds from various M_1-plants and cultivated under continuous illumination (2000 Lux) at 24 ±2,5°C. The experiments with the early and middle-early races were finished after 30 days, the experiments with the late race after 66 days. The results are given in the following table:

Race		$\bar{x} \pm 3 \cdot s_{\bar{x}}$	v%	$\bar{y} \pm 3 \cdot s_{\bar{y}}$	v%	n
BP 4-1	Control	16,0 ±3·0,09	14,0	6,2 ±3·0,02	7,0	638
	Treated	15,3 ±3·0,16	16,5	6,6 ±3·1,00	15,1	257
ST 56	Control	19,9 ±3·0,06	10,4	7,9 ±3·0,02	7,7	1394
	Treated	18,7 ±3·0,12	17,8	8,0 ±3·0,05	18,1	796
Zb 25	Control	42,3 ±3·0,48	22,3	15,3 ±3·0,33	42,7	388
	Treated	34,7 ±3·1,46	38,3	14,8 ±3·0,48	32,5	87

$\bar{x}$: the mean number of days from the germination to the formation of flower primordia;
$\bar{y}$: the mean number of rosette leaves.

The given changes in the earliness show on the average a trend to lower the number of days which are needed for the transition to the generative stage, especially in the late race Zb 25; the number of leaves is even lower here. The within races variance of the M_2 as compared with the control increases significantly.

The percentage of plants remaining after 30 or 66 days in the vegetative stage increases in the M_2 in all three races. The difference between the M_2 and the control is significant, too. The experiments are being continued.

Genes for late flowering

J.H. van der VEEN

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Single-gene differences for early versus different degrees of late have been reported by e.g. HÄRER (1951), DIERKS (1958), and NAPP-ZINN (1962). Modifiers may play a role, in some environments more than in others. NAPP-ZINN (1962) assumes for the combination of the races Limburg (Li-5) x Stockholm (St)(early x very late) a minimum of 4 unidentified loci.

For h e t e r o t i c crosses of early x (less) early races like Enkheim (En) x Catania (Ct), DIERKS (1958) concluded to one major gene (from Ct) and an unknown number of heterosis inducing modifiers (from En) NAPP-ZINN (1963) adopts the same explanation for his heterotic early x (medium) late cross Li-5 x Zürich (Zü).

From the F_2 of the heterotic early x (less) early cross Dijon (Di) x Li-2, I could select 2 classes of new homozygotes, an early class (not heterotic with either parent) and a late class (giving 1 early : 3 late with both parents). Greenhouse data (in days) are:

	aabb Sel.96	AAbb Di	aaBB Li-2	AaBb F_1	AABB Sel.189
Midsummer 1964 (sunlight only)	22	25	30	37	47
Autumn 1964 (assim.+photop. light added)	27	29	38	55	73

Proof for t w o complementary-like m a j o r g e n e s was obtained by comparing (in autumn!) position of peaks in F_2-frequency distribution with those of the 4 homozygous, all F_1's, and the 4 backcrosses. This led to 6 phenotypes of the theoretical F_2 ratio (4 : 3) : (4 : 2 : 2 : 1). In the second group, A acts stronger than B, but in the first group, only B-b can be classified.

A third gene C is being traced now, which would be complementary to B again, but not to A. Under this hypothesis B is the basic gene for late flowering.

More (preliminary) details will be presented at the 1st International Arabidopsis Symposium held at Göttingen in April 1965.

References:
DIERKS,W.: Z.Pflanzenzücht. 40, 67-102 (1958)
HÄRER,Luise: Beitr.Biol.Pflanzen 28, 1-35 (1951)
NAPP-ZINN,K.: Z.Vererbungslehre 93, 154-163 (1962);Beitr.Biol. Pflanzen 38, 161-177 (1963)

Induced mutations for flowering time

H.A.S.HUSSEIN and J.H. van der VEEN

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In a model experiment on "micro-mutation breeding", BHATIA and van der VEEN (1964) treated RÉDEI's very early type Landsberg-erecta with EMS, and found no selection response for earlier (perhaps due to a "physiological limit"), but did succeed in obtaining 11 fully vigorous and fertile selections, which flower 4 - 5 days later and have 6 more rosette leaves. After vernalization, they closely resemble the parent type, which did not respond.

To study the problem "easy versus difficult direction" in mutation breeding, and to see in how far this relation varies with different mutagens and different genotypes, we have started to treat the 11 selections, having selfed these for 10 generations. These selections clearly cannot be considered to represent a physiological limit.

At the same time we will try to analyse the flowering time - leaf number genotypes of the 11 selections, and of the second cycle selections-to-be, a.o. by introducing gene B from Limburg-2 (cf. van der VEEN 1965)

References:
BHATIA,C.R., and J.H.van der VEEN: Techn.Meeting on Use of Induced Mutations in Plant Breeding, Rome 1964 (in press)
VEEN,J.H. van der: Arabid.Inf.Serv. 2, 5 (1965)

Induced quantitative mutations in Arabidopsis thaliana

R.D.BROCK

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A number of mutagenic agents have been used to induce mutations in flowering time and plant weight in the early-flowering race Estland. Over all the mutagenic treatments the M_2 means shifted towards

later flowering and reduced log. fresh weight. The M_2 distributions were skewed towards lateness and towards reduced plant weight, but when the morphological mutants were removed from the data the distributions became more symmetrical.

The distribution and magnitude of the induced genetic variation and the high heritability values indicate that selection should quickly regain the loss due to the shift in the mean, and that real progress would be predicted for both characters. Selections have been made and are currently being tested.

Genetic correlation between the two characters was high in the total data (-0.7), but when the morphological mutants were removed this correlation was much reduced (-0.2).

The mutagenic agents tested were thermal neutrons, gamma rays, ethyl methanesulphonate (EMS), 2-chloro-ethylmethanesulphate, nitrous acid and di-ethylsulphate. The most efficient mutagens for inducing quantitatively inherited mutations were the ionizing radiations and EMS.

Observations on variations of megasporogenesis

G.P.RÉDEI

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VANDENDRIES in 1909 published a beautiful treatise on the embryology of some crucifers including Arabidopsis thaliana (Sisymbrium thalianum). According to his observations the young ovule contains a multicellular archesporium in which only a single archesporial cell (cellule primordiale) enlarges and develops into a functional megaspore mother cell (cellule-mère définitive). My observations on our Columbia wild type are in complete agreement with his description. In a material containing factor Gf also a different type of differentiation was observed. In some ovules not single megaspore mother cells but twin sporocytes were present, rarely even twin embryosacs developed. According to VANDENDRIES in Cardamine pratensis and Sisymbrium taraxacifolium multiple tetrads are formed regularly in each ovule; only one embryosac develops however. It appears that the pattern of megasporogenesis may be under a relatively simple genetic control. In Arabidopsis thalianum (GAY & MONN.) from Saharanpur, India, single-celled archesporium was reported by MISRA (1962). It would be interesting to know whether this is really a new type of megasporogenesis or just information on the earliest stages has not been collected. If the type of sporocyte development is different from that observed first by VANDENDRIES, the genetic basis of the difference would be of interest from evolutionary point of view.

References:

MISRA,R.C.: Agra Univ.J.Res.,Sci., 11, 191-200 (1962)
VANDENDRIES,R.: La Cellule 25, 414-459 (1909)

Differential recombination in mega- and microsporogenesis in the presence of factor Gf

G.P.RÉDEI

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It gas been reported (RÉDEI 1964) that a gametophyte-factor (new designation Gf) in linkage group 2 caused abnormal segregation. Since the unusual female gametophyte factor fails to transmit through the megaspores (Probability of 1% transmission if 0.001) by using appropriate markers e.g. (Gf + / + py) x (py / py) [py = pyrimidine requiring] the frequency of the recombinant strand (+,+) in the egg nuclei can be easily ascertained by the aid of the inherent selectivity of the conditional lethal system (half tetrad). Through the male side all four products of the meiosis can be recovered. The male transmission of Gf is, however, only ca. 32 per cent. Simple test cross using Gf carrier as pollen parent does not reveal the genetic constitution of each strand because Gf does not have visible phenotype; it merely affects the transmission of the carrier strand. For a complete analysis an additional progeny test is needed beyond the test cross generation. A satisfactory estimate of the recombination frequency can be obtained, by a simple manipulation: e.g. in a (py as / py as) x (Gf + / + py) test cross from the number of pyrimidine requiring individuals, the recombination fraction obtained in the female was substracted and this way the frequency of the male transmitted Gf^+ py strands was derived. Only the py^+ class of the test cross was subjected to an additional progeny analysis. From the cross-overs only the number of the Gf^+ py^+ strands was used. If the recombination in female and male is identical the outlined procedure should yield information on linkage intensity in the male identical with that observed in the female. The experimental results obtained by this technique were the following:

Exp.No.	Recombination fraction in female		in male	
	No.	%	No.	%
1	24	20,3	1	6,7
2	16	13,6	9	26,5
3	26	17,3	4	25,0
4	16	12,6	8	27,0
5			8	20,5
6			35	26,7
7			17	23,9
8			4	13,8
9			10	23,3
10			25	26,8
Average recombination		16,0%		25,5%

The variation among experimental series is quite considerable. Recombination is unfortunately a highly variable phenomenon in all organisms. It is clear, however, that in the male low recombination frequencies overlapping the values observed in the female occured only in the two smallest populations. The difference is

significant at the 0,01 per cent level. Sex differential recombination in plants was rarely reported so far. In animals crossing over is very rare in the heterogametic sex; in monoecious plants if any difference exists, recombination occurs with lower frequency in megasporogenic meiosis (cf. RHOADES 1941). There is no information from Arabidopsis whether sex differential recombination would take place also in the absence of Gf.

References:
RÉDEI,G.P.: Arabid.Inf.Serv. 1, 11 (1964)
RHOADES,M.M.: J.Amer.Soc.Agron. 33, 603-615 (1941)

Progress in studies of induced mitotic recombination

Y.HIRONO

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Since the first demonstration of induced premeiotic recombination in higher plants (HIRONO and RÉDEI 1964) three additional cases of mitotic exchange of linked markers were detected (Table 1). If irradiated plants heterozygous for appropriate markers displayed visibly different stems, seed was harvested from all branches adjacent to the sector. The progeny of the yellow sector plant No.1 contained also green plants because of the chimeric nature of this stem. The progenies of the yellow sectors of the other three plants were free from non-yellow individuals. In all cases the yellow stem progenies of plants No.1, 1224-1 and 1100-6 displayed an excess of gi while the opposite situation was obvious in plant No.1111-63. The information obtained from the analysis of plant No.1224-1 is of special interest. Unlike the other three cases here in the progeny of the yellow sector the recombined strand appears normal and the parental chromosome carries a defect. Thus direct evidence is available now that the appearance of the yellow sector was not due to simultaneous deletion of the ch^+ and pa markers. So far, though several, but only circumstantial evidence supported this interpretation. Analysis of adjacent green sectors has shown that abnormalities occur concomitantly with the appearance of the yellow parts. The progenies from the green stem sectors of plants No.1, 1111-63 and 1224-1 contained an excess of pa plants. In all cases the frequency of gi plants was higher than expected in these green sector progenies. In the case of plant No.1 it was ascertained that a small chromosomal defect between ch and pa loci caused the distortion of the phenotypic ratios.

The probability for finding four coincidences of the appearance of a yellow sector accompanied by an adjacent anomalous green sector is $(10^{-2})^4$. (The independent occurence of anomalies affecting transmission of a specific strand after similar irradiation is about 10^{-2}.) The premeiotic recombination was clearly demonstrated by the information obtained from the yellow sector. It is

Table 1

Apparent reciprocal products of the premeiotic marker exchange exemplified by the progeny test of stem sectors

Plant No.	Original genotype	Type of sector	Phenotypes observed +++	gi^2++	+ch^1+	++pa	gi^2ch^1+	gi^2+pa	+ch^1pa	gi^2ch^1pa	Total observed
1	$\frac{gi^2\ ch^1\ +}{+\ +\ pa}$	yellow	40	11	120	16	126	1	0	0	314
		green	85	23	0	128	3	17	0	1	257
1111-63	$\frac{gi^2\ +\ pa}{+\ ch^1\ +}$	yellow	0	0	90	0	13	0	0	0	103
		green	48	21	10	5	1	34	0	0	119
1224-1	$\frac{gi^2\ +\ pa}{+\ ch^1\ +}$	yellow	0	0	21	0	36	0	0	0	57
		green	7	1	1	41	0	21	0	1	72
1100-6	$\frac{gi^2\ +\ +}{+\ ch^1\ +}$	yellow	0	0	19	0	15	0	0	0	34
		green	107	56	28	0	5	0	0	0	196

not known by what means does this premeiotic exchange come about but a mechanism identical with meiotic crossing-over appears unlikely.

Complementory products of the exchange have not been identified so far but their occurence is suggested by some facts.

Reference:
HIRONO,Y., and G.P.RÉDEI: Arabid.Inf.Serv. 1, 15-16 (1964)

A major mutation in Arabidopsis thaliana

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In the F_2 generation of plants x-rayed in the zygote stage a strange mutant arose. Besides other altered characteristics the mutant shows the loss of family characteristics of the Cruciferae. The number of sepals, petals, anthers and carpels is often increased, but occasionally the ovary consists of one carpel only:

Sepals		Petals		Anthers		Carpels	
number	%*	number	%*	number	%*	number	%**
		3	4,7			1	0,6
4	42,2	4	53,1	6	20,3	2	9,0
5	45,3	5	40,6	7	47,0	3	24,1
6	10,9	6	1,5	8	26,5	4	53,6
7	1,5			9	1,5	5	12,0
				10	1,5	6	0,6
				11	1,5		
				14	1,5		

Per cent of 64 (*) or 166 (**) flowers investigated

Normal and abnormal flowers are present in the inflorescence of one plant. All pods of the mutant even those originating from a normal ovary with two carpels show a structure different from that of a normal Arabidopsis pod.

Crossings of the mutant with the original strain Enkheim as well as reciprocal crossings are fertil. The mutant remains constant after self-pollination. The breeding of the crossings has not yet been finished. It seems that we are dealing with a mutant in one gene only.

Flower malformations in mutants as a means of partitioning the developmental process

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During morphogenesis of flowers two phases can be distinguished with regard to the temporal succession of differentiation: 1. the total h i s t o l o g i c a l p a r t i t i o n of the apical shoot meristem into definite areas producing flowers with a specific number of whorls, and 2. the partition of the growing points of the flowers into zones of different f u n c t i o n . These two independent processes normally closely coordinate the 5 whorls of a cruciferous flower with their specific function. In consequence of gene mutations this coherence of spatial and functional partitioning, however, can be disorderd, and the basic independence of both processes may be demonstrable (cf. for literature : GOTTSCHALK 1964). This, in fact, holds for three instructive X-ray induced mutants of Arabidopsis, race Antwerpen, segregating from heterozygous plants in the X_2-generation in monogenic ratios. These mutants prooved to be entirely steril, and thus could not be maintained. Therefore their following descriptions will give the protocoll designations only.

Mutant 24/17: Vegetatively the mutant develops an entirely normal rosette. The inflorescence appears only little later than normal, but the leaves at the inflorescence shaft remain narrow with growth deformations and brownish patches resulting from cellular disorganisation in the mesophyll. The shaft branches early and intensively, but elongates only to half the height of the normal. The main defect, however, manifests itself at the shoot apex (Fig. 1a). The primary ridges for the flower formation are growing all around the apical cone in a number of nearly the double of the usually formed flowers. But they cease the development with a short filiform tip without any indication of leaf. This makes the shoot tip look l i k e a d i s s e c t e d a p i c a l c o n e of a hydrophyte (e.g. Elodea). Histologically the cells of the growing point of the adult mutant are no longer meristematic, but fully grown with large vacuoles. Apparently in the mutant the speed of the cellular differentiation relative to the division frequency is accelerated to such an extent, that a precocious termination of growth does result in the mutant.

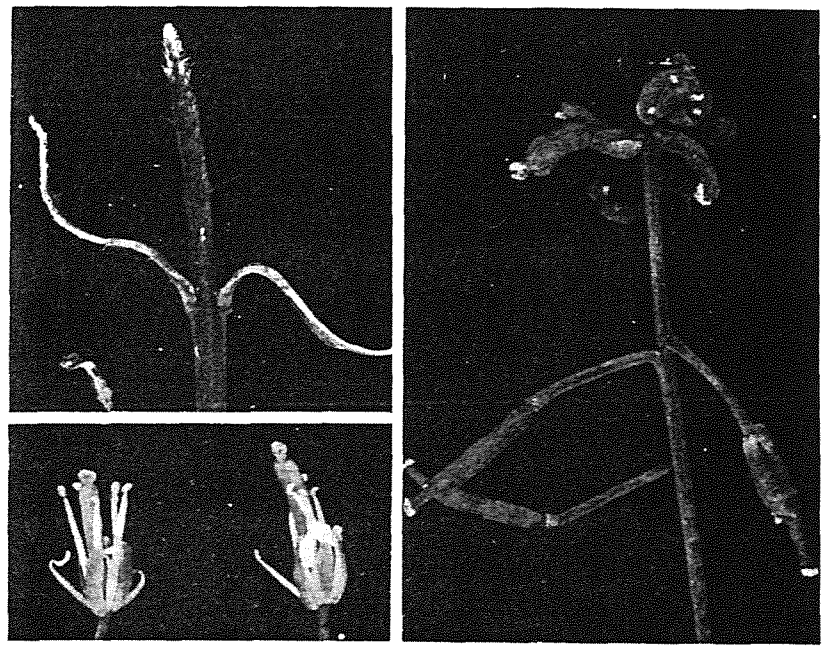

Figure 1

In the following two mutants a gene dependent functional change in flower development results in a shift of the sexual dioecy to the female and male side respectively.

Mutant 8/76: The growth of rosettes is rather delayed, the plants remain smaller, the inflorescence low, but extensively branched and bushy. The first flowers look normal, only dropping their sepals and petals at an early blossoming stage. The terminal flower, however, consists of totally e f f e m i n a t e d o r g a n s only (Fig. 1b and one flower dissected in Fig.2). In every whorl each leaf shows a papillose stigma and a carpel-like rolling. Sometimes the margin of even anthers or petals form small white ovules. But these as well as those in the medium, morphologically normal pistils are not apt for fertilization. Similarly the lower pods on the inflorescence do not yield any vital seed.

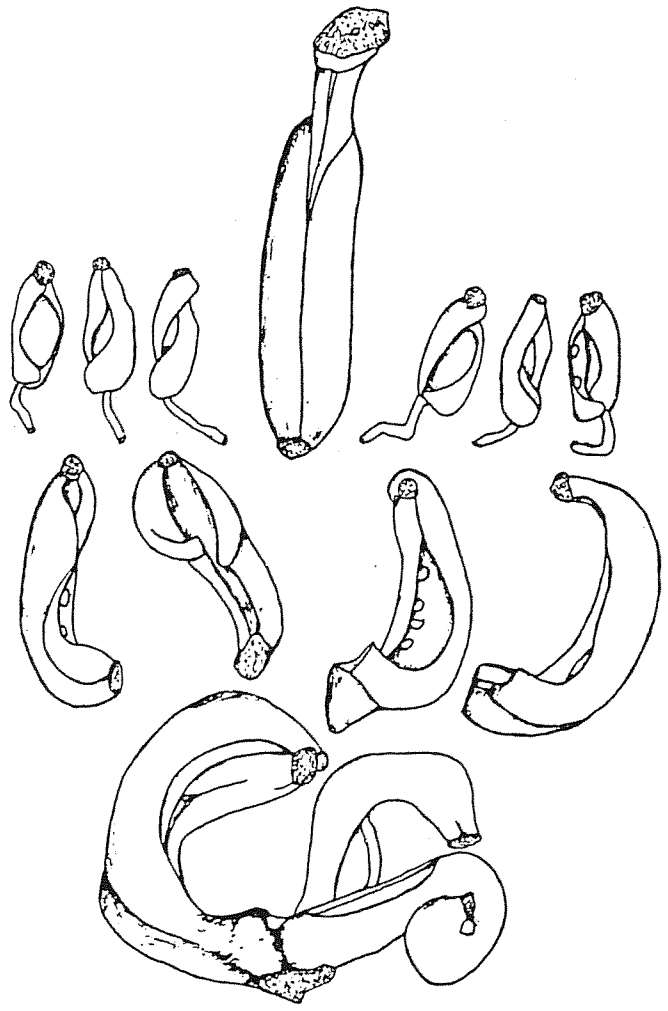

Figure 2

Mutant 16/31: Here the sex determination of flowers is s h i f t e d t o w a r d s m a l e n e s s . Rosettes and inflorescences are hardly inhibited, and sepals, stamens and carpels exhibit no peculiarities in structure. The sepals only are staminodially modified. Their lower part regularly consists of a stalk, the upper one may be petal like white or outward more or less identical with an anther. Histologically there is no archespore, but only mesophyll. The pollen of the normal stames are fertil. But even with additional pollination no fertile seeds were received, though the pods nearly reach normal size.

Reference:
GOTTSCHALK,W.: Botan.Studien (Jena), H.14, 144-167 (1964)

Action of X rays on the apical meristems of diploid and polyploid Arabidopsis

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Previous experiments have indicated a lower radiosensitivity of tetraploid plants of Arabidopsis thaliana, in comparison to diploid ones, but a higher sensitivity of hexaploid (and octoploid) forms.

In order to understand the origin of the seedling lethality, apical meristems of diploid, tetraploid and hexaploid forms have been observed, on axial sections, after X irradiation. One day after the treatment, a number of meristematic cells became deeply stained with hematoxylin. These destroyed cells were not distributed at random in the whole apex, but were very numerous in the second layer of the tunica and much sparser in the first one. Some degenerated cells were isolated, others were clumped. Their frequency and density were especially high in the middle of tunica 2 and at the top of the corpus.

Three different experiments have given the following rates of degenerated cells:

Age of seedlings	Number of seedlings	Tunica 1	Tunica 2	Corpus	Total
5 days: 2x	6	17,3 %	68,8 %	22,1 %	33,7 %
4x	12	13,7 %	78,1 %	25,3 %	38,0 %
6x	11	8,3 %	76,1 %	17,1 %	31,4 %
11 days: 2x	30	1,8 %	19,9 %	5,4 %	8,1 %
4x	18	2,0 %	36,5 %	13,8 %	15,4 %
6x	16	3,9 %	48,4 %	18,3 %	20,8 %
11-14 days: 2x	16	6,3 %	34,7 %	10,7 %	15,5 %
4x	12	4,0 %	48,6 %	13,3 %	19,0 %
6x	18	4,6 %	47,8 %	18,8 %	21,1 %

Five day seedlings (5 days after sowing) have received a dose of 15.000 r, older plants have received 20.000 r . In the third experiment (20.000 r), diploid and tetraploid plants were 11 day old, and hexaploid were 3 days older, in order to observe the seedlings nearly at the same stage before inflorescence initiation. In each experiment, the three forms were sowed (except the 6 x in the third case), cultivated, irradiated and fixed at the same time.

The percentages given above are the proportions of degenerated cells occuring in the two layers of the tunica and in the corpus. They show the higher sensibility of tunica 2 and the resistance of the epidermal layer. The percentage of degenerated cells appears to be higher in polyploids, at least for the 11 day old plants. On the other hand, the data given for the second and third experiments are clearly different for diploid and tetraploid seedlings.

Chimeric structure after EMS treatment of seeds

J.H. van der VEEN and M.GERLACH

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Seeds of the race Dijon treated with EMS (10 mM, unbuffered, 24 hrs, 24°C) gave final germination and survival-to-flowering ratios almost equal to the control. Of 360 M_1-plants, 128 were sterile (or almost so), and 101 yielded easy-to-score cotyledon mutants.The seeds of the first 12 fruits on the main stem were sown per fruit on agar, and topographies of the fruit positions were made from the dried (!) plants.

To calculate sector size, only plants were considered, of which

at least 10 fruits gave at least 12 seedlings each, thus avoiding non-representation of sectors and statistical escape of mutants within fruits. From 25 such plants, the average mutant sector size was calculated as 24,8% (it is probable that no neighbouring sectors with identical mutant phenotype occured in these 25 plants). This figure does, of course, n o t necessarily imply that the sporocyte tissue derives from 4 meristematic cells present at the start of treatment.

Mutant sectors like (33 : 1,32 : 7,25 : 5) were found, which indicates chimerism of borderline fruits.

The details are included in a preliminary report read in 1964 for the Genetical Society of the Netherlands (to be published in "Genen en Phaenen", 1965). In the meantime MÜLLER (1964) has reported detailed studies on MNU induced chimerism, and we completely agree with his statement that often reduced fertility goes in sectors like mutant segregation does. Since sterility and mutant segregation seem to be distributed more or less independently over sectors, it would perhaps follow for mutation-breeding, that only f u l l y fertile fruits (or sectors or plants) may be harvested, and that the mutagen concentration should not be so high as to make such fruits (etc.) rare. However, the first author continues to study reduced fertility, as more critical data are needed to see in how far this probably oversimplified picture holds.

Reference:
MÜLLER, A.J.: Züchter 34, 102-120 (1964)

Somatic sectoring after X-irradiation and ethyl methanesulfonate treatment

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Plants homozygous for ch (HIRONO 1964) when treated with ethyl methanesulfonate (EMS) produced many yellow sectors of a phenotype similar to that of tissues homo- or hemizygous for ch. None of these sectors were deficient in chlorophyll b and actually this was expected. X-irradiated wild types only very rarely displayed yellow sectors of considerable size.

Plants heterozygous for ch produced yellow sectors both when irradiated or treated with EMS. The sectors which appeared after the irradiation were all similar inasmuch as the chlorophyll b content was reduced. Only one exception was found among 30 sectors examined. About 1/3 of the sectors produced by EMS treatment had reduced chlorophyll b content and in the majority of the sectors the total pigment content appeared lower and this was the cause of the pale green or yellowish color.

The mechanism by which the yellow sectors arose could be studied if shoots developed from the sector. Sixteen stem sectors produced by X-irradiation on plants heterozygous for ch were progeny tested. In four cases yellow sector was brought about by a premeiotic marker exchange. In two cases deletion of ch^+ allele produced the yellow sectors. Because of the small size of the shoot sectors or because of the low fertility (possibly due to the physiological effect of the irradiation) ten plants failed to yield enough seed to clarify the mechanism underlying the sector formation. The information available from the majority of this last

group of sectors rules out the non-disjunctional origin. Deletion, mutation, or premeiotic exchange between homologous chromatids may be responsible for these cases.

Reference:
HIRONO,Y.: Arabid.Inf.Serv. 1, 25,26 (1964)

Effect of low levels of X-irradiation on seed dormancy

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Seed dormancy in Arabidopsis prevents immediate germination of harvested seeds. The mechanism of dormancy is not well understood. Inspite of all favourable conditions of temperature, light etc. dormant seeds show a very low percentage of germination or may fail to germinate entirely. This, to a limit extent, depends on ecotype and temperature conditions during seed development. Following a cold treatment of moist seeds at 2°C or below for 5-6 days, nearly 100 % germination is obtained. In one of the experiments, increased germination was recorded following low dosages of X-irradiation. This led to planning an experiment to study the effect of low levels of X-irradiation on seed dormancy. Freshly harvested seeds of race Landsberg, type "erecta", were used. The results are summarised below:

Dosage	Germination percentage		
	Dry seeds equiliberated over P_2O_5 for 48 hours	Seeds soaked for 8 hours	Soaked seeds cold treated at 2°C for 6 days
0	3,5	3,8	98,8
50 r	3,4	24,7	98,4
100 r	5,6	58,3	98,8
200 r	8,3	60,4	98,3

Stimulatory effect of X-irradiation was marked on germination of soaked seeds. However, no such effect was observed on subsequent growth of the seedlings, neither they showed any morphological aberrations. The doses used were very low in comparison to the LD-50 dose in the range of 30-40 kr for soaked seeds.

Repression of genetic activity has been recently hypothesised as the cause of dormancy in potato tuber buds (TUAN and BONNER 1964). Increased germination in Arabidopsis following kinetin treatment (MAHESHWARI and IYER 1964) suggests possibility of a similar mechanism preventing seed germination. Presuming that dormancy is due to some sort of genetic repression, it is hypothesised that low levels of irradiation may work by way of derepressing the genetic activity. This could explain some of the controversial results on the effect of low levels of radiations.

The repression of genetic activity could be due to lack of DNA synthesis or preventing the transfer of genetic information from the DNA to the sites of protein synthesis through messenger RNA. No information on the effect of low levels of radiation on molecular level seems to be available and this certainly appears to be worth looking for.

References:
TUAN,D.J.H., and J.BONNER: Plant Physiol. 39, 768-772 (1964)
MAHESHWARI,Nirmala, and R.D.IYER: Arabid.Inf.Serv. 1, 8 (1964)

Lethal effects of ultra violet rays in Arabidopsis

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Effect of ultraviolet rays of 2537 nm on seedlings was examined. Seeds were sown on wet filter paper in petri dishes. About 5 mm long seedlings were exposed to 1 - 100 min of 100 erg/mm^2/sec intensity and were kept in a dark room for 24 hours for the elimination of photoreactivation effect of UV effect fixation. Killing effectiveness of UV rays was very high; about half of the treated seedlings died at 10 min treatment and almost all died out at 100min treatment as shown in the table below.

UV-Treatment		Number of seedlings			
Min.	No. of seeds	Total	Surviving	With growth arrested	Dead
0	202	199	189	10	0
1	199	195	99	91	5
3	198	197	35	142	20
5	199	198	27	126	45
7	198	195	49	89	57
10	201	198	22	71	105
20	196	194	30	52	112
30	199	196	46	35	115
50	200	194	17	18	159
100	200	200	11	11	178

Moreover, retardation of growth was also marked, about half of the seedlings having still only cotyledons without any further development two weeks after the treatment in the 1 min lot. Such seedlings are expected to die out soon. The photoreactivation effect will be examined in the next experiment, but I do not know whether the severe killing effect coincides or not with the induction of gene mutations, because the stem of young seedlings is very soft and transparent and it seems that UV rays act on the stem rather than on the growing point.

RBE in Arabidopsis treated with 1,5 MeV fission and 14,1 MeV fast neutrons to gamma-rays

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RBE values of 1,5 MeV fission and 14,1 MeV fast neutrons to gamma-rays in Arabidopsis were studied by specific loci method. The flower of this plant is small and it is somewhat difficult to obtain many F_1 seeds. Therefore, F_2 seeds from the cross between a single recessive hairless and a wild strain (Landsberg) were used.

Table 1. Segregation ratio and mutation rate in irradiated F_2 populations

Treatment	No.of seeds	% of survivals	Haired	Hairless	Chi-square	P	Expected number of het.zygot.	No.of plants with hairless sectors (%)
Control	960	88,4	646	203	0,5971	0,20-0,50	430,6	0 (0,00)
γ-ray (^{60}Co) 25kr	920	93,0	639	217	0,0560	0,50-0,95	426	3 (0,70)
γ-ray (^{60}Co) 35kr	960	85,8	636	188	2,0970	0,10-0,20	424	8 (1,89)
Neutron (1,5MeV) 1,5krad	960	89,5	640	219	0,1121	0,50-0,95	426,6	3 (0,70)
Neutron (1,5MeV) 2,5krad	950	87,8	644	190	2,1887	0,10-0,20	429,3	5 (1,16)
Neutron (1,5MeV) 3,5krad	960	91,0	652	222	0,0748	0,50-0,95	434,6	13 (2,99)
Control	990	94,4	691	244	0,5992	0,20-0,50	460,6	0 (0,00)
γ-ray (^{137}Cs) 30kr	992	91,4	682	225	0,0180	0,50-0,95	454,6	4 (0,87)
γ-ray (^{137}Cs) 50kr	976	92,9	683	224	0,0444	0,50-0,95	455,2	11 (2,41)
Neutron (14,1MeV) 0,9krad	979	92,0	678	223	0,0298	0,50-0,95	452	4 (0,88)
Neutron (14,1MeV) 2,0krad	1984	93,2	1417	431	2,9229	0,05-0,10	944,6	10 (1,05)
Neutron (14,1MeV) 2,9krad	952	93,1	675	211	0,6636	0,20-0,50	450	6 (1,33)
Neutron (14,1MeV) 4,9krad	983	91,3	692	205	2,2032	0,10-0,20	461,2	13 (2,81)

Dry seeds were subjected to 1,5 2,5 and 3,5 krad of 1,5 MeV fission neutrons in Oak Ridge National Laboratory and also to 0,9, 2,0, 2,9 and 4,9 krad of monoenergetic 14,1 MeV fast neutrons in Hiroshima University. 20 to 50 kr of gamma-irradiations from ^{60}Co and ^{137}Cs were also carried out in each neutron experiment in Oak Ridge National Laboratory and National Institute of Genetics, respectively. About 1.000 seeds in each lot were sown in sterilized sand and experiments were carried out under continuous artificial light in an air-conditioned room at 25 $\pm 4^{o}C$. Around 90 per cent of seeds germinated in the controls and all irradiated lots, and almost all of seedlings survived. Irradiations up to 50 kr of gamma-rays and 5 krad of neutrons had no effect on germination or survival rate.

During the growth of seedlings, segregation ratio of hairless homozygotes was examined. Hairless homozygotes segregated according to the ratio of 3 : 1 in all lots, as expected, and segregation fitted, according to chi-square test, the simple Mendelian ratio. Therefore, haired plants must have consisted of homo- and heterozygotes, namely two thirds could be assumed to be heterozygotes. Should somatic mutation from dominant haired to recessive hairless occur in heterozygotic seeds, it had to appear as hairless sectors for which they were examined. Number of such plants with such sectors were counted. Mutation rates increased with increasing dosage in each kind of radiation and the relation was non-linear. Mutation rates were almost the same at 25 kr of ^{60}Co gamma-rays and 1,5 krad of 1,5 MeV fission neutron and 0,80 per cent were obtained in both lots. From the results, RBE value of 1,5 MeV fission neutron to gamma-rays was about 16. On the other hand, mutation rate in the lowest dosage lot of 14,1 MeV fast neutrons was somewhat high when it is compared with the other lots. But, RBE value of 14,1 MeV fast neutrons may be estimated as about 13 from the curve of mutation rates and dosages.

Futile attempts to induce recessive lethals by 5-bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IUdR)

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Dry seeds (ds) of Arabidopsis thaliana were soaked for 18 h at 24°C in 3,2 x 10^{-3} M BUdR. The frequency of recessive lethals was determined by the embryo test (cf. MÜLLER 1963, 1964). In each M_1 plant 150 embryos in five successive pods of the main inflorescence were tested. No increase of mutation frequency could be found (see Table 2).

In other experiments germinating seeds and seedlings (6) were treated. The seeds were placed in petri dishes on filter paper, soaked with aqueous solutions of BUdR or IUdR of various concentrations. The solutions were changed daily. After 2 days the seeds germinated, after 7 days the seedlings were planted into soil. In Table 1 some data on growth inhibition after these treatments are summarized. The frequency of recessive lethals did in no case significantly exceed the spontaneous mutation frequency (Table 2). Even for IUdR 10^{-6} M (m_c = 0,75 per cent) a P-value of 0,05 was calculated. All mutants detected in the treated series were embryonic lethals, whereas in the control 5 pod progenies with chlorophyll mutants (m_b = 0,13, m_c = 0,03 per cent) were found.

Table 1:

	Control	BUdR				IUdR	
		10^{-4}M	$1,6 \cdot 10^{-5}$M	$3,2 \cdot 10^{-6}$M	10^{-6}M	10^{-5}M	10^{-6}M
Av.length of prim.roots(mm)	7,5	0	1,5	2	5,5	1,6	5
% seedlg.with second.roots	0	0	96	22	3	98	4
% survivors after planting	63	0	18	41	58	16	52

Table 2:

Treatment	Number of tested M_1-plants	Number of tested pod progenies	m_b	m_c
Control	800	4000	0,53	0,11
BUdR $3,2 \cdot 10^{-3}$M (dS)	369	1845	0,54	0,17
BUdR $1,6 \cdot 10^{-5}$M (G)	100	500	0,60	0,05
BUdR $3,2 \cdot 10^{-6}$M (G)	152	760	0,79	0,05
BUdR 10^{-6}M (G)	826	4130	0,48	0,08
IUdR 10^{-5}M (G)	180	900	0,89	0,18
IUdR 10^{-6}M (G)	206	1030	3,10	0,75

m_b = frequency of segregating pod progenies (%)

m_c = frequency of mutants (%)

Since BUdR and IUdR are known to be incorporated into the DNA of higher plants this results may be interested in two ways:
1) the base analogue have not reached the shoot meristem, or
2) the incorporation does not induce a significant increase of the frequency of recessive lethals (the test does, of course, not exclude that other mutation types are induced in higher plants). Further experiments allowing a decision between these two alternatives would be of great interest.

References:
MÜLLER,A.J.: Biol.Zbl. 82, 133-163 (1963)
MÜLLER,A.J.: Züchter 34, 102-120 (1964)

Acridine effects on Arabidopsis plants

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Among substances related to nucleic acid metabolism, acridine has been tested for physiological effects in M_1 and for mutagenic activity in M_2. Acridines bind to DNA by intercalation between

adjacent base pairs, and induced phage mutants have been isolated.

We have cultivated plants in dishes on perlite supplemented with 40 ml of a mineral medium containing 1,25 - 12,5 γ acridine ($C_6H_4CH:C_6H_4:N$) per ml under continous light and a constant temperature of 25°C. The results are the following:

Acridine treatment (γ/ml) 100 seeds each	Seeds germinated after 10days (%)	Plants surviving after 23days (%)	Plants harvested (%)
Control	92	92	92
1,25	77	88	64
2,50	66	84	58
5,00	66	68	44
7,50	50	58	10
12,50	40	52	6

In particular the experiment demonstrated 1) an inhibition of germination in relation to the concentrations used, 2) a lot of physiological effects : dwarf plants, rhomboidal rosette leaves, petioles enlarged, midrib prominent, 3) a lethality very pronounced, measured at harvesting, and 4) a low seed-set in M_1 though seeds were well developed. Among 384 M2 seedlings no chlorophyll or morphological mutants have been detected. - New experiences are now underway using acriflavine, acridine orange and proflavine.

Effect of pH, copper and zinc ions on mutagenic action of EMS *

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Availability of Cu and Zn ions during EMS treatment was reported to enhance the frequency of chromosomal aberrations (MOUTSCHEN-DAHMEN 1963). Information on genetic effects of EMS in combination with metallic ions is not available in literature. We therefore planned an experiment to study the interaction of Cu and Zn ions with EMS, at three levels of pH, on chlorophyll mutation rate in Arabidopsis. The results are presented below. Details are being published elsewhere. The following additions to the EMS-solution were used:

Zn 10^{-3}mM as $ZnSO_4$ EMS 0,12%

Cu 10^{-3}mM as $CuSO_4$ for 24 hrs at 24° C

EMS-treatment	M_1-families scored	Mutation rate: Segregating M_1-families (%)	Mutation rate: Mutants per 100 M_2-plants
Deionized water	134		
Buffer pH 5	128		
Buffer pH 7	132		
Buffer pH 9	140	0,76	0,01
Buffer pH 5 + Zn	132		
Buffer pH 7 + Zn	143		
Buffer pH 9 + Zn	142		

(next page continued)

EMS-treatment	M_1-families scored	Mutation rate	
		Segregating M_1-families (%)	Mutants per 100 M_2-plants
Buffer pH 5 + Cu	147		
Buffer pH 7 + Cu	147		
Buffer pH 9 + Cu	132		
Buffer pH 5 + EMS	124	57,26	7,14
Buffer pH 7 + EMS	133	46,62	5,56
Buffer pH 9 + EMS	148	37,84	4,55
Buffer pH 5 + Zn + EMS	139	63,31	8,70
Buffer pH 7 + Zn + EMS	129	55,04	8,36
Buffer Ph 9 + Zn + EMS	162	43,21	7,32
Buffer pH 5 + Cu + EMS	139	55,40	7,58
Buffer pH 7 + CU + EMS	136	52,94	7,68
Buffer pH 9 + Cu + EMS	155	52,00	8,63

Reference:
MOUTSCHEN-DAHMEN,J., and M.:Experientia 19, 144-145 (1963)

* Part of the research carried out at the Department of Genetics, Agricultural University, Wageningen, The Netherlands.

A survey on agents tested with regard to their ability to induce recessive lethals in Arabidopsis

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The following survey deals with results of experiments carried out at the Institut für Kulturpflanzenforschung, Gatersleben. Recessive lethals (embryonic lethal mutations and lethal chlorophyll mutations) identifiable by the embryo test (cf. MÜLLER 1963,1964a) were used as indicator of mutagenic effectivity of the agents. For all tested agents only the highest concentration allowing an evaluation of M_2-progenies is given. Besides this concentration (aqueous solution) duration of treatment and treatment temperature are specified. Treated were either dry seeds (dS), presoaked seeds (pS) or germinating seeds and seedlings (G). Symbol r means reduction of root length in per cent (cf. MÜLLER 1964a,b). Lm means average number of recessive lethals mutations per treated germ line cell. ("-" symbolizes that the mutation frequency after treatment was not significantly higher than the spontaneous mutation frequency.)

Agent and treatment conditions	r	Lm
1. Mustards, sulphonic esters and ethylenimines		
Di-2-chloroethylmethylamine HCl (HN2) - dS, 2 mM, 18 h, 24°	51	0,29
Tri-2-chloroethylamine HCl (HN3) - dS, 10 mM, 18 h, 24°	47	0,27
2-Chloroethyldiethylamine HCl - dS,500 mM,18 h,24°	41	-

Di-2-chloroethylmethylamine oxide (Nitromin, Mitomen) - dS, 25 mM, 18 h, 36°	32	0,06
Di-2-chloroethylamine-phosphamide ester (Endoxan, Cytoxan) - dS,200 mM, 18 h, 36°	44	-
Ethyl methanesulphonate (EMS) - dS,25 mM,18 h,24°	62	3,42
1,4-(Dimethanesulphonoxy)-butane (Myleran) - dS,1 mM, 18 h, 36°	57	0,03
Triethylenemelamine (TEM) - pS,400 mM, 3 h, 24°	12	-
2. N-Nitroso compounds		
N-Nitroso-N-methylurea - dS,0,12 mM, 18 h, 24°	42	4,82
N-Nitroso-N-ethylurea - dS,1 mM, 18 h, 24°	65	2,65
N-Nitroso-N-methylurethane - pS,0,16 mM, 3 h, 36°	53	3,48
N,N'-Dinitroso-N,N'-dimethyloxamide - pS,sat.sol.,6 h, 36°	6	0,32
N,N'-Dinitroso-N,N'-dimethylterephtalic acid diamide - dS,sat.sol.,60 h, 36°	8	-
N-Nitroso-N-methyl-N'-nitroguanidine - dS,0,5 mM, 18 h, 24°	39	3,06
3. N- and S-Hydroxymethyl and N-C-N compounds		
N-Hydroxymethyl-benzimidazole - pS,sat.sol.,8 h, 36°	5	-
N,N'-Bis-hydroxymethyl-dioxypiperazine - pS,sat.sol.,24 h, 36°	0	-
2-(Hydroxymethyl-mercapto)-imidazole - pS,sat.sol.,8 h, 36°	6	-
N-(Piperidino-methyl)-benzimidazole - pS,sat.sol.,24 h, 36°	5	-
N-(Morpholino-methyl)-succinimide - pS,400 mM, 24 h, 36°	0	-
4. Base analogues		
5-Bromodeoxyuridine (BUdR) - dS,3,2 mM,18 h, 24°	20	-
G,0,016 mM, 7 days, 24°	+	-
5-Iododeoxyuridine (IUdR) - G,0,01 mM, 7 days,24°	+	-
Nebularine - dS,40 mM, 24 h, 24°	0	-
G,0,06 mM, 6 days, 24°	+	-
Hypoxanthine - G,0,1 mM, 6 days, 24°	+	-
5. Other compounds		
Maleic acid hydrazide - dS, 25 mM, 18 h, 24°	75	-
Ethyl alcohol - pS, 1000 mM, 18 h, 36° ($+O_2$)	37	-
G,100 mM, 6 days, 24°	+	-
Acetone - dS, 500 mM, 18 h, 24°	30	-
2,4-Dinitrophenol - pS, 0,1 mM, 3 h, 36°	0	-
Potassium cyanide - pS, 10 mM, 3 h, 36°	0	-
Sodium azide (NaN_3) - pS, 10 mM, 3 h, 36°	5	-

Hydroxylamine HCl - dS, 5 mM, 18 h, 24°	12	-
Chloramphenicol - pS, 5 mM, 3 h, 36°	0	-
Sulfathiazol - pS, 0,2 mM, 3 h, 36°	0	-
Ethylenediamine tetraacetic acid, Na-salt - pS, 20 mM, 6 h, 36°	0	-
Sodium nitrite - dS, 10 mM, 18 h, 24°	28	-
Amethopterin - G, 0,001 mM, 6 days, 24°	+	-
6. X-rays (for comparison) - pS, 25 kr	92	0,82

References:
MÜLLER,A.J.: Biol.Zbl. 82, 133-163 (1963)
MÜLLER,A.J.: Züchter 34, 102-120 (1964a)
MÜLLER,A.J.: Kulturpflanze 12, 237-255 (1964b)

Production of thiamineless mutants

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Since our last report on this subject (FEENSTRA 1964) 45 more mutants in which the thiamine synthesis is blocked were found, 19 requiring at least the pyrimidine moiety of the vitamin molecule for growth, 12 the thiazole part and 14 the complete vitamin. Up to now in total 69 independently arisen mutants have been obtained. Based on the results of crossings the presence of 10 groups of mutually allelic mutant genes could be shown, 59 of the mutants could be assigned so far to a group. Four groups of thiamine requiring mutants were found, with 2, 3, 8 and 1 mutants falling in them, none of them being allelic with the early thiamineless mutant of LANGRIDGE (1958). Growth requirements of the first group are also fulfilled by a mixture of pyrimidine and thiazole.

The distribution of pyrimidineless mutants in three groups, as mentioned by FEENSTRA (1964) was confirmed, 13 of the mutants belong to group I, and show allelism with the mutant py of RÉDEI (1960), 4 mutants belong to group II; the latter, when crossed by a representative of group I, give a (usually semi-) wild type F_1. Group III contains 13 mutants which give mutant type F_1's both when crossed to group I and group II. For the mutant genes in groups I, II and III the symbols py-1, py-2 and py-d are suggested (cf. this volume, page 25).

Comparable distribution was found for the thiazoleless mutants: one group of 4 mutants which are allelic with RÉDEI's mutant tz - for which now the denotation tz-1 is suggested-, a second series of 8 (tz-2) and a third of 3 mutants (tz-d), with complementation pattern analogues to that found among the three pyrimidine groups.

References:
FEENSTRA,W.J.: Arabid.Inf.Serv. 1, 23 (1964)
LANGRIDGE,L.: Austral.J.Biol.Sci. 11, 58-63 (1958)
RÉDEI,G.P.: Genetics 45, 1007 (1960)

The site of the block in LANGRIDGE's thiamine mutant 1018/6

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It was reported (LANGRIDGE 1958) that mutant 1018/6 is completely restored by thiamine, and the pyrimidine and the thiazole moieties of the vitamin are ineffective. Recent biochemical studies indicate that the synthesis of thiamine from the specific pyrimidine and the specific thiazole involves several steps (cf. BROWN and REYNOLDS 1963). Thiazole is phosphorylated before it is coupled to pyrimidine. Though LANGRIDGE's mutant responds best to thiamine, 4-methyl-5-ß-hydroxyethyl thiazole alone is also beneficial. It appears therefore that the mutation affects an intermediate step between thiazole synthesis and the coupling of thiazole-monophosphate to pyrimidine-pyrophosphate. The expression of 1018/6 is quite variable; the results of two experiments were the following:

Supplement	Exp. No.1		Exp. No.2	
	No.of plants	Fresh weight (mg) M	No.of plants	Fresh weight (mg) M
None	8	5,1	20	15,6
Pyrimidine	9	5,4	17	14,0
Thiazole	14	15,9	18	19,2
Pyrimidine + Thiazole	14	11,5	16	16,7
Thiamine	9	25,5	17	24,3

References:
LANGRIDGE,L.: Austr.J.Biol.Sci. 11, 58-63 (1958)
BROWN,G.M., and J.J.REYNOLDS: Ann.Rev.Biochem. 32, 419-462 (1963)

Genetic studies on genes for 4-amino-5-hydroxy methyl-2-methyl-pyrimidine and for 4-methyl-5-(ß-hydroxyethyl) thiazole synthesis

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As mentioned in this volume, page 24, genes for pyrimidine synthesis fall into 3 groups based on complementation relations. A possible explanation is that py-1 and py-2 belong to adjacent cistrons, and that mutants of the class py-d possess a deletion covering parts of both the cistrons to which py-1 and py-2 belong. To test whether recombination can occur between py-1 and py-2, crosses were made between mutants out of these series, and the hybrid was crossed both with the wild type (A), and with the mutant py-d (B).

With (A), the first backcross generation is wild type, and only after selfing of these plants the recombination frequency can be estimated in the next generation from the number of non-segregating families. 270 families were tested, none of these were non-segregating, which means that F_1 did not yield ++ gametes.

With (B), plants originating without recombination are all deficient for pyrimidine synthesis, only after recombination wild type plants can occur. No wild types were found among 2673 test-cross plants. If recombination can occur, the frequency is expected

to be smaller than approx. 7×10^{-4}.

At the moment crossing according to scheme B is being repeated at a larger scale, to obtain further information.

Recombination between genes tz-1 and tz-2 for thiazole synthesis so far has been tested only according to scheme A; 139 progenies tested failed to show recombination.

Regulation of plastid differentiation in mutant im by visible light

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Some characteristics of mutant im have been reported (RÉDEI 1964) and some possible mechanisms of the variegation have been suggested. Recent studies indicate that visible light is one of the most important agents affecting the phenotype of im. It appears that the photosensitive substance has an absorption maximum in the red region of the spectrum. In continuous illumination under a blue filter the leaf pigment content raises several fold above to that of the control (continuous spectrum) while under a red filter pigment production is suppressed considerably.

Pigment γ/gr	Chlorophyll		Carotenoids	Total
Filter	a	b		
neutral	77	25	23	125
red	50	18	14	82
blue	241	85	60	386

Cysteine provides some protection against red light and increases the beneficial effect of blue light. At low temperatures, short daily illumination (8 hs) promotes plastid differentiation considerably. At higher temperatures this beneficial effect is manifested only if the 8 hs illumination is not continuous but it is interrupted in several cycles. The 8 x 1 hr daily illumination is also very effecient in promoting flowering and in reducing hypocotyl elongation in contrast to the 1 x 8 hr light treatment. Since the photoperiodic response or stem elongation is not affected by the im mutation it appears that a receptor system different from the phytochrome is involved. It is not yet known what steps of gene function are affected by the various inducers and inhibitors. Experiments are underway to test the most effective spectral regions, and to investigate the site of the photosensitive system by using selective inhibitors and antimetabolites.

Reference:
RÉDEI,G.P.: Arabid.Inf.Serv. 1, 11 (1964)

Normalisation of mutant-phenotypes on glucose medium

Jiřina ŠVACHULOVÁ, J.VELEMÍNSKÝ and V.POKORNÝ

(Institute of Experimental Botany, Prague, Czechoslowakia)

The nutritional requirements of several lethal chlorophyll mutants were studied, using sterile cultures under continuous illumination. Among 39 different mutants tested, seven of the chlorina type reverted to the normal phenotype on a minimal agar-medium

containing 2% glucose. There were some slight differences in the speed of normalisation among these mutants, but they all reached normal or nearly normal phenotype at the 4.-5. leaf-pair stage . On all other media, either minimal or maximal according to JACOBS (1964) but without glucose, all these mutants died without any change of phenotype. The rest of the examined mutants (12 chlorina, 15 xantha and 4 albina) was able to grow and develop rosettes on the minimal medium + 2% glucose without any increase of the pigment content. No requirement for other subtances like amino acids, vitamins, purines and pyrimidines were detected among all the mutants tested. Principally the same effect as that of glucose was observed by the authors and by SCHROBSDORFF and RÖBBELEN (unpubl.) with sacharose.

It seems, that for a part of lethal chlorina, which on the minimal medium or in the soil are able to synthetize only small quantities of chloroplasts pigments and later get bleached and die, only a source of energy supply suffices for their normalisation. Nevertheless it remains to be answered, whether the blocks in these mutants concern functions associated with photosynthesis only, as presumed by WALLES (1963), or whether the supplied energy is consumed directly for the chlorophyll-protein complex etc.

References:
JACOBS,M.: Arabid.Inf.Serv. 1, 36-37 (1964)
WALLES,B.: Hereditas 50, 317-344 (1963)

Influence of glucose feeding on the free amino acid content of chlorophyll mutants

Jiřina ŠVACHULOVÁ and J.VELEMÍNSKÝ

(Institute of Experimental Botany, Prague, Czechoslowakia)

In previous papers (VELEMÍNSKÝ et al.1963, 1965) it was reported, that an abnormal accumulation of amids and basic amino acids like lysine, arginine and histidine was found in lethal chlorophyll mutants growing on soil in the greenhouse. It was postulated that this accumulation is a consequence of proteolysis after the energy store of seeds gets exhausted. Using the chlorina mutant (No. 42), which does not normalize on an agar medium containing 2% glucose, the correctness of this hypothesis was confirmed : No greater accumulation of amids and basic amino acids, mentioned in comparison to green control plants, was observed under these feeding conditions.

References:
VELEMÍNSKÝ,J., T.GICHNER, V.POKORNÝ and Jiřina ŠVACHULOVÁ: Arabid. Inf.Serv. 1, 21-22 (1964)
VELEMÍNSKÝ,J., Jeřina ŠVACHULOVÁ and T.GICHNER: Proceedings Symp. "Induction of Mutations and the Mutation Process", Praha, 27.-29.sept.1963, pp.57-61 (1965)

Free amino acid composition of Arabidopsis seeds

M.JACOBS

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Seeds of race Wilna have been analysed for their amino acid composition by using a Beckman-Spinco apparatus. 100 mgs seeds

have been utilised.

Amino acid	mg/gm fresh weight	% of proteins	γ/one seed
Lysine	11,22	5,56	0,198
Histidine	5,03	2,49	0,089
Arginine	15,84	7,84	0,280
Aspartic acid	18,14	8,98	0,321
Threonine	8,95	4,43	0,158
Serine	9,14	4,53	0,161
Glutamic acid	42,83	21,21	0,758
Proline	11,28	5,59	0,199
Glycine	12,20	6,04	0,215
Alanine	8,60	4,26	0,152
Cysteine	2,07	1,02	0,036
Valine	11,00	5,45	0,194
Methionine	3,98	1,97	0,070
Isoleucine	9,06	4,49	0,159
Leucine	14,93	7,39	0,264
Tyrosine	7,32	3,62	0,129
Phenylalanine	10,31	5,11	0,182
Total:	200,19		3,565

The results show that the concentrations used in culture media are clearly higher than seed content. Extensive analysis of morphological mutants for qualitative and quantitative content of amino acids are now underway.

B. TECHNIQUES

Storage conditions, dormancy and germination of seeds

G. RÖBBELEN and Herta KERSTEIN

(Institute of Agronomy and Plant Breeding, University of Göttingen, Germany)

Formerly in the Institute in Göttingen seed-stocks were kept in a cellar-room at 16-18°C in a locker (80x50x60 cm) on the bottom of which each week newly dried silicagel had been spread in a flat layer. After some time, however, it showed that the seeds so stored had a germination capacity inferior to those left in the greenhouse for the same time-interval. The normal germination activity was regained only after a 14 days' storage in the greenhouse. This finding brought about the following investigations.

As is well known (KUGLER 1951, LAIBACH 1956) freshly harvested seeds of many of the Arabidopsis races exhibit a distinct dormancy. Seeds of the race Enkheim (En-2) derived from ripe shedding pods were placed on wet filterpaper (or soil) for various days after harvest. The half of the total number of petridishes was transferred twice in a refrigerator for breaking of dormancy by an intermittend cold-treatment (20°C - 4°C - 20°C - 4°C - 20°C each for 12 h). Then cultivated at 20°C for alltogether 10 days under continuous illumination the following germination percentages were received in an average of 6 replications of 100 seeds each:

Sowing - days after harvest	Cold - treated		Without cold-treatment	
	Filterpaper	Soil	Filterpaper	Soil
1	22	9	10	0
14	45	12	19	11
21	89	45	35	23
28	91	48	38	27
42	95	82	79	76
56	100	95	95	96

The race En-2 investigated thus shows a dormancy up to about 50 days (cf. deviating results of REINHOLZ 1947). The cold-treatment used shortens this period of time to nearly the half. In the parallel sowing on soil in the greenhouse with cultivation conditions as similar as those for plants sown on filterpaper, the initial sprouting inhibition proved to be much more pronounced.

In a second experiment 14 days old seeds of En-2 were stored for various periods of time at 20°C in evacuated desiccators over the following saturated solutions establishing the different humidities of 5% with P_2O_5, 10% with $ZnCl_2$, 58% with NaBr, 86% with KCl, and 88% with K_2CrO_4. Seeds were removed from these desiccators after different storage times and sown on filterpaper; after 10 days at 20°C the following germination percentages were counted from 3 x 100 seeds:

Rel.humidity during storage	Storage in the desiccator for 69 days	198 days
5%	12	36*
10%	54	81
58%	74	96
86%	5	0
88%	7	0
Control stored in the greenhouse	98	100

* In a parallel test with the same sample of seeds the germination was increased by cold-treatment to 73%.

Desiccated seeds stored for 69 days under 5 and 10% rel. humidity resp. were transferred into the desiccator with 58% and after different intervals sown on filterpaper. The germination percentages after 10 days then were the following:

First storage at 5% rel. humidity:

Days in 58%	0	5	10	15	20	25	30
Germination %	12	14	17	32	37	46	64

First storage at 10% rel. humidity:

Days in 58%	0	5	10	15	20	25	30
Germination %	54	47	37	74	75	81	94

From the foregoing experiments it might be concluded that the after-ripening processes in Arabidopsis-seeds are retarded under very dry conditions. Similarly storage in high humidity prevents seeds from gaining a sufficient germination capacity at least in the time-span tested. This is in accord with the common observation that fresh seeds immediately sown will not sprout even when seeds of the same source stored under medial moisture conditions have long reached their full germination capacity. A rel. humidity in the atmosphere of about 40-50% which can be adjusted easily with a small air-conditioning machine, therefore seems to be suitable for the after-ripening processes as well as for the maintenance of a good readiness for immediate germination of Arabidopsis seeds. This is also indicated with the following data of a germination test with seeds (race En-2) of different age (number of seedlings 10 days after sowing in %; for comparison cf. NAPP-ZINN 1964, table 1):

Age of seeds (years)	5	4	3	2	1
Year of harvest	1960	1961	1962	1963	1964
Germination %	19	86	69	92	87

References:
KUGLER,Ida: Beitr.Biol.Pflanzen 28, 211-243 (1951)
LAIBACH,F.: Naturwiss. 43, 164 (1956)
NAPP-ZINN,K.: Ber.dtsch.bot.Ges. 77, 235-242 (1964)
REINHOLZ,Erna: Fiat Report 1006, 1-70 (1947)

Uniform cultures in soil

J.H.van der VEEN

(Department of Genetics, Agricultural University, Wageningen, The Netherlands)

For many purposes it is desirable to reduce non-genetical variation to a minimum. Two phases are important: a) synchronizing g e r m i n a t i o n , for which a trace of KNO_3 is known to be a powerful means, and b) unhampered s e e d l i n g g r o w t h after transplanting, which I found to be promoted greatly by t r a n s p l a n t i n g e t i o l a t e d s e e d l i n g s w i t h a g a r b l o c k s.

Though the influence of many factors is widely known, I list my procedure step by step. It has been arrived at either by trial and error, or by systematic variation of factors. This was done partly in collaboration with my collegue Dr. W.J. FEENSTRA.

It should be noted that the factor-dosage given below is based on "safety first" and "for all cases" considerations: e.g. a shorter cold-treatment may be sufficient for breaking dormancy (depending on variety, age of seeds, etc.). One should,however, be also aware that 100% germination does not necessarily imply simultaneous germination.

a) Synchronizing germination

1. After-ripening up to 3 weeks in the dark at 32°C. depends on variety, maternal and external ripening conditions, stage of seeds at harvest, etc. Seeds left one day more on mother-plant, I occasionally found to require less storage time. Of course, one is anxious not to loose seeds from crossing by splitting open of fruits. A non-splitting mutant would be welcome, also for large quantity harvest per plant. Afterripening is still the weakpoint in the procedure and suggestions would be helpful.

2. Seeds are laid out on a 4 mm layer of agar (see below): 0,75% Oxoid No. 3 in tapwater with 100 mg/1 KNO_3 (the roots do not penetrate). To break seed dormancy, the petridishes are put in refrigerator (dark 2-4°C) for 5 days. - N.B.: If seeds have to be dried for mutagen treatment, prior coldtreatment is on water-saturated filterpaper in petridishes, and afterwards seeds are put on agar as above and transferred to light (a3)

3. Transfer to light, e.g. 300W/m^2 at 50 cm. Temperature within (!) dishes 24°C.

Any neglect of the factors afterripening-cold treatment-light + KNO_3, may increase range of germination (first seeds to last seeds) up to several days.

After 24 hrs under light, germination is not yet visible. At this moment the seeds are well synchronized to enter vernalization treatment (cf. NAPP-ZINN's statement that germination is a preriquisite for successful vernalization). I would add: good synchronizing is a preriquisite for unambiguous vernalization results. For unvernalized plants one proceeds after 24 hrs under light as follows:

b) Seedling growth

1. Transfer to dark (24°C). Here visible germination follows. After 2 or 3 days hypocotyles are uniformly, e.g., 1-1 1/2 cm long.

2. Then transfer petridishes to light (as a3) for 1 day (vigorous seedlings) or better 2 days. Cotyledons turn green.

3. Soil culture in, e.g., greenhouse. Put seedlings with blocks of agar (use spatula) into small holes. Agar is incised (e.g. with glass tube) before etiolation. Press some soil over agar. During the first few days the seedlings are very sensitive to drying out (a main cause of disuniformity). Agar acts as a water reservoir and decays after some time. Soil splashing often is detrimental to uniformity, and etiolation now enables the cotyledons to be well free from the soil. Nevertheless use a fine spray in the beginning. Sheltering against bright sunlight is with a wood lattice.

4. When seedlings are well established, hard soil surface or algal growth can spoil much, and can be avoided by a well-balanced sterilized leaf-soil mixture.

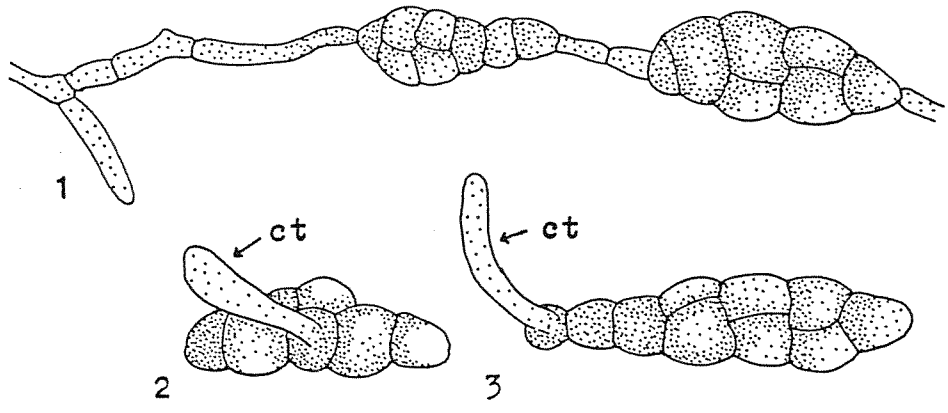
5. Uniformity of field of additional light is important, specially in winterseason. Philips TL 55 daylight type is very satisfactory for germination (see A3), formative processes, seed setting and ripening, but for long-day simulation incandescent bulbs are required.

Epidemic appearance of Alternaria brassicae on Arabidopsis growing in green-houses

Brigitte BERGER

(Institute of Agronomy and Plant Breeding, University of Göttingen, Germany)

Green-house cultures of Arabidopsis thaliana and some related species (A.suecica, A.arenosa) can be destroyed completely by Alternaria brassicae. Older leaves lying near the soil are usually the first to be affected. The symptomes are yellowish-brown spots which later fuse. The leaves then become brown and dry. The infection spreads to younger leaves and follows up to stem. Such heavily affected plants are unable to survive. It often happens, that the stamens dry up even in the closed buds and so do the developing seeds. The characteristic conidia and the septate and branched mycelium could be observed under the microscope on both sides of the infected leaves.



Alternaria brassicae. 1. Conidial chain, 2-3 Germinating conidia, ct conidial tube

A. brassicae is of common occurence and a very injurious fungus on Cruciferae. It belongs to the order Moniliales (family Dematiaceae) of Fungi imperfecti. The parasite is transmitted as mycelium or as conidia hibernating in soil, infected plant debris and also on seeds. Humid and warm conditions favour the transmission of the fungus. Chemical methods of controlling the infection are rarely practiced. Some success seems to be attained with spraying the accustomed Cu - preparates or zinc carbamates ("Fuclasin Ultra"-SCHERING). In any case it is necessary to work with sterilized (steamed) soil.

Remarks on sterile culturing

W.J.FEENSTRA

(Department of Genetics, Agricultural University, Wageningen, The Netherlands)

S u b s t r a t e : Fine grain perlite for sterile culture in petridishes. Dishes + dry perlite can be sterilised in an oven; sterile nutrient solution added afterwards in a sterile cabinet. Solution should be poured in slowly. The composition of the medium can be changed at a later moment by adding a (concentrated) solution of additional compounds, e.g., a minimal medium may be altered into a complete one.

S e e d s t e r i l i s a t i o n : Hydrogen peroxyde/ethanol (LANGRIDGE 1957) gave better results than 3 1/2% calcium hypochlorite solution.

S e e d p l a n t i n g : If planting of individual seeds is required, "vacuum tweezers" are useful, especially on perlite surface. Apparatus can be made by drawing out piece of capillary glass tubing. Entrance diameter 1/2 - 3/4 of seed diameter. Other side of tube is inserted in piece of rubber vacuum tubing, which has a hole in the wall, in which a small piece of glass tubing is inserted (side tube). The other end of the rubber tubing is connected (preferably by a rather flexible piece of tubing) to a vacuum pump (water jet pump). By covering and uncovering of the side tube by a finger, vacuum can be made and released resp. Dry or wet seeds can be picked up very easily even from a wet surface (e.g., glass-sinter filter used for seed sterilisation).

C o n d i t i o n s d u r i n g g r o w t h : When petridishes are illuminated, care should be taken to keep the temperature i n s i d e at the desired level. Cooling may be necessary, this can be achieved by placing the dishes on a zinc tray floating on water, the temperature of which is controlled. Cooling from below has the advantage that no condensation takes place on the lids.

In order to allow normal flower stem formation, the lids are removed when the flower buds become visible, and the bottom-dishes are placed in glass jars (preserving-jars are cheap), which are covered by the lid of a larger sized petridish. Although rel. humudity is 100%, reasonable seed-set takes place.

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

An emasculation technique

W.J.FEENSTRA

(Department of Genetics, Agricultural University, Wageningen, The Netherlands)

Emasculation is carried out under a stereo-microscope (10x) by means of a small pipette connected to a water jet pump. The pipette is drawn from a piece of thin-walled glass tubing, the entrance should be wide enough to let the largest anthers pass, but otherwise the tube should be as small as possible, to inflict as little damage upon the flowers as possible. A hypodermic needle, from which the pointed end has been cut off, will also act. The air flow is controlled by a pedally operated valve.

Callus culture of Arabidopsis

J.R. LOEWENBERG

(Department of Botany, University of Wisconsin, Milwaukee,Wisc., U.S.A.)

While genetic studies on Arabidopsis are progressing rapidly, physiological and metabolic studies appear to be lagging. One of the reasons for this situation is the size of the organism. In order to obtain an appreciable mass of this material, an investigation to grow Arabidopsis as callus culture has been started. For the past six months Arabidopsis thaliana callus, derived from seedlings, has been subcultured at monthly intervals and continues to grow vigorously. 0,8% agar-medium on which the cultures are growing contains in addition to mineral salts per liter:

sucrose	30 g	6-furfurylaminopurine (kinetin)	0,4 mg
yeast extract	1 g	para-chlorophenoxyacetic acid	1,0 mg
tryptone	1 g	calcium pantothenate	0,5 mg
inositol	100 mg	nicotinic acid	0,5 mg
glutamic acid	75 mg	choline chloride	0,5 mg
asparagine	75 mg	pyridoxine	0,5 mg
adenine	25 mg	thiamine	0,2 mg

The complete medium is adjusted to pH 5,8 before it is autoclaved.- The callus cultures being studied require yeast extract and tryptone; however, these two materials can be replaced by 1% coconut milk and also by 1% tomato juice.- Studies to improve and define the medium are in progress.

Tissue culture

Nancy K. ZIEBUR

(48 Kendall Avenue, Binghamton, New York, U.S.A.)

A tissue culture of Arabidopsis was established from strain Estland and has been serially subcultured five times at intervals of about 2 months. The original tissue piece, a section of hypocotyl, was grown on a medium containing 2,4-D (3 mg/l) and coconut milk (10% by volume), as well as minerals, 2% sucrose, and 0,9% agar, with the pH adjusted to about 5,7 before autoclaving. Subsequent cultures have been maintained on a similar medium, with the coconut milk replaced by the liquid in commercial, canned, sweet corn.

Growth of subcultures is apparent to the unaided eye in 2-3 weeks; after about 2 months the fresh weight is about 50 times that at the start.

The results of one experiment are given, comparing the effects of coconut milk and corn liquid. The tissues were weighed after 8 weeks.

	Individual tissue fresh weights(mg)
control medium	10, 12, 13, 14
10% coconut milk	197, 255, 346, 368
10% corn liquid	149, 172, 279

Other experiments suggest that at a more suitable (higher) concentration, corn liquid may be fully as effective as coconut milk. Dietetic corn has been used for the most part, to avoid the complication of the NaCl ordinarily added to corn. This precaution is probably not necessary.

The mineral solution was that used by LANGRIDGE (1957), except that iron was supplied as Sequestrene 330 Fe. One trial using the major minerals in the revised medium of MURASHIGE and SKOOG (1962), indicates that this medium is little, if any, more favorable than the other. With either mineral solution virtually no growth occured unless coconut milk or corn liquid were present.

References:

LANGRIDGE,L.: Austr.J.biol.Sci. 10, 243-252 (1957)
MURASHIGE,T., and F.SKOOG: Physiol.Plantarum 15, 473-497 (1962)

C. MATERIAL

Stocks Wanted

It has to be announced the request of Prof. J.A.M. BROWN, Biology Department, New York University, University Heights, New York, N.Y. 10453, U.S.A. for seeds of induced late flowering mutants together with the initial forms. Small samples available may be sent directly or by the way of "Arabidopsis Information Service".

The LAIBACH Standard Collection of Natural Races

G.RÖBBELEN

(Institute of Agronomy and Plant Breeding, University of Göttingen, Germany)

It is already 60 years since the student Friedrich LAIBACH collected seeds of an inconspiciously small Crucifer and determined its chromosome number to be as low as n=5. From that time, especially in the late thirties, Prof. LAIBACH brought together multiple races in order to assemble a versatile collection of Arabidopsis thaliana for developmental and genetical research. Most of these stocks and records, however, were lost in the last war's chaos. But LAIBACH did really spare no pains to fill the gaps, and the collection again arrived at a size of more than 100 different types.

Since the exponential growth of Arabidopsis research, LAIBACH generously distributed seeds of these races to everyone. His collection thus gained the rank of a standard sortiment in a short time. Therefore it appeared desirable to place a comparative description of its main types at everyones disposal.

With this in view 120 races from 89 places were sown at the 28.-29.1.1964 on soil in the greenhouses of the Institute of Agronomy and Plant Breeding, Göttingen (together with 29 forms collected from 20 places by the author). They were transplanted 2-3 weeks later and examined all over their lifetime for morphological and developmental characters. The following tables represent a compilation of these data completed by notes kindly contributed by Prof.LAIBACH.

From the beginning the races were named after the place of their origin. For s y m b o l i z a t i o n the earlier uses have been modified, and every newly collected type is now denoted with the f i r s t and t h i r d l e t t e r of its name (this conditioning more combinations!). The original strain (population) gets the suffix "0", while lines segregating out of this initial collection are signed with consecutive numbers.

The purpose of the following presentation is threefold:

1. to make the reader familiar with the enormous variability within this collection enabling him to select more specifically those races specially suited for his experimental requirements,
2. to eliminate deviating types being propagated somewhere under the original designation though heavily mixed up (please compare your races with the descriptions and in case of differences send some seeds to the editor of AIS for comparison!), and
3. to stimulate the collection of new races from natural locations wherever available, for complementing the present assortment. Everyone is kindly asked to note for each new sample collected as precisely as possible: the place of origin, its exposition and inclination, type of soil, association (plant community) and dominant anthropogenic factors.

57 new races have kindly been placed at the disposal of AIS in 1964 by several active members, such as BÜRGER, DAHLGREN, LAIBACH, MARTEUS, MÜSCH, RATCLIFFE, RÖBBELEN and JEREMIAS. These races will be included into the standard collection after comparative culture in 1965. Seeds of all races, the older as well as the new ones, can be received at request by the way of Arabidopsis Information Service.

Descent of races of the LAIBACH standard collection

RASSE Race	HERKUNFTSORT (COUNTRY) Origin	STANDORT Location	ERHALTEN Received	SAMMLER (SORTIMENT) Collector
Ak-1*	Achkarren/Freiburg,Br. (D)***	Weinberg	1955	Röbbelen(R)**
An-1,2,3	Antwerpen (B)	-	1954	Bot.Garten(R)
Bch-1,3,4	Büchen/Lauenburg (D)	Aufgeschütteter Sand	1957	v.Prollius(R)
Bd-0	Berlin-Dahlem (D)	Bot.Garten	1939	Noack (L)
Be-0,1	Bensheim/Bergstr. (D)	-	1940	Spilcher (L)
Bl-1	Bologna (I)	-	1940	Bot.Garten(L)
Bla-1,2,3 4,5,6	Blanes/Gerona (E)	-	1954	Laibach (L)
Bla-10	"	-	1956	Laibach (L)
Br-0	Brünn (CS)	-	-	Bot.Garten(L)
Bs-1	Basel (CH)	-	1940	Bot.Garten(L)
Bs-2,5	"	-	1954	Bot.Garten(R)
Bsch-0	Buchschlag/ Frankfurt (D)	Gärtnerei, Nähe Bhf.Buchschlag	1940	Laibach (L)

* The numbers refer to different races

** Derived from the sortiment of Prof. Laibach (L) and Dr.Röbbelen (R) respectively

*** Country of origin:

A	Austria	N	Norway
B	Belgium	NL	Netherlands
CH	Switzerland	P	Portugal
CS	Czechoslowakia	Pl	Poland
D	Germany	S	Sweden
Dk	Denmark	SF	South-Finland
E	Spain	SU	Soviet-Union
F	France		Japan
I	Italy		Lybia

RASSE Race	HERKUNFTSORT (COUNTRY) Origin	STANDORT Location	ERHALTEN Received	SAMMLER (SORTIMENT) Collector
Bsch-2	Buchschlag/ Frankfurt (D)	Bahnstrecke Buchschlag-Langen	1943	Laibach (L)
Bu-0	Burghaun/Rhön (D)	-	1941	Laibach (L)
Chi-0,1,2	Chisdra (SU)	-	1943	Zickler (L)
Co-1	Coimbra (P)	-	1955	Bot.Garten(L)
Co-2,3	"	-	1954	Bot.Garten(R)
Ct-1	Catania (I)	-	1942	Boss (L)
Da-0	Darmstadt (D)	-	1940	Spilcher (L)
Di-0	Dijon (F)	-	1937	Bot.Garten(L)
Dr-0	Dresden (D)	Bot.Garten	1939	Söding (L)
Ei-2,4,5,6	Eifel (D)	400m ü. N.N.	-	Neugebauer(L)
El-0	Ellershausen/ Göttingen (D)	Muschelkalk-verwitterungs-boden,Südhang	1959	Ehrlich (R)
En-1	Enkheim/Frank-furt (D)	-	1940	Aufmuth (L)
En-2	"	Feldrand	1939	Laibach (L)
Ep-0	Eppenhain/ Taunus (D)	-	1941	Laibach (L)
Er-0	Erlangen (D)	trockener, sandiger Weg auf d.Burg-berg,mittle-rer Keuper, 320m ü. N.N.	-	Mägdefrau (L)
Est-0	Estland (SU)	sandiger Hü-gel beim Kirchspiel Ma-ria Magdalena von Välgi	1940	- (L)
Est-1	"	Eisenbahn-böschung bei Pinsa	1940	- (L)
Fe-1	Freiburg/Br. (D)	Bot.Garten	1954	Röbbelen (R)
Fr-2,3,4	Frankfurt (D)	Bot.Garten	1940	Laibach (L)
Ga-0	Gabelstein/ Limburg (D)	-	-	Laibach (L)
Gd-1	Gudow/Mölln(D)	Straßenrand	1957	v.Prollius(R)
Gie-0	Gießen (D)	beim Philo-sophenwald	1939	Funk (L)

RASSE Race	HERKUNFTSORT (COUNTRY) Origin	STANDORT Location	ERHALTEN Received	SAMMLER (SORTIMENT) Collector
Gö-0	Göttingen (D)	Gartetal, sonnige Wegböschung	1939	Steiner (L)
Gö-2	"	nördl.der Stadt,unweit Autobahn	1958	Röbbelen (R)
Gr-1,2	Graz (A)	-	1939	Buschmann (L)
Gr-3,4,5	"	am Rande eines Kornfeldes,äußere Ragnitz	1939	Buschmann (L)
Gr-6	"	-	1954	Bot.Garten (R)
Gü-0,1	Gückingen/ Limburg (D)	-	1947	Laibach (L)
Ha-0	Hannover (D)	Bot.Garten	1939	Schacht (Tüxen) (L)
Hi-0	Hilversum (NL)	eine stille Straße	-	Arens (L)
Hm-0	Hannoversch-Münden (D)	Bot.Garten	1939	Jahn (L)
Hs-0	Hann.Ströhen (D)	-	1961	Rusche (R)
In-0	Innsbruck (A)	-	1939	Bot.Garten (L)
Is-0	Isenburg (D)	-	1939	Burk (L)
Is-1	"	Feld in Richtung Gravenbruch	1940	Laibach (L)
Je-0	Jena (D)	beschatteter Buntsandsteinfelsen	1939	Herzog (L)
Kä-0	Kärnten (A)	bei Weißensee	1957	Laibach (L)
Kn-0	Kaunas (SU)	beim Dorf Varnius	1942	Dapp (L)
Ko-2	Kopenhagen (DK)	-	1937	Bot.Garten (L)
Kl-0	Köln (D)	bei Zollstock	1939	Sierp (L)
Kl-1	"	bei Dellbrück	1940	Hülsbruch (L)
Kl-2	"	Bot.Garten	-	Hülsbruch (L)
Kl-3,4	"	bei Thilenbruch,etwa 1,5km v.Bot. Garten entfernt	-	Hülsbruch (L)

RASSE Race	HERKUNFTSORT (COUNTRY) Origin	STANDORT Location	ERHALTEN Received	SAMMLER (SORTIMENT) Collector
Kr-0	Krefeld (D)	-	1940	Bot.Garten (L)
La-0,1	Landsberg/ Warthe (D)	bei Zilenzig,Krs. Osternburg	1939	Michel (L)
Lg-0	Leiden (NL) (Lugdunum)	-	-	Bot.Garten (L)
Li-1	Limburg/Lahn (D)	Autobahnbrücke ü.d. Lahn,weißer Helgenstock, derselbe Standort a.d. 1905 Stempel f.Chromosomenuntersuchungen an A. thal.v.Laibach gesammelt wurden	1940	Laibach (L)
Li-2	"	unterhalb d. Schleuse	1940	Laibach (L)
Li-3	"	oberhalb d. Schleuse	-	Laibach (L)
Li-5	"	Straße Limburg.Dietkirchen,Nähe Autobahn	-	Laibach (L)
Li-6	"	Bahndamm bei Böhnes Brauerei	1944	Laibach (L)
Li-7	"	Grundstück Laibach,aufgeschütteter Boden	1954	Laibach (L)
Li-8	"	Brachacker, oberhalb Autobahn,zw. Dierkircher Weg u.Weißer Helgenstock	1953	Laibach (L)
Li-10	"	-	-	Laibach (L)
Li-11	"	Brückenvorstadt,nach Weißer Helgenstock	1961	Laibach (L)
Li-12	"	Straße"Am Rosenhang",vor d.Garten von Laibach	1961	Laibach (L)

RASSE Race	HERKUNFTSORT (COUNTRY) Origin	STANDORT Location	ERHALTEN Received	SAMMLER (SORTIMENT) Collector
Lm-2	Le Mans (F)	Weizenfeld	1943	Dorn (L)
Lö-1,2	Lörrach/Baden (D)	-	1955	Hügin (R)
Lu-1	Lund (S)	-	1939	Bot.Garten (L)
Ma-0,2	Marburg/Lahn (D)	Bot.Garten	-	Claußen (L)
Mr-0	Monti Rossi (I)	1000 bis 1500m ü.N.N.	1952	Staatssammlung München (L)
Mrk-0	Märkt/Baden (D)	-	1955	Hügin (R)
Mt-0	Martuba/Cyrenaika (Lybien)	-	1942	Boss (L)
Na-1	Nantes (F)	-	-	Bot.Garten (L)
Np-0	Nieps/Salzwedel (D)	sandiger Boden, Bahndamm	1963	Bürger (R)
Nw-0,1,2	Neuweilnau (D)	-	1943	Laibach (L)
Nw-3,4	Taunus (D)			
Öst-0	Östhammar/Uppland (S)	-	1956	Almquist (L)
Ø-0	Øystese/Hardanger Fjord (N)	-	1957	Laibach (L)
Pa-1,2,3	Palermo (I)	Bot.Garten	1955	Röbbelen (L)
Pf-0	Pfrondorf/Tübingen (D)	430m ü. N.N., Acker, sonnige Lage	-	Hummel (L)
Pi-0,2	Pitztal/Tirol (D)	oberhalb Trenkwald 1450m ü.N.N, planierte Stelle neben d. Straße	1942	Laibach (L)
Po-0,1	Poppelsdorf/Bonn (D)	trockener, sandiger Standort, Südwestseite d. Poppelsdorfer Schlosses im Bot.Garten	1939	Wiesmann (L)
Pr-0	Praunheim/Frankfurt/M. (D)	Lehmboden bei einer Ziegelei	1941	Kolbe (L)
Pt-0	Pötrau/Lauenbg. (D)	sandige Brache	1957	v.Prollius (R)

RASSE Race	HERKUNFTSORT (COUNTRY) Origin	STANDORT Location	ERHALTEN Received	SAMMLER (SORTIMENT) Collector
Rd-0	Rodenbach/Rhön (D)	-	1941	Laibach (L)
Rou-0	Rouen (F)	Weg in einer Parkanlage	1943	Kiehne (L)
Rsch-0,4	Rschew/Starize (SU)	-	1942	Hülsbruch (L)
Ru-0	Ruppachtal/ Taunus (D)	-	1958	Laibach (L)
Sg-1,2	St.Georgen/ Freiburg/Br. (D)	Brachacker	1954	Röbbelen (R)
Si-0	Siegen (D)	am Wegrand beim Hermes-bacher Fried-hof	1940	- (L)
St-0	Stockholm (S)	-	1939	Arnidtson (L)
Stw-0	Stolbowa,Orel (SU)	dürftiger,san-diger Brach-acker	1943	Hülsbruch (L)
Ta-0	Tabor (CS)	-	1937	- (L)
Te-0	Tenala (SF)	-	1956	Collander (L)
Tsu-0	Tsu (Japan)	-	1940	- (L)
Tu-0,1	Turin (I)	-	1940	Cappolotti (L)
Uk-1,2	Umkirch/Frei-burg/Br. (D)	Dreisamufer, aufgeschütte-ter Damm	1954	Röbbelen (R)
Uk-3	"	Rieselgut	1954	Röbbelen (R)
Uk-4	"	Ort Umkirch	1954	Röbbelen (R)
Vi-0	Vilbel/Taunus (D)	Dorfausgang nach Dortelweil	1940	Laibach (L)
Wa-1	Warschau (PL)	-	1937	Bot.Garten (L)
Wil-1,3	Wilna (SU)	bei Towniskaia	-	Johuber (L)
Wil-2	"	im Zakret-Park	-	Johuber (L)
Ws-0	Wassilewskije (SU)	sandiges Roggen-feld Retschije, ostwärts v. Dnijeper	1943	Hülsbruch (L)
Wü-0	Würzburg (D)	aufgefüllter Sandboden b. Meinheim (Altmühltal)	1939	Zeidler (L)
X-0	unbekannt	-	-	- (R)
Zü-0	Zürich (CH)	-	-	Bot.Garten (L)

Characteristics of races of the LAIBACH standard collection

Rasse Race	Rosette Rosette			Blätter Leaves						Blüte (Tage) Flowering (days)		
	Blatthaltung Leaf posture	Größe Size	Dichte Compactness	Blattansatz Attachment	Breite Width	Breite:Länge Width:Length	Behaarung Hairiness	Farbe Colour	Rand Margin	1.Blüte 1.open flow.	Vollblüte Full fl.ing	Ernte Harvest
Ak-1	3	2	3	2	2	1	2	2	4	70	112	118
An-1	3	3	3	2	2	2	3	2	4	65	70	105
An-2	3	3	2	2	1	2	2	2	4	77	79	118
An-3	3	2	3	2	2	1	3	3	4	71	75	118
Bch-1	2	4	3	2	2	2	3	3	4	72	80	111
Bch-3	3	3	1	2	1	1	2	2	3	71	72	118
Bch-4	3	3	3	2	2	1	3	1	3	63	78	118
Bd-0	3	2	3	2	2	1	1	3	4	70	73	111
Be-0	4	1	2	2	2	1	2	2	6	75	79	121
Be-1	4	2	3	2	2	1	2	2	5	70	80	118
Bl-1	4	2	2	2	1	2	4	3	7	77	84	118
Bla-1	4	3	1	2	1	2	3	1	3	68	72	111
Bla-2	4	2	1	2	1	1	3	1	4	68	72	111
Bla-3	3	3	1	2	1	2	3	1	3	69	72	111
Bla-4	3	2	2	2	2	2	3	2	5	69	82	107
Bla-5	3	3	1	2	1	2	3	1	3	69	74	111
Bla-6	2	3	2	2	2	1	3	1	3	75	82	118
Bla-10	3	3	3	2	2	1	3	1	3	68	70	118
Br-0	4	2	2	2	1	2	4	3	7	79	89	125
Bs-1	3	3	3	2	2	2	4	3	7	77	79	121
Bs-2	4	2	4	2	2	1	3	2	7	65	71	111
Bs-5	3	2	3	2	2	1	3	2	7	69	80	118
Bsch-0	4	2	2	2	2	1	2	2	7	68	77	113
Bsch-2	4	2	3	2	2	1	2	2	3	68	72	113
Bu-0	2	3	3	1	2	2	3	3	7	77	82	121
Chi-0	3	3	3	2	2	2	3	2	5	81	84	125
Chi-1	4	3	3	2	1	1	3	3	4	68	72	113
Chi-2	2	3	4	2	2	1	3	3	4	70	77	118
Co-1	3	3	2	2	1	1	4	2	5	57	63	97
Co-2	3	2	2	2	2	1	2	2	4	65	78	103
Co-3	4	2	1	2	1	1	3	2	4	67	75	105
Ct-1	3	3	2	2	1	1	2	3	7	69	77	118
Da-0	3	3	2	2	1	1	2	3	7	69	75	118
Di-0	3	2	3	2	1	2	2	2	4	62	82	113
Dr-0	3	2	2	2	2	1	1	2	4	70	84	118
Ei-2	3	2	3	2	2	2	2	2	4	72	77	118
Ei-4	4	3	4	2	1	1	3	2	3	72	84	121
Ei-5	5	4	3	2	2	1	1	2	2	66	69	121
Ei-6	3	2	2	2	2	2	3	1	7	55	59	111
El-0	3	3	3	2	1	2	3	3	4	68	72	111
En-1	3	3	3	2	1	1	2	3	5	65	68	107
En-2	3	2	3	2	1	1	3	3	7	70	76	113

Rasse Race	Rosette Rosette			Blätter Leaves						Blüte (Tage) Flowering (days)		
	Blatthaltung Leaf posture	Größe Size	Dichte Compactness	Blattansatz Attachment	Breite Width	Breite:Länge Width:Length	Behaarung Hairiness	Farbe Colour	Rand Margin	1.Blüte 1.open flow.	Vollblüte Full fl.ing	Ernte Harvest
Ep-0	2	3	3	2	2	2	2	2	3	73	77	118
Er-0	2	3	3	2	2	2	1	2	5	79	82	125
Est-0	2	3	4	2	2	1	4	3	4	73	77	118
Est-1	3	3	3	2	1	1	3	2	5	75	82	125
Fe-1	3	3	3	2	1	1	2	3	5	69	73	125
Fr-2	3	2	4	2	2	1	2	2	5	70	73	118
Fr-3	3	3	4	2	1	1	2	3	4	73	79	118
Fr-4	3	2	3	2	1	1	3	2	6	75	84	121
Ga-0	4	3	4	2	1	2	1	3	5	69	77	113
Gd-1	2	3	4	2	2	1	2	2	5	79	87	121
Gie-0	4	2	2	2	1	1	3	3	7	65	68	113
Gö-0	3	3	3	2	1	2	3	2	3	59	65	102
Gö-2	3	3	3	2	1	2	2	3	4	73	75	113
Gr-1	4	3	3	2	2	1	1	2	5	71	77	121
Gr-2	3	3	2	2	2	1	1	2	4	75	80	121
Gr-3	3	2	3	2	2	1	1	2	3	69	70	118
Gr-4	3	2	4	2	2	1	1	3	4	62	82	118
Gr-5	3	2	4	2	2	2	2	3	5	68	70	113
Gr-6	4	2	2	2	2	2	3	2	6	75	87	118
Gü-0	3	3	3	2	1	2	3	3	5	68	71	104
Gü-1	3	1	3	2	1	2	2	1	7	62	65	104
Ha-0	2	3	3	2	2	2	3	2	5	70	77	113
Hi-0	3	2	4	2	1	2	2	2	6	68	71	113
Hm-0	3	2	2	2	2	1	2	2	4	78	82	118
Hs-0	4	2	3	2	1	1	3	2	3	65	72	111
In-0	3	1	5	2	1	1	1	2	3	69	79	113
Is-0	4	2	2	2	1	1	3	3	5	70	81	118
Is-1	4	1	3	2	2	1	2	3	6	68	70	118
Je-0	2	3	3	2	2	2	2	2	4	68	100	125
Kä-0	4	1	4	2	2	1	1	2	6	65	71	105
Kn-0	3	2	5	2	1	1	2	2	4	68	72	111
Ko-2	1	3	3	1	2	2	1	2	6	96	111	139
Kl-0	3	3	2	2	2	1	2	2	4	68	71	118
Kl-1	3	1	3	2	2	1	2	2	5	68	73	118
Kl-2	2	2	2	2	2	1	1	2	6	99	107	142
Kl-3	3	2	2	2	1	1	3	2	4	70	75	111
Kl-4	4	2	3	2	1	1	3	2	4	71	81	113
Kr-0	4	2	2	1	2	2	2	2	2	75	80	118
La-0	3	2	1	2	2	2	1	2	3	55	65	118
La-1	3	2	4	2	2	2	1	2	4	77	92	125
Lg-0	2	3	3	1	2	2	2	2	6	79	82	121
Li-1	3	2	4	2	2	1	3	1	4	75	83	121
Li-2	2	2	4	2	2	1	4	3	4	81	92	125
Li-3	3	2	2	2	2	2	2	2	3	69	77	113

Rasse Race	Rosette Rosette			Blätter Leaves						Blüte (Tage) Flowering (days)		
	Blatthaltung Leaf posture	Größe Size	Dichte Compactness	Blattansatz Attachment	Breite Width	Breite:Länge Width:Length	Behaarung Hairiness	Farbe Colour	Rand Margin	1.Blüte 1.open flow.	Vollblüte Full fl.ing	Ernte Harvest
Li-5	3	4	2	2	2	2	2	2	4	75	79	121
Li-6	3	3	3	2	2	2	1	2	2-4	89	104	139
Li-7	3	3	3	2	2	1	3	2	4	65	68	113
Li-8	2	3	4	2	1	1	3	3	4	73	77	117
Li-10	2	3	4	2	2	1	2	2	2-3	86	127	139
Li-11	2	2	1	2	2	1	2	1	6	98	105	-
Li-12	2	3	2	2	2	1	3	2	4	62	69	113
Lm-2	4	2	4	2	1	1	2	3	5	61	65	97
Lö-1	3	2	3	2	2	1	2	2	4	75	82	121
Lö-2	3	2	4	2	2	1	2	2	4	-		
Lu-1	2	3	3	1	2	3	2	2	2-3	100	105	121
Ma-0	3	2	5	2	1	1	3	3	4	69	71	107
Ma-2	3	2	3	2	2	1	2	2	4	62	71	111
Mr-0	2	4	5	2	2	2	3	2	5	91	94	139
Mrk-0	4	2	2	2	1	1	2	2	7	79	82	121
Mt-0	4	3	5	2	1	1	2	3	5	68	70	113
Na-1	3	1	3	2	2	1	3	2	5	74	92	118
Np-0	3	2	2	2	2	1	3	2	5	69	71	113
Nw-0	2	3	3	2	2	1	2	2	4	71	75	125
Nw-1	3	3	1	2	2	1	2	2	4	75	79	125
Nw-2	3	3	3	2	2	1	2	2	6	70	101	125
Nw-3	3	2	3	2	2	1	2	2	7	62	79	113
Nw-4	3	2	3	2	2	1	3	3	5	65	68	113
Öst-0	2	3	3	2	2	2	2	2	4	112	121	147
Øy-0	3	2	3	2	1	1	2	2	6	120	129	139
Pa-1	3	2	2	2	1	1	2	2	4	51	61	89
Pa-2	3	2	2	2	2	1	2	2	4	68	75	111
Pa-3	3	3	2	2	2	1	2	2	5	57	62	97
Pf-0	3	2	4	2	2	1	3	3	4	68	72	118
Pi-0	3	2	4	2	1	1	2	3	4	70	79	121
Pi-2	3	2	2	2	1	1	1	2	5	55	63	105
Po-0	4	2	3	2	2	1	2	3	4	68	73	113
Po-1	4	2	2	2	1	1	1	2	7	74	79	118
Pr-0	3	3	2	2	1	1	1	2	5	56	75	125
Pt-0	3	3	5	2	2	2	2	2	3	82	93	133
Rd-0	3	2	2	2	1	1	2	2	3	79	84	128
Rou-0	2	3	2	2	2	1	3	2	4	79	82	125
Rsch-0	3	3	3	2	2	1	3	2	4	68	75	113
Rsch-4	3	2	4	2	2	1	3	2	7	79	80	125
Ru-0	3	3	3	2	2	1	1	2	5	75	77	118
Sg-1	2	3	4	2	1	2	3	2	4	68	82	118
Sg-2	3	3	3	2	1	2	2	3	5	75	82	118
Si-0	3	3	3	2	1	1	2	2	4	75	78	125
St-0	3	2	4	2	2	1	2	3	5	78	86	139

Rasse / Race	Rosette: Blattthaltung / Leaf posture	Rosette: Größe / Size	Rosette: Dichte / Compactness	Blätter / Leaves: Blattansatz / Attachment	Blätter / Leaves: Breite / Width	Blätter / Leaves: Breite:Länge / Width:Length	Blätter / Leaves: Behaarung / Hairiness	Blätter / Leaves: Farbe / Colour	Blätter / Leaves: Rand / Margin	Blüte (Tage) / Flowering (days): 1.Blüte / 1.open flow.	Blüte (Tage) / Flowering (days): Vollblüte / Full fl.ing	Blüte (Tage) / Flowering (days): Ernte / Harvest
Stw-0	3	2	5	2	1	1	2	3	4	67	68	113
Ta-0	4	2	3	2	1	1	1	2	3	71	75	111
Te-0	1	3	3	2	2	2	2	2	3	91	125	152
Tsu-0	3	2	4	2	2	2	2	3	5	71	80	121
Tu-0	3	2	4	2	2	1	2	3	5	75	84	118
Tu-1	3	2	5	2	1	1	2	2	5	75	84	118
Uk-1	4	2	3	2	1	1	3	2	3	77	84	121
Uk-2	4	2	3	2	2	2	3	2	4	68	101	118
Uk-3	3	2	4	2	1	1	3	3	5	78	92	125
Uk-4	2	3	3	2	2	2	1	2	7	75	82	121
Vi-0	3	2	3	2	2	1	3	3	4	71	73	113
Wa-1	2	3	5	1	2	2	2	2	4	72	75	111
Wil-1	1	2	5	2	1	1	1	3	5	79	84	118
Wil-2	1	3	5	2	2	2	4	3	1-5	77	82	118
Wil-3	3	2	4	2	1	1	4	3	4	68	72	111
Ws-0	3	2	3	2	1	1	1	2	7+5	82	87	125
Wü-0	3	2	4	2	2	1	2	3	4	68	71	113
X-0	4	2	1	2	1	1	2	2	3	77	79	118
Zü-0	4	1	4	2	2	1	3	2	5	77	82	121

Legend for the foregoing table

	Blatthaltung	Leaf posture
1	flach	flat
2	ziemlich flach	incompletely flat
3	halbaufrecht	half upright
4	aufrecht	upright
5	stark aufrecht	strongly upright

	Rosettengröße	Rosette size
1	sehr groß	very large
2	groß	large
3	mittelgroß	medium
4	klein	small

	Rosettendichte	Rosette compactness
1	sehr locker	very loose
2	locker	loose
3	normal dicht	normal
4	dicht	compact
5	sehr dicht	highly compact

	Blattansatz	Leaf attachment
1	abgesetzt	abrupt
2	herablaufend	transitional
	Blattbreite	**Leaf width**
1	schmal	narrow
2	breit	broad
	Blattbreite:Länge	**Leaf width:length**
	breiteste Stelle:	broadest diameter:
1	über der Mitte	distal to the medial
2	Mitte	the medial
3	unterh.d.Mitte	proximal to the medial
	Behaarung	**Leaf hairiness**
1	schwach behaart	weakly hairy
2	behaart	hairy
3	stark behaart	strongly hairy
4	unbehaart	glabrous
	Blattfarbe	**Leaf colour**
1	hellgrün	light green
2	grün	green
3	dunkelgrün	dark green
	Blattrand	**Leaf margin**
1	ganzrandig	smooth
2	wellig	sinnate
3	schwach gesägt	weakly serrate
4	gesägt	serrate
5	scharf gesägt	strongly serrate
6	gezähnt	dentate
7	weit gezähnt	sparsely dentate

D. NEWS

Symposium on Arabidopsis Research

Göttingen, April 21-24, 1965

An International Symposium on Arabidopsis Research will be held at the Institute of Agronomy and Plant Breeding, University of Göttingen, in April 21-24, 1965. This symposium will be especially devoted to discussions on methodological problems arising in Arabidopsis research work. The programme is as follows:

22.4.:	8.30	Inaugural Session
	10.00	Section I : Variation and Taxonomy
	15.00	Section II : Development
23.4.:	8.00	Section III : Genetics
	10.30	Section IV : Mutant Analysis
	15.00	Section V : Mutagenesis
24.4.:	9.30	Business Meeting and Demonstrations
	13.00	Departure for Excursion

The lectures including discussion will be published in a supplementary issue of "Arabidopsis Information Service". Everyone interested in Arabidopsis research work will at request receive more detailed informations on the symposium from the organizer Doz.Dr.G.Röbbelen, Institut für Pflanzenbau und Pflanzenzüchtung, Universität Göttingen, von-Siebold-Str. 8, Deutschland.

Laboratory Research Communications

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Belgium

B r u x e l l e s : M.JACOBS

Isolation and studies of b i o c h e m i c a l m u t a n t s , utilisation in schedule of reverse mutations test, fine structure, biosynthetic pathways and relations between biochemical, developmental and morphological effects of a gene.

Investigations on the activity, mechanism of action and specificity of chemical m u t a g e n s , particularly alkylating agents, HNO_2, nitrosamine, alkaloids, base analogues and biological macromolecules.

L o u v a i n : J.BOUHARMONT

Study of the stem apex of Arabidopsis : distribution of the mitosis in the meristematic layers, observed after treatment with colchicine. M i t o t i c a c t i v i t y is studied at different stages of the seedling development.

Seeds and seedlings of 2x, 4x, and 6x Arabidopsis were treated by ^{60}Co in order to study the r a d i o s e n s i t i v i t y of the three types. Chlorophyll mutants are identified and the occurence of micromutations is investigated by comparison of the variability of progenies.

Czechoslowakia

B r n o : I.CETL, and J.KUČERA

Investigations on n a t u r a l p o p u l a t i o n s . Genetical basis of d e v e l o p m e n t a l and q u a n t i t a t i v e c h a r a c t e r s . Effect of r a d i a t i o n and c h e m i c a l m u t a g e n s on developmental and quantitative characters.

P r a g u e : T.GICHNER, J.ŠVACHULOVÁ, J.VELEMÍNSKÝ and V.POKORNÝ

C h e m i c a l m u t a g e n e s i s : The recent experiments are concerned with the comparison of various mono- and polyfunctional sulphonates; the results will be presented at the Mutation Symposium organized in honour of G.MENDEL in Prague, August 1965. Experiments on the mutagenic action of the nitrosourea-group are also underway.

P h y s i o l o g y of l e a f - c o l o u r m u t a n t s : The normalization-phenomenon of chlorina mutants on the glucose-agar media is investigated in relation to the chlorophyll and free amino acid contents.

France

G r e n o b l e : K.NAPP-ZINN

Studies underway concern:
1) the relations between r e s p i r a t i o n and a f t e r - r i p e n i n g in Arabidopsis seeds (together with Michèle SAGNARD),
2) the regeneration phenomena in Arabidopsis l e a f c u t t i n g (together with Danielle BERSET),
3) the genetical bases of the requirements of a f t e r r i p e - n i n g and l i g h t in the seeds of certain Arabidopsis strains (together with Maryse GRÉ).

Germany

G a t e r s l e b e n : A.J.MÜLLER

The studies underway are concerned mainly with problems of m u t a g e n e s i s . In this connection investigations on the mode of action of N-nitroso compounds, on the control of mutagen sensitivity of seeds (including action of metabolic inhibitors) and on repair mechanisms are carried out.

G a t e r s l e b e n : R.HAGEMANN

Studies about the possibility of phenotypic n o r m a l i s a t i o n of chlorophyll lethals by means of aseptic culture.

G ö t t i n g e n : G.RÖBBELEN and Brigitte BERGER

Besides the programmes cited in Arabid.Inf.Serv. 1, 29 (1964) the following experiments have been set up:

1. Crossing of Arabidopsis thaliana with related species for clarifying the taxonomical classification.
2. Morphogenetical (esp. chloroplast structure) and pigment-analytical studies with leaf-colour-mutants growing under "monochromatic" light.

Netherlands

W a g e n i n g e n : W.J.FEENSTRA

I n t e r m e d i a t e s i n t h i a m i n e b i o s y n t h e s i s . — So far four non-allelic mutants have been found which grow on a medium with thiamine, but fail to grow on one with "pyrimidine" or "thiazole". In yeast, "pyrimidine" -mono- and diphosphates and "thiazole"-monophosphate are intermediates in the biosynthesis of the vitamin. In order to establish whether these compounds are likewise active in a higher plant experiments are being carried out on the synthesis and isolation of the pure compounds and on the feeding of mutants on them. Preliminary results indicate that some of the mutants do show growth.

W a g e n i n g e n : J.HEYTING and W.J.FEENSTRA

I s o l a t i o n o f b i o c h e m i c a l m u t a n t s . Experiments using the accumulation technique developed by the second author, so far only yielded mutants in which the synthesis of thiamine is blocked. Indications that other mutants might have been isolated (see Arabid.Inf.Serv. 1, 23, 1964) proved to be false. A period of starvation and the use of a "complete" medium containing only a limited number of organic compounds might have given rise to the above mentioned results. Large scale experiments in which the starvation period is omitted, and in which the complete medium contains yeast extract, are being carried out now.

U.S.A.

K n o x v i l l e : S.BELL and T.C.BONDURANT

Seeds have been treated with ethyl methanesulfonate and m u t a n t s for physiological and genetic studies are now being isolated. - Information for l i n k a g e studies is also being sought.

U p t o n (Brookhaven National Laboratory) : H.H.SMITH and C.R.BHATIA

G e n e t i c e f f e c t s of i n c o r p o r a t e d t h y m i d i n e a n a l o g u e s : Earlier work in this laboratory using ^{125}I labelled 5-iododeoxyuridine has shown the incorporation of this thymidine analogue in the megagametophyte cells and also in the pollen mother cells. Incorporation as observed by grain count was more in the nuclei than the cytoplasm and it was postulated that the site of analogue incorporation is at the DNA level.

A change in base pairing brought about by incorporated unnatural bases is expected to bring a change in the triplet code which would result in a mutation, unless the mechanism for maintenance of genetic information is much more complex. Experiments are underway in which plants of every alternate generation are treated with the analogue. Even after three cycles of treatment we have not observed any chlorophyll or morphological mutant. The frequency of lethals is high in the progeny of treated plants.

Single base pair changes accumulated over several generations are more likely to be expressed in quantitative characters controlled by a number of genes. With this objective, variation for flowering time, a quantitative character, is being followed with selection of the extreme types. The results, to date, show increased variation in population derived from treated plants but selection has so far failed to fix the variation.

E l e c t r o p h o r e t i c s t u d i e s w i t h s o l u b l e l e a f a n d s e e d p r o t e i n s : Technics to electrophoretically separate leaf and seed proteins on polyacrylamide gels are being worked out. The objective is to isolate genetic variations in the protein pattern. Attempts will be made to locate important enzymes and their electrophoretic variants will be looked for. It is also planned to study variation in proteins of different mutant types.

A r a b i d o p s i s c u l t u r e o n D e u t e r i u m O x i d e : It is planned to culture Arabidopsis on progressively increasing concentrations of D_2O. Very little information is available on the growth of higher plants with this heavy stable isotope of hydrogen. Arabidopsis by virtue of its short cycle and easy culture in chemically defined media furnishes an ideal material for such studies. Morphological and genetical effects of deuteriation will be studied. The main objective, however, is to obtain seeds (if possible) in which water is replaced by D_2O. Radiation sensitivity studies with deuteriated seeds may yield useful information on the role of water in the modification of radiation sensitivity.

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