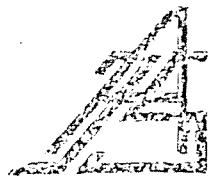


A R A B I D O P S I S

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

No. 1

In collaboration with

F. Laibach, Limburg/Lahn
A. J. Müller, Gatersleben
G. P. Rédei, Columbia
J. Velemínský, Praha

edited and published by

G. Röbbelen, Göttingen

March 1, 1964
G ö t t i n g e n

A R A B I D O P S I S

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

No.1 Göttingen March, 1964

A. Brief Notes

B. Laboratory Research Communications

A. PREFACE

On the occasion of the XI. International Congress of Genetics in Scheveningen in 1963 an exchange of ideas and information was initiated among the research workers interested in Arabidopsis. Since many valuable and interesting suggestions were forthcoming, it appeared necessary in the interest of all the research workers, to give the existing data a systematised and unified expression. It should hence be the duty of the "Arabidopsis Information Service" to circulate effectively the available information conducive to Arabidopsis research. No prospectus was designed earlier for such a scientific newsletter. This issue has therefore been composed merely on the basis of several contributions kindly sent to the editor by various research workers. The present format may however be a proposal for the future issues.

Needless to mention here the versatility of Arabidopsis in scientific experiments; readers are referred to the contents and especially to the bibliography which the editor attempted to compile as complete as possible. This wide field of research, for which this "botanical Drosophila" is so specially suited, should be covered in the "Arabidopsis Information Service". Genetics will indeed take the main part, but notes on ecology, morphology, development, physiology or biochemistry should as well be appreciated. The future success and fate of this initiative endeavour depends upon the aspect of active cooperation of all those occupied with any Arabidopsis research.

In the first instance the "Arabidopsis Information Service" proposes to report brief information on the current interests and studies underway at various research centers. These informal "Laboratory Research Communications" (Sect.B) are the most important concern of the newsletter. They serve to communicate general information to all coworkers and in no case be treated as publications. Of equal importance are all suggestions, queries or criticisms, communicated for consideration or discussion (cf.Sect.D). This "Correspondence" demands your active cooperation assistance and maximum attention!

Secondly the "Arabidopsis Information Service" should represent the central organisation where the adresses of Arabidopsis research laboratories are registered and recent literature is compiled. Some coordination is being attempted in problems such as the naming the genes (cf.p.38), establishment of stock collections and exchange of material (cf.p.37).

Finally the "Arabidopsis Information Service" will publish at request "Brief Notes" on original observations (cf.Sect.A) not exceeding one typewritten page. The manuscripts should be in final shape for printing and be made as short and clear as possible. While English will be the official language, contributions might also be accepted in German or French, in case lingual difficulties should otherwise prevent from cooperation. In order to ensure rapid publication no proofs can be sent. While the responsibility for the reported observations rests with the author, any mistake and error of composition are the editors. No reprints can be supplied.

As the "Arabidopsis Information Service" will be issued

yearly, all contributions and manuscripts should reach the editor not later than Feb. 1 of each year at the following address:

Gerhard Röbbelen, Institut für Pflanzenbau und Pflanzenzüchtung, 34 Göttingen, von-Siebold-Str.8, Deutschland

In presenting this first issue it is a pleasure to acknowledge my indebtedness and gratitude to Professor Arnold Scheibe for his generous support in many stages of the preparation of this newsletter. I am especially indebted to Miss Dr. Satya Nirula for reading the manuscript and to Miss Oda Wolf for preparing the typescript. But I would be remiss if I did not acknowledge the many suggestions and contributions given by all the authors enlisted in the following pages. I wish that the present issue will be a small substitute for their efforts and a stimulus for further cooperation.

Gerhard Röbbelen

A. BRIEF NOTES

(For citation cf. the bibliography in Sect.E)

ADAMS, M.V.: Studies on growth and development of several races of Arabidopsis thaliana

Hypocotyl growth rate - mean values and variation. Material: Selfed progeny of single plants coming from seed lots received from LANGRIDGE, Canberra. Races: Catania (Sicily); Tsu (Japan); Graz (Austria); Langen (Germany); Martuba (North Africa); Stockholm (Sweden). Procedure: Post-dormancy seeds were wetted, placed in petri plates, cold-treated at -5°C for three days, and placed on 2% sterile nutrient agar at 18°C in the dark for the duration of the experiments, except for periodic removal for hypocotyl measurements. Forty seeds of each lot were measured. The results were as follows:

Table 1: Mean length of hypocotyl in mm and coefficients of variation

Race		Hours after appearance of root radicle				
		8	16	24	32	40
Catania	$\bar{X}$	1.67	2.56	4.04	5.56	6.66
	C.V.	22.74	14.29	13.85	15.40	13.45
Tsu	$\bar{X}$	4.03	6.53	7.81	8.61	9.00
	C.V.	9.59	5.60	5.08	4.81	5.06
Graz	$\bar{X}$	1.39	2.77	4.90	6.26	7.22
	C.V.	20.60	12.41	11.18	15.24	15.20
Langen	$\bar{X}$	2.11	3.79	5.19	5.75	5.98
	C.V.	16.74	12.16	9.53	10.27	11.48
Martuba	$\bar{X}$	2.29	3.98	5.83	6.99	7.78
	C.V.	23.22	12.51	11.17	11.39	10.55
Stockholm	$\bar{X}$	4.34	6.53	7.40	7.78	7.92
	C.V.	10.73	9.23	11.07	12.23	13.10

		48	56	64	72	80
Catania	$\bar{X}$	7.12	7.23	7.34	7.39	7.40
	C.V.	13.77	13.74	13.96	14.43	14.45
Tsu	$\bar{X}$	9.04	9.08	9.08	9.08	9.08
	C.V.	5.16	5.26	5.26	5.26	5.26
Graz	$\bar{X}$	7.98	8.12	8.20	8.28	8.28
	C.V.	16.77	17.23	17.18	17.78	17.95
Langen	$\bar{X}$	6.17	6.20	6.20	6.21	6.21
	C.V.	13.29	13.15	13.12	13.21	13.21
Martuba	$\bar{X}$	8.27	8.54	8.56	8.57	8.57
	C.V.	11.17	11.97	12.17	12.20	12.20
Stockholm	$\bar{X}$	7.95	7.96	7.96	7.96	7.96
	C.V.	13.28	13.16	13.16	13.16	13.16

Comparison of hypocotyl growth rates and coefficients of variation of homozygous lines and some of their F_1 hybrids. (Procedure as before, except this was another experiment; so data are not the same for the lines). The results were as follows:

Table 2: Hypocotyl length in mm measured number hours after root radicle appeared

Race	8 hrs	16	24	48	72
Catania	0.65	1.46	2.05	4.90	6.31
Tsu	0.80	1.95	3.25	5.83	6.84
Graz	0.69	1.42	2.08	5.58	7.16
Langen	0.82	1.67	2.72	5.24	6.50
Martuba	0.43	1.01	1.56	4.62	5.66
Stockholm	0.51	1.20	1.43	5.82	7.56
Hybrids					
Cat. X Tsu	1.15	2.74	3.82	7.62	9.18
Cat. X Graz	0.53	1.03	1.75	4.51	6.33
Cat. X Lang.	1.50	3.27	4.64	8.16	9.66
Cat. X Mart.	1.45	3.03	4.24	6.87	7.38
Tsu X Graz	0.77	1.79	2.72	6.23	7.05
Tsu X Lang.	1.18	2.41	3.63	6.74	8.00
Tsu X Mart.	0.82	2.03	3.15	5.90	6.84
Graz X Lang.	0.86	1.81	2.61	6.45	7.75
Graz X Mart.	0.67	1.64	2.40	5.14	6.03
Lang. X Mart.	0.82	1.73	2.67	5.30	6.19

Table 3: Some data on stem anatomy of five lines of A.thaliana

Race	Number of plants	Average number of vascular bundles per stem	Average number of xylem vessels per bundle
Catania	5	8	20.1
Tsu	3	13	25.3
Graz	2	9	39.4
Langen	3	10	28.6
Martuba	4	13	28.5

The above represents in part work done in collaboration with Dr.W.T.CHANG (deceased December 15,1963) at Michigan State University in 1961-62.

MAHESHWARI,N.,and R.D.IYER: Developmental conditions in Arabidopsis

Material of Arabidopsis thaliana procured from Saharanpur, India, and from Netherlands is grown aseptically in test tubes on solid nutritive medium. The seeds are exposed to 8°C for 48 hours for optical germination. Kinetin (10^{-6} mol.) and red light have been found to enhance germination. The critical photoperiod is 8 hours, and 5 cycles are needed to induce flowering. However primordia appear 12-15 days after germination of seeds and the entire life cycle is completed within 21 days. Kinetin promotes flowering at 10^{-1} and 10^{-8} mol. The cold requirement of seeds cannot be replaced by gibberellins or auxins.

RATCLIFFE,D.: Observations on the physiology of flowering

In the last few years strains of Arabidopsis have been collected from various parts of the British Isles, particularly Scotland, for some visual observations on their flowering physiology. The main difference between the strains is the length of cold treatment of germinating seeds necessary to induce normal flowe-

ring; that is, fairly rapid flowering under high light intensity, long days, reasonably high temperatures. If a short period (3-4 weeks) of vernalisation is given the strains vary very much in the length of time to the appearance of flowers and the size of the plant at flowering time. These differences show a general correlation with the climatic conditions of the area of origin: the strains from warmer areas flower more readily than those from colder areas.

All British strains obtained are winter annual and as far as the author knows no summer annual strains have been found in Britain. The author has in his possession reasonable quantities of seed of about 25 strains mostly from Britain but also from France and Spain (strains from the latter countries also seem to be winter annual).

It might be suggested that Arabidopsis from western and north western Europe is likely to be winter annual, since these areas have a maximum of rainfall during (usually) a mild winter, and that Arabidopsis from central Europe, with more extreme winters and a summer maximum of rainfall, will usually be summer annual.

HIRONO,Y.: Utilization of the increased vigor obtained through prolongation of the vegetative stage

Under the standard culture conditions in Columbia (continuous illumination) many weak mutants fail to produce satisfactory amounts of seed and crosses are rarely successful. Short day treatment (8 hrs daily illumination) help to overcome these difficulties. The transfer of these genes, causing poor viability, to a late genetic background is even more helpful. Generally they then produce good pollen which makes the crosses feasible and set enough seed to carry out all kinds of genetic analyses. As an example:

ch^1 mutant yields generally only a few hundred seeds under continuous illumination;

ch^1 gi^2 double mutant, however, gave a seed output of several thousand. Mutant pa can hardly be used as the female in crosses, but gi^2 pa is an excellent female or male parent.

REDEI,G.P.,and Y.HIRONO: Linkage studies

Only negative observation on linkage has been reported in Arabidopsis so far (cf. KUGLER 1951). Since the availability of properly mapped chromosomes is an elementary prerequisite of most critical genetic studies, the fragmentary information gathered in our laboratory will be presented here. Linkage intensities have been estimated in F_2 populations by the product method of FISHER and BALMAKUND with the aid of the tables constructed by F.R.IMMER (Genetics 15,81-98,1930,and ibid 19, 119-136,1934). This technique gives almost as accurate information as a test-cross if close linkage is tested in coupling. Since the production of test-cross material may be very limited in the case of weak mutants, while thousands of plants can be easily studied in F_2, the product method proved to be very useful. Collecting linkage information is not a very exciting and a rather tedious job thus most of our data resulted only as a byproduct of other studies. This also explains some of the incompleteness.

So far the number of linkage groups in Arabidopsis is higher, than that of the basic chromosome number. Present report will include information about the better known groups.

Linkage group 2.

G a gametophyte factor, no female transmission, relatively fair male transmission
er erecta type, slightly rounder rosette leaves, shorter petioles, compact inflorescence, blunt fruits. Good, clear marker.
hy excellent seedling marker, hypocotyl more than twice as long as normal, yellow green (pleiotropy).
py 2, 5-dimethyl, 4-aminopyrimidine requiring
vr_2 virescent
re veins are darker than interveinal tissues
as rosette leaves, especially the first are asymetric and lobed.
su yellow
se serrated leaves

Approximate map distances and gene order:

G 15.5 er 1.5 hy 5.5 py 2.0 vr_2 4 re 3 as 10 su

se has been tested so far with only er and in a F_2 population of a few hundred individuals no double mutants were detected. Some of the distance e.g. between leaf colour mutants have been estimated indirectly. Interference has not been observed. Additivity is reasonably normal.

Linkage group 3.

In group 3 seven genes were found to be linked. Most of them have not yet been studied in coupling and information about the linear order is not available.

Linkage group 4.

According to somatic recombination studies the centromere appears to be on the left of xv.

xv yellow-green, colyledons are yellow the leaf margin is yellowish. 3 m.u. from gi
gi^1, gi^2 affect photopheriodic response. The vegetative stage is prolonged 2 or 3 times in gi^1 and 4 or 6 times in gi^2 which results in giant rosettes. 25 m.u. from ch.
P pollen abortion factor. The heterozygotes for P produces about 25-30% abortive pollen and the homozygote is normal. About 3% recombination with gi and 12% with ch
ch^1, ch^2 Chlorophyll b is absent from ch^1 and reduced in ch^2.
Gm male gametophyte factor. No transmission in male and poor transmission in female. Between ch and pa.
pa dwarf and dark green. 8 m.u. from ch.

Besides these ed (leaf and flower shape) and tz (thiazole requiring) may be in the same linkage group.

REDEI,G.P.: A pollen abortion factor

A genetic factor (P) is responsible for the appearance of nearly 30 percentage shrunken pollen in a mutant line when heterozygous. Homozygotes for this factor are not distinguishable from the normal individuals and under good culture conditions shed pollen which stains well in over 99 percent. Heterozygotes segregate according to the ideal expected haploid ratios e.g. $P^+ : P = 504 : 505$. This indicates that factor P does not have any deleterious effect

on the chromosome which carries it and does not alter marker frequencies in F_2. These facts give some clues why the phenotypic ratio of the pollen itself fails to comply with 1 : 1 expectation. P effect can not be due to gene mutation or deficiemcy. Linkage to fourth chromosome markers indicates that crossing-over in an inverted segment can not account either for the 30 percentage pollen lethality. P factor has a suppressing effect on crossing-over in linkage group four. It appears to be a chromosomal interchange and it is under further genetic and cytological studies. P seems to be useful tool in various genetic techniques.

REDEI,G.P.: A female gametophyte factor

Factor G when present in repulsion with several second linkage group markers gives a distorted F_2 ratio. In case of close linkage the mutant class outnumbers that of the wild. In coupling with the same markers the mutant class is extremely reduced, only cross-overs show up. G itself does not have any other phenotypic effect. Transmission studies indicate that ovules do not transmit G while its male transmission may approach 30 percent. This in itself is unusual since most abnormalities generally have higher female transmission. In case of the most general type of megaspore development (monosporic, chalazal) 50 percent seed abortion would be the result if one of the two parental spores would fail to transmit. Actually, however, about 64 percent seed set could be established i.e. 28 percent more than expected. Second devision segregation with megaspore substitution (Renner effect) in the two basal products of the tetrad could produce, however, just the observed phenomenon. Preferential segregation would have also the same effect. Further analysis is in progress.

REDEI,G.P.: An episome resembling factor in Arabidopsis

The nature of an episome is not completely understood. It is claimed however, that it may exist in two states (integrated and autonomous) and it is an addition to the genome. Mutant im seems to satisfy these criteria. By various external factors the phenotype of the cells may be changed from white to green and vice versa as if a special element would be transient from one stage to another. The recessive gene responsible for this alternating function is chromosomal since tight linkage with another marker, vc_2 has been demonstrated. Irradiation with x-rays resulted in an increased greening as if the white stage would be an integrated state. Removal of the element suppressing the locus appears to restore normal chlorophyll production (autonomous state). The variegation can not be permanently removed, however, neither by irradiation nor by any other treatments of considerable phenotypic effect. It appears as if the radiation would dislodge temporarily a single controlling element or it would cause thinning out of several suppressor units. This displaced episome may later be reintegrated or the decreased number of the independent elements may reach the original level after several replications. The phenomenon may also be interpreted as a V-type position effect. The two interpretations are not mutually exclusive. Experiments designed to uncover the basis of this variegation are underway.

RÖBBELEN,G.: A gene induced plastom mutant

The plastom has been defined by RENNER as the sum of the extrachromosomal autonomous heritable elements within the plastid. In Arabidopsis, just as has been found for some other plants, plastom mutations can be induced by a nuclear gene in its homozygous recessive condition. Such a mutant that has been termed albomaculata (am) arose as a segregant in a second generation after X-irradiation of mature pollen grains with 32 kr, giving a ratio of 96 . variegated plants to 312 green ones. According to expectation about 60% of the latter green again segregated in the following generation after natural selfing, giving the same ratio of 3:1. The offspring of the variegated mutants without exception was variegated again, the portion of the white leaf area,however,in these mutant descendants was enlarged significantly, sometimes up to that extent, that entirely white seedlings were formed. These did never occur in the segregating progeny of a heterozygous green plant; here the homozygous recessiv individuals did not show that albomaculatio until the later stages of plant development.

The second observation, pointing towards the possibility of an extrachromosomal, plastomatic factor involved in this variegation, was, that its pattern within a leaf is strictly correlated to the margin of a cell descendence. Out of entirely white cells in the course of leaf development only white ones originate. Within these cells after staining with Rhodamin B in the light microscope nothing but these faintly coloured, vesicle like plastids were found without any inner differentiation. At the border between white and green leaf sectors,however, mixed cells were frequently seen with these small mutated plastids and the larger normal chloroplasts side by side. The same clear-cut difference between both plastid types could be demonstrated in electron microscope pictures, where normal, mutated as well as both plastid types jointly could be traced in one cell. No intermediates between both types occured.

The third evidence has been worked out in hybridization experiments, proving that this chloroplast mutation is independent of the genom, after it once has been induced in one plastid by the mentioned recessiv nuclear gene. Crossing the mutant reciprocally with the normal basic variety Enkheim (En), the status albomaculatus is inherited to the heterozygous hybrid strictly maternaly. No variegation occurs with the mutant as pollen parent. The following hybridization succession was done in order to establish whether or not the mutated status of the plastids may be maintained for several generations irrespective of the nuclear constitution. The variegated mutant with green and white plastids and the gene am in a homozygous recessive condition has been crossed as a female with a normal "En" plant. The heterozygous F_1 gives lethal white, green and variegated plants, that depends on the composition of the zygote. After crossing to normal, heterozygous and dominant homozygous appear 1:1. That means that likewise 50% of the variegated plants should carry the homozygous dominant allel of am, i.e. am^+. To show this, a random sample of these variegated plants is used as pollen parents in crossing with normal plants. According to the expectation, this F_1 is green. On the one side about 50% of these individuals, that is to say the heterozygous ones, give raise to segregating F_2-offsprings, whereas

on the other side really no albomaculatio appeared. The selfed progenies of the latter $\underline{am}^+$ $\underline{am}^+$ parent plants then were followed for four generations up to now. And though apparently no new plastom mutation had been induced because of the normal dominant nuclear constitution, the variegation could be maintained unchanged.

REDEI,G.P.: Crossing experiences with polyploids

Some aneuploids and polyploids have been found. With an autotetraploid (K-16), an approximately autohexaploid (M-10) and our standard diploid wild (W) some crosses were made resulting in the following observations.

Genotype or cross	No.of flowers pollinated	No.of fruits examined	Number of seeds Total	Normal	Percentage of normal seed
K-16	--	5	170	168	98.8
M-10	--	6	58	49	84.5
♀ K-16 x ♂ W	19	15	371	340	91.6
♀ W x ♂ K-16	30	26	292	8	2.7
♀ M-10 x ♂ W	21	19	139	6	4.3
♀ W x ♂ M-10	6	5	24	0	0.0
♀ M-10 x ♂ K-16	16	14	44	38	86.4
♀ K-16 x ♂ M-10	22	22	55	23	41.8

This incomplete information indicates that the cross compatibility of Arabidopsis polyploids is similar to that of other species, namely the seed set is better if the female is the higher chromosome number parent. It may be noted furthermore that the hexaploids are separated from the diploids with an almost complete sterility barrier while tetraploids and hexaploids can be intercrossed readily. It appears thus that gene transfer from diploids to higher ploids will be easier stepwise.

HIRONO,Y.: A genetic method for localization of chromosome defects

The distance between a marker and a chromosomal defect which affects the transmission of the involved strand can be estimated on the basis of the observed phenotypic ratio and the inherent transmission of the defect. If two markers are used the defect can be located and its extent may be determined. The transmission of defect may be different in the two gametophytes and homozygous condition may involve lethality.

Formulas have been constructed for F_2, based on the principle of maximum likelihood, in order to make possible the estimation of linkage intensities for such defect factors. The recovery and the lethality of the homozygotes have been considered both in coupling and repulsion phase for defect and markers concerned. Charts have been constructed which facilitate the estimation. If any two of the three variables (transmission, phenotypic ratio, map distance) is known the third can be directly read. Using the phenotypic ratio of the two

known marker genes, with a new mathematical formula the map of defect and markers can be constructed without direct laborious transmission tests.

REDEI,G.P.: An attempt to analyse the genetic fine structure of chromosomes

Electronmicroscopic studies reported from various laboratories indicate that the chromosomes of higher organisms contain several (up to 128) strands of deoxyribonucleic acid. In bacteriophage it is estimated that about 40 percent of the DNA is genetically active. It is not known, however, how much of the DNA of the cells of the higher organisms carries genetic information. If several of the multiple strands are genetically active we may expect sectors, variegation by the sorting out of mutated elements in mitosis. Variegation in somatic cells really can be observed both spontaneously and after treatments with chemical mutagens. If transversal multiplicity of the genetic strands is a reality we may expect delayed appearance of mutation especially after treatment with delicate agents. Bromouracil as a possibly appropriate mutagen has been chosen to test this hypothesis. The analogue has been incorporated into the agar medium and five consecutive generations were examined for somatic variegation or mutation. The summary result of the experiments is the following.

Generations	No. of single seed progenies	Mutants obtained	
		Stable	Variegating
B_2	137	--	--
B_3	1,278	1	2
B_4	17,655	3	--
B_5	38,015	2	--
Total	57,085	6	2

The data so far obtained do not allow very definite conclusions. There is no direct evidence that bromouracil is incorporated into the nucleus though this seems likely. It appears, however, that bromouracil is not highly mutagenic; the mutations observed in later generations are apparently not due to delayed segregation but rather to spontaneous mutation. Although the DNA may be arranged in multiple parallel strands apparently only one helix carries genetic information or any change in one of the "transversely multiple genes" has the same effect as an alteration of the entire bundle.

STEINITZ-SEARS,L.M.: Cytological studies in Arabidopsis

The chiasma frequency at MI in normal diploid Arabidopsis was found to be 1.83 per chromosome, or 82% ring and 18% rod bivalents.

The line K 16, a spontaneous autotetraploid, had 2n=20 chromosomes, determined at TI, and at least one individual had 2n=20 + 1. Meiotic configurations have not yet been worked out in detail.

MIO is very variable line containing hexaploids. Of six individuals analysed one each had 2n=17,18,22,and 30 while the

other two had more than 30 chromosomes. The meiotic configurations were complex: one individual had $4^{IV}\ 2^{II}$ in most of its PMC's but 10^{II} in some. Another plant had $2^{IV}\ 1^{III}\ 3^{II}$ in several cells. The following data were obtained incidentally in the search for the five primary trisomics.

Chromosome numbers in PMC's of 96 offspring ($F_2 + F_3$)
(from 1 cross 4n x 2n)

2n =	10	11	12	13	14	15	16	17	18	19	20
Frequency	40	31	5	1	3	3	4	3	2	1	3

The plants analysed were not sampled at random; on the one hand care was taken to include all seedlings marked "different from normal" in the sample, on the other more plants were sampled than analysable meiotic preparations obtained.

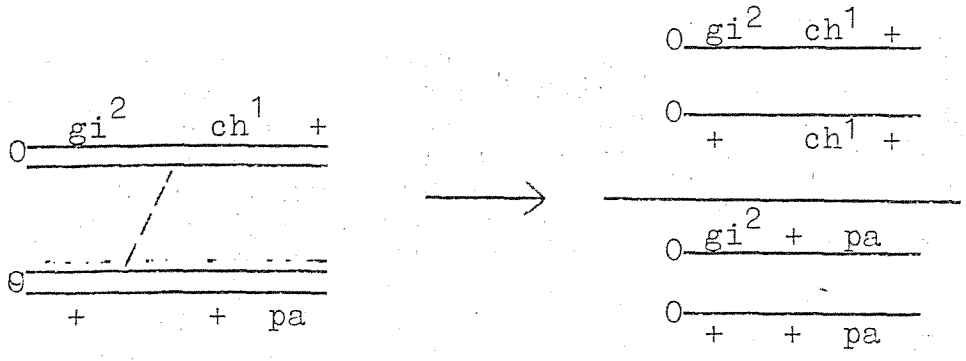
The paucity of seedlings with higher chromosome numbers may be due to the following three factors:

1. Pairing in triploids is often irregular with consequent lagging and loss of chromosomes.
2. Pollen containing the normal chromosome number of 5 is vastly favored over that with extra chromosomes.
3. Seedlings with unbalanced chromosome constitution may not survive long enough to be sampled in meiosis.

HIRONO,Y.,and G.P.REDEI: Somatic recombination induced by x-rays

Plants heterozygous for fourth linkage group markers with the genetic constitution $\left|\frac{gi^2\ ch^1\ +}{+\ +\ pa}\right|$ have been irradiated.

Some sectors have been subjected to genetic tests. The progeny of one yellow green sector (ch^1) segregated for gi^2 and gi^+ but pa was absent. Such a phenomenon could be due to somatic recombination at the four strand stage:



$gi^2\ ch^1$ and $gi^+\ ch^1$ were observed in nearly equal number indicating a lower transmission of the $gi^+\ ch^1$ strand. This may be interpreted as an unequal chromatid exchange. Though the end result of such a somatic recombination is similar to that of crossing over, the mechanism by which this originates is probably different.

Though the appearance of somatic sectors may be due to several causes, the evidence available may satisfactorily exclude the following possibilities:

1. Deficiency of the ch1 - pa region extending to 8 map units may result in a yellow sector (ch^1 uncovered in hemizygous) and would explain the absence of the pa marker. If the distance between left breakage point and gi^2 would be 29 m.u., and there would be no transmission of the defective strand (reasonable for a 8 m.u. long deficiency) an approximately 1:1 segregation for gi$^+$: gi^2 may be expected as a result of meiotic recombination in the tested progeny. In the second generation of some of the yellow sector progeny an abnormal phenotypic ratio of gi$^+$: gi^2 was observed, indicating some transmission of the affected strand. Since the actual distance between gi and ch loci is 24.8 ± 1.2 units and the defective strand has some transmission the hypothesis of claiming a deletion of the whole ch - pa region appears unwarranted.
2. Failure of disjunction of sister chromatids before mitotic anaphase (non-disjunction) may result in ch sectors but in such a case its progeny should not segregate for markers which were in repulsion in the original strands.
3. Translocation of the ch$^+$ pa region to a non-homologous chromosome which would segregate to the same pole as the non-translocated ch$^+$ pa strand may result in hemizygosity for ch^1, and could produce a yellow-green segment. Such translocation results in deficiency as #1, which has already been discussed.
4. A forward mutation from ch$^+$ to ch^1 and a simultaneously a reversion from pa to pa$^+$ and in addition the appearance of a factor causing distorted ratios would explain the observed facts but would require a coincidence of such a low probability that this hypothesis can hardly be considered seriously.

   Four additional cases under study appear to support the chromatid exchange interpretation. Somatic recombination has not been observed to occur spontaneously.

ARNOLD,C.G.: Diploid androgenesis

Androgenetic plants arise by development of a sperm nucleus in the egg plasm without participation of the egg nucleus. Androgenesis can either be produced by crossing different species or by killing the egg nucleus for instance by x-rays. As the plasm is more resistant against x-rays than the nucleus it may be possible to kill the nucleus leaving the plasm alive. An untreated sperm nucleus brought into such an irradiated plasm would have the chance to develop androgenetically. If the resulting androgenetic plant shows any differences they must be attributed to the irradiated plasm. Androgenesis thus could be a means for producing plasmatic mutants.

Haploid androgenetic plants are, of course, for this project unsuitable. It is desirable to produce diploid androgenetic plants, caused by pollinating with diploid pollen of a tetraploid plant. To receive tetraploid plants shoot apices of seedlings were treated with colchicine solutions in different concentrations from 0,02 to 0,1%. The effect was measured by counting the chromocentres in the resting nuclei of young petals. Treatment with a 0,08% solution of colchicine produced the greatest number of chimeras.

To find out the lethal irradiation dose for the egg nucleus

emasculated flowers were x-rayed with different intensities and soon after irradiation pollinated with haploid pollen. 2, 4, 6, and 8 days after irradiation the development of the embryos was checked. At 7000 r only few embryos reach the stage of the controls. The investigation will be continued raising the irradiation doses until the sperm nucleus develops by itself. Then irradiated flowers will be pollinated with diploid pollen.

ARNOLD,C.G.: An x-ray mutant with eight chromosomes

In X_1 (x-rayed as zygotes) among other plants which varied from the normal Arabidopsis thaliana one plant was extremely different. The mutant had smaller leaves and petals, nine extraordinary thin stems; it was nearly sterile and produced only few seeds, even after pollination with normal pollen. Cytological control showed 8 chromosomes. Further studies of the X_1-progeny will follow.

BOUHARMONT,J.: Action of x-rays on an autopolyploid series in Arabidopsis

Tetra-, hexa- and octoploid forms have been induced by colchicine in a diploid local variety of Arabidopsis. The reaction of the four chromosomal races was studied after irradiation of seedlings by several doses of X-rays (5, 10, and 20kr). Growth retardation and morphological aberrations of the treated plants were more marked after stronger irradiations and some differences appeared between the various forms. The dosis of 20 kr alone was partly lethal for diploid and tetraploid forms, while all hexaploid and octoploid plants died with 20 kr and some others with 10 kr. The frequency of chlorophyll mutants is investigated at the second generation. The first observations indicate a decrease of the number of the mutant seedlings with increasing chromosome number.

AUSTIN,R.B.: Heritable variation after phosphorus deficiency

The amounts of phosphorus in pea and watercress (Pisum and Roripa spp.) seed as affected by the nutrition of the parent plants were found to influence the growth and yield of the plants raised from such seed, both on phosphorus deficient and normal media. It further appeared that the plant-to-plant variability (as measured by $\frac{S(x-\bar{x})^2}{n}$, when x = log fresh weight of plant) was greater in populations grown from phosphorus deficient than from normal seeds, both in deficient and normal media.

These results were thought to warrant further testing in order to discriminate between the possibilities that (1) heritable variation was generated by the phosphorus deficiency treatments and (2) plants grown in conditions of phosphorus stress revealed latent genetic variation in response to phosphorus deficiency. Arabidopsis thaliana, strain ESTLAND, is being employed for this work, and it is being grown in a glasshouse in pots containing vermiculite and supplied with Hewitt's nutrient solution modified to contain either 0.4 or 4.0 milligram equivalents of phosphorus per litre. The cultures are not aseptic.

RÖBBELEN,G.: Sensitivity pattern of germination in chemomutagenesis

After presoaking dry seeds of Arabidopsis thaliana (race Enkheim) on wet filter paper for different intervals the effect of mutagenic treatment with some alkylating chemicals, urethans and X-rays was measured with the M_2-chlorophyll-mutation test. According to our expectation the mutation rate was increased with increasing time of presoaking, though in the chemical treatments certainly dry seeds would absorb the maximum amount of the mutagen. The maximum of this intensifying effect of presoaking, however, appeared at a different point of the curves in the three cases mentioned. With ethyl methanesulfonat (EMS) as well as with other alkylating agents (diethyl sulfate, or ethylene imine) the highest mutation frequency occured after 15 hrs presoaking, while phenyl ethyl urethans had a maximum efficiency after 48 hrs and X-rays after 24 hrs of the same pretreatment. This is indicafive for a different sensitivity pattern in the course of germination, when mutagens with different mechanism of reaction are applied. A more detailed analysis of this effect is thought to be valuable for a better understanding of the mutation process in higher plants. $\bar{x}$ (PEU)

Table: Influence of presoaking on the number of segregating M_2-families (in % of the total) after mutagenic treatment

Interval of presoaking (hr)	Treatment		
	EMS (1%;2hr)	PEU (0.09%;8hr)	X-rays 16kr
0	3.3	2.2	0.5
3	3.1	0.9	1.9
6	4.1	0.6	2.1
9	16.3	1.5	3.1
12	44.2	1.4	3.8
15	58.7	1.2	4.7
18	56.3	0.9	5.0
20	55.9	1.8	4.8
24	48.1	1.6	5.9
36	39.6	1.9	4.9
48	36.4	3.2	4.0
60	40.6	2.1	3.9
72	38.0	2.3	4.3

VELEMÍNSKÝ,J., T.GICHNER, V.POKORNÝ, and J.ŠVACHULOVÁ: Mutation induction in Arabidopsis

In the various mutation experiments the frequency of induced mutations has been scored according to the method of MÜLLER (1963) identifying the embryonic and chlorophyll abnormalities in the immature pods of M_1-plants. The main results are shown in the following table.

Mutagen	Concentration or dose	Segregating M_1-plants (%)	Mutant seeds (%)
EMS	0.025-0.1%	11-93	1.03-29.0
EDTA	10^{-2} mol	1	0.5
EMS + EDTA	0.025-0.1% + 10^{-2} mol	10.3-95.9	1.5-22.0
Lasiocarpine	2×10^{-2} mol	14	2.8
Monocrotaline	2×10^{-2} mol	9	2.5
CB 3025 *	10^{-1} mol	4	0.7
CB 1592 **	3×10^{-2} mol	73	25.9
NaN_3	$2,5 \times 10^{-3}$ mol	1	0.4
DNP	10^{-3} mol	2	0.4
Methyl nitroso-urea (MNU)	3×10^{-4} mol	98.6	55.2
Ethyl nitroso-urea (ENU)	3×10^{-4} mol	95.0	45.7
MNU (15°C)	3×10^{-4} mol	96.9	30.2
ENU (15°C)	3×10^{-4} mol	16.0	2.3
MNU + NaN_3	3×10^{-4} mol + $2,5 \times 10^{-3}$ mol	94.3	25.8
ENU + NaN_3	3×10^{-4} mol + $2,5 \times 10^{-3}$ mol	12.1	1.4
X-rays (dry seeds)	20-72 kr	11.7-35.9	4.7-23.7
X-rays (24 hrs pre-soaked seeds)	5-18 kr	19-42	5.01-22.4
Control (H_2O)	-	1.2	0.4

Duration of treatment: 24 hrs; temperature: 25°C, if not otherwise stated.

* CB 3025 NN-di(2-chloroethyl)amino-l-phenylalanine
** CB 1592 S-2-chloroethylcysteine hydrochloride

JACOBS,M.: Studies on the activity and specificity of some chemical mutagens

EMS is the most efficient mutagen giving 5-10% of chlorophyll mutations (for 1000 M_2 plants). The effect of different concentrations has been studied especially by measuring the fertility of the M_1-plants.

MMS at the concentration of 0.2% and 2hrs treatment is a very poor mutagen increasing the mutation frequency only to 4-10 times the spontaneous rate. Apparently it is, however, not so toxic in M_1 as supposed in literature.

For EMS and MMS different mutation spectra (chlorophyll mutations) have been recorded; this points towards an alkyl group specificity in these mutagens.

In preliminary experiments BUDR and IUDR have been shown to be incorporated into the nucleus and to have mutagenic effects. New large scale cultures are in M_2 at present.

ARNOLD,C.G.: Preliminary tests with Contergan (N-phtalyl glutamic acid imid)

To test whether Contergan is a mutagenic substance and has an influence also on the development of plants, the following experiment was carried out: Flowers and pods of Arabidopsis thaliana, race Enkheim, were treated with a solution of Contergan (1%). The stems were split and one half was cut at the base and the end dipped into the solution for 42 hrs (cf. RÖBBELEN 1962c). Controls were treated with water. The development of the embryos treated with Contergan was in some instances retarded and sometimes stopped in early stages. Young embryos seem to be more sensitive than older. The treated plants produced fewer seeds than the controls. As the number of plants was low the experiment has to be repeated.

RÖBBELEN,G.: Futile attempts of mutation induction with some base analogues and antimetabolites

With reference to the experiences in microorganism some years ago mutation experiments had been conducted, the negative result of which might be of value for those interested in similar subjects. Base analogues and antimetabolites have been applied in concentrations up to sublethal ones (about 10% survival), using treatments of dry seeds or buds, the latter with the tongue slit method (cf. RÖBBELEN 1962c).

Table:

Mutagenic substance	Seed treatment			Bud treatment		
	Concentrat. of mutagen	Number of M_2-families tested: Total	Segregating %	Concentrat. of mutagen	Number of M_2-families tested: Total	Segregating %
6-Methoxy-purine	0,1 %	597	0.2	0.1	741	-
Caffeine	1/10 mol	583	0.5	1/50 mol	870	3.9
Hypoxanthine				0.07 %	593	0.3
5-Bromouracil	0,2%	284	1.2	0.1%	677	-
8-Azaguanine	1/500 mol	277	2.8	1/1000 mol	808	2.1
8-Azaxanthine				1/200 mol	1013	-
Benzimidazole	1/100 mol	594	0.3	1/1000 mol	1008	0.2

Though the primary action on the M_1-plant development was more or less drastic, the increase of the spontaneous mutation rate (this being about 0.1% measured in the M_2 chlorophyll-mutation test) was only low and in no instance specific. These results, being summarized in the following table apparently point towards the fact, that in higher plants the pool of DNA precursors can hardly be depleted in this way, to achieve specific genetic effects.

HIRONO,Y.,and G.P.REDEI: Somatic screening technique for alterations at specific loci

According to the literature, Arabidopsis has been used in relatively extensive experiments to study the overall mutagenic effect of various agents.

In our laboratory attempts were made to investigate mutation rate at a specific locus, ch. The following genetic set up proved to be useful:

+	ch	+
gi	25 + 8	pa

Genetic alterations at the ch locus may appear in leaf sections extending sometimes to half leaves, or even to shoots. With a simple paper chromatographic test, or spectrophotometric analysis the identity of the sector can be easily established. Since the distance between pa and ch is short, if seed progeny can be obtained from the mutated sector, the old and the new allele can be easily separated. Such a technique is of special value in comparing the specificity of x-rays and ethylmethane sulfonate. According to the experience obtained in our material ethylmethane sulfonate induces many yellow green sectors but from 16 analyzed cases none was ch, but some kind of dominant effect of unknown loci. All x-ray produced yellow green sectors examined in this material turned out to be chlorophyll b deficient i.e. locus specific. It must be noted, however, that majority of these sectors did not arise through a mutational event. This can be determined only by progeny test which distinguishes somatic recombination, deletion, non-disjunction etc. from mutation. Seeds treated under identical conditions give rise to sectors of similar size (half leaf or one fourth leaf) and from this the number of functional cells in the apical meristem can be estimated. The pattern of differentiation is also monitored by the development of the sector.

VELEMÍNSKÝ,J., T.GICHNER, V.POKORNY,and J.ŠVACHULOVÁ: Structural and chemical analysis of chlorophyll mutants

In several lethal chlorophyll mutants (phenotype: albina, xantha, chlorina) the ultrastructure of plastids has been studied electrone microscopically in cooperation with Dr.DÖBEL, Gatersleben. The following mutants with different blocks in the course of plastid development have been analyzed:

1. Mutants with blockade before the development of prolamellar body (2 albinas).
2. Mutants forming the prolamellar body as a last step of development (3 xanthas).

3. Mutants forming only long non-differentiated lamellae running through the whole plastid (3 xanthas and 1 chlorina).
4. Mutants forming abnormal grana-like structures (1 chlorina).

In group 2 two different types of prolammellar bodies were observed, differing in the shape and constituents from which they were formed. The prolamellar body of xantha 2695 is very similar to that already known in the literature. It is predominantly composed of vesicles which also form chains running out from the prolamellar body. The prolamellar body of xantha 1432 is formed by short and long tubuli (lamellae?), markedly grouped in clusters, often surrounded by a continuous lamellae and thus sharply separated from the stroma. The reason of these differences is being studied at present.

II. The content of the free amino acids was studied in about 40 different chlorophyll mutants of the albina, chlorina or xantha type each lethal in the cotyleadon stage. No qualitative differences were found. In the majority of mutants an abnormal accumulation of amides, especially asparagine or the basic amino acids arginine, histidine and lysine, valine, tyrosine etc., has been observed. Some differences, however, between mutants have been found in the quantitative content of some amino acids. Extensive analyses on the occurence of these differences are underway.

RÖBBELEN,G.: A chlorophyll mutant without plastid grana

Generally the deficient synthesis of leaf pigments in the so-called "chlorophyll mutants" coincides with a more or less drastic reduction in chloroplast differentiation. According to some initial electrone microscopical investigations RÖBBELEN (1959) established a model including the various blockades in the reaction chain of development of chloroplast ultrastructure known up to that time. This model proved to be valid for several other chlorophyll mutants investigated in Arabidopsis in the meantime.

A principally new aspect, however, resulted from the electrone microscopical analysis of a new xantha mutant. In this genotype,V 81,chloroplasts were found for the first time with a genic blockade at the last stage of differentiation, i.e. just before grana formation. The chloroplasts, regular with regard to all the residual structural elements, are traversed by entirely uniform lamellae. These lammellae exist in the same number as known for the normal chloroplast, but only fail to organize grana- and intergrana areas.

The mutant chloroplasts are photosynthetically active; under certain conditions of plant growth starch granules can be deposited. At any rate the mutant can grow up to maturity, though rather delayed. A more thorough ultrastructural and physiological analysis of this genotype appears to be of special interest for an understanding of development and function of the grana in chloroplasts of higher plants.

JACOBS,M.: On the induction of biochemical mutants

Biochemical mutants have been obtained by means of sterile culture of M_2's after mutagenic treatment. The special requirements (amino acids, vitamins, purins and pyrimidines) are

being individually established. Mutants resistant to copper have also been found; the test for the genetical basis of resistance shows a single gene dependence. Moreover mutants for resistance to DDT have been selected after spraying or dipping the young plants in a 1:500 solution of the commercial product.

FEENSTRA,W.J.: Genetical investigations with induced biochemical mutants

Biochemical mutants were induced by treatment of seeds with ethylmethane sulfonate. Of the total of seedlings, growing on sterile mineral media, in M_2 as many abnormal ones as possible were raised on a "complete" medium. In M_3, and, if necessary, in following generations, it was tried to establish the specific requirements of the mutants obtained. In this way it could be established with certainty, that 8 mutants were deficient for thiamine production; four of these grew if the pyrimidine part of the molecule was added to the substrate, one needed the thiazole moiety, whereas three only grew when the complete vitamine was given.

Tests of allelism were carried out by making the heterozygous combinations of the different mutants. As could be expected, always a non-deficient F_1 resulted when mutants, having different requirements, were crossed. Within the thiamine group two different loci could be established. The pyrimidine group has not been completely investigated as yet; the situation, however, seems somewhat complicated, as two mutants, if crossed with a third one, both yielded a deficient F_1, but if crossed with each other gave a non-deficient offspring. Possibly, adjacent cistrons and deletions of different size play a role here.

Next to the thiamine mutants, it may be that a few mutants were obtained in which the biosynthesis of uracil, phenylalanine or nicotinic acid is blocked. Further study is needed.

In view of the relatively numerous occurrences of thiamine deficiency it was decided to select more particularly for this kind of mutants. In the M_2 plants are selected based on their phenotype when growing on mineral medium (lack of chlorophyll in the first leaves), and then raised on a thiamine containing substrate. In the M_3 it is tested whether true mutants are obtained, and, if so, what is their specific requirement. In this way 9 pyrymidine-less, 5 thiazole-less and 2 thiamine-less mutants have been obtained. At the moment allelic relationships among these are being studied; at the same time the search for more mutants of this kind is continued.

REDEI,G.P.: D-alanine, a specific inhibitor

The L forms of amino acids usually occur in the tissues but in general, plants can utilize both enantiomorphs nearly equally. Alanine is an exception, however. The L form alone may function as a poor nitrogen source, and also may slightly promote growth if inorganic nitrogen is supplemented with it. D-alanine, however, is a powerful inhibitor if added to an inorganic nitrogen containing medium. The D-alanine inhibition is completely released by L-alanine and the commercially available D, L-batches behave more or less identically with the L enantiomorph.

The result of a typical experiment:

Alanine (mg per l) supplemented to the medium	Fresh weight %
basal, none	100.0
40 L	109.9
40 D	16.8
40 L and 40 D	99.5
20 L and 40 D	69.2
10 L and 40 D	50.2
5 L and 40 D	40.9
2 L and 40 D	32.8

No other amino acid fully reverted the inhibition but several allowed partial release. A preliminary paper chromatographic test indicated that Arabidopsis cultured on a medium with D-alanine as the sole N-source accumulated large amount of free alanine while several other free amino acids were absent or greatly reduced. It appears that D-alanine may be useful in studies of protein synthesis of Arabidopsis.

REDEI,G.P.: New bioassay for vitamin B_1

The discovery of mutants responding to thiamin and to both the pyrimidine and thiazole moieties respectively makes it possible to assay directly thiamin + pyrimidine and thiamin + thiazole with py or tz mutants respectively. Differential response of the two types of mutants toward an unknown extract permits the calculation of all three substances independently. An Arabidospis bioassay offers the following advantages over a microbial technique.

1. Culture technique and the medium are extremely simple
2. Sensitivity is higher than that of fungi and not lower, perhaps even higher than that of lactobacilli (5 millimicrogram B_1 is detectable) with Arabidopsis.
3. Almost no equipment is needed.
4. Contamination or backmutation does not endanger the reliability.
5. Very little effort is needed to maintain the stocks. The seed can be stored conveniently for two years.
6. Evaluation of the results can be done over a longer period of time.

Possible disadvantages:

1. A test requires about two weeks
2. Arabidopsis may be more sensitive to certain toxic substances in the crude vitamin extract than fungi or bacteria.
3. If the extract contains thiamin, and nearly equal amounts of both pyrimidine and thiazole only the sum of these substances can be estimated.

In conclusion: under various circumstances an Arabidopsis bioassay may be superior to the animal or microbial tests available.

REDEI,G.P.: Additional thiamin requiring mutants

It has been already reported briefly (REDEI 1960,1962a) that a pyrimidine and also a thiazole requiring mutant have been found. Since, two more thiazole requiring mutants have been identified in 5-bromouracil treated material. It is unlikely that the analog played any part in the induction, while contamination is unlikely because the material was genetically marked. The three thiazole requiring mutants are allelic. More recently a new allelic pyrimidine mutant was also detected in an x-rayed progeny. Thus the total number of our thiamin responding mutants is 5. The pyrimidine locus belongs to linkage group 2, the thiazole locus is perhaps loosely linked to one of our fourth linkage group markers. In higher plants this material provides unique opportunities to study such phenomena as reverse mutation, pseudoallism, allelic complementation etc.

HIRONO,Y.: Effect of plant age on pigment content of chlorophyll b deficient mutants

The chlorophyll content per fresh weight of wild type and two ch mutants (ch^1 and ch^2) of different ages were measured. The plants cultured in pots were exposed to sunlight during the day and illuminated with 200-watt incadescent bulbs during the night (December - January). In the greenhouse the temperature ranges were about 20^0 - 30^0C.

For chlorophyll analysis whole plants without root and flower were used. The results are shown in the Table. The amount of chlorophyll per fresh weight is higher in the younger stage than in the old stage.

	Age in days	Chlorophyll (microgram/g. fresh weight) a M	a S.E.	b M	b S.E.	Ratio a : b. M	S.E.
Wild	20	1,131	± 92	422	± 49	2.75	± 0.11
	30	736	± 51	255	± 23	2.91	± 0.09
	40	766	± 58	260	± 26	2.95	± 0.09
ch^1	20	495	± 23	*8	± 4		
	30	416	± 29	*0	± 4		
	40	396	± 8	*6	± 3		
ch^2	20	657	± 53	72	± 4	9.26	± 0.63
	30	560	± 50	67	± 9	8.56	± 0.42
	40	547	± 21	74	± 7	7.63	± 0.49

The pigment content was measured by the technique of RÖBBELEN (1957a). *This small amount is not considered as real evidence for the presence of the pigment and it is rather an unavoidable error of this kind of technique.

HIRONO,Y.: Effect of glucose on chlorophyll content per fresh weight

The chlorophyll content per fresh weight of the wild-type and two ch mutants (ch^1 and ch^2) grown in test tube under natural light increases with the glucose concentration of the medium. The ratio between chlorophyll a and b is fairly constant.

The dry matter percentage also increases with the glucose concentration (the last column of Table).

The chlorophyll content based on dry weight of plant is not much affected by glucose if it is supplied in concentrations below 2 percent.

	Glucose (%)	Chlorophyll (microgram/g.fresh weight) a M	a S.E.	b M	b S.E.	Ratio a : b M	Ratio a : b S.E.	Percentage of dry matter M	Percentage of dry matter S.E.
Wild	0	780	± 21	264	± 11	2.96	± 0.06	7.5	± 0.1
	0.5	794	± 12	283	± 6	2.80	± 0.02	7.8	± 0.1
	2.0	1,095	± 54	385	± 20	2.85	± 0.01	10.1	± 0.3
	4.0	1,386	± 31	559	± 10	2.78	± 0.02	17.1	± 0.4
ch^1	0	540	± 10	*7	± 1			7.6	± 0.2
	0.5	567	± 15	*2	± 5			8.3	± 0.2
	2.0	703	± 20	*8	± 3			10.7	± 0.4
	4.0	805	± 23	*3	± 1			15.4	± 0.9
ch^2	0	577	± 7	39	± 1	15.11	± 1.31	8.2	± 0.1
	0.5	601	± 15	43	± 1	14.28	± 1.10	8.6	± 0.1
	2.0	764	± 13	53	± 5	14.71	± 1.13	10.3	± 0.3
	4.0	930	± 43	80	± 6	11.77	± 0.85	15.1	± 0.4

The pigment content was measured by the technique of RÖBBELEN (1957a).* This small amount is not considered as real evidence for the presence of the pigment and it is rather an unavoidable error of this kind of technique.

B. LABORATORY RESEARCH COMMUNICATIONS +)

Australia

C a n b e r r a C i t y : R.D.BROCK

Induced quantitative variation in Arabidopsis thaliana. A number of mutagenic treatments (gamma rays, thermal neutrons, EMS, 2 Cl-EMS, DES, nitrous acid and nebularine) are being compared for their efficiency in inducing mutations, affecting quantitatively inherited characters (growth rate and flowering time). These studies are also aimed at confirming and extending the general hypothesis developed with other species, viz. that the response of any quantitatively inherited character to random mutation is an increase in the variance and a shift of the mean away from the direction of previous selection.

Radiation sensitivity of Arabidopsis races. Differences in sensitivity to gamma radiation have been detected among races of Arabidopsis thaliana. An investigation of the genetic basis of the sensitivity differences is in progress.

Belgium

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

Investigations on the differential activity and specificity of some chemical mutagens (cf. p.19), in particular on the mode of action of a treatment with HNO_2.

Research concerning the genetical basis of radiosensitivity and mutability; striking differences between different races have been observed.

Studies on biochemical mutants (cf. p.22)

L o u v a i n : J.BOUHARMONT

In the course of mutation experiments with an autopolyploid series, the following points will be envisaged : occurence of different types of mutants, viz. chlorophyll deficient, sterile types etc., heredity of polyploid chlorophyll mutants, causes of the differences in sensitivity, action of gamma-rays and radiomimetic agents. The characteristics of non irradiated polyploids will be compared (cf. p.17)

Canada

H a l i f a x , N.S. : O.P.KAMRA

Different mutation spectra after treatment with various chemical mutagens have been realized.

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Czechoslovakia

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

Chemical mutagenesis. In pursuit to the mutation experiments reported on page 18 we are studying the effcet of some chemomutagens (mainly of the urea group) in combination with metabolic inhibitors (DNP, NaN_3) as well as the action of their decomposition products.

Biochemical mutants. The studies referring to this topic are concerned with artificial nutrition, identification and possibility of normalisation of nutritionally deficient mutants from the group of chlorophyll lethals. The genetic relations between mutants of this group are being studied by hybridisation of heterozygotes (cf. p.21).

B r n o : I.CETL

From natural populations of Arabidopsis, collected at several localities in Moravia, ČSSR, lines are being extracted differing in developmental characters and some more quantitative features. With these lines problems of quantitative inheritance will be investigated, partly even with making use of mutagens.

Germany

B e r l i n : J.BARTHELMESS

Correlation in quantitative characters. After crossing two natural races the correlation with regard to flowering time and number of branches of the inflorescence has been studied in different selection lines.

B o n n : W.GOTTSCHALK

Investigations will be done on the problem of homologous mutations.

E r l a n g e n : C.G.ARNOLD,D.CRUSE

Induction of mutations and diploid androgenesis (cf. pp.16, 17 and 20).

G a t e r s l e b e n : A.J.MÜLLER

Comparative investigations on the efficiency, biological effects and mode of action of various mutagens have been conducted. The mutagenic activity is measured mainly by the frequency of recessive lethals (embryonic lethals and chlorophyll mutations). In this connection genetical investigations with lethal factors, studies on the chimerical structure of M_1-plants and on the diplontic selection are carried out. Using x-rays the effects of moisture, storage, and oxygen post-treatment on the mutation frequency were studied. Experiments were performed to determine the radiosensitivity at several stages of development (including developing embryos). In studies with chemical

mutagens the following compounds have successfully been used so far: EMS, HN 2, HN 3, myleran, 1-methyl-3-nitro-1-nitrosoguanindine, and methylnitrosourea. After treatment with $NaNO_2$, nebularine, JUdR, BUdR, amethopterine, hydroxylamin and streptomycin no significant increase in the lethal mutation frequency was observed. In addition to other methodological investigations some questions on the physiology of germination were studied.

G ö t t i n g e n : G.RÖBBELEN

The studies underway are concerned with:
Mutation induction with different chemicals during various stages of plant development recorded with the chlorophyll mutation test (cf. p. 18).
Mapping of chlorophyll genes by hybridisation and cytogenetic techniques (trisomics).
Investigations on interaction between different chlorophyll genes at the level of morphogenesis and developmental physiology.
Studies on structure and development of chloroplasts in chlorophyll mutants by means of electrone microscope (cf. p.22).
Experiments on the mutability and nature of the plastom (cf. p.12).

H a n n o v e r : H.RUNDFELDT

A computon is used for solving biometrical problems in plant breeding. Arabidopsis seems to be a suitable object in such pilot experiments.

H a n n o v e r : H.GLUBRECHT, W.SCHEUERMANN

The investigations deal with physiological and morphogenetic effects of low doses of ionizing radiations.

K ö l n : K.NAPP-ZINN

The recent experiments are concerned with analyses:
n the genetical basis of vernalization requirement (number, dominance, epistasy and linkage of the responsible genes),
on the distribution of the requirements of after-ripening and vernalization in natural populations,
on the relation between seed age and plant development.

L i m b u r g / Lahn: F.LAIBACH, F.J.KRIBBEN

The interspecific cross compatibility between A.thaliana, A.suecica, A.pumila and Cardaminopsis arenosa is being examined.

M a i n z : B.HACCIUS, H.REICHERT

X-ray dependent teratogenesis in embryos of Arabidopsis is being investigated in comparison to that in other species of dicotyleadons. Histological observations of the x-ray damaged shoot apex provided new aspects for an understanding of the x-ray induced embryo abnormalities, studied by REINHOLZ (1959).

Great Britain

A b e r d e e n , Scotland: A.D.MC KELVIE

Research on linkage. Using segregation results from many crosses a linkage map is being built up.

B a n g o r , North Wales: H.A.P.INGRAM

Experiments on ecological interference with some mutants are proposed.

B e l f a s t , North Ireland: D.J.CARR

Linkage research on the genes pd ap se no , which from Dr. MC KELVIE's data would appear to exist in one linkage group of the strain Estland.

An attempt to adapt the same strain to different edaphic conditions and then to investigate the genetic changes which have taken place. There has been a good deal of recent work on natural populations of other plants in which it has been shown that strains or races exist which are adapted to peculiarities of the soil, but no genetical work has been done on this apart from the pioneer investigation of Hutchinsia by MELCHERS.

R o s l i n , Scotland: D.RATCLIFFE

Observations on the physiology of flowering (cf. p.8)

W e l l e s b o u r n e , Warwick: R.B.AUSTIN

Heritable variation after phosphorus deficiency (cf. p.17).

India

N e w D e l h i : N.MAHESHWARI, R.D.IYER

Work with chemical mutagens, analogues of purines and pyrimidines is contemplated. Further cf. page 8.

Italy

P a v i a : R.CIFERRI

Several strains of Arabidopsis thaliana are used as test-plant to measure the activity of growth-depressing chemicals, like "Phosfon D" and "CCC".

P i a c e n z a : C.LORENZONI

Investigations on the effects of treatments with physical and chemical mutagens on quantitative characters are planned.

Netherlands

W a g e n i n g e n : C.R.BHATIA

Influence of genotype on mutagen induced variation for quantitative characters. The different races of Arabidopsis thaliana are the product of natural selection over long period and differ widely in their genotypes, especially with respect to flowering. Days to flower is a quantitative character, comparatively easy to score and under controlled conditions the environmental variation is very small. It is planned to use single plant seed samples of a number of races, differing appreciably in days to flower, under the same photoperiodic and other physiological conditions. Following treatment with EMS and X-rays, only normal looking plants in M_2 and M_3 generation will be scored for flowering, thus eliminating all chlorophyll and morphological mutants. Statistical parameters of each population will be estimated. Extreme plants on + and - side of the mean will be selected to extract lines on both sides of the mean. - Aim of the experiment is to illicit information if the phenotypic release of genetic variability for quantitative characters, following treatment with mutagens, is more in one direction, and if so, whether the "easy direction" is correlated with the genotype of the parent material.

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

Studies with induced biochemical mutants (cf. p.23).

W a g e n i n g e n : J.H. VAN DER VEEN

Arabidopsis is used as an object in studies on the effect of various selection methods in breeding of self pollinators. The main character investigated is vernalization response. After crossing two lines, which flower 7 weeks after sowing in F_2 large variation arose indicating that this character can be treated as quantitative.

W a g e n i n g e n : L.ZELLES

Several mutant strains are being selected to test gene-dependent differences in plant reaction to day length and vernalization temperature.

U S A

C o l u m b i a , Missouri: G.P.REDEI, L.M.STEINITZ-SEARS, Y.HIRONO

The studies underway are the following:

Physiological genetic studies on nutritional mutants. Further attempts are being made to locate more precisely the genetic blocks of thiamin synthesis in the thiazole and pyrimidine requiring mutants available. Linkage information is being sought.

Physiological investigations on the functional anomalies of a gene causing somatic variegation. Previous studies

demonstrated that the two extreme phenotypes of mutant im are not due to mutation of the gene but rather to an alteration of its function. It appears that the change is mediated through an independent regulatory element. Chemical and physical factors affecting the change and the possibility of a complete removal of that element is being investigated.

Analysis of gametophyte factors. Genetic damage of the chromosomes generally result in poor male transmission and provides means for estimating the extent of the defects if used with multiple markers. A female gametophyte factor is being investigated aiming to detect megaspore competition and to study the possibility of half tetrad analysis.

Somatic recombination. Marker exchange at the four strand stage has been demonstrated in our laboratory. Investigations are underway to estimate the frequency and the basic mechanism of the event.

Studies on gene structure. Multiple allelic loci have been detected in Arabidopsis and with appropriately marked chromosomes intragenic recombination is being studied at two loci.

Mutational mechanism. Genetic analysis of chromosome fine structure, "multiple strandedness" is being attempted by using base analogue mutagens.

Genetic and physiological studies on chlorophyll b deficient mutants. Multiple alleles at the ch locus are under study to explore eventual allelic complementation. Role of chlorophyll b in photosynthesis will be investigated with special regard to H^+ donors in photophosphorylation.

Map construction. Continuous efforts are being made to develop Arabidopsis into a more useful genetic tool. New mutants are studied for linkage. Trisomics are also adopted to facilitate this effort.

Trisomics. Continuation of the study and characterization of trisomics with the possible development of secondary trisomes.

Chromosomal aberrations. Analysis of chromosomal aberrations in genetically suspect lines. Two translocations have been found in one line.

Gametophyte factor. Histological and cytological study of E.M.C.'s and embryo sacs in the line with a gametophyte factor.

Pachytene. Study of the earlier stages of mitosis and development of a pachytene technique with special regard to heterochromatin and the relationship of the centromere to heterochromatin.(cf. pp.9 ff.).

E a s t L a n s i n g , Michigan: M.W.ADAMS

Studies on growth and development of several natural races (cf. p.7).

R a l e i g h , North Carolina: D.F.MATZINGER

Herbarium collections have been looked through to find specimens which have been collected in North Carolina. According to these data this spring seed collection shall be made in the field preparatory to initiating genetic studies.

S y r a c u s e , N.Y.: F.A.VALENTINE

For studies on cell wall formation instead of trees Arabidopsis mutants are to be selected with changes in cellulose and other cell wall constituents.

S a l i n a s , California: E.J.RYDER

Up to now data are gathered with regard to the behavior of Arabidopsis strains under the local green house conditions. Future research is planned in the field of quantitative inheritance.

W a s h i n g t o n , D.C.: W.SHROPSHIRE

This laboratory is working on photoperiodic responses in Arabidopsis thaliana and the physiology of germination induced by photomimetic substances.

C. TECHNIQUES AND MATERIAL

Sterile culture of Arabidopsis on agar medium

J.VELEMÍNSKÝ, and T.GICHNER

The cultivation of Arabidopsis thaliana on inorganic agar medium under aseptic conditions is a very useful method for physiological genetics. After the first detailed description of this method (LANGRIDGE 1957b) the cultivation of Arabidopsis on artificial medium became routine in many laboratories. It is, however, evident that all these laboratories have their own modifications which are given both by the experience of the research workers and by their equipment. In addition to this, in laboratories, dealing with the identification of nutritionally deficient mutants, different modifications of the maximal medium are used. The method used in our laboratory is essentially derived from the above mentioned work of LANGRIDGE, as well as from KVITKO (1960) and MÜLLER (unpubl.).

The recipe for the preparation of the nutrient medium first sets the following 7 stock solutions:

A) KNO_3	20 g
B) $Mg\ SO_4.7H_2O$	3 g
C) $Ca\ (NO_3)_2.4\ H_2O$	10 g
D) $K_2\ HPO_4$	3 g
E) $Fe\ SO_4.\ 7\ H_2O$	1.4 g
F) EDTA (Na,-salt)	10 g
G) trace elements, consisting of:	
$H_3\ BO_4$	1.5 g
$Mn\ SO_4.\ 7\ H_2O$	0.9 g
$Zn\ SO_4$	0.13 g
$Cu\ SO_4.\ 5\ H_2O$	0.05 g
$(NH_4)_6\ Mo_7\ O_{24}$	0.5 g

Stocks A to G each made up to 1 litre with aqua dest. The solution E of $Fe\ SO_4.\ 7\ H_2O$ must be prepared just before the preparation of the nutrient solution. The other 6 solutions (A-D and F-G) may be kept in a refrigerator for several months.

The final nutrient solution should be made with the following amounts and in the following sequence:

50 ml of each A + B + C + D + E + 5 ml F + 1 ml G

To the solution obtained 8 g of agar is added and the total made up to 1 litre with bidestilled water. If the agar is powdered no special care is necessary, but if in chunks, it must be broken into small pieces. The solution is then autoclaved (15 to 30 min; 1.5-2.5 atm) and while still hot poured

into sterile glass vessels or test tubes. 50 ml of the nutrition solution is used in Petri dishes, jars or Erlemmeyer flasks with a diameter of the bottom of 9 cm, whereas in test tubes (15 x 170 mm) 5 ml of the solution is dispensed. The test tubes, used for individual cultures, and the Erlenmeyer flasks, used for large scale cultures, are plugged with Kapsenberg's plugs or cellulose wadding covered with cellophane. The jars are covered with lids. LANGRIDGE (1957b) packed the space round the lid with cotton wool pretreated with cethyl piridinium chloride (0,1 per cent in chloroform solution) to keep the cultures aseptic. For cultivation of plants to maturity in addition to test tubes, big Erlenmeyer flasks and jars are used the lids of which are removed when the larger flower stalks grow too high. The slight sterility frequently observed due to pollen abortion is higher in tubes than in the larger vessels (JACOBS, unpubl.). Petri dishes are appropriate for the cultivation of plants to butonisation, but even here the plants may be kept up to maturity under suitable conditions.

For sterilization dry seeds are immersed in a 2% solution of chloramine B for 5 to 10 min, then washed in sterile water in a sterile vessel. The washing as well as the following procedures is best done in a sterile box (sterilized chemically with e.g. Zephirol and/or with UV-light) or in a Hansen's chamber. As another simple method of seed sterilization LANGRIDGE (1957b) used a mixture of absolute ethanol + 20% H_2O_2 (1:1) for 10 min and RÖBBELEN (unpubl.) 1% bromine-water for 5-10 min both without any washing after the treatment, JACOBS (unpubl.) 3,5% Calcium hypochlorite for 5 min.

The seeds are then planted on the solid agar by a Pasteur's pipette with an air ball in a sterile box under UV-light. The planting, however, may also be done without a sterile box if the sterility demanded may be lower. The seeds are sucked up with a small amount of water and released to the agar. This no doubt works faster than planting with a platin loop. The diameter of the pipette, however, must only be a little larger than the diameter of the seeds to avoid any surplus of water which would inhibit germination. To escape from the same complication RÖBBELEN (unpubl.) uses 45° slope agar in his tubes so that the superfluous water runs down. To stimulate germination sometimes it is recommended to keep the cultures in cold (0-5°C) for 24 hrs after planting, but with some material this can be certainly omitted. Wooden blocks are used as testtube holders with holes as high as the agar medium, as an exposure of the roots to light may inhibit growth (LANGRIDGE 1957b).

When a phytotron or other growth chambers with some regulatory systems are at hand, the following cultural conditions should be arranged (LANGRIDGE 1957b): temperature 25°C with continuous illumination of 800 - 12.000 f.c., i.e. 8.600 - 13.800 lux and a relative humidity above 60%. Fluorescent light tubes are convenient light sources. In less equipped conditions the cultivation of Arabidopsis is possible at temperatures between 10 and 30°C, but a temperature of about 20°C is needed for good germination. Just so continuous illumination is not obligatory but a 16 hrs day-length is also very suitable for plant growth. Even a light intensity of about 3.000 lux is sufficient.

Nutrient recipes for selection and growing of biochemical mutants

M. JACOBS

I. The minimal medium is basically that of HOAGLAND-ARNON

A. Major elements:

$Ca(NO_3)_2$	0.820 g
$K NO_3$	0.505 g
$Mg SO_4 . 7 H_2O$	0.480 g
$K H_2 PO_4$	0.136 g

in 1.000 ml aqua bidest.

B. Iron: 13.05 g EDTA (Na-salt) in 134 ml of n K OH + 12.45 g $Fe SO_4 . 7 H_2O$ adjust to 500 ml with aqua bidest. and shake for 24 hrs

C. Minor elements:

$Mn Cl_2 . 4 H_2O$	1.81 g
$Zn SO_4 . 7 H_2O$	0.222 g
$Cu SO_4 . 5 H_2O$	0.079 g
$H_3 Bo_3$	2.860 g

in 1.000 ml aqua bidest.

D. Molybdenum: $Mo O_3$ 150 mgrs in 10 ltr 1/10 n H_2SO_4

Global formulation : 1000 ml A + 1 ml B + 1 ml C + 1 ml D.

II. Substrates

1. Agar — 0.8-1% Difco Bacto-Agar

2. Silica-gel — obtained through the passage of a sodium silicate solution in a glass column filled up with Amberlite IR 120 (Röhm and Haas, USA). Adjust to pH 6.2 with Na OH. The gel is very suitable after autoclaved sterilization.

3. Quartz-sand poured in Soxlet thimbles for easy regulation of water supply and these placed in sterile test tubes (RÖBBELEN, unpubl.)

4. Perlite or Vermiculite for plant culture.

III. Complete medium

A. Glucose : 0.5% in minimum medium
B. Casein hydrolysate : 0.01%
C. Yeast extract (if necessary) : 0.01-0.1%
D. Purines and pyrimidines : see table 1
E. Vitamins : see table 2
F. Amino acids : as used by LANGRIDGE (1957b). Hydroxy proline may be adjoined at the rate of 10 mg/ 1.000 ml medium.

All the supplements are dissolved in water except guanine. The complete medium is autoclaved (120°C , 15 min) or filtered through Millipore.

Table 1: Solution of purines and pyrimidines

	Amount per ltr in stock solution	Stock solution used per 1ltr medium
Adenine	0.30 gr	5 ml
Cytosine	0.15	5
Thymine	0.15	5
Xanthine	0.10	15
Hypoxanthine	0.15	5
Uracile	0.15	5
Guanine	0.03gr/ltr of 1/100 n HCl	50

Table 2: Solution of vitamins

Pyridoxine-HCl	0.2 gr	0.2 ml
Nicotin amide	1.0	0.2
Folic acid	0.2	0.2
Ca.pantothenate	0.2	0.2
Ascorbic acid	4.0	0.2
Thiamine HCl	0.2	0.2
Choline chloride	0.2	0.2
i-Inositol	40.0	0.2
p-Aminobenzoic acid	0.2	0.2
Biotin	0.1	0.02
Riboflavin	0.1	0.25

Genetic material

This shall be the place for giving in future issues of Arabidopsis Information Service lists of gene symbols, mutant descriptions and genetic stocks available for distribution in different laboratories. Kindly take notice of the corresponding note of Dr. MÜLLER, Gatersleben, on page 38 .

Stocks available: For the next number of Arabidopsis Information Service a describing list of all natural races being propagated in the original sortiment of Professor F.Laibach, Limburg/Lahn will be prepared. On this occasion it might be valuable to include as many races as possible, that might be picked up at other localities. Everyone being in the possession of natural races not originating out of the Laibach sortiment is kindly requested to send to the editor some seeds and a description of the habitat, morphology and development of these forms for comparison and enrolment in this standard sortiment.

Stocks wanted: It has to be announced the request of Dr. L. Mühlhäuser, 706 Schorndorf, Silcherstr.67, Deutschland for seeds of an early flowering glabrous Arabidopsis mutant together with the initial hairy form. Small samples available may be sent directly or by the way of the editor.

D. LETTERS TO THE EDITOR

M.JACOBS, Bruxelles

This letter gives some suggestions towards future duties of the Arabidopsis Information Service, mentioning the following points:

1. Instructions regarding the best way of defining geographical origin and phenotypical differences among supposed "races" of Arabidopsis.
2. Presentations of the many aspects of Arabidopsis culture, viz. sowing technique, light requirement, breaking of seed dormancy, propagation through cuttings etc.
3. Discussions about the methodological points of view in mutation research, especially when done with chemicals e.g. EMS, concerning time of treatment, concentration, use of buffer, effect of temperature, pre- and post-sowing conditions, statistical treatment of results and standard estimation of mutation rates.

G.P.REDEI, Columbia, Mo.

"When in The Hague you proposed the News-letter idea it was unanimously welcome, but there was no request about the cost of production. I believe we all could contribute a little from our research funds if this would be needed, or perhaps we could get a special fund if this will be necessary."

Comment of the editor: You will be interested in knowing that the expense incurred in connection with assembling, mimeographing and mailing of this issue amounts in the order of DM 350,-, viz. DM 2.50 each. No prospect of any fund for financing the next issue is at present held out to the editor. Thus donations payed to the account of "Arabidopsis Information Service", Konto-No. 2069 at Städtische Sparkasse, Hauptstelle, Göttingen,will as well be appreciated as suggestions for future financial sources.

A.J.MÜLLER, Gatersleben

Proposals for a nomenclature of mutants and genes in Arabidopsis.

1. Principally the recommendations of the "International Committee on Genetic Symbols and Nomenclature" (Un.Int.Sci. Biol. Ser.B.30, 1-6,1957) should be applied.
2. To avoid that the same denotation or symbol will be used for different mutants, a list of mutants and genes should be prepared. An Arabidopsis worker should be delegated to permanently conduct this register. Everyone intending to publish a new denotation or symbol need send his proposals to the mentioned personal. He receives immediately an information, whether or not the proposed symbols or denotations have already been disposed. The date of receipt will decide the priority.
3. To increase the value of mutant nomenclature and to avoid confusion through duplicated denotation it might be desired that a definite nomenclature scheme has to be followed,

especially with regard to characters with a high phenotypic and genotypic variability. Such a nomenclature pattern has already been proposed by MÜLLER (1963) for lethal mutations and by LAMPRECHT (Agri Hort.Genetica 18,135-168,1960;cf. BLIXT: ibid 19,402-447,1961) for sublethal and viable chlorophyll mutants. For instance each mutation causing yellow cotyleadons should be named as xantha. Any newly arisen mutation might be differentiated by denoting as xa_1, xa_2 and so on. Other existing mutants, for example, the yellow-green variants are not to be termed xantha.

4. If crossing results prove two similar phenotypes to be allelic in nature, the symbols will acquire the same suffic but different exponents. For instance, if xa_1 and xa_5 are shown to be allelic they get the new denotation $\boxed{xa_1^1}$ and $\boxed{xa_1^2}$. Two independently arisen mutants should only be claimed identical after having been proved allelic in proper crossing experiments.

5. Mutants which are no longer avaible for comparison should be removed from the list. The denotations and symbols thus falling vacant could again be used only for phenotypes similar to the earlier mutant. Arabidopsis Information Service will publish a complete list of existing mutants including the original reference and the adresses of the respective seed supplying station. If a worker is unable to maintain his stocks on announcement in Arabidopsis Information Service another working-team should undertake this responsibility.

6. New denotation of mutants and symbols should not be introduced if these are not intended to be conserved for further experiments. In this case a simple numbering should be preferred.

7. Arabidopsis Information Service will periodically publish a complete review on those genes that either could be ascribed to a linkage group or for which crossing-over data have been worked out.

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F. ADRESSES OF ARABIDOPSIS RESEARCH WORKERS

Adams,M,W.: Dept.Crop Sci.,Michigan State Univ.,East Lansing, Mich.,USA

Anand,Miss R.: Dept.Bot.,Univ.,New Delhi 6,India

Arnold,C.G.: Botanisches Institut,Universität,852 Erlangen, Schloßgarten 4,Deutschland

Austin,R.B.: Plant Physiol.Sect.,Nat.Vegetable Res.Station, Wellesbourne,Warwick,England

Barthelmeß,Fräulein I.: Institut f.Vererbungs-u.Züchtungsforschung,1 Berlin-Dahlem,Albrecht-Thaer-Weg 6,Deutschland

Bell,S.: Dept.Bot.,Univ.Tennessee,Knoxville,Tenn.,USA

Bhatia,C.R.: Lab.v.Erfelijkheidsleer,53 Generaal Foulkesweg, Wageningen,Nederland

Boardman,N.K.: Division Plant Industry,Commonwealth Sci.a.Indust. Res.Org.,P.O.Box 109,Canberra City,Australia

Bouharmont,J.: Inst.Carnoy,24 Rue du Canal,Louvain,Belgique

Bradshaw,A.D.: Dept.Agricult.Bot.,Univ.Coll.North Wales,Memorial Buildings,Bangor,Caernarvonshire,England

Brock,R.D.: Div.Plant Industry,Commonwealth Sci.a.Indust.Res.Org., P.O.Box 109,Canberra City,Australia

Brown,J.A.M.: Technic.Office,Commonwealth Sci.a.Indust.Res.Org., P.O.Box 109,Canberra City,Australia

Buiatti,M.: Istituto di Genetica,Università,Via S.Epifanio 14, Cas.Post 99,Pavia,Italia

Carr,D.J.: Dept.Bot.,The Queen's Univ.,Belfast 7,Ireland

Carter,J.F.: Dept.Agronomy,North Dakota State Univ.,Fargo,N.D., USA

Cetl,I.: Dept.Plant Physiol.a.Genet.,Purkyně-Univ.,Kotlářská 2, Brno,ČSSR

Ciferri,R.: Istituto ed Orto Botanico,Università,Via S.Epifanio 14,Cas.Post 99,Pavia,Italia

Cruse,Fräulein D.: Botanisches Institut,Universität,852 Erlangen, Schloßgarten 4,Deutschland

Daly,K.: San Fernando Valley State Coll.,18111 Nordhoff Street, Northridge,Calif.,USA

Dommergues,P.: Station centrale de Genetique,Centre National de Recherches Agronomiques,Versailles,France

Duvigneaud,P.: Lab.de Genetique des Plantes Super.,Université, 1850 Ch.de Wavre,Bruxelles,16,Belgique

Dyer,A.F.: Dept.Bot.,Royal Bot.Garden,Inverleith Row,Edinburgh 3, Scotland

Elliott,C.G.: Dept.Bot.,Glasgow Univ.,Glasgow W.2.,Scotland

Fausto,L.: Istituto Botanico,Parma,Italia

Feenstra,W.J.: Lab.v.Erfelijkheidsleer,53 Generaal Foulkesweg, Wageningen,Nederland

Fujii,T.: Dept.Induced Mutation,Nat.Inst.Genetics,Yata 1111, Sizuoka-ken,Misima,Japan

Gajewski,W.: Dept.Generaal Genet.,Al.Ujazdowskie 4,Warsaw,Poland

Gichner,T.: Ústav Experimentální Botaniky, ČSAV,Praha-Dejvice, Na cvičišti 2,ČSSR

Gilbert,N.: John Innes Horticult.Institution,Bayfordbury,Hertford, Herts,England

Glubrecht,H.: Institut f.Strahlenbiologie,Techn.Hochschule, 3 Hannover,Herrenhäuserstr.2,Deutschland

Gottschalk,W.: Institut f.Landw.Botanik,Universität,53 Bonn, Meckenheimer Allee 176,Deutschland

Gregory,F.G.: Res.Inst.Plant Physiol.,Imperial Coll.Sci.Technol., London,England

Griffing,B.: Div.Plant Industry,Commonwealth Sci.a.Indust.Res.Org., P.O.Box 109, Canberra City,Australia

Haccius,Frau B.: Botanisches Institut,Universität,65 Mainz,Saarstraße 21,Deutschland

Harberd,D.J.: Dept.Agricult.,Univ.,Leeds 2,England

Harper,J.L.: Dept.Agricult.,Univ.,Oxford,England

Hastie,A.C.: Dept.Bot.,Queen'sColl.,Dundee,Scotland

Hill,J.: A.R.C.Unit Biometric.Genet.,Dept.Genetics,Univ., Birmingham,England

Hirono,Y.: Univ.Missouri,304 Curtis Hall,Columbia,Mo.,USA

Hylander,N.: Inst.Systematic Bot.,Univ.,Uppsala,Sweden

Ingram,H.A.P.: Dept.Agricult.,Univ.Coll.,Memorial Buildings, Bangor,England

Iyer,R.D.: Div.Bot.,Indian Agric.Res.Inst.,New Delhi 12,India

Jacobs,M.: Lab.de Genetique des Plantes Super.,Université, 1850 Ch.de Wavre,Bruxelles 16,Belgique

Kamra,O.P.: Dept.Biol.,Dalhousie Univ.,Halifax,N.S.,Canada

Kappert,H.: Institut f.Botanik,Universität,44 Münster,Schloßgarten 4,Deutschland

Kelly,P.: The Nuffield Foundation,Sci.Teaching Project (Biol.Sect.), Nuffield Lodge,Regent's Park,London N.W.1,England

Kermicle,J.: Dept.Genet.,Univ.Wisconsin,Madison 6,Wisc.,USA

Kock,P.C.de: Plant Physiol..Dept.,The Macauly Inst.Soil Res., Craigiebuckler,Aberdeen,Scotland

Kondo,S.: Nat.Inst.Genet.,Yata 1111,Sizuoka-ken,Misima,Japan

Kribben,F.J.: Amts-Apotheke,6250 Limburg/Lahn,Grabenstr.32, Deutschland

Kvitko,K.V.: Katědra genetiki leningradskovo Universitěta, Bioloqičeskij Fakultět,Leningrad V-164,SSSR

Laibach,F.: Biologisches Forschungsinstitut,6250 Limburg/Lahn, Am Rosenhang 1,Deutschland

Langridge,J.: Div.Plant Industry,Commonwealth Sci.a.Indust.Res. Org.,P.O.Box 109,Canberra City,Australia

Lauer,F.J.: Inst.Agricult.,Univ.Minnesota,St.Paul 1,Minn.,USA

Lawrence,C.: Wantage Res.Lab.(A.E.R.E.),Wantage,Berks.,England

Lorenzoni,C.: Istituto di Genetica Vegetable,Fac.Agraria,Università cattolica,Piacenza,Italia

Mabbey,D.G.: Biol.Dept.,Fettes Coll.,Edinburgh 4,Scotland

Maheshwari,N.: Div.Bot.,Indian Agric.Res.Inst.,New Delhi 12,India

Maheshwari,S.C.: Dept.Bot.,Univ.,New Delhi 6,India

Matzinger,D.F.: Dept.of Genetics,School of Agricult.,North Carolina State Univ.,Raleigh,N.C.,USA

McKelvie,A.D.: Agricult.Bot.Dept.,North Scotland Coll.Agricult., Crown Maurious,41 1/2 Union Street,Aberdeen,Scotland

Meijer,G.: Philips Res.Lab.,Eindhoven,Nederland

Mentzer,C.: Musée Nationale d'Histoire Naturelle,Chimie Appliquée aux Cornes Organises,63 Rue Buffon,Paris,France

Mickey,G.H.: New Engl.Inst.f.Medical Res.,P.O.Box 308,Ridgefield, Conn.,USA

Morishima,H.: Dept.Applied Genet.,Nat.Inst.Genet.,Yata 1111,Sizuoka-ken,Misima,Japan

Mühlhäuser-Härer,Frau I.: 706 Schorndorf,Silcherstr.67,Deutschland

Müller,A.J.: Institut f.Kulturpflanzenforschung,Gatersleben,Krs. Aschersleben,Bez.Halle,Deutschland

Napp-Zinn,K.: Botanisches Institut,Universität,5 Köln-Lindenthal, Gyrhofstr.15,Deutschland

Oka,H.I.: Nat.Inst.Genet.,Sizuoka-ken,Misima,Japan

Osone,K.: Fac.Agricult.,Tokyo Univ.,Tokyo,Japan

Pokorný,V.: Ústav Experimentální Botaniky,ČSAV,Praha-Dejvice, Na cvičišti 2,ČSSR

Ratcliffe,D.: Scott.Plant Breed.Stat.,Pentlandfield,Roslin, Midlothian,Scotland

Reddy,G.M.: Dept.Bot.,Univ.California,Los Angeles 24,Calif.,USA

Rédei,G.P.: Univ.Missouri,117 Curtis Hall,Columbia,Mo.,USA

Rehwaldt,Ch.: State Univ.Coll.Forest.,Syracuse Univ.,Syracuse 10, N.Y.,USA

Reinholz,Frau E.: Max-Planck-Institut f.Biophysik,6 Frankfurt/Main Süd 10,Forsthausstr.70,Deutschland

Röbbelen,G.: Institut f.Pflanzenbau u.Pflanzenzüchtung,Universität, 34 Göttingen,von-Siebold-Str.8,Deutschland

Rundfeldt,H.: Institut f.Angewandte Genetik,Techn.Hochschule, 3 Hannover,Herrenhäuserstr.2,Deutschland

Ryder,E.J.: Crops Res.Div.,U.S.Dept.Agricult.,P.O.Box 98,Alisal Branch,Salinas,Calif.,USA

Scossiroli,R.: Istituto di Genetica,Via S.Epifanio 14,Pavia, Italia

Shropshire,W.jr.: Div.Radiat.a.Organism,Smithsonian Institution, Astrophys.Observatory,Washington 25,D.C.,USA

Simmond,N.W.: John Innes Inst.,Bayfordbury,Hertford,Herts.,England

Skok,J.: Div.Biol.Med.Res.,Argone Nat.Lab.,Box 299,Lemont,Ill.,USA

Smith,H.H.: Biol.Dept.,Brookhaven Nat.Lab.,Upton,L.I.,N.Y.,USA

Sosna,M.: Ústav Experimentální Botaniky,ČSAV,Praha-Dejvice,Na cvičišti 2,ČSSR

Swaminathan,M.S.: Div.Bot.,Indian Agric.Res.Inst.,New Delhi 2, India

Scheuermann,W.: Institut f.Strahlenbiologie,Techn.Hochschule, 3 Hannover,Herrenhäuserstr.2,Deutschland

Schwanitz,F.: Biologisches Institut,Atomforschungszentrum des Landes Nordrhein-Westfalen,517 Jülich,Deutschland

Steinitz-Sears,Mrs.L.M.: Univ.Missouri,108 Curtis Hall,Columbia, Mo.,USA

Tabernacle,Miss P.A.: Dept.Agricult.Bot.,Univ. Coll.North Wales, Memorial Buildings,Bangor,Caernarvonshire,England

Thomas,R.: Lab.de Genetique des Plantes Super.,Université, 1850 Ch.de Wavre,Bruxelles 16,Belgique

Valentine,F.A.: Dept.Forest Bot.,Coll.Forestry,Univ.,Syracuse, N.Y.,USA

Vaughan,J.G.: Dept.Bot.,Chelsea Polytechnic,Manresa Road,London SW 3,England

Veen,H.J.van der: Lab.v.Erfelijkheidsleer,53 Generaal Foulkesweg, Wageningen,Nederland

Velemínský,J.: Ústav Experimentální Botaniky,ČSAV,Praha-Dejvice, Na cvičišti 2,ČSSR

Walker,S.: Dept.Genet.,Univ.,Liverpool,England

Zelles,Mrs.L.: Institut voor de Veredeling van Tuinbouwgewassen, Postbus 16,Wageningen,Nederland

Errata of "Arabidopsis Information Service" No. 1

Page 21, lines 17-20 should read correctly:
According to the experience obtained in our material ethylmethane sulfonate induces many yellow green sectors also in the wild type but from 16 analyzed cases none was ch, but some kind of dominant effect of unknown loci.