ARABIDOPSIS INFORMATION SERVICE

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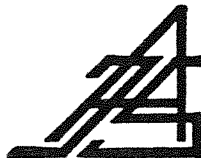

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A R A B I D O P S I S

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

No. 10 Göttingen March, 1973

CONTENTS

A. Brief Notes

RÉDEI, G.P.: Genetic and nutritional control of the glycolytic pathway in Arabidopsis, Cardaminopsis and Hylandra 3
RÉDEI, G.P.: Fructose-1,6-diphosphate aldolase isozymes in Arabidopsis, Cardaminopsis and Hylandra 5
CHUNG, Shu-ching, and G.P. RÉDEI: OMP decarboxylase in the wild type and the im mutant of Arabidopsis 6
JACOBS, M., and F. SCHWIND: Genetic variation in the acid phosphatase isozymes of Arabidopsis thaliana 7
CORCOS, A., B. PIPER, and R. LEWIS: Redifferentiation of normal Arabidopsis plants from callus culture 10
VAN DER VEEN, J.H., and Hetty BLANKESTIJN - DE VRIES: Double reduction in tetraploid Arabidopsis thaliana, studied by means of a chlorophyll mutant with a distinct simplex phenotype 11
GRESSHOFF, P.M., and C.H. DOY: Induction and differentiation of haploid Arabidopsis thaliana 12
RÉDEI, G.P.: Considerations on the genetic mechanisms in organelles of Arabidopsis 13
DIEKMANN, H., and A.R. KRANZ: Interactions between phytochrome (P_{fr}), gibberellic acid (GA_3) and cyclic adenosine monophosphate (C-AMP) in seed germination of heteroallelic genotypes of Arabidopsis thaliana (L.) HEYNH. 14
FERRO, K., and A.R. KRANZ: Gene action of two ch-loci on the primary carotenoids in leaves of Arabidopsis thaliana (L.) HEYNH. 16
DEMCHENKO, S.I., V.A. AVETISOV, and P.G. BUTENKO: An enquiry into chlorophyll chimerae in Arabidopsis in M_1 by means of tissue culture 17
ABDULLAEV, H.A., P.D. USMANOV, S.V. TAGEEVA, and Yu.S. NASYROV: On the homological variability of the chloroplast membrane system 18
USMANOV, P.D., and O.V. USMANOVA: On the genetic control of the chloroplast size and number in mesophyll cells of species of Arabidopsis 20
YULDASHEV, O.H., A.V. IVANOV, P.D. USMANOV, and L.B. RUBIN: The effects of the capacity and density of the ruby laser irradiation on the seeds of Arabidopsis thaliana (L.) HEYNH. 21
SHEVCHENKO, V.V., L.I. GRINIKH, E.M. PROTOPOPOVA, and G.A. GRIGORIEVA: A study of the mutagenic action of 1,4-bisdiazoacetylbutane and 8-ethoxycaffeine on Arabidopsis thaliana 22
FERSHTAT, A.M., and O.G. STEPANENKO: The influence of ionizing radiation on growth correlations of Arabidopsis thaliana (L.) HEYNH. 23
BOUNIAS, M.: Effects primaires et mutagènes de l'irradiation gamma des graines d'Arabidopsis 24
BOUNIAS, M.: Equipement pigmentaire des cotylédons d'Arabidopsis après irradiation "gamma" des graines 26
BOUNIAS, M.: Détermination de l'equipement enzymatique général chez Arabidopsis (Témoins et mutants) 28
BOUNIAS, M.: Incorporation de Leucine-^{14}C dans les pigments photosynthétiques, les sucres et amino-acides libres de plants d'Arabidopsis : Témoins et mutants viridis 29
SREE RAMULU, K., and J.H. VAN DER VEEN: Sulphydryl protection against ionizing radiations 30
IVANOV, V.I.: Estimation of induced mutation rate in Arabidopsis 31
CORCOS, A.: An arginine mutant 32
OOSTINDIER-BRAAKSMA, Fietje J., and W.J. FEENSTRA: Nitrate reduction in Arabidopsis thaliana 33
FEENSTRA, W.J., Dieuwke L. DE HEER, and Fietje J. OOSTINDIER-BRAAKSMA: Negative results of treatment with bacterial DNA on "repair" of mutants of Arabidopsis 33
COREY, L.A., and D.F. MATZINGER: Diallel crosses among races of Arabidopsis thaliana 34

(continued)

RELICHOVÁ, Jiřina, Helena FISCHEROVÁ, and I. CETL: The localization of recessive lethal genes into linkage groups 34
CETL, I., Zina MAŇOUŠKOVÁ, and Jiřina RELICHOVÁ: Variability and heritability of a developmental character in mountain populations of Arabidopsis 35

B. Techniques

CHEUNG, Kin-Wah: The aseptic culture of Arabidopsis thaliana (L.) HEYNH. in liquid medium 37
BOUNIAS, M.: Culture aseptique d'Arabidopsis sur sable et liquide minéral "minimum" 38
BOUNIAS, M.: Détermination de l'acide indolyl-3-acétique dans les rosettes de feuilles d'Arabidopsis 40

C. Informations

Announcement 42
Graduate assistantship 42
Correction 43
Important editorial change 43

D. Bibliography

Fifth addition to the list compilated in A.I.S. No. 5 43
In the press 44

It is a pleasure to acknowledge the dedicated and efficient services of Miss Elisabeth BISKUP, Mrs. Liesel BUSCH and Miss Friederike BUGGERT in the assembly and typing of this Newsletter.

G. RÖBBELEN

A. BRIEF NOTES

Genetic and nutritional control of the glycolytic pathway in Arabidopsis, Cardaminopsis and Hylandra

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Data of last year (RÉDEI, 1972) have shown that growing Arabidopsis, Cardaminopsis and Hylandra on autoclaved fructose containing media results in reduced levels of fructose-1,6-diphosphate aldolase.

Continuation of the study with additional enzymes working in a sequence (Fig. 1) revealed noteworthy trends (Table 1). For the sake of simplification of the extensive data, the information collected with the three species is condensed to averages. Enzyme activities are expressed nanomoles of substrate cleaved or product formed, as applicable, per mg protein per minute. It is remarkable that the activities of enzymes depending on each other so closely, displayed such large differences. Triosephosphateisomerase activity exceeded ca. 4,000 fold the activity of phosphofructokinase. There is only one enzymatic step between the product of the latter enzyme and the substrate of the former one. Similar, although not of quite that magnitude, differences are obvious among several other activities in the plants grown on all three media. One may wonder on the rationale of this apparent absence of coordination within a single pathway. It is conceivable that the in vitro activity may not reflect the status inside the cells of intact tissues, yet the differences are quite impressive.

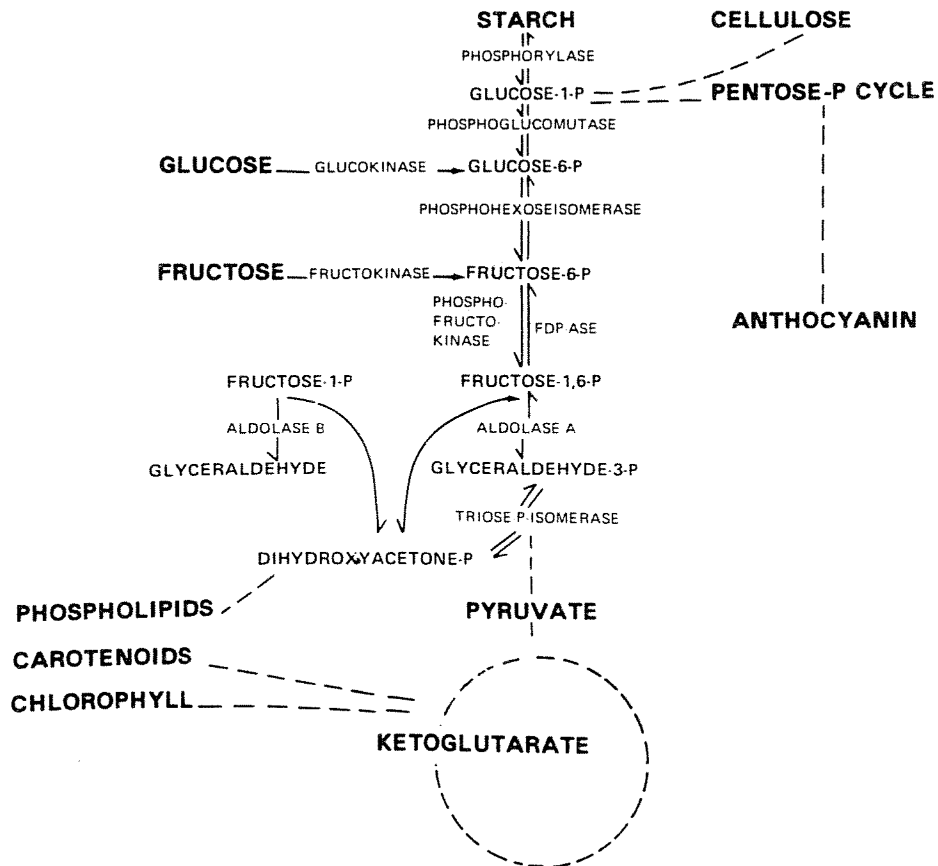
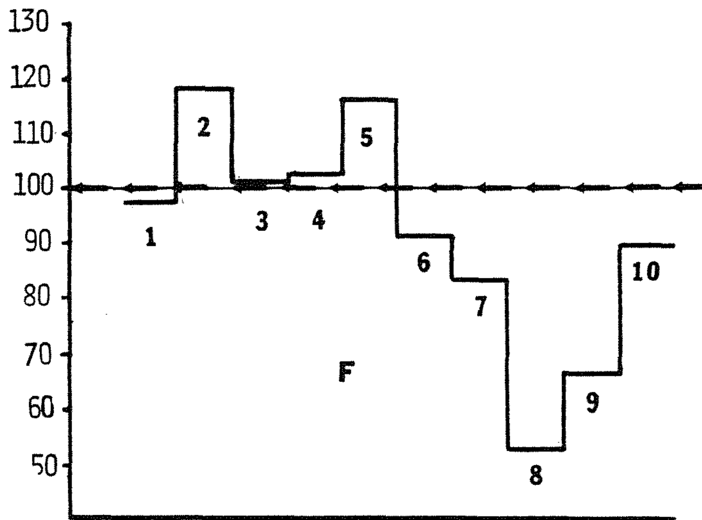


Figure 1: The Embden-Meyerhof glycolytic pathway and some of its contributions to the metabolic pool relevant to this study

Table 1: Activities of glycolytic enzymes in nanomoles / mg protein / minute in Arabidopsis, Cardaminopsis and Hylandra grown aseptically under continuous illumination

Enzyme	No. of expts.	Autoclaved culture media		
		No sugar	2% Glucose	2% Fructose
1 Glucan phosphorylase	9	11.5 (100)	10.9 (95)	11.1 (97)
2 Phosphoglucomutase	15	144.4 (100)	144.3 (100)	169.8 (118)
3 Phosphohexoseisomerase	18	4,882.2 (100)	4,995.0 (102)	5,001.7 (102)
4 Glucokinase	9	9.7 (100)	12.5 (129)	10.0 (103)
5 Fructokinase	9	14.0 (100)	17.9 (128)	16.3 (116)
6 Phosphofructokinase	6	3.4 (100)	3.0 (88)	3.1 (91)
7 Fructose-1,6-diphosphatase	6	113.3 (100)	93.5 (83)	94.7 (84)
8 FDP-aldolase condensation	9	147.8 (100)	128.4 (87)	78.1 (53)
9 FDP-aldolase cleavage	18	95.5 (100)	94.5 (99)	67.7 (71)
10 Triosephosphateisomerase	15	12,230.0 (100)	11,633.0 (95)	11,666.6 (95)

If the activity of the enzymes in the plants grown on the two different hexose media is expressed as a percent of that in the extracts of plants raised on sugar-free solutions an interesting tendency becomes obvious. The relative activity of enzymes involved in deposition of carbohydrates into starch, cellulose or in the diversion of them into the pentose phosphate cycle is either unchanged or somewhat increased under the condition of nutrition on autoclaved hexose media. The activity of enzymes involved in the processing of sugars through energy yielding reactions is somewhat decreased, however. This reduction is especially obvious in the activity



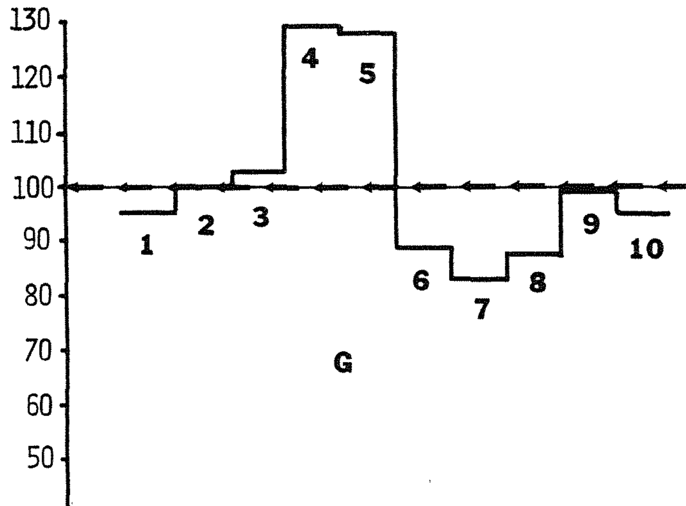


Figure 2:
The activity of some glycolytic enzymes extracted from plants grown on autoclaved fructose (F) and autoclaved glucose (G), relative to that obtained from plants raised on sugar-free media. The average of the experiments condensed in Table 1. The numbers refer to the sequence of enzymes as listed in Table 1.

of fructose-phosphate aldolase when the plants were grown on fructose media. Upon autoclaving, fructose rapidly yields hydroxymethyl furfural, hydroxyacetyl furan and a series of other compounds, highly toxic to these plants. The breakdown of glucose is much less under the conditions prevailing.

This trend of enzyme activities was nicely reflected by the increased starch, cellulose and anthocyanin production under sugar nutrition. The same time, in accordance with the expectations based on enzyme activities, a shortage in pyruvate, keto glutarate, phospholipids, chlorophylls and carotenoids was obvious. Thus the genetically determined level of enzymes could be modified to some extent by the conditions of nutrition of the plants. Addition of autoclaved fructose to the incubation mixture does not affect the activity of aldolase. The activity of the hexokinases is higher, however, in the presence of their substrates in the extraction mixtures.

Reference:
RÉDEI, G.P.: Arabidopsis Inf.Serv. 9, 40 (1972)

Fructose-1,6-diphosphate aldolase isozymes in *Arabidopsis*, *Cardaminopsis* and *Hylandra*

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Arabidopsis and related species are very sensitive to the degradation products of fructose, detectable in autoclaved nutrient media containing this hexose. It has been shown (RÉDEI 1972) that fructose aldolase level is reduced in the plants grown on such media. KAKLIJ and NADKARNI (1970) have claimed that in bacteria cultured on ribose media there was a selective repression of aldolase isozymes. In the human congenityl anomaly, hereditary fructose intolerance, fructose-1-phosphate aldolase activity is deficient (FROESCH 1972). It was of interest to know the isozyme spectrum in *Arabidopsis*, *Cardaminopsis* and *Hylandra* under nutrition with autoclaved fructose. The number of aldolase bands in *Cardaminopsis* and *Hylandra* is the same, 4. In *Arabidopsis* one isozymic band was absent, otherwise the spectrum was identical. The conditions of nutrition did not influence qualitatively the composition of this enzyme in a form detectable by the method used (PENHOET, RAJKUMAR and RUTTER 1966). The number of aldolase isozymes in another crucifer, radish, was found to be 2 (TAKEO 1969). The gene responsible for the third aldolase band seems to be absent in *Arabidopsis*. If *Hylandra* is realy a hybrid of *Cardaminopsis* and *Arabidopsis*, there seems to be no dosage effect in this function.

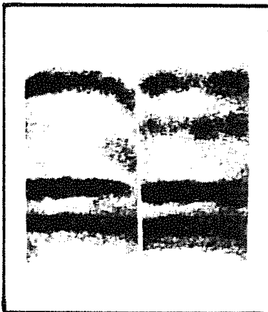

Figure 1: Cellulose polyacetate electrophoretic separation of FDP-aldolase isozymes. Left *Arabidopsis*, Right *Hylandra* (←origin). Note that *Hylandra* has an extra band.

References:
FROESCH, E.R.: Essential fructosuria and hereditary fructose intolerance. In: The Metabolic Basis of Inherited Disease, Pp. 131-148. Stanbury, J.B., Wyngaarden J.B., and Frederickson, D.S. eds., McGraw-Hill, New York (1972)
KAKLIJ, G.S. , and NADKARNI, G.B.: Arch.Biochem.Biophyss. 140, 334-40 (1970)
PENHOET, E., T. RAJKUMAR, and W.J. RUTTER: Proc.Nat.Acad. Sci. U.S. 56, 1275-1282 (1966)
RÉDEI, G.P.: Arabidopsis Inf.Serv. 9, 40-41 (1972)
TAKEO, K.: Phytochemistry 8, 2127-2131 (1969)

OMP decarboxylase in the wild type and the im mutant of Arabidopsis

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The effect of 6-azauracil or its riboside, 6-azauridine, on the repression of variegation of im was reported earlier by RÉDEI (1967a). Conversion of orotic acid to ribonucleic acid was inhibited by azauracil in leaf discs of the cocklebur plant, Xanthium pennsylvanicum Wall (ROSS 1964). Considerable evidence indicated an interference with decarboxylation of orotidylic acid (OMP) by OMP decarboxylase. The existence of OMP decarboxylase and OMP pyrophosphorylase in plant leaves was demonstrated by WOLCOTT and ROSS (1967). The presence of these two enzymes which catalyze sequential steps in the de novo biosynthesis of uridine-5'-monophosphate (UMP) indicated that the normal pathway of pyrimidine nucleotide synthesis in higher plants proceeded through orotic acid and OMP.

The activity of OMP decarboxylase was measured as the rate at which the enzyme extract released $C^{14}O_2$ from OMP-7-C^{14}. The reaction mixture typically contained in 0.5 ml buffer 10 mμ mole of OMP (containing 0.01 μ Ci of OMP-C^{14}), and enzyme extract. The reaction was carried out at room temperature (20-22°C) for 10 minutes in weighing bottles in which was placed a small vial containing 0.3 to 0.5 ml of 3% KOH to collect the $C^{14}O_2$ released from OMP-C^{14}. Two 0.1 ml portions of the KOH was taken for scintillation counting. The protein content was determined by the method of LOWRY et al. (1951). One unit of enzyme was defined as that amount which converted 1 mμ mole of OMP per hour per mg protein.

Incorporation of 6-azauridylic acid in the incubation mixture inhibited OMP-decarboxylase activity in Arabidopsis and it has been shown that this drug is a competitive inhibitor of this enzyme also in other organisms.

Interestingly, the cellular level of OMP-decarboxylase increases in plants cultured aseptically on azauracil media (Table 1).

Table 1: The level of OMP-decarboxylase in mutant and control grown on minimal and 6-azauracil containing media

Genotype:	Mutant (im)		Control (im^+)	
Medium:	Minimal	AzU	Minimal	AzU
Activity:	24.3	32.6	16.9	31.7
Index:	(143)	(193)	(100)	(187)

The level of OMP decarboxylase in white tissue of im was found to be higher than the wild type and the green tissue of im:

Table 2: OMP decarboxylase activity in different tissues

Genotype	Tissue	Specific activity
wild type	green	12.37
"im"	green	14.09
"im"	white	20.45

Further experiments with control and 6-azauracil treated plants of both genotypes showed that the activity of this enzyme is dependent on the stage of development:

Table 3: OMP decarboxylase activity changes during development

	Specific activity		
Genotype	Control	6-azauracil treated	Age (days)
gi^2xv gl^2	2.7	16.2	18
	32.8	36.3	21
	20.0	18.2	24
	12.1	30.7	27
	25.1	45.9	30
im gi^2	10.6	36.2	18
	34.5	57.0	21
	18.4	31.3	24
	24.2	41.6	27
	39.3	52.4	30

In both genotypes the effect of 6-azauracil is apparent. When treated with 6-azauracil there is an increase in the level of cellular OMP decarboxylase activity except in the 21 and 24 day plants of gi^2xv gl^2. In these two cases, the expected difference of enzyme activity in "im" and wild type is not entirely clear. This experiment is done only once yet the data of Table 3 are in reasonably good agreement with those of Tables 1 and 2.

The present study indicates that the action of 6-azauracil may be due to its conversion to 6-azauridylic acid which then inhibits the activity of OMP decarboxylase. The accumulation of precursors in the pathway may in turn induce the development of increased levels of OMP decarboxylase. KROOTH (1971) reported that when human diploid cell strains of any genotype were grown in the presence of barbituric acid, 5-azaorotic acid or 6-azauridine, they exhibited elevated activity of the final two enzymes of the UMP path. The drugs inhibited UMP synthesis and this caused accumulation of substrates which in turn increased the cellular level of the enzymes, i.e. the drugs eventually induced more catalytic activity than the drugs could inhibit.

The effect of 6-azauracil on the improvement of plastid differentiation in the mutant im is considerable. The leaf pigment production in the mutant grown on azauracil media of optimal concentrations may increase 20 fold. In the im^+ type, even when homozygous for other genes causing chlorophyll deficiency, such an effect cannot be seen.

Azauracil or derivatives are not natural metabolites of the cell, thus these normally poisonous drugs can only indirectly be involved in the partial remedy of the genetic defect.

The increased OMP decarboxylase level in the im mutant, relative to the wild type, appears to be a natural reaction to compensate for increased RNA degradation observed in the plants (RÉDEI 1967b). The beneficial effect of boosting this "defense" mechanism seems plausible.

The im mutants do not appear to be concerned with a deficiency or with serious alterations in structural genes, since the plants can exhibit an almost normal plastid differentiation under certain conditions. This locus seems rather to be involved in a regulatory role which it may perform by coordinating anabolic and catabolic functions. In the wild type the azauracil-induced increase in OMP decarboxylase level may be only a "luxury" as far as plastid differentiation is concerned, in the im mutants this increase may fill a genuine need, however. The primary consequence of mutations at the im locus requires further studies.

References:

KROOTH, R.S.: Molecular models for pharmacological tolerance and addiction. Ann. New York Acad.Sci. 179, 548-560 (1971)

LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR, and R.J. RANDALL: Protein measurement with the Folin phenol reagent. J.Biol.Chem. 193, 265-275 (1951)

RÉDEI, G.P.: Suppression of a genetic variegation by 6-azapyrimidines. J.Heredity 58, 229-235 (1967a)

RÉDEI, G.P.: Biochemical aspects of a genetically determined variegation in Arabidopsis. Genetics 56, 431-443 (1967b)

ROSS, C.W.: Influence of 6-azauracil on pyrimidine metabolism of Cocklebur leaf discs. Biochim.Biophys.Acta 87, 564-573 (1964)

WOLCOTT, J.H., and C. ROSS: Orotidine-5'-phosphate decarboxylase and pyrophosphorylase of bean leaves. Plant Physiology 42, 275-279 (1967)

Genetic variation in the acid phosphatase isozymes of Arabidopsis thaliana

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We have previously described the acid phosphatase polymorphism occuring in Arabidopsis and the genetic analysis of electrophoretic variants (JACOBS and SCHWIND, 1972; JACOBS and SCHWIND, 1973). We report in this issue other aspects of the combined biochemical and genetical analysis of acid phosphatases.

1. Survey of the electrophoretic variation

We have already mentioned the AP-1 group of acid phosphatase isozymes, characterized by a high activity level and the occurrence of four bands in each homozygous variant. The AP-1 variants are under the control of three codominant alleles (AP_1^S, AP_1^I , AP_1^F) at the AP_1 locus.

We have also observed the existence of two other isozyme groups, AP-2 and AP-3. The AP-2 system which includes three faint bands after electrophoresis on starch-gel (see, JACOBS, 1971) is characterized by a weak activity level and a faster migration rate to the anode than the AP-1 enzymes. The pattern also vary according the geographical races, but independently of the AP-1 bands. For instance, Zü represents a race distinct from two other races Chi and Wil by the particular migration of the second AP-2 band (Fig. 1). This absence of correlation between AP_1 and AP_2 variants leads to the concept of a particular genetic control of AP-2 system which is now analyzed. This AP-2 group can be resolved in a high number of bands after isoelectric focusing on polyacrylamide gels.

The AP-3 is represented by two faint bands migrating to the cathode. These bands are only detectable in plants cultured in liquid medium, supplemented with 2% glucose (RÉDEI, 1971). These cathodal isozymes could represent a third genetically controlled acid phosphatase enzyme.

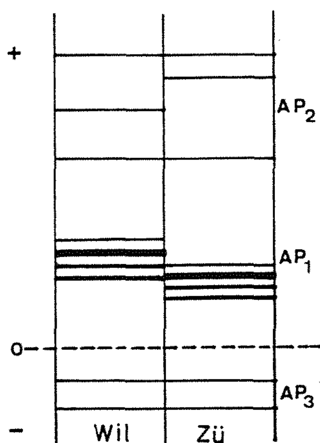

Figure 1: Schematic representation of acid phosphatase isozymes in leaf extracts of Arabidopsis.
AP^{I}- 1 type = Wil, AP^{S}- 1 type = Zü

In this type of submerged culture, betaglycerophosphate ($5 \cdot 10^{-4}$M) as the only source of phosphate in the medium can assure the growth of Arabidopsis plants. Zymograms obtained from such plants do not show modifications in acid phosphatase patterns.

2. Control of the AP-1 set of isozymes

One of the possible reasons to obtain a set of four isozymes might arise from the existence of different aggregates of acid phosphatase monomers. In order to test this hypothesis, electrophoresis was performed in 10% and 15% starch concentration according to SMITHIES (1962). The retardation coefficient calculated for each band shows approximately the same value, 43%. These results indicate that the multiple molecular forms do not probably represent distinct multimeric proteins.

To confirm this assumption, a gel filtration of leaf extract was performed in Biogel P 150. Results are shown in Fig. 2 in which protein spectrum and acid phosphatase

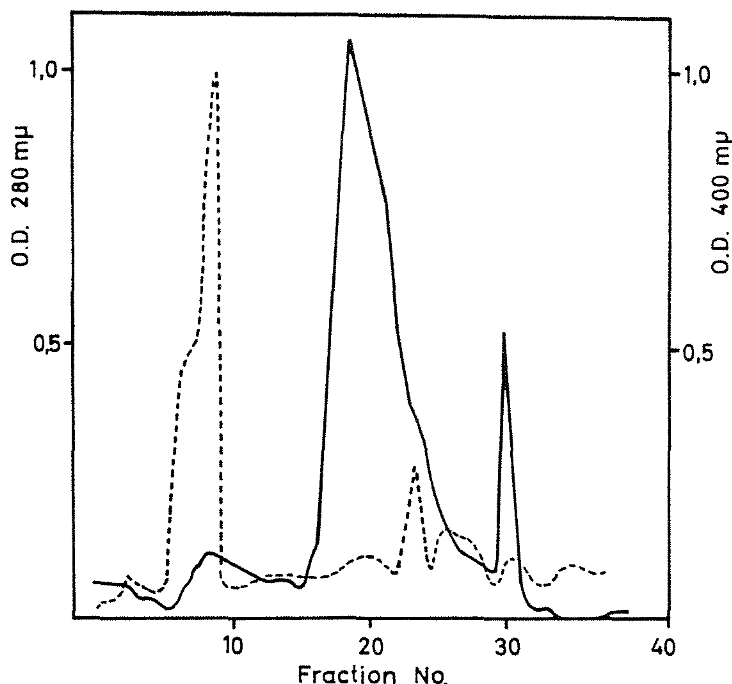

Figure 2: Gel filtration of Arabidopsis leaf extract (1 ml) on a column of Bio-Gel P 150 equilibrated with a 0.005 M acetate buffer pH 4.8. The flow rate was 10 ml/hr. Fractions of 2.5 ml were collected. The dashed line represents the phosphatase activity measured in presence of 0.001 M p-nitrophenylphosphate at 400 mμ; the solid line represents the protein spectrum measured at 280 mμ

activities are represented. The most active fraction was electrophoretically tested and the zymogram obtained displays the typical AP-1 four bands pattern. The separation of acid phosphatase isozymes is now tested by DEAE cellulose chromatography.

3. Organ-specific variation

The zymograms obtained with extracts from various organs of Arabidopsis show the same four bands-pattern of the AP-1 group, except for dry seeds (Fig. 3). The four isozymes are however represented in varying concentrations, in comparison with the activity distribution observed in the leaves. In roots for instance the two first bands are present in a very low concentration.

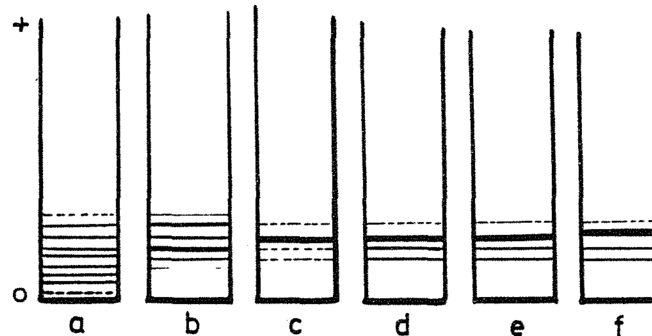

Figure 3: Acid phosphatase pattern in various organs of Arabidopsis (race Wil, AP^{I}-1 type). a: dry seeds, b: 64 hr-germinated seeds, c: roots, d: leaves, e: stems, f: flowers

The Wil seed zymogram displays nine bands; four of them correspond to the Wil leaf isozymes and four others are less anodal. The activity, distributed equally among the nine bands in dry seeds, shifts gradually as the germination proceeds to the bands which shall remain active throughout the life cycle. Preliminary investigations on the genetic control of seed bands have shown that no strong absolute correlation exists between the AP_1 variants defined using leaf extracts and dry seed electrophoretic patterns of the corresponding types. It is thus possible that a specific type of genetic control occurs in the seeds.

4. Genetic control of the acid phosphatase activity

Ethylmethanesulfonate (80 mM-3h) was used as mutagenic agent to induce mutations in the activity of the acid phosphatase-1 gene-enzyme system. The search for induced mutants was performed by subjecting to starch gel electrophoresis extract from individual plants in the progeny of a M_1 treated plant. Until now 1500 M_2 plants were scored for changes in the activity of /and changes of electrophoretic pattern. Figure 4 shows four different types of activity distribution among the bands. Type c appears with a relatively high frequency (0.45%) and type d was scored only once. The mutants are now characterized and a spot test assay (adapted from BELL et al., 1972) is now used to allow the screening of large numbers of mutagenized plants with regard to their activity.

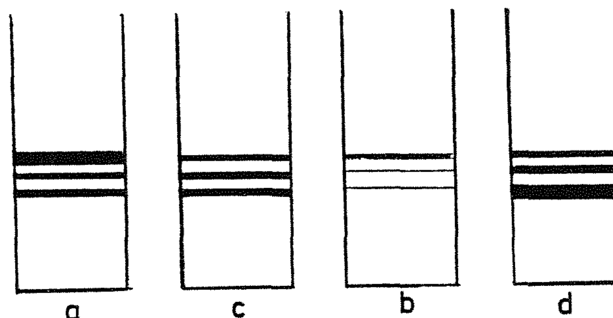

Figure 4: Types of activity distribution among AP-1 isozymes detected in progenies of EMS-treated plants.
a: control activity pattern, b: plant with reduced phosphatase activity
c: equal distribution of the activity among the bands
d: inverted activity distribution with regard to control pattern

Moreover, a change in the electrophoretic pattern was observed in a progeny of AP_1^I, type seeds. Multiple enzyme bands reappear but with electrophoretic mobilities corresponding to those of the AP_1^F type. The spacing between isozymes of the set is not altered. These data lead to the conclusion that the multiple AP-1 bands are all products of the same gene.

References:

BELL, J.B., R.F. MAC INTYRE, and A.P. OLIVIERI: Biochem.Genetics 6, 205-216 (1972)
JACOBS, M.: Arabid.Inf.Serv. 8, 20-21 (1971)
- , and F. SCHWIND: Arabid.Inf.Serv. 9, 11-12 (1972)
- , - : Plant Science Letter 1, 95-104 (1973)
RÉDEI, G.P., and C.M. PERRY: Arabid.Inf.Serv. 8, 34 (1971)
SMITHIES, O.: Arch.Biochem.Biophys.Suppl. 1, 125 (1962)

Redifferentiation of normal Arabidopsis plants from callus culture

A. CORCOS, B. PIPER and R. LEWIS

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NITSCH (1967) reported that hypocotyl sections from Arabidopsis in the presence of kinetin (1 mg/l) and adenine (10^{-4}) produced adventitious buds which produced inflorescence in long days. YOKOYAMA and JONES (1965) succeeded in slowly proliferating cotyledon, leaf, root and stem tissue on Nickell's medium containing coconut milk and kinetin. Redifferentiation was also reported in their abstract. STEWARD, KENT and MAPES (1967) in a study of sequential and synergistic effects of growth regulating substances observed differentiation. Basal medium (B) supplemented with 1.25-5.00 ppm naphtalene acetic acid (NAA) promoted very active growth of the callus, whereas basal medium plus coconut milk (CM) contributed to organization. When the media were sequentially changed as follows (B+CM+NAA)⟶(B+NAA)⟶B, there was a clear improvement in morphogenetic development.

Figure 1: Redifferentiation of normal Arabidopsis plants from callus culture

We have been able to obtain an abundant formation of growth of callus in a medium containing 2-4D, vitamins, kinetin, glucose, and minerals (CORCOS and LEWIS, 1971). Preliminary work indicated that a simple transfer of callus on solid media containing I.A.A. (0.03 mg/l) and kinetin (1 mg/l) permits the formation of roots, shoots, flowers and seeds. A study in the Spring of 1972 was made to ascertain if this was the best combination of the two hormones for differentiation from Arabidopsis callus, or it was shown for tobacco callus (SKOOG, 1957).

Hypocotyl callus was grown on 9 different media. The basal medium was the one of VELEMÍNSKÝ and GICHNER (1964) to which was added the concentrations of kinetin and IAA given in Table 1. For each treatment, 20 replicate 100 ml test tubes with steel caps were used. The test tubes were kept under constant light, at room temperature for a period of four months, the time necessary for the callus to differentiate roots, leaves and stems, flowers and pods. Table 1 summarizes the results.

Table 1: Percentages of test tubes with roots, leaves, flowers and pods are indicated

Media	Kinetin mg/l	IAA mg/l	% tubes with roots	with leaves	with flowers	with pods
1	0.05	0.003	70	0	0	0
2	0.05	0.030	95	0	0	0
3	0.05	0.300	83	0	0	0
4	1.00	0.003	75	5	0	0
5	1.00	0.030	100	77	62	8
6	1.00	0.300	74	63	42	31
7	1.50	0.003	75	58	58	16
8	1.50	0.030	84	74	74	21
9	1.50	0.300	60	50	50	15

Root differentiation occurred independently of the concentration of kinetin or IAA.

However, stems and leaves, flowers and pods differentiated only when the concentration of kinetin was 1.0 to 1.5 mg/liter and IAA 0.03 to 0.3 mg/liter. The number of pods and seeds (as seen in the pods) was greater when the concentration of IAA was 0.3 mg/liter which is ten times greater than the concentration that SKOOG used.

We can conclude that differentiation of callus into normal plants is possible in media containing glucose (20 g/liter), 1.0 to 1.5 mg of kinetin 0.03 to 0.3 mg of IAA liter without transfer to sequential media.

Double reduction in tetraploid Arabidopsis thaliana, studied by means of a chlorophyll mutant with a distinct simplex phenotype

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A dozen tetraploid M2-lines were kindly provided by Miss BALKEMA in this Department (cf. BALKEMA, 1971, 1972). She had put seeds of the early flowering pure line Landsberg-'erecta' on filterpaper (in Petridishes), soaked with a solution containing 10 mMol EMS and 0.2% colchicine. After 24 hrs at 24°C in the dark, the seeds were rinsed and put to germinate on agar at 24°C under TL, and on the 7th day the seedlings were transplanted to the greenhouse (22°C). Harvesting on the M1-plants was from side-branches (or from a higher part of the main inflorescence), which were non-chimeric tetraploid in the sporocyte tissue (judged from pollen size), and which in addition segregated albina's (embryo test).

We confirmed from the M_2-seedlings that these segregations were duplex-type (AAaa selfed), i.e. the mutations were induced on the "chromosome level" (G_1). Of course, mutations induced on the "chromatid level" (G_2) will have escaped detection, since in triplex-type segregations (AAAa selfed) recessives (aaaa) derive from double reduction and consequently are rare.

As also observed by BOUHARMONT, the tetraploids are of excellent vigour (cf. BALKEMA, 1965), and are very stable (cf. BALKEMA, 1967), i.e. off-types are rare in successive generations.

Compared with the diploid control, seedset is 40-60%, which explains the shorter siliquae. This reduction is almost entirely due to non-fertilized ovules. Triploids, obtained by reciprocal crosses between the diploid control and the tetraploid line ultimately selected by us (see below), have 20-30% (!) seedset, which reduction is, compared with the tetraploid, equally due to less ovules per fruit and an increased fraction of non-fertilized ovules. The seeds of these triploids contain 30-40% embryonic lethals (seedcoat prematurely brown, embryotest). Cf. 1-2% for the diploid and 2-3% for the tetraploid parent.

From M_2 till M_5 we carried out line and subline selection, to improve uniform vitality and fertility, to select against simultaneously induced mutants (including embryonic lethals) and to decide which albina had best fenotypic expression both as embryo and as seedling. Ultimately, a very satisfactory line was chosen, which also has no recessive deficit in the embryo-test (although germination of the albina's is sometimes incomplete), and which has the u n i q u e f e a t u r e of distinct simplex expression: young Aaaa plants are light green, AAaa plants normal green. Simplex embryo's and seedlings cannot be distinguished. Attempts with nutrient medium in perlite to accellerate simplex expression seem promising. In this selected line, also a fusca-mutant (purplish discoloration in the embryo's) is carried on. This non-germinating recessive segregates independently of the albina.

Denoting the coefficient of double reduction by c, the frequencies of AA, Aa, aa gametes are

for triplex (AAAa)	resp.	(2+c)/4 ,	2(1-c)/4,	c/4
for duplex (AAaa)	resp.	(1+2c)/6,	4(1-c)/6,	(1+2c)/6
for simplex (Aaaa)	resp.	c/4 ,	2(1-c)/4,	(2+c)/4

From theoretical considerations and from data in literature, a reasonable maximum value for c is 1/8.

The most direct evidence for double reduction and consequently a direct estimate for c comes from triplex-plants. Selfing gives maximally 1/1024 aaaa, which even with the embryo-test is laborious scoring. Crosses with the (lethal) nulliplexes are not possible. However, the distinct simplex phenotype greatly increases the efficiency of estimation (for c = 1/8, triplex produces 1/32 aa gametes, resp. 1/32 simplex offspring).

The results of our embryo-tests on simplexes and duplexes are presented below. BOUHARMONT's (1969) data have been added (locus denoted here by B-b).

Selfing of	Number of plants	Embryo's		Expected albina's		Estimate of c
		Total	Albina's	for c = 0	P	
Aaaa (M4)	80	8389	2224	2097	< 0.01	0.06
Aaaa (M5)	55	12364	3279	3091	< 0.01	0.06
Bbbb	-	12229	2473	3057	-	-
AAaa (M4)	76	8091	355	225	< 0.01	0.13
AAaa (M5)	94	19306	724	536	< 0.01	0.08
AAaa (M6)	12	6108	234	170	< 0.01	0.09
BBbb	-	13213	361	367	n.s.	-

The double reduction estimates from duplexes are somewhat higher, for which we have no ready explanation. It is clear that BOUHARMONT's mutant suffers from a recession deficit and therefore is unsuitable for the estimation of c. Of course, in our material the estimates of c may be somewhat deflated by (e.g. gametic) selection. To study this, reciprocal crosses were made between 41 pairs of duplex

and simplex plants. The fraction simplex among non-albina plants is $4(1-c^2)/16-2c-c^2$), which ratio is insensitive to c (see expectations for c = 0 and c = 1/8 below). The albina's are not included, since their germination is variable.

Cross	Plants		Expected		P
	all green's	light green's	c = 0	c = 1/8	
AAAa x Aaaa	1548	367	387.0	387.4	0.20-0.30
Aaaa x AAAa	1383	321	345.8	346.1	0.20-0.10
				Jointly	0.06

We conclude that selection, if any, plays only a minor role. If gametic, than it seems equal for female and male gametogenesis.

Selfing-seed from the same 41 triplex plants was sown and resulted in:

12211 normal green, 276 light green, 8 albina

(for c = 0 only normal green's are expected). Neglecting the few albina's this leads via the fraction c (1-c)/4 light green's to c = 0.10. For this c-value the number of expected albina's is 8 (a perfect fit).

Since no crosses triplex x nulliplex can be made, a possibility to study the selective difference, if any, between female and male gametogenesis resp. gametes is the reciprocal cross triplex x diploid. Extensive scoring is under way. First the expression of the triploid simplexes (Aaa) had to be studied. For this purpose 10 pairs of simplex and diploid plants were reciprocally crossed (the number of progeny reflects the differences in seedset between 2n and 4n):

AA x Aaaa gave 351 normal green and 365 light green,
Aaaa x AA gave 142 normal green and 156 light green.

As even the joint data do not significantly deviate from 1:1 (expected for c = 0), there is no point in estimating c.

The distinction light green versus normal green is in triploids by far not as clear as in tetraploids, and in diploids (from 2n x 3n and 3n x 2n) phenotypic distinction between Aa and AA becomes uncertain. Therefore, it seems that apart for the number of A-alleles, also the ratio between A and a-alleles affects the expression of leaf colour.

References:

BALKEMA, G.H.: Chimerism and diplontic selection (Ph.D. Thesis, Wageningen), pp. XV + 173 (1971)
- : Radiation Botany 12, 51-55 (1972)
BOUHARMONT, J.: In "Arabidopsis Research" (Rep.Int.Symp., Göttingen), 31-36 (1965)
- : Arabid.Inf.Serv. 4, 3-4 (1967)
- : In "Induced mutations in plants" (Proc. IAEA/FAO Symp., Pullman), 603-610 (1969)

Induction and differentiation of haploid Arabidopsis thaliana

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Haploid callus and plants were cultured from anthers of diploid *A. thaliana* races considering (1) removal of anthers at late prophase-metaphase I of meiosis, (2) selecting a genotype favourable for callus formation from dividing microsporocytes on a moderate auxin-moderate kinetin fully defined medium and (3) a developmental sequence leading towards the complete plant via a callus stage rather than direct embryoid induction.

Mineral salts (GAMBORG and EVELEIGH, 1968) supplemented with vitamins, trace-elements, iron-chelate, sucrose (2%), Difco-agar (0.8%), IAA (6 mg/l) and kinetin (1.5 mg/l) (GRESSHOFF and DOY, 1972) were used to induce callus growth from excised anthers. Within 2-3 weeks of incubation in the dark at 27°C and high relative humidity, yellow callus grew through the anther wall of 3 out of 18 inbred races (received from Dr. R.D. BROCK). These successful races were: Wassileswkija (WS); Estland (EST), Martuba (MT). We do not claim that the other races will never produce haploid callus, however, they will not with the small range of media and hormones that we tested.

Differentiation of plants from callus was induced following transfer of callus to BLAYDES' mineral salts (BLAYDES, 1966) supplemented with vitamins, trace elements, iron chelate, sucrose (2%), Difco-agar (0.8%). NAA (0.5 mg/l) and kinetin (10 mg/l), which was then incubated at 27°C in a 16 hr - 8 hr light-dark cycle (GRESSHOFF and

DOY, 1972). Plants were sterile as expected for their haploid condition, but they developed normally otherwise (Fig. 1).

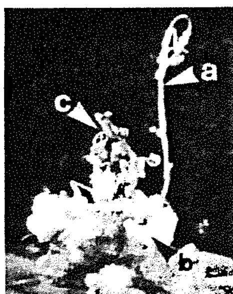

Figure 1: Dying A. thaliana flowering shoot (a) with callus (b) regenerating new flowering shoots (c)

After one year of culture, these callus cell lines have remained hyploid.

References:
BLAYDES, D.F.: Physiologia Plant. 19, 748 (1966)
GAMBORG, O.L., and D. EVELEIGH: Can.J.Biochem. 46, 417 (1968)
GRESSHOFF, P.M., and C.H. DOY: Aust.J.Biol.Sci. 25, 259-64 (1972)

Considerations on the genetic mechanisms in organelles of Arabidopsis

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Extranuclear inheritance received increased attention in recent years. This area of genetics is well suited for study with higher plants because of their complex cells. Arabidopsis may become of special interest because the complexity of the cellular structure is accompanied with the smallest amount of cellular DNA among higher plants (SPARROW, PRICE and UNDERBRINK, 1972). Several other higher plants contain 50-90 times as much DNA as Arabidopsis. The DNA content of the chloroplasts varies relatively little among the various green plants from algae to angiosperms, and it is in the range of that of T4 bacteriophage (BASTIA, CHIANG, SWIFT and SIERSMA, 1971).

Chloroplast mutability in Arabidopsis, like in several other plants, apparently may be autonomous (RÖBBELEN, 1962) or it may be controlled by nuclear genes (RÖBBELEN, 1966; RÉDEI, 1973). Though autonomous mutability may be the result of a number of different events, for the sake of simplicity it is generally assumed that it is due to an alteration in the template of the organelle. Template alteration in the organelle must be involved also in the nuclear gene induced mutability because the mutations can be fixed, maintained, and transmitted in the absence of the inducer (RÖBBELEN, 1966; RÉDEI, 1973). The mutator gene may induce single or relatively few types of alterations (RÖBBELEN, 1966) or a large number of viable and lethal mutations may occur in its presence (RÉDEI, 1973). If only a single site in the plastid would respond to the mutator activity, one would expect that the direct or indirect product of the inducer gene carries an extremely sophisticated information, capable of recognition of nucleotide sequences consisting probably of many residues. At the moment, we know about very few such macromolecules. Perhaps the repressors isolated from bacteria would qualify for such a job. The detection of such a repressor, if it exists in Arabidopsis, would probably be feasible by technics known. In the case of the general type of mutability in the organelles, induced by nuclear genes, there may be a number of equally plausible additional mechanisms. The nuclear gene may produce a chemical mutagen. The probability for the existence of such a compound does not seem very high because the presence of the plastid - targeted mutator genes known in Arabidopsis so far, does not involve increased mutability in the nucleus, in the mitochondria, or in other detectable cytoplasmic entities. The nuclear gene may be just as specific in the case of the generalized mutator (e.g. chm) as in the specialized type (e.g. am) but while in the latter case it would have affinity for the regulatory site of a plastogene, in the former case, it may be involved in the synthesis of the organellar ribosomes, presumably transcribed on plastid DNA, and used for translation of organellar genetic information. It is also conceivable that the generalized type of mutator controls the synthesis of the plastid DNA-polymerase. The various alternatives are amenable to experimental tests.

The cytoplasmically targeted nuclear mutators may contribute information to cellular mechanisms of evolution. The symbiotic origin of the cells of green plants and other higher organisms have been the topic of numerous essays recently, yet only speculations could be offered either for or against the hypotheses. It may be of interest to note that cytoplasmically targeted nuclear genes are known in a number of higher plants (cf. KIRK and TILNEY-BASSET, 1967) yet I am not aware of any major cytoplasmic factor which would determine nuclear mutability in a fashion comparable to the nuclear mutators controlling plastid mutability. Some of the mutators located in the nucleus affect several organelles simultaneously (e.g. WETTSTEIN, 1961) thus one may gain an insight into the independence and interdependence of the hierarchy of multiple genomes within the cell.

Some very fundamental questions concerning the formal genetics of the cytoplasmic systems need clarification. The number of chloroplasts in the cells of Arabidopsis generally varies between 20-80, depending on the tissue. Are we dealing here with polysomy or polyploidy? The fact that we can find cells with many apparently normal green plastids and single white one, makes it difficult to interpret the observation in these terms. The individual plastids are generally assumed to be haploid. We do not know whether the mutants we obtain are recessive or dominant. Dominant mutations in the nuclear genes are very rare yet the abundant mutations induced by the chm locus appear rather of the dominant type. Presumably the genetic information of the plastome within single cells is not at all unique yet no available observation indicate epistasis or genetic interaction within the "polysomic" system. Pleiotropy or linkage apparently exists, however, since sterility and certain types of plastid alterations are generally concomitant, and other traits appear independent. Evidence for crossing over in the plastome of higher plants is not available. In Arabidopsis the plastome is transmitted only maternally, yet the mutator system may place numerous alterations within single cells. Thus in principle, chances for recombination may be available and it may be observed when the methods of detection are adequate. The population genetics of the plastids is far from sufficiently understood. Neither the "one sided sorting out" (MICHAELIS, 1967) nor the preferential segregation (TILNEY-BASSET, 1970) models are comforting REDEI, 1973).

There is a respectable number of circumstantial evidence that the cytoplasmically inherited genetic information is located in one or the other organelle but so far the most direct experimental data (SCHIFF, 1971) still can be interpreted by alternative assumptions.

References:
KIRK, J.T.D., and R.A.E. TILNEY-BASSET: The Plastids Pp. 608. Freeman, San Francisco (1967)
BASTIA, D., K.-S. CHIANG, H. SWIFT, and P. SIERSMA: Proc.Nat.Acad.Sci. U.S. 68, 1157-1161 (1971)
MICHAELIS, P.: Nucleus 10, 1-14 (1967)
REDEI, G.P.: Mutation Res. (in press) (1973)
RÖBBELEN, G.: Z.Vererbungslehre 93, 25-34 (1962)
– : Z.Pflanzenphysiol. 55, 387-403 (1966)
SCHIFF, J.A.: Stadler Symp. 3, 89-113 (1971)
SPARROW, A.J., H.J. PRICE, and A.G. UNDERBRINK: Brookhaven Symp. Biol. 24, 451-494 (1972)
TILNEY-BASSET, R.A.E.: Genet.Res. 16, 49-61 (1970)

Interactions between phytochrome (P_{fr}), gibberellic acid (GA_3) and cyclic adenosine monophosphate (C-AMP) in seed germination of heteroallelic genotypes of Arabidopsis thaliana (L.) HEYNH.

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The seed germination of the genotypes ch+/ch+, ch^1/ch^1, ch^2/ch^2 and ch_3/ch_3 (KRANZ, 1970) produced from the r-mutants of REDEI (ch^1, ch^2) and RÖBBELEN (ch_3) and the wildtype En-2 (ch+) has been examined. The germination decreases significantly in the seeds grown for several generations under abnormal conditions of yellow light (HEHL and KRANZ, 1971). In order to explain the causes of these reactions, the seeds have been treated with GA_3, red (R=660 nm) and far-red (FR=700 nm) light. We got the following results:

1) In ch+/ch+ and ch_3/ch_3 light is obligatory, but in ch^1/ch^1 and ch^2/ch^2 facultative for seed germination.
2) The genotypes are not qualitatively defective in the phytochrome system, but a lower P_{fr}-content is apparently present in the seeds of the mother plants grown under yellow light (Mc COLLOUGH and SHROPSHIRE, 1970).
3) The active form of phytochrome (P_{fr}) cannot trigger complete germination in the seeds for which light is obligatory. Certain amounts of GA_3 must be added to get germination of all seeds.

4) Permanent treatment with yellow light results in "Dauermodifikation" of the seed germination in the following generations. Apparently the decrease of native gibberellic acid causes this behaviour.
5) We suppose, that in the wildtype the de-novo synthesis of gibberellic acid is completely blocked, but in the other genotypes this block is partial and therefore quantitative.

The behaviour in seed germination induced by these treatments can be demonstrated by the example of the third inbred generation (I_{3y}) grown under yellow light (Table 1). GA_3 and R (P_{fr}) promote the germination in the four genotypes. While the maximum

Table 1: Number (p.c.) of germinated seeds in four genotypes (I_{3y}) of A.thaliana treated to GA_3, FR and R.
(FR: far red light, 56.4 x 10^3 erg/cm^2·sec; R: red light, 52.4 x 10^3 erg/cm^2·sec, temperature 2.25 ± 2.5°C)

Treatment	Genotypes			
	ch+/ch+	ch^1/ch^1	ch^2/ch^2	ch_3/ch_3
Dark; H_2O	0	11	4	6
" ; GA_3 10^{-3}M	0	21	12	33
H_2O; 5' FR	0	62	77	-
" 5' FR + 5' R	20	79	99	26
" 5' R + 5' FR	0	79	80	7
GA_3 10^{-3}M; 5' FR	0	82	50	-
" ; 5' FR+5'R	85	96	96	71
" ; 5' R +5'FR	40	94	84	63

of germination can only be induced by GA_3 plus R, R alone induces this maximum in ch^2/ch^2, i.e. GA_3 substitutes just under the action of R. From this result we suppose, that P_{fr} acts by GA_3 on the germination.

But, how does the P_{fr} promote the seed germination? To explain this the seeds of the four genotypes produced under normal light were additionally treated with C-AMP or 5'- AMP. Figure 1 shows the reactions of those seeds for which light is obligatory. Evidently C-AMP with and without R promotes the germination in ch+/ch+, but under these conditions 5'-AMP stops the seed germination of both genotypes, especially when GA_3 is absent. Comparatively GA_3 is most effective, as it can be seen when the seeds are treated with GA_3 alone.

Therefore the reaction chain in the seed germination of A.thaliana can be formulated as follows:

Our results confirm the supposition repeatedly stated (MOHR, 1972; SHROPSHIRE, 1972) that C-AMP operates as a "second messenger" of P_{fr} in the seed germination.

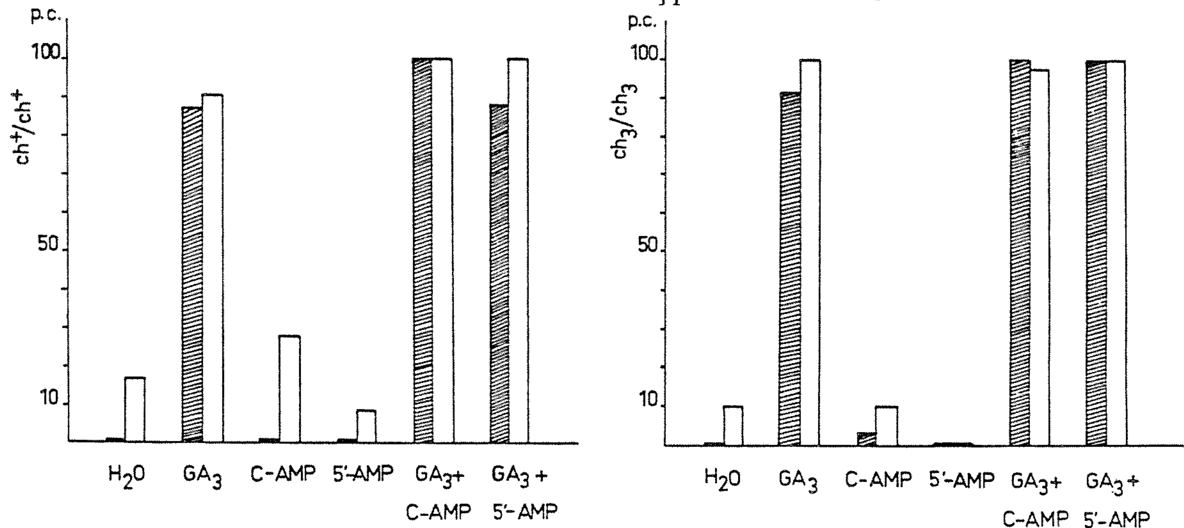

Figure 1: Seed germination of ch+/ch+ and ch_3/ch_3 for which light is obligatory after treatment with FR plus R, GA_3 10^{-3}M, C-AMP 10^{-3}M and 5'-AMP 10^{-3}M (hatched column: dark; white column: 5 minutes FR + 5 minutes R)

References:
HEHL, M., and A.R. KRANZ: Arabid.Inf.Serv. 8, 16-17 (1971)
KRANZ, A.R.: Arabid.Inf.Serv. 7, 28-30 (1970)
McCOLLOUGH, H.J.M., and W. SHROPSHIRE jr.: Plant Cell Physiol. 11, 139-148 (1970)
MOHR, H.: Lectures on Photomorphogenesis, Springer-Verlag, Berlin (1972)
SHROPSHIRE, W.: Photophysiology 7, 33-72 (1972)

Gene action of two ch-loci on the primary carotenoids in leaves of Arabidopsis thaliana (L.) HEYNH.

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Several investigations in the ch-mutants of RÉDEI and RÖBBELEN have shown fundamental results in the genetic control of the chlorophyll biosynthesis. Further, the two mutated gene loci act on the total amount of the carotenoids (KRANZ 1970). Therefore, we are studying the pleiotropic effect of two heteroallelic ch-genes on the primary carotenoids (PC).

Analysing the PC, ß-carotene (ß-C), lutein (L), neoxanthine (N) and lutein-5,6-epoxide (LE) by the thin layer chromatographic method (TC) of HAGER and MEYER-BERTENRATH (1966, 1967) plants of the wildtype (ch+/ch+) and three mutant genotypes (ch^1/ch^1, ch^2/ch^2, ch_3/ch_3) were used which had been cultivated from seed germination to flowering in a glass house under standard conditions (KRANZ, 1968). Further difference spectra computed from in-vivo remission spectra of leaves (DR) were analysed by the method of KRANZ (1972, 1973). The plants of this material were cultivated in a growth chamber under controlled light conditions (KRANZ, 1968).

The PC studies probably do not participate in the metabolism of the secondary carotenoids (SC). But N is a secondary product of ß-C, L and LE descents from α-carotene (α-C) (CYGAN, 1967; GOODWIN, 1971). The four genotypes reach the maxima of these PC at different time. Table 1 shows that delayed development and pigment reduction are in all cases positively correlated in the sequence ch+/ch+ > $ch^2/ch^2 \geq ch^1/ch^1 > ch_3/ch_3$.

Table 1: Days after seeding and maxima of primary carotenoids (mg/g dry weight) in four genotypes of A.thaliana

Genotype	ß-Carotene		Neoxanthine		Lutein		Luteinepoxide	
ch+/ch+	24	0.145	22	0.169	25	0.370	24	0.077
ch^1/ch^1	30	0.112	24	0.066	30	0.213	28	0.044
ch^2/ch^2	27	0.097	24	0.079	27	0.172	27	0.045
ch_3/ch_3	34	0.015	28	0.035	33	0.054	30	0.010

In order to detect exact differences between the genotypes the analysis of the regressions of the TC-values between the PC-derivatives are carried out. Figure 1 ascertains that about one third less molecules N per mol ß-C are admitted by the

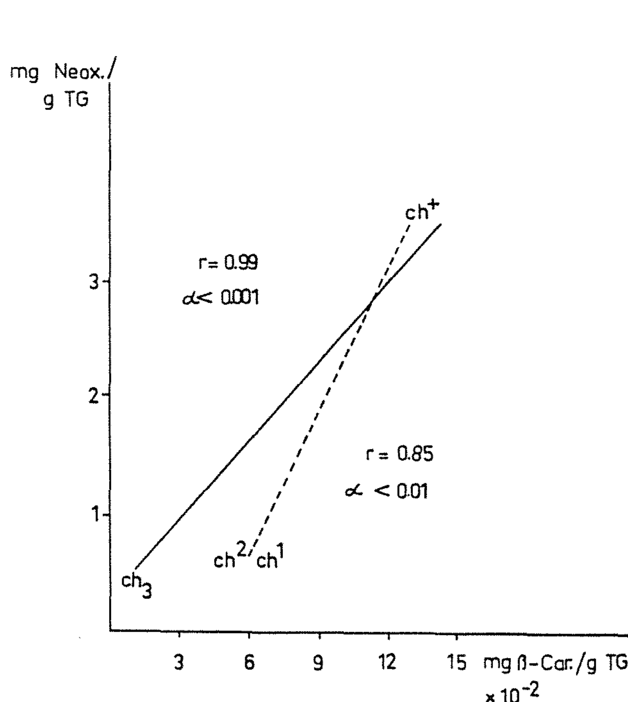

Figure 1: Regression lines and correlation coefficients (r) between ß-C and N in the wildtype (ch+) and the genotypes of the first locus (ch^1, ch^2) and the genotype of the second locus (ch_3). (α: probability of the error for r)

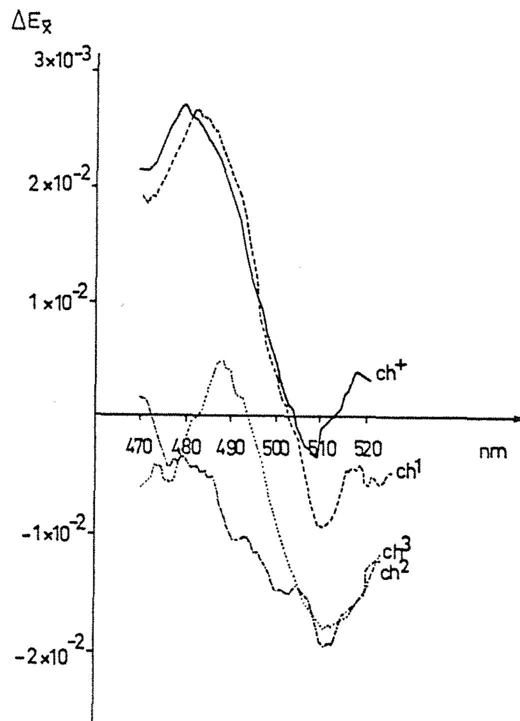

Figure 2: DR-spectra of leaves in four genotypes of A.thaliana. ($\Delta E = E_{Ri} - E_{Rj}$, E_{Ri}: In-vivo remission after dark period E_{Rj}: In-vivo remission after 10 minutes light)

alleles ch^1 and ch^2 of the first gene than by the allel ch_3 of the second gene; in other words the production of N from ß-C is more reduced by the first ch-locus mutations than by the second locus mutation. Further one step in the PC-biosynthesis is controlled by the two genes before α-C is separated from ß-C, because L, the descendant of α-C, is reduced in the mutant genotypes (see table 1). OGAWA et al. (1966) and BOARDMAN and ANDERSON (1967) detected that L and N are predominantly localized at the grana and ß-C at the intergrana membranes. Therefore the first ch-gene reduces the PC of the grana stronger than the PC of the intergrana membranes; the second ch-gene reduces the PC of both membrane systems equally.

In order to analyse the photoinduction and photodestruction of the PC DR-spectra are measured between λ = 465 to 530 nm after treatment with light for 1, 10 and 60 minutes. Figure 2 shows that there is one PC-complex characterized by $\lambda_{max} \sim 510$ nm, which increases after 10 minutes light; i.e. this complex is photoinduced in the sequence $ch+/ch+ < ch^1/ch^1 < ch^2/ch^2 = ch_3/ch_3$. But the other complex characterized by $\lambda_{max} \sim 485$ nm decays by light in the sequence $ch+/ch+ = ch^1/ch^1 > ch_3/ch_3$; in ch^2/ch^2 this complex is absent or not photodestructed. Further experiments are necessary to differentiate the components of these two complexes.

References:
BOARDMAN, N.K., and J.M. ANDERSON: Biochim.Biophys.Acta 143, 187-203 (1967)
CYGAN, F.C.: Ber.dtsch.Bot.Ges. 8, 627-644 (1967)
GOODWIN, T.W.: Aspects of Terpenoid Chemistry and Biochemistry. Academic Press, London 1971
HAGER, A., and T. MEYER-BERTENRATH: Planta 69, 198-217 (1966)
- , - : Planta 74, 138-172 (1967)
KRANZ, A.R.: Arabid.Inf.Serv. 5, 56-57 (1968)
- : Arabid.Inf.Serv. 7, 28-30 (1970)
- : Arabid.Inf.Serv. 9, 19-21 (1972)
- : Z.Pflanzenphysiol., in preparation, 1973
OGAWA, T., et al.: Biochim.Biophys.Acta 112, 223-234 (1966)

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An enquiry into chlorophyll chimerae in Arabidopsis in M_1 by means of tissue culture

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According to our earlier reports (DEMCHENKO et al., 1972), we hoped to clarify the nature of chimerae in M_1 by tissue culture experiments. The transition of the changed somatic cells through the non-differentiating callus growth and subsequent redifferentation into plantlets was chosen as approach to analyse the type of alteration which has taken place in the sectors. For the same purpose DULIEU (1971) redifferentiated chimerae of tobacco plants from chlorophyll deficient sectors by the use of tissue culture.

The results of our first experiments are as follows. We examined chlorophyll chimerae in M_1 arising in the race En-1 of Arabidopsis thaliana HEYNH. after treatment with N-nitrosomethyl urea. Chlorphyll-deficient sectors of leave tissue were isolated aseptically and pieces of 2-5 mm^2 were transferred onto culture media for the sake of induction of callus growth. Similar explants taken from normal green leaves of untreated plants were used as control. The control and the tested explants responded likewise to modifications in the composition of the nutritional media, which definitively affected the explants' further development. We used agar media MS (MURASHIGE, SKOOG, 1962) supplemented with 3% sucrose, kinetin and NAA (1 mg/l) mesoinositol (80 mg/l) and vitamin B_1 (0.4 mg/l) and media G (GAMBORG et al., 1968), supplemented with 3% sucrose, Caseine hydrolyzate (400 mg/l), mesoinositol (80 mg/l) and vitamins after Staba.

Callus develops from the explants in 8-14 days. A combination of NAA and kinetin in medium MS induced callus formation more efficiently than a combination of IAA and kinetin in medium G. Unlike medium G, medium MS caused abundant rhizogenesis in the explants. This observation holds true for both normal and defective tissues though in the latter growth and differentiation were inferior compared to the normal. On the control medium G, after a month time stem buds appeared; after 2 1/2 month the number of tubes displaying redifferentiation amounted to 83% of the total. On medium MS NAA stem buds differentiation was inhibited. In case of chlorophyll

deficiency stem bud regeneration was reduced, and on GAMBORG's medium it reached only 25%.

The regenerated plants arising both from the defective or control explants differed in morphogenesis from the normal plants grown in test-tubes from seed. They were smaller, poorly foliated, often without rosette leaves, and they had only flowers. While plants redifferentiated from normal tissue hardly differed from the plants grown from seeds in the rate of development including organogenesis and sporogenesis, the plants redifferentiated from the chlorophyll deficients sectors displayed disturbances in both micro- and macrosporogenesis (Table 1). In the control, about 80% of the regenerants yielded normal seeds; no seeds were obtained from the plants redifferentiated from the chlorophyll deficient tissue, virespective of the albina, chlorina, viridis or xantha colour of the explants.

Table 1: Characteristics of morphogenesis in Arabidopsis seedlings regenerated from tissues compared with plants cultured from seeds

	Stages of morphogenesis	Control	"Spots"	Seed plants
1	Callus formation	+ +	+	
2	Induction of roots from callus	+ +	+	
3	Growth of roots from callus	+ +	+	
4	Induction of stem buds from callus	+ +	+	
5	Stem growth	+ +	+	+ + +
6	Development of roots	+ +	+	+ + +
7	Growth of roots	+ +	+	+ + +
8	Flower primordia	+ +	+	+ + +
9	Ovules	+ +	±	+ + +
10	Pollen	±	−	+ + +

Most interesting is the finding that regenerants from some explants retained their original colour. Namely, among 4 regenerants grown from callus derived from viridis explants three had uniformly viridis colour and one was normal green. Segregation into the original defective colour and normal "wild" green was noted in all viridis and chlorina cases. Albino explants normally gave rise to chlorophyll-deficient regenerants, only once green plants were also obtained. No other colour nor variegated phenotype were ever observed. That chlorophyll-deficient tissue growing on culture media rich in kinetin showed no repair of colour may be treated as evidence for the genetic rather than physiological nature of the variegation. The emergence of green regenerants on "defective" callus may be due to several reasons (admixture of green cells in the original explants, reversion). Our further exploration will be aimed at understanding the reasons for segregation of the colour in the regenerants and the identification of the cell genotype in chimeric M_1 plants.

Sterility of the tested regenerants may not be an intractable obstacle. The possibility should not be ruled out that subjected to the proper conditions regenerants may pass through sporogenesis. Since there was 20% sterility among the regenerants in the control, we believe that besides genetics, also the suboptimal cultural condition are responsible for the infertility.

A similar tissue culture technique can be applied in the future for the analysis of anthers and haploid tissues.

References:

DEMCHENKO, S.I., R.Ya. SEROVA, and V.A. AVETISOV: Arabid.Inf.Serv. 9, 31-33 (1972)
MURASHIGE, T., and F. SKOOG: Physiol. Plantarum 15, 473 (1962)
GAMBORG, O.L., R.A. MILLER, and K. OJIMA: Exptl.Cell Res., 50, 151 (1968)
DULIEU, H.: In: Morphogenesis in plant cell, tissue and organ cultures, Delhi, p. 42 (1971)

On the homological variability of the chloroplast membrane system

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The analysis of the great number of mutants of Arabidopsis thaliana (L.) HEYNH. revealed several main morphotypes of the membrane systems. These morphotypes have been compared to the chloroplast membrane system of some existing taxons (Fig. 1). The scheme demonstrates the similarity between the variability of mutation of Arabidopsis thaliana chloroplasts and the membrane system of some other plants. The chloroplast membrane system of the well investigated plants (Chlamydomonas reinhardi, Hordeum vulgare, Pisum sativum, Zea mays, Lycopersicum esculentum, Nicotiana tabacum, Oenothera) is much alike to the morphotypes of the membrane system in Arabidopsis thaliana. This information suggests that chloroplasts of the well investigated plants have homological variability in their membrane systems. This homology makes worthy a study of the wide range of the plastom mutants to seek answer to the problem

whether the morphotypes of their membranes would be similar to those of the nuclear mutants.

Such a study may shed light on the origin and evolution of the chloroplast membrane system in the plant world.

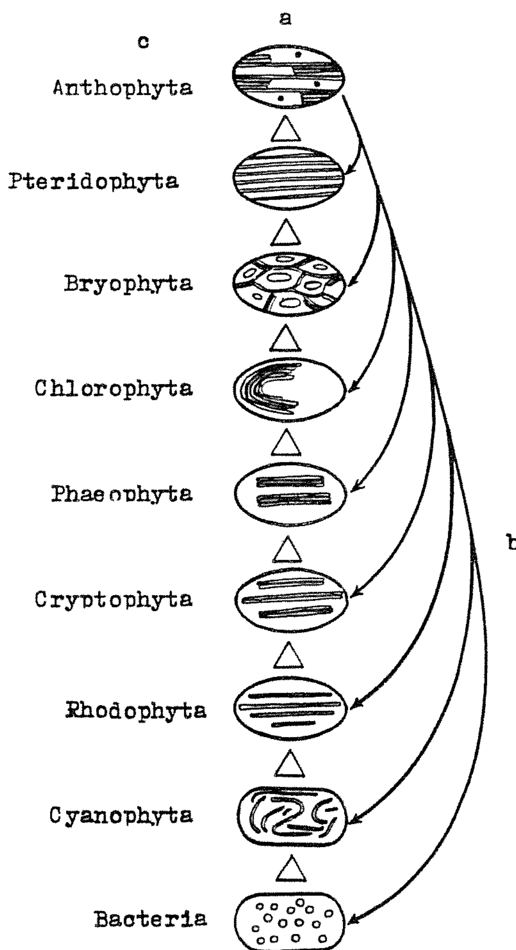

Figure 1: The main morphotypes of the membrane systems of the photosynthesizing organisms (a shortened list).
a. The imagined direction of the historical evolution of photosynthetic membranes.
b. Morphotypes of the membrane systems in chloroplast mutants of Arabidopsis thaliana.
c. The main groups of plants are arranged in the phylogenetic order.

Reference:
H.A. ABDULLAEV, and S.V. TAGEEVA: Theses "Geneticheskie Aspekty Photosynteza", Dushanbe, 1972

On the genetic control of the chloroplast size and number in mesophyll cells of species of *Arabidopsis*

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It is a common knowledge now that formation and activities of the photosynthetic apparatus are controlled both by the chromosomes and by the extrachromosomal systems.

In this connection, the main point of investigations on the genetics of photosynthesis now is the elucidation of the role of these systems in the process of chloroplast formation and function.

Our investigations were designed to study the effect of genotype (nuclear mutations and a set of chromosomes) on the size and the number of chloroplasts in mesophyll cells in plants of the genus *Arabidopsis*.

Chloroplasts of the primary two expanded leaves were studied in 5 species of *Arabidopsis* (YUNUSOV, KASYANENKO, USMANOV, 1970) and 14 mutant strains of race Enkheim *in vivo* (USMANOVA, 1972).

Figure 1 shows the variations in the chloroplast number and the diameter in the cells of the plants studied compared to an increasing number of chromosomes. Coefficient of regression ($b = 0.38 \pm 0.098$) curve of the chloroplast number differs from zero (at $t_d = 4$; df = 3; P = 0.025). Consequently, an increasing number of chromosomes correlates positively to the number of chloroplasts.

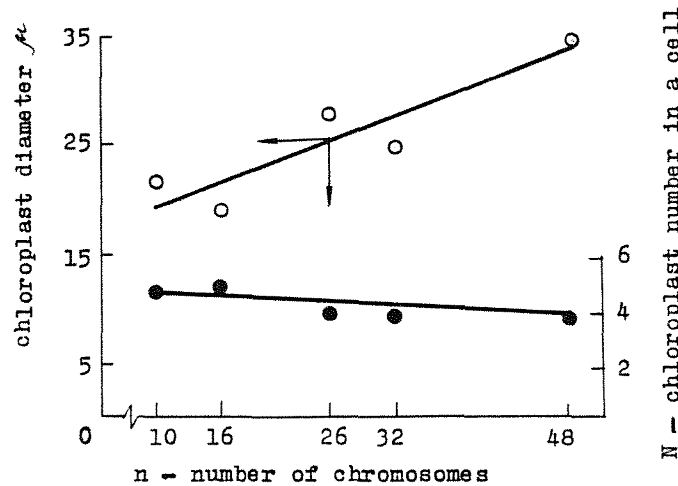

Figure 1: The relationship between n-number of chromosomes, diameter of chloroplasts (dark circles) and N-average number of chloroplasts (light circles) in mesophyll cells of 5 species of *Arabidopsis*

Coefficient of regression of the curve for diameters 0.014 ± 0.013 ($t_d = 1.08$; df = 3; P = 0.38) indicates that in all species studied an increase of the chromosome number does not affect the size of chloroplasts. This led us to the concept that chloroplast size is under the control of the extrachromosomal systems.

A study of the 14 mutant strains revealed that nuclear mutations statistically influenced the size and number of chloroplasts.

Figure 2 represents the curve only for the chloroplast size: $b = 0.070 \pm 0.005$; $t_d = 14.7$; df = 13; P = 0.0002.

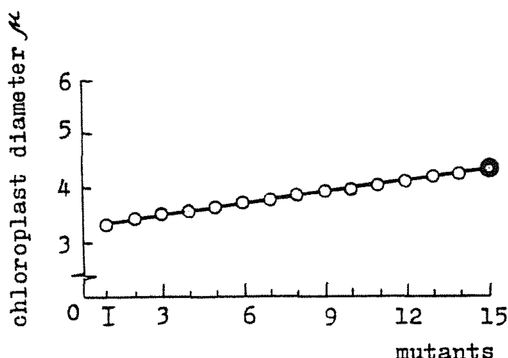

Figure 2:
Diameter of chloroplasts in 14 chlorophyll mutations of *A.thaliana* compared to the normal; 1-14 mutants are arranged in the order of increasing average diameter.
15 - diameter of chloroplasts in the wild type

On the basis of our results and the numerous data of the literature (obtained for different plants: wheat, barley, maize, sugar-beet, potatoes etc.) the general conclusion is made that the average number of chloroplasts in the cell is controlled by the genetic factors localized in the nuclear apparatus.

Since the data of the literature are contradictory we cannot make a valid suggestion concerning the localization of the genetic system controlling the size of chloroplasts.

References:
YUNUSOV, S.Yu., A.G. KASYANENKO, and P.D. USMANOV: Arabid.Inf.Serv. 7, 34 (1970)
USMANOVA, O.V.: Genetic Aspects of Photosynthesis. Abstracts, 72-73, Dushanbe, 1972

The effects of the capacity and density of the ruby laser irradiation on the seeds of *Arabidopsis thaliana* (L.) HEYNH.

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For the interpretation of the biological effects of laser irradiation the following parameters are to be considered: wave length, percussion wave, energy density and heat. The role of heat and energy density were analized in this paper.

Air-dried seeds of *Arabidopsis thaliana* (L.) HEYNH. were irradiated by a ruby laser (λ = 6943 A) in conditions of free generation - C (thermal factor is clearly expressed), and with modulated quality - Q (thermal factor is less expressed).

The survival of plants to the stage of the first two leaves formation has been determined.

Figure 1 shows, that the rate of plant destruction under Q-conditions (counts period $\tau = 10^{-9}$ sec.) is considerably higher than under C-conditions (counts period $\tau = 10^{-3}$ sec.). The observations on the limits of tolerance determined for both irradiation conditions are comparable. 100% destruction of plants subsected to C-conditions was observed at the dose 24 J/cm^2, and under Q-conditions - at 10-11 J/cm^2. Thus laser irradiation generated under Q-conditions has a greater biological efficiency. Consequently, heat effects are not the most important among the biological effects of laser irradiation.

Figure 2 demonstrates significant differences between the biological effects of the concentrated and fractioned irradiation of seeds (no additivity!).

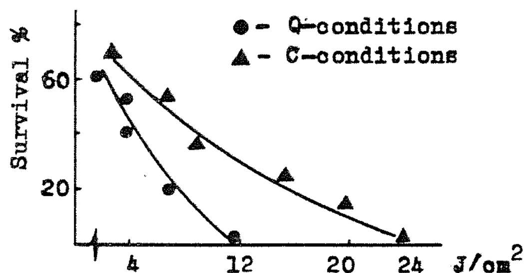

Figure 1:
The survival of *A.thaliana* plants at different doses of laser irradiation

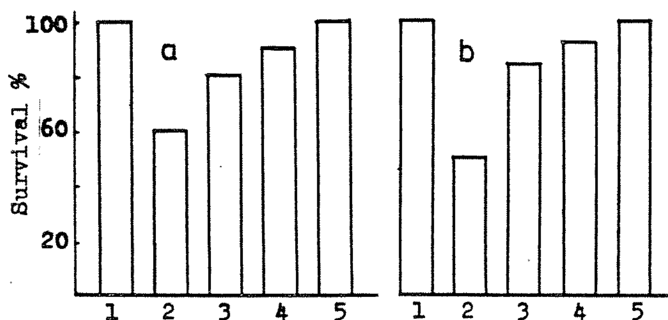

Figure 2:
The effects of energy density on the survival of *A.thaliana* plants.

a. Q-conditions: (1) control; (2) 4.4 J/cm^2; (3) 2.2+2.6 = 4.8 J/cm^2 (4) 1.4+1.2+2.4 = 5.0 J/cm^2; (5) 1.2+1.2+0.8+0.8+1.6 = 5.6 J/cm^2

b. C-conditions: (1) control; (2) 11.4 J/cm^2; (3) 5.7+6.5 = 12.3 J/cm^2 (4) 3.3+3.5+3.8 = 10.6 J/cm^2; (5) 3.5+2.3+2.3+3.4 = 11.5 J/cm^2

Experiments on the effects of energy density revealed the threshold of effective doses of laser irradiation, at which the biological effects begin: in C-generation conditions the effect begins at impulse energy 3.5 J/cm^2, in Q-conditions - at 1.6 J/cm^2.

Reference:
STARTSEV, G.A., and O.H. YULDASHEV: Geneticheskie Aspekty Photosinteza. Abstracts. Dushanbe, 1972

A study of the mutagenic action of 1,4-bisdiazoacetylbutane and 8-ethoxycaffeine on Arabidopsis thaliana

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It has been reported that 1,4-bisdiazoacetylbutane (BDAB) induced mainly gene mutations (RAPOPORT and ZOZ, 1962) and 8-ethoxycaffeine (EOC) induced mainly chromosome aberrations (EHRENBERG, 1956). We expected that applying of these mutagens would be useful for the elucidation of the nature of embryonic lethals.

Seeds presoaked for 8 hrs were treated for 18 hrs with BDAB (1%) at 25°C or with EOC (0.025 M) at 12°C. Seedlings were treated for 18 hrs with BDAB (0.15%) or with EOC (0.025 M). Aseptic and soil cultures were grown under continuous luminescent illumination at 24-26°C. Percentage of survival after BDAB treatment of seeds was 72%,that of seedlings 77%; after EOC treatment - 98% and 71%, respectively (control 98%). Five siliquae per plant were scored according to the procedure of MÜLLER.

The siliquae which contained underdeveloped ovules were devided into 3 classes according to the ratio of underdeveloped ovules to the total number of the ovules: I - $\geqslant$1/10; II - 1/11-1/20; III - $<$1/20. The total spontaneous frequency of plants which had siliquae with underdeveloped ovules was 60%; the total spontaneous rate of underdeveloped ovules was 0.75%; 70% of these belonged to class III, 30% - to class II and only 1-2% - to class I. After the treatment of seeds and seedlings with BDAB and EOC the rate of class II and III ovules did not increase.

The table demonstrates that BDAB and EOC induced low frequency of lethals of class I - 2.3 and 2.6% per plants and 0.18 and 0.14% per ovules, respectively. The hereditary nature of the lethals of class I was confirmed in M_3. Because of the low mutagenic effect of BDAB and EOC we cannot make the conclusion about the nature of embryonic lethal mutations.

Table 1: The effects of BDAB and EOC on seeds and seedlings of Arabidopsis thaliana

Variant		Scored	Plants Number	Plants % ± m	Siliquae Number	Siliquae % ± m	Ovules Number	Ovules % ± m
BDAB	seeds (aseptic culture)	total number	199	100	981	100	33571	100
		lethals	7	3.6±1.33	13	1.3±0.35	66	0.20±0.024
	seedlings (aseptic culture)	total number	194	100	959	100	35047	100
		lethals	6	3.1±1.24	10	1.0±0.32	86	0.20±0.017
EOC	seeds (soil culture)	total number	65	100	321	100	14553	100
		lethals	2	3.1±2.14	4	1.2±0.61	24	0.17±0.033
	seedlings (aseptic culture)	total number	77	100	374	100	12191	100
		lethals	2	2.6±1.81	2	0.5±1.16	18	0.15±0.035
	seedlings (soil culture)	total number	61	100	303	100	14016	100
		lethals	3	4.9±2.76	3	1.0±0.56	23	0.16±0.033
Control	aseptic culture	total number	153	100	762	100	25468	100
		lethals	1	0.6±0.62	1	0.1±0.12	6	0.02±0.009
	soil culture	total number	101	100	503	100	22615	100
		lethals	1	1.0±0.99	1	0.2±0.20	8	0.02±0.030

In details see SHEVCHENKO et al. (1973)

References:

EHRENBERG, L., A. GUSTAFSSON, and U. LUNDQUIST: Acta Chem.Scand. 10, 492-494 (1956)
RAPOPORT, I.A., and N.N. ZOZ: Tsitologiya, 330-334 (1962)
SHEVCHENKO, V.V., L.I. GRINIKH, E.M. PROTOPOPOVA, and G.A. GRIGORIEVA: Genetika 9 (1973) in press

The influence of ionizing radiation on growth correlations of Arabidopsis thaliana (L.) HEYNH.

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The correlation between the root growth and other physiological processes of plants have not been studied completely. Researches of CHVOSTOVA (1959) showed a positive correlation between the inhibition of the root growth of barley and the extent of mitotic damage after irradiation. CONTANT and DANKERT (1968) indicated a high correlation (r = 0.91) between the root length and the survival. The connection between the decrease of root elongation measured 100 hours after germination and flowering time of the irradiated plants was much less clear. In the investigations with Arabidopsis the root length and the appearance of the cotyledons (3-rd day after planting) and the appearance of the rosette leaves (7-th day after emergence) is often used as an index of radiation injury to seeds (MÜLLER, 1964; SANINA, 1970).

The aim of this paper is to estimate the length of the root in various phases of the development of irradiated plants relative to the control. Arabidopsis thaliana race Enkheim was the experimental material. Before the sowing the seeds were irradiated by gamma-rays at doses of 10, 30 and 60 krad at the dose rate of 3 x 10^3 rad/min. A portion of the control and of the irradiated seeds were sown in the Petri dishes for observations of root length, the rest was sown in the test-tubes to observe the influence of the irradiation on the root growth and other developmental characters. The germination of the seeds in the Petri dishes and the test-tubes was simultaneous irrespective of the dose of irradiation. The root growth was depressed and the development of the rosette was delayed with an increase of the dose of the irradiation (Table 1).

Table 1: The influence of gamma-rays on the root length and on the time of appearance of the rosette

Observations	Control		10 krad		30 krad		60 krad	
	$M+m_m$	$C+m_c$	$M+m_m$	$C+m_c$	$M+m_m$	$C+m_c$	$M+m_m$	$C+m_c$
1.	9.4±0.23	37.6±1.96	7.8±0.33	47.6±4.16	3.5±0.14	44.4±3.24	1.8±0.07	46.3±3.34
2.	23.3±0.36	23.8±1.15	18.4±0.40	25.9±1.64	8.2±0.27	39.2±2.23	4.0±0.15	46.5±3.36
3.	5.9±0.10	11.4±1.12	6.5±0.10	10.5±1.02	7.9±0.17	15.2±1.55	8.3±0.19	15.9±1.57

Table 1 gives the statistical parameters of the following characters: 1 - the root length on the 3-rd day after germination, 2 - the root length on the 7-th day after germination, 3 - the time of appearance of the rosette.

A correlation matrix analysis (Table 2) indicates that in the control plants and in the group which received only 10 krad irradiation there is no correlation between criteria 1 and 2, but at the higher doses there is a good correlation between estimates no. 1 and 2. The time of appearance of the rosette is closely correlated with the root length of the control and irradiated plants. At the same time the appearance of the rosette has a positive connection with the other development phases (FERSHTAT, SAVIN and STEPANENKO, 1971).

Table 2: Correlation matrix

Observations	Control		10 krad		30 krad		60 krad	
	2	3	2	3	2	3	2	3
1.	0.34±0.13	0.23±0,14	0.26±0.13	-0.36±0.13	0.42±0.12	-0.83±0.04	0.87±0.03	-0.85±0.04
2.		-0.41±0.12		-0.84±0.04		-0.39±0.12		-0.90±0.03

The results indicate that an observation of the root growth at the beginning of the rosette development provides a practical estimate of the radiation damage.

References:

CHVOSTOVA, V.V., and N.L. DELONE: Tsitologiya 1,3,320 (1959)
CONTANT, R.B., and R. DANKERT: Arabid.Inf.Serv. 5, 41 (1968)
MÜLLER, A.J.: Kulturpflanze, 12, 237 (1964)
SANINA, A.V., V.I. IVANOV, and E.A.TIMOFEEVA-RESSOVSKAY: Radiobiologiya 10, 4, 629 (1970)
FERSHTAT, A.M., V.N. SAVIN, and O.G. STEPANENKO: Arabid.Inf.Serv. 8, 3 (1971)

Effects primaires et mutagènes de l'irradiation gamma des graines d'Arabidopsis

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Avant d'aborder l'étude biochimique de quelques mutants chlorophylliens radio-induits chez Arabidopsis (BOUNIAS, 1972), nous avons analysé les effets primaires des rayons γ du cobalt-60 appliqués sur les graines sèches. Les irradiations ont été effectuées en 1966 et 1967 en "Gammacell" et en 1968 en salle d'irradiation, à raison de 1240 et 1230 rad/mn respectivement. Les résultats concernent la germination, la croissance en phase végétative et en phase florale, ainsi que les taux de mutations en 2ème génération. (F_2).

1) G e r m i n a t i o n - La fig. 1 montre que les taux de germination décroissent linéairement en fonction des doses. Par extrapolation, la germination serait totalement inhibée vers 1100 Krad, ce qui traduit une r a d i o - r é s i s t a n c e c o n s i d é r a b l e.

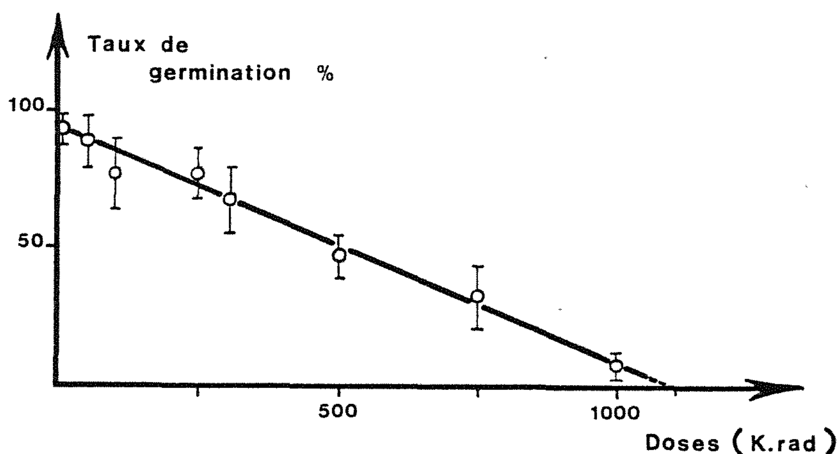

Figure 1: Influence de l'irradiation γ des graines sèches sur la germination, chez Arabidopsis thaliana

2) C r o i s s a n c e v é g é t a t i v e - Les plantules agées de 10 jours, issues de graines irradiées présentent une réduction de hauteur par rapport aux témoins (tableau 1). Cette diminution est linéaire en fonction des doses croissantes entre 0 et 30 K.rad et entre 30 et 60 K.rad (Corrélation : r = - 1 ; N = 10) mais pas de 0 à 60 K.rad. D'autre part, les feuilles cotylédonaires, qui forment un plan horizontal chez les témoins, s'incurvent après irradiation et l'angle ainsi formé se ferme progressivement en fonction des doses croissantes.

Tableau 1: Effets de l'irradiation γ des graines d'Arabidopsis au niveau de la hauteur des plantules et de l'angle cotylédonaire (N = 10 mesures)

Doses (Krad)	0	5	10	20	30	40	50	60
Hauteur des plantules (mm)	10,0 ± 1,0	9,2 ± 1,2	8,2 ± 0,4	6,6 ± 0,7	5,0 ± 1,2	4,6 ± 0,7	4,4 ± 0,2	3,4 ± 0,3
Angles des cotylédons	180°	-	180°	177°	163°	123°	74°	-

3) C r o i s s a n c e e n p h a s e f l o r a l e e t f e r t i l i t é
Une étude systématique de 5 en 5 Krad a montré que les hampes florales deviennent stériles vers 125 Krad, mais que leur formation n'est totalement inhibée que vers 200 Krad.

La fig. 2 montre l'influence des doses croissantes de rayons γ sur le nombre moyen de graines par plant.

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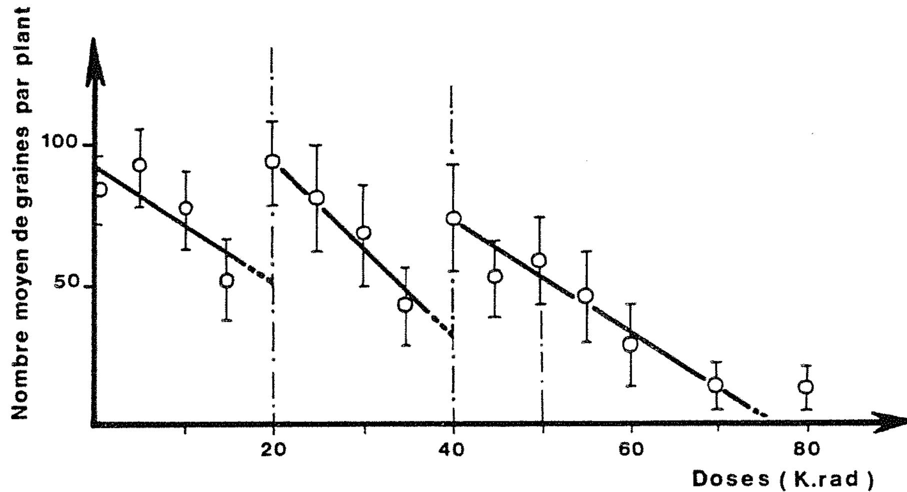

Figure 2: Niveau de croissance atteint par les plants issus de graines irradiées : Nombre de graines par plant (N = 20 plants par dose (2 répétitions))

Il est apparu que la courbe dose/effet ne constitue pas une droite dans son ensemble. Cependant, le calcul statistique montre qu'elle peut se décomposer en trois fractions linéaires (tableau 2) dont les origines se situent chacune à un seuil comparable au niveau de croissance atteint par les témoins (BOUNIAS, 1967).

Tableau 2: Décomposition de la courbe donnant le nombre moyen de graines par plant en fonction des doses (N = 20 mesures)

Intervalles de doses	Nature de la régression	pente de la droite	Coefficient de corrélation
0 à 15 K.rad	linéaire	- 1,85	- 0,75
20 à 35 K.rad	linéaire	- 3,00	- 0,97
40 à 70 K.rad(1)	linéaire	- 1,85	- 0,94
0 à 80 K.rad	non linéaire	-	

(1) 50 à 70 K.rad dans le cas de la hauteur moyenne des hampes (fig.3)

4) E f f e t s   m u t a g è n e s - R e l a t i o n F_1/F_2

La courbe traduisant l'augmentation des taux de mutations en F_2 en fonction des doses, présente une allure opposée à celle représentant les effets de l'irradiation sur la croissance en F_1, avec deux minima situés vers 20 et 50 K.rad correspondant aux seuils maxima de croissance des hampes florales (fig. 3).

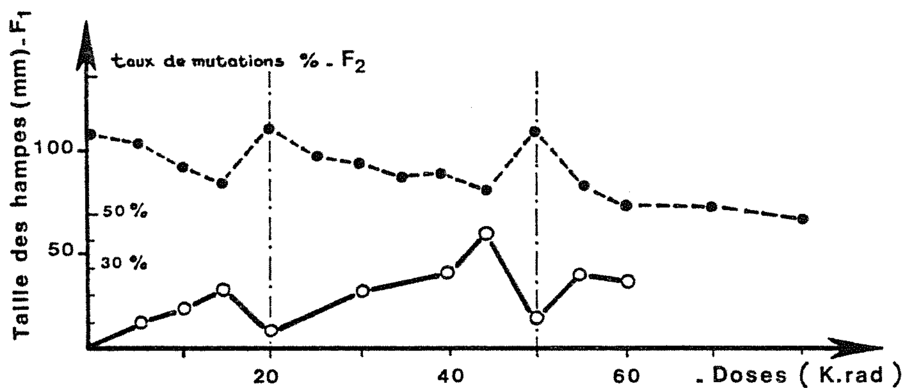

Figure 3: Influence des doses d'irradiation γ sur la croissance des hampes en F_1 (--•--) et sur les taux de mutations en F_2 (—o—) chez Arabidopsis

Ces résultats ont permis de montrer que les taux de mutations obtenus en F_2 sont inversement proportionnels à la croissance des plants en F_1, ce qui concorde avec les résultats de BROCK (1967) et GICHNER (1966). Pour un échantillon de 12 couples de mesures, le calcul donne les résultats suivants:

coefficient de corrélation : r = - 0,92 ; Régression linéaire
Equation de la droite : taux de mutations % = - 0,31 h + 51
(h = hauteur moyenne des hampes (mm))

5) D i s c u s s i o n - L'effet des rayons γ appliqués à doses croissantes sur les graines sèches d'Arabidopsis se traduit au niveau de la germination puis de la phase végétative par une décroissance continue, linéaire dans le premier cas. Cependant, la courbe dose/effet présente une allure discontinue après le stade floral (hauteur des hampes et production de graines) : elle peut se décomposer en 3 droites successives ayant respectivement pour origines les seuils : 0 (Témoins) - 20 K.rad - et 40 à 50 K.rad. Pour chacun de ces seuils, les niveaux de croissance atteints par les plants sont statistiquement comparables (BOUNIAS, 1967).

HACCIUS (1963) a mis en évidence chez Arabidopsis des phénomènes de restauration cellulaire après action des rayons X, et nous avons émis l'hypothèse, dans le cas présent, d'un mécanisme complexe de restauration mettant en jeu successivement trois types distincts de cellules méristématiques embryonnaires présentant des radiosensibilités décroissantes (SPARROW et EVANS, 1961).

Un tel mécanisme ne peut se traduire de facon perceptible sur la croissance que si le méristème embryonnaire comporte un très petit nombre de cellules, ce qui est le cas chez Arabidopsis.

S u m m a r y
Gamma rays of ^{60}Co applied on dry seeds of Arabidopsis induced a linear decrease of germination, between 5 to 1000 K.rad. The stem emergence was completely inhibited after 200 K.rad irradiation and no seed production was obtained above 125 K.rad. The relationship between stem growth or seed production and dose rate was not linear between 5 to 80 K.rad, but was composed of three discontinuous linear curves, each of them starting at a growth-level very closed to that of control plants. It has been suggested the hypothesis of a cellular-regulation mechanism involving the action of three successive embryonic meristematic cells of decreasing radio-sensitivity.

Références:
BOUNIAS, M.: Thèse Doctorat ès sciences - LYON, 250 p. (1972)
- : DEA - LYON, 12 p (1967)
BROCK, D.R.: Rad.Bot. 7, 193-203 (1967)
GICHNER, T.: Biol.Plant. 8, 209-212 (1966)
HACCIUS, B., and H. REICHERT: Planta 60, 289-306 (1963)
SPARROW, A.H., and EVANS: Brookhaven Symp.Biol. 14, 83 (1961)

L'auteur remercie Monsieur SAUBIN qui a eu pour têche d'effectuer les irradiations et de vérifier la dosimétrie.

Equipement pigmentaire des cotylédons d'Arabidopsis après irradiation "gamma" des graines

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Au cours de l'étude des effets primaires des rayons "gamma" chez Arabidopsis, une très grande radio-résistance des plantes a été mise en évidence après irradiation des graines sèches (BOUNIAS, 1967). Les cotylédons issus de graines irradiées présentent un aspect et un poids comparables à ceux des témoins pendant les premiers jours qui suivent la germination. Ceci nous a incité à étudier plus précisément l'équipement pigmentaire des cotylédons après irradiation jusqu'à la limite de viabilité des graines (BOUNIAS, 1973).

1) T e c h n i q u e s - Les irradiations ont été réalisées en "Gamma cell" et les doses vont jusqu'à 10^3 K.rad à raison de 1230 rad/mn. Les pigments chlorophylliens et caroténoides ont été analysés grace à une microméthode quantitative de chromatographie en couche mince (BOUNIAS, 1969). Les plants étudiés sont âgés de 5 jours et pèsent chacun 0,33 $\pm$ 0,05 mg.

2) R é s u l t a t s - Le tableau 1 traduit l'influence des doses croissantes de rayons γ sur l'équipement pigmentaire photosynthétique des plantules.

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Tableau 1: Composition pigmentaire des cotylédons d'Arabidopsis après irradiation γ des graines. Teneurs exprimées en Moles x 10^{-9}/mg de poids frais - incertitude : $\pm$ 16%

Doses (K.rad)	0	35	250	500	750	10^3
Nombre de plants	68	50	50	50	50	12
[Germination %] (1)	(89)	(78)	(77)	(49)	(34)	(9)
Chlorophylle (a)	0,26	0,25	0,23	0,22	0,18	0,20
Chlorophylle (b)	0,19	0,21	0,23	0,17	0,13	0,13
ß Carotène	0,06	0,09	0,18	0,06	0,05	0,05
Lutéine	0,13	0,11	0,17	0,09	0,08	0,10
Violaxanthine	0,07	0,07	0,13	0,05	0,05	0,05
Néoxanthine	0,08	0,05	0,14	0,03	0,03	0,08
Pigment rouge	0	++	++++	Trace	-	-

(1) BOUNIAS (1973)

L'équipement pigmentaire ne diminue légèrement qu'aux très fortes doses. Les réductions ne sont significatives que dans le cas de la Néoxanthine (sauf à 10^3 K.rad), de la violaxanthine à partir de 500 K.rad, de la lutéine et de la chlorophylle (b) à 750 et 10^3 K.rad. Les teneurs en chlorophylle (a) et en ß carotène ne sont jamais diminuées de façon significative; ce dernier tend même à se concentrer aux doses les plus faibles.

Un pigment rouge est apparu chez les plants issus des graines irradiées aux doses les moins fortes. Son analyse n'a pu être approfondie faute d'une quantité suffisante pour permettre sa purification. Son Rf. sur couche mince d'alumine (solvant : Benzène-cyclohexane-Acétone-Méthanol 25-25-3-1) est légèrement inférieur à celui du ß carotène (tableau 2).

Tableau 2: Caractéristiques chromatographiques du pigment supplémentaire apparu dans les plants issus de graines irradiées à 250 K.rad

Composés	Rf	Longueur d'onde maximum d'absorption sur plaque
ß Carotène	0,816	440 à 465 nm
Pigment rouge	0,764	490 à 520 nm

3) D i s c u s s i o n - Il est ainsi apparu que l'équipement pigmentaire photosynthétique des cotylédons issus de graines irradiées jusqu'à des doses extrêmes (10^6 rad) ne subit pas d'altérations très importantes. Les hydroxy-caroténoides subissent de légères diminutions et un pigment particulier apparaît à 250 K.rad sans corrélation avec l'augmentation des doses. Au-delà de 250 K.rad, les plants peuvent survivre plusieurs mois à l'état de rosettes aux feuilles toujours vertes mais de taille réduite.

Ces résultats confirment donc la remarquable radiorésistance d'Arabidopsis sous l'action des rayons gamma. Cette propriété, déjà mise en évidence par GOMEZ CAMPO et DELGADO (1964) ainsi que DEVI et al. (1964) à l'aide des rayons X, est attribuée par SPARROW et EVANS (1961) au très faible volume nucléaire de cellules d'Arabidopsis.

S u m m a r y

Gamma-rays of ^{60}Co applied on dry seeds of Arabidopsis from 35 to 10^3 K.rad induced no qualitative modifications of the chlorophylls and carotenoids concentrations in 5-days cotyledons. Only chlorophyll (b) and hydroxycarotenoids-concentrations were slightly but significantly reduced at higher dosages (750 and 1000 K.rad).

Références:

BOUNIAS, M.: D.E.A., LYON, 12 p. (1967)
- : Arabid.Inf.Serv. 10, 24-26 (1973)
- : Chimie Analytique, 51, 76-82 (1969)
GOMEZ-CAMPO, C., and L. DELGADO: Rad.Bot., 4, 479-483 (1964)
DEVI, S.K., K. PRASAD, and RILEY: Rad.Bot., 4, 347-353 (1964)
SPARROW, A.H., and EVANS: Brookhaven Symp. in Biology 14, 83 (1961)

Détermination de l'equipement enzymatique général chez Arabidopsis (Témoins et mutants)

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Au cours de recherches sur la caractérisation biochimique de mutants chlorophylliens de végétaux supérieurs, l'équipement enzymatique ou zymogramme d'un mutant chlorophyllien (Vir-20) a été comparé à celui des plants témoins.

1) T e c h n i q u e - La technique utilisée dérive de celle mise au point par PLANTEVIN, NARDON et LAVIOLETTE (1968) pour la détermination des zymogrammes tissulaires chez les Insectes. Les extraits de feuilles sont incubés en présence d'un substrat chromogène dans des réservoirs capillaires en plastique d'une contenance de 0,1 cm^3, constitués d'une feuille de substance absorbante inerte placée entre deux parois de plastique dont l'une est transparente. (Les réservoirs capillaires sont délimités par emboutissage à chaud). Après deux à trois heures d'incubation à 30°C, les résultats des réactions enzymatiques sont estimés à l'oeil nu par lecture de la réaction colorée. Le tableau 1 énumère les divers substrats employés ainsi que les réactifs de colorations.

Tableau 1: Substrats et réactifs utilisés pour la détermination des zymogrammes chez Arabidopsis

Enzymes recherchées	Substrats spécifiques	Réactifs diazo-bleu	Réactifs soude
Phosphatases alcalines	Phtaléine di-Phosphate	-	+
Phosphatases alcalines	Naphtyl-Phosphate	+	-
Phosphatases acides	Naphtyl-Phosphate	+	-
Estérase acétique	Naphtyl-acétate	+	-
Estérase butyrique	O. nitro-phényl-butyrate	-	-
Estérase laurique	ß Naphtyl-Laurate	+	-
Lipase palmitique	ß Naphtyl palmitate	+	-
Lipase stéarique	ß Naphtyl stéarate	+	-
Lipase nonanoique	ß Naphtyl nonanoate	+	-
Lipase myristique	ß Naphtyl myristate	+	-
DL-Alanyl-Amino-peptidase	Alanyl-Naphtylamide	+	+
L-Leucyl-amino peptidase	Leucyl-Naphtylamide	+	+
Valine-ß amino peptidase	Valine-Naphtylamide	+	+
ß Galactosidase	O. nitro phényl galactoside	-	-
Glucosaminidase	Naphtyl-Acétyl-Glucosaminide	+	+
Glucuronidase	p. Nitro Phényl Glucuronide	+	+
Glucosidase	Bromo-6-Naphtyl-2-ß-Glucopyranoside	+	+

2) R é s u l t a t s - Le tableau 2 indique les activités enzymatiques décelées chez les mutants et les témoins. Ces activités sont présentées dans l'ordre décroissant, selon une échelle d'estimation d'intensité allant de 0 à 5.

Tableau 2: Zymogramme provenant des extraits de feuilles d'Arabidopsis Témoins et Mutées (Echelle d'intensité arbitraire : de 0 à 5)

Enzymes	Tém.	Mut.	Enzymes	Tém.	Mut.
Phosphatases acides	4	4	Glucosidase	1	1
Phosphatases alcalines	3	4	Galactosidase	1	1
Estérase acétique	3	3	Estérase nonanoique	1	1
Estérase butyrique	2	2	Estérase myristique	1	1
DL-alanyl-amino-peptidase	2	2	autres estérases	0	0
L-leucyl-amino-peptidase	2	3	Glucosaminidase	0	0

3) D i s c u s s i o n - Cette technique, d'un emploi aisé, permet de déterminer rapidement les zymogrammes comparés de mutants et de témoins à partir de faibles quantités de tissus (100 mg de poids frais). Dans le cas présent, le mutant viridis (Vir. 20) présente une augmentation d'activité phosphatasique alcaline et de leucyl-amino-peptidase. Des travaux ultérisurs ont montré l'importance de ces observations, et le rôle de la leucine sur l'activité phosphatasique (BOUNIAS, 1972).

S u m m a r y

The rosette-leaves zymogram of an Arabidopsis viridis mutant is compared to that of control plants. The mutant shows the same qualitative equipement as control plants, but an increased alkaline phosphatase and leucyl-amino-peptidase activity.

Références:

BOUNIAS, M.: Arabid.Inf.Serv. 9, 15-17 (1972)
- , and H. PACHECO: C.R.Acad.Sc. 275, 201-204 (1972)
PLANTEVIN, G., P. NARDON et P. LAVIOLETTE: IInd Intern.Coll.Invert.Tissue culture - Tremezzo (1967)

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Incorporation de Leucine-^{14}C dans les pigments photosynthétiques, les sucres et amino-acides libres de plants d'*Arabidopsis* : Témoins et mutants viridis

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Des travaux antérieurs ont montré que la L-leucine peut jouer un rôle important dans la régulation de l'appareil photosynthétique en stimulant l'équipement pigmentaire lorsqu'elle est administrée aux plantes à faible dose et en l'affaiblissant à concentration élevée (BOUNIAS et PACHECO, 1972). Ce phénomène a permis d'obtenir un reverdissement chez plusieurs mutants chlorophylliens de type viridis (BOUNIAS, 1972a).

Nous avons analysé l'incorporation de cet amino-acide dans les pigments et cofacteurs photosynthétiques, les sucres libres et les amino-acides libres des feuilles d'*Arabidopsis* (témoins et mutants), à l'aide de L-leucine-(u)-^{14}C administrée par les racines.

1) T e c h n i q u e s - La leucine-(u)-^{14}C est administrée aux plantes dans les conditions suivantes:
- Dose inhibitrice = 1,5 µMole/cm^3 (1,9 µMole/plant)
- Dose activatrice = 0,8 µMole/cm^3 (0,85 µMole/plant)
- Activité spécifique : (Act. sp.) 500 µCi/mMole
- Durée de contact : 5 heures

Les métabolites sont analysés par micro-chromatographie quantitative en couche mince (BOUNIAS, 1969) et l'activité spécifique des composés analysés est déterminée à l'aide d'une microméthode autoradiographique (BOUNIAS, 1972b).

2) R é s u l t a t s - Les résultats présentés ci-dessous correspondent à l'incorporation de leucine (u)-^{14}C à dose inhibitrice chez les témoins et à dose activatrice chez les mutants, ce qui a pour effet de rapprocher sensiblement leur niveau pigmentaire : l'écart initial, de 45% dans des conditions normales de culture, est alors ramené à 15%.

Tableau 1: Activité spécifique des Pigments et Co-facteurs photosynthétiques d'*Arabidopsis*, Témoins et Mutants Viridis, après incorporation de leucine-(u)-^{14}C-(BOUNIAS, 1972a) - Expression des résultats en µCi x 10^{-6}/µMole

Pigments	Act.sp. tém.	Act.sp. Mut.	Co-Facteurs	Act.sp. tém.	Act.sp. Mut.
Chlorophylle (a)	43	76,5	Tocophéryl-chroménol	18	5,8
Chlorophylle (b)	10,5	17,5	Chroménol-II	8,5	10
ß Carotène	168,5	93,5	Chroménol-III	11,5	6,5
Lutéine	16	42	Plasto-chroménol	(+)	1,8
Violaxanthine	79	36,5	Plastoquinone-A	-	10

La leucine-^{14}C s'incorpore largement dans la plupart des pigments photosynthétiques, ce qui confirme les travaux de FALUDI-DANIEL et al. (1969) sur le mais. La voie de biosynthèse paraît être celle de l'acéto-acétyl coenzyme A conduisant aux chaines isoprénoides. Cependant, le métabolisme des pigments (excepté la lutéine) reste plus lent chez les mutants que chez les témoins.

Tableau 2: Activité Spécifique des sucres d'*Arabidopsis*, témoins et mutants, après incorporation de leucine-(u)-^{14}C (Expression en µCi x 10^{-6}/µMole

Sucres	Maltose	Glucose	Fructose
Act. Sp. Témoins	-	39,1	3,8
Act. Sp. Mutants	55,2	40,3	50

Les sucres sont nettement plus marqués chez les mutants, ce qui paraît correspondre à une rétro-synthèse accrue par intermédiaire de la voie de l'Acétyl-Coenzyme A, destinée à compenser le défaut de photosynthèse.

Dans la plupart des cas, l'incorporation de leucine (u)-^{14}C est plus importante chez les mutants chlorophylliens: ceci montre que les augmentations de teneurs en amino-acides libres observées chez ces mutants (BOUNIAS, 1972a) ne résultent pas d'un simple ralentissement d'utilisation ou d'une accumulation passive.

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Tableau 3: Activité spécifique des amino-acides libres d'Arabidopsis témoins et mutants après incorporation de leucine-(u)-^{14}C- (Expression en uCi x 10^{-6}/ uMole)

Amino-acides	Activité spécif. Tém.	Activité spécif. Mut.	Amino-acides	Activité spécif. Tém.	Activité spécif. Mut.
Ornithine	70,3	64,3	Thréonine	5,35	21,6
Lysine	100,4	222	Alanine	-	35,8
Histidine	74,7	23,8	Ac. γAmino-Butyr.	-	316,4
Arginine	77,6	61	Valine	-	123,1
Ac. Aspartique	77,8	58,5	Isoleucine	-	1,6
Asparagine	65,8	59,1	Leucine	2134	45
Sérine	+	58,9	Phényl-alanine	-	275
Glycocolle	9,0	83,8	Tryptophane	-	3263
Ac. Glutamique	76,9	154,2			
Glutamine	44,4	100,5			

3) D i s c u s s i o n - La leucine-(u)-^{14}C s'incorpore bien dans la plupart des pigments et cofacteurs photosynthétiques, des sucres et des amino-acides libres. Dans les mutants, malgré la stimulation de l'appareil photosynthétique par la leucine à faible dose, les pigments chlorophylliens restent moins marqués que chez les témoins, de même que l'acide aspartique (et son amide), très liés à la photosynthèse (HATCH et SLACK, 1970). Par contre, les autres amino-acides et les sucres libres sont plus radioactifs chez les mutants que chez les témoins, ce qui semble traduire une accélaration compensatrice du métabolisme non photosynthètique.

S u m m a r y
L-Leucine-(u)-^{14}C is well incorporated in chlorophylls, carotenoids, cofactors, free sugars and free amino-acids of Arabidopsis mutant and control rosette-leaves. The -^{14}C-label of chlorophylls is lower in mutants,but their non-photosynthetic metabolism seems to be accelerated as a compensation.

Références:

BOUNIAS, M.: Chimie Analytique 51, 76-82 (1969)
- , et H. PACHECO: C.R.Acad.Sc. Paris, 275, 201-204 (1972)
- : Thèse Doctorat ès sciences, LYON 250 p (1972a)
- : Arabid.Inf.Serv. 9, 36 (1972b)

FALUDI-DANIEL, A., A.H. NAGY et A. NAGY: Progr. in Photosynthesis, Vol. II, 592-598 (1969)

HATCH, M.D. et C.R. SLACK: Progr. in Phytochemistry 2, 35-106

Sulphydryl protection against ionizing radiations

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Nearly all studies on sulphydryl protection against radiation induced g e n e t i c damage in higher plants, focus on chromosomal aberrations observed in root tips. Genetic parameters like M_2-mutant percentage were studied by ANDO (1972) who for M_2 chlorophyll mutants found no protective effect when posttreating gamma-irradiated rice seeds with cysteine, and by MALVAREZ et al. (1965) who for M_2 chlorophyll mutants found with X-irradiated barley seeds no protective effect when cysteamine was applied as posttreatment, and an increase rather than a decrease when it was applied as pretreatment.

On the basis of preliminary experience (VAN DER VEEN et al., 1969) we chose the sulphydryl compound 1,4-dithiotreitol (DTT), which was found by BICK and JACKSON (1968) to give a 68% reduction of chromosomal aberrations of "the two-hit type" in X-ray treated marsupial leucocyte cultures. We applied the Arabidopsis embryo-test on siliquae 4 and 5 of the main stem, and expressed M_2-mutant percentage as percentage of embryonic mutants (embryonic lethals and chlorophyll's) among fertilized ovules.

Seeds of Landsberg-'erecta' were first kept on wet filterpaper during 5 days at 2^oC (to break dormancy), and redried. Prior to (4 hrs) and during irradiation the seeds were kept submers in water or DTT-solution (1.2%) at 24^oC, and afterwards they were rinsed and put to germinate at 24^oC under TL. The seedlings were transplanted to an air-conditioned greenhouse (22^oC). X-irradiation (200 r/min.) and fast neutron irradiation (75 r/min) was carried out at the ITAL (Wageningen). For replacement of air by nitrogen, the gass was bubbled (vacuum pump) through the solution at the beginning of pretreatment.

We had found that post-treatment has no protective effect, and that it made no difference for the protective effect whether DTT was applied only before, only during or both before and during irradiation. With DTT concentrations higher than 1.2% the protective effect was not much enhanced, whilst toxic effects (germination delay, seedling survival) became noteworthy.

With respect to the X-ray dose response curve, we found that nitrogen does not affect the exponential nature of the curve throughout the dose range, whilst DTT changes the curve towards linearity, which indicates 1) that DTT preferentially protects against those types of chromosomal damage which can lead to two-hit events, and 2) that the effect of DTT is q u a l i t a t i v e l y different from that of nitrogen, so that an "additive" effect of DTT and nitrogen might be expected, which however was not the case as is illustrated by the following data on mutant % (200 resp. 135 plants scored per item for X-rays resp. FN):

Atmosphere	air	air	nitrogen	nitrogen
% DTT	0.0	1.2	0.0	1.2
X-rays (24 kR)	10.3	6.0	6.3	6.8
Fast neutrons (3 kR)	6.9	3.0	4.3	4.7

From these data, it is seen that DTT not only gives considerable protection with both types of irradiation, but also that 1) DTT gives no extra protection under hypoxia, and 2) DTT alone can give m o r e protection than nitrogen alone (see fast neutrons). We therefore advance as an explanation that DTT is blocked in its action by hypoxia, i.e. certain metabolic processes may be a prerequisite for DTT to reach its target site. The complete absence of protection found at low pH (2.8) points in the same direction.

In addition, our data do not indicate a close connection of DTT protection with the oxygen effect as 1) with fast neutrons, which are known to have a comparatively low oxygen effect, protection is as least as effective as with X-rays (see above), and 2) with the present semi-anaerobic treatment (seeds submers) protection was found to be no less than with aerobic treatment (seeds on filterpaper), viz. 7 kR-equivalents at 24 kR in both cases, though with aerobiosis X-ray damage is about twice as high. Therefore, it is quite well possible that DTT not so much acts in competition with oxygen or by scavenging oxygen mediated free radicals, but that it acts mainly through a direct protective effect in the living cell, i.e. protection of the nucleo-protein complex or of the DNA repair enzymes (cf. the discussion on sulfur-containing radioprotectors by BACQ and GOUTIER, 1967).

A full account will be published elsewhere.

References:
ANDO, A.: In "Induced Mutations and Plant Improvement", Symp. IAEA/FAO, Buenos Aires, Pp. 491-500, 1972
BACQ, Z.M., and R. GOUTIER: Brookhaven Symp.Biol. 20, 241-262 (1967)
BICK, Y.A.E., and W.D. JACKSON: Nature 217, 479-480 (1968)
MALVAREZ, E.M., et al.: Boletin Genet. 1, 3-16 (1965)
VAN DER VEEN, J.H., et al.: Arabid.Inf.Serv. 6, 23 (1969)

Estimation of induced mutation rate in Arabidopsis

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In search for relationships between empirical data on mutant segregation and the number of mutations induced in embryonic meristem of autogamous plants, plausible assumptions are required concerning (a) the mean number of the germ line cells in the embryonic meristem, (b) the pattern of distribution of mutations in the initial cells, and (c) the genetic structure (either chimeric or not) of the gametogenic tissue of single M_1-flowers, within which the selfpollination takes place.

All the available data indicate that the number of germ line cells (n) in embryonic meristem of Arabidopsis seeds is more than 1, which makes possible the chimerism of M_1-plants (MÜLLER, 1965). Independent estimations of different authors give $\bar{n}$ close to 2 (LANGRIDGE, 1958; LI and RÉDEI, 1969; NIKOLOV and IVANOV, 1969; IVANOV, 1971; RELICHOVA, 1972).

Single flowers are generally not chimeric, however. This may be inferred from the close agreement with the 3:1 expectation of the segregation in M_2 of induced chlorophyll mutations, e.g. among 1783 embryos in mutation bearing pods we found 1368 normal

and 415 mutant embryos, i.e. 3.3:1. In order to obtain an unbiased estimate of segregation ratio, only data on pod progenies from M_1-plants bearing s i n g l e induced mutations were included.

Considering the induced mutations in meristematic cells of Arabidopsis, it seems reasonable to expect that the distribution of mutations follows the Poisson series as proposed by A.J. MÜLLER (1965).

It is to be pointed out, that a non-chimerical structure of single flowers is an important additional assumption for the direct application of the Poisson distribution of induced mutations in M_2, when formulae

$$M = -\ln(1 - m_b) \text{ and}$$

$$M = -\ln(1 - m_c)/\bar{f} \quad \text{(MÜLLER, 1965a)}$$

are deduced, where M is the mean number of mutations per initial cell, m_a-frequency of M_2 plants with mutants in the first five siliquae, m_b-frequency of segregating M_1 pod-progenies, m_c - M_2 mutant frequency, and $\bar{f}$ - mean frequency of mutants in progeny of heterozygous (non-chimeric) plants.

Assuming $\bar{n} = 2$, it may be found, that $M = -\ln(1 - m_a)/\bar{n} = -0.5 \ln(1 - m_a)$, where m_a is the frequency of segregating M_1 plant-progenies.

With overall mutation rate being low, the Poisson mean (M) does not differ significantly from the frequency of mutation bearing cells (m). Then, instead of the above formulae, the following approximate relationships may be applied:

$$m = m_b \quad \text{(MÜLLER, 1965)}$$

$$m = m_c/\bar{f} \quad \text{(FRYDENBERG, 1963), and}$$

$$m = m_a/\bar{n} = 0.5\ m_a.$$

The latter relationship is esentially similar to those proposed by J. LANGRIDGE (1958) and S.L. LI and G.P. RÉDEI (1969).

References:

FRYDENBERG, O.: Radiation Bot. 3, 135 (1963)
IVANOV, V.I.: Arabid.Inf.Serv. 8, 29 (1971)
LANGRIDGE, J.: Austral.J.Biol.Sci. 11, 58 (1958)
LI, S.L., and G.P. RÉDEI: Radiation Bot. 9, 125 (1969)
MÜLLER, A.J.: Induction of Mutations and the Mutation Process. Proc.Symp. Praha, p. 46 (1965)
- : Arabidopsis Research. Rep.Symp. Göttingen, p. 192 (1965a)
NIKOLOV, C.V., and V.I. IVANOV: Genetika 5, 166 (1969)
RELICHOVA, J.: Arabid.Inf.Serv. 9, 28 (1972)

An arginine mutant

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Seeds of the variety Estland were treated with ethyl methanesulfonate (10 mM, 22°C) for 16 hours. Among the M_2 seedlings, one nutritional mutant was rescued in a complete liquid medium, asexually propagated and tested on different solid media. It was soon discovered that this nutritional mutant was an amino-acid mutant which was later identified as an arginine mutant. It grows well in presence of arginine, citrulline, ornithine, glutamic acid and proline.

Nitrate reduction in Arabidopsis thaliana

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In order to isolate nitrate reductase-less mutants of Arabidopsis more chlorate resistant mutants were isolated. Besides the chl-2 type (previously reported, OOSTINDIER-BRAAKSMA and FEENSTRA, 1972) one more nitrate reductase-less mutant was isolated. Further experiments will have to reveal whether this mutant concerns the gene chl-2 or not.

The 16 other new chlorate resistant mutants appeared to have a nitrate reductase activity equal to or higher than wildtype. Complementation tests were carried out with 5 of these mutants, all showed no complementation with the chl-1 mutant, which suggests that these mutations probably affect the same gene.

Linkage experiments showed that chl-1 is linked with an giving 14% recombination, and that chl-2 is linked with er, giving 12% recombination. Therefore chl-1 belongs to linkage group 1, and chl-2 to linkage group 2 of RÉDEI.

To study the induction of synthesis of nitrate reductase, submerged culturing (as described by RÉDEI and PERRY, 1971) on different nitrogen sources was tried. The experiments showed that growth of plants in liquid media depends on the presence of nitrate, although it was possible to starve plants of nitrogen for a few days without loss of activity of the control enzym glucose-6-phosphate dehydrogenase (G6PD). When plants of 7 days old (in nitrate medium) were starved for nitrogen the nitrate reductase activity dropped within 36 hours to about 0, and after addition of nitrate the normal activity was recovered within 6 hours. Submerged culturing in liquid medium seems promising to further investigations on induction and the effect of inhibitors there on.

References:
OOSTINDIER-BRAAKSMA, Fietje J., and W.J. FEENSTRA: Arabid.Inf.Serv. 9, 9-10 (1972)
RÉDEI, G.P., and C.M. PERRY: Arabid.Inf.Serv. 8, 34 (1971)

Negative results of treatment with bacterial DNA on "repair" of mutants of Arabidopsis

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LEDOUX, HUART and JACOBS (1971) have reported reversion of mutant to wild phenotype as a consequence of treatment of seeds of Arabidopsis with solutions of DNA from bacteria. Since both bacterial transformation and genetics of Arabidopsis are research topics in our laboratory we were interested in repeating these experiments.

After having carried out several experiments we now want to report that we have not been able to obtain positive results. Since "transformation" of higher plants with bacterial DNA is a novel and potentially usefull phenomenon, it seems worthwhile to inform other workers possibly active in this field of the fact that we did not succeed in obtaining it.

In the following the data of our last experiment are given. DNA was isolated from Escherichia coli and Bacillus subtilis cultures according to the modified Kirby procedure of VENEMA et al. (1965). The B.subtilis DNA had normal biological activity when tested in bacterial transformation. It was divided into two portions, one of which underwent an additional precipitation with ethanol, after which it was redissolved in DSC (0.015 M NaCl + 0.0015 M trisodium citrate).

The E.coli DNA and the two samples of B.subtilis DNA were dialized against DSC for several days, until phenol free. DNA concentrations determined by the diphenylamine reaction (BURTON, 1956), were, in γ per ml: E.coli about 900, B.subtilis about 650, B.subtilis, precipitated and redissolved, about 600.

Seeds of the thiamineless Arabidopsis mutant V131 (FEENSTRA, 1964), requiring the pyrimidine moiety of thiamine for growth, were sterilised using hydrogen-peroxide / ethanol, and dried.

About 100 seeds were placed in a well of a spotplate and immersed in 0.04 ml of DNA solution. Spotplates were placed in Petri dishes over DSC, to prevent evaporation of the solvent. Each type of DNA solution was used to incubate 10 times 100 seeds; the same amount of seeds was treated with DSC, as a control. Petri dishes were kept at 25°C and continuously illuminated for 48 hours. Both DNA and DSC treated seeds germinated partly during the incubation period. After incubation seedlings and not

germinated seeds were laid out in Petri dishes on perlite moistened with a mineral nutrient solution; the dishes were then continuously illuminated at 25°C.

Mutant seedlings on mineral medium die off within 10 to 15 days, showing chlorophyll-less rosette leaves (FEENSTRA, 1964); "repaired" plants are expected to grow, and to exhibit more or less normal green colour. None of the latter were found. The number of seedlings after each treatment are given below.

Source of DNA	Number of seedlings after treatment of about 1000 seeds
E.coli	380
B.subtilis	390
B.subtilis, precipitated and redissolved	330
DSC (control)	440

References:
BURTON, K.: Biochem.J. 62, 315-323 (1956)
FEENSTRA, W.J.: Genetica 35, 259-269 (1964)
LEDOUX, L., R. HUART, and M. JACOBS: Informative molecules in biological systems, L. LEDOUX, ed., North Holland Publ.Cy., 1971: 159-175
VENEMA, G., R.H. PRITCHARD, and T. VENEMA-SCHRÖDER: J.Bacteriol. 89, 1250-1255 (1965)

Diallel crosses among races of Arabidopsis thaliana

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Six races of *Arabidopsis* from Europe, Asia, and Africa and one race collected in North Carolina served as parents in a diallel analysis. The races and all possible F_1 hybrids, including reciprocals, were evaluated in a growth chamber with continuous light of 1600 ft.-c. at 25°C. Individual seeds were sown in test tubes on an aseptic mineral agar. Two levels of nutrients were used; one, the standard for *Arabidopsis* culture and the other, 1/12 standard level.

Three characters evaluated were germination time, length of the primary root 12 days after seeding, and mass green weight of the entire plant 18 days after seeding. Significant differences were obtained among races and among hybrids within each nutrient level and combined over nutrient levels. The hybrids outperformed the parents for all races except the race from Tsu Islands, in which case the performance of the Tsu Islands race surpassed the hybrids.

From the diallel analysis of the hybrids, variance components for general combining ability, specific combining ability and specific reciprocal differences were not significant. However, a large component of variance existed for average maternal effects, suggesting that a large portion of the variance among genotypes was due to maternal seed effects or to the cytoplasm.

The localization of recessive lethal genes into linkage groups

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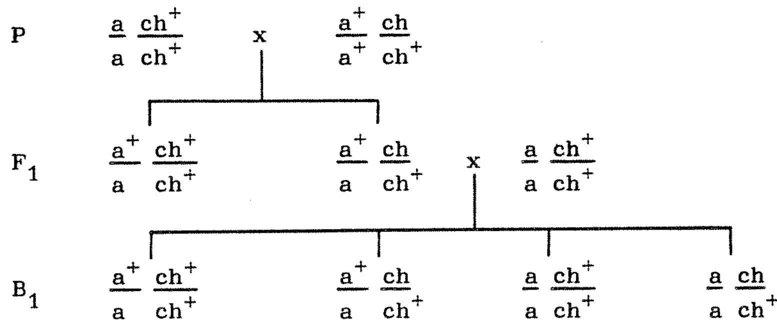
The study of linkage between two different recessive mutant alleles is difficult because they are in repulsion; for this reason, double mutants are not available so that an analytical back-crossing is impossible. The detection of linkage in the F_2 is also disadvantageous (HIRONO and RÉDEI, 1964; McKELVIE, 1965). In addition, further difficulties arise if lethal genes should be localised. In *Arabidopsis*, however, this disadvantage turns into preference if we have to do with embryonic and chlorophyll lethal genes which can be detected by means of a MÜLLER's "Embryonentest" (MÜLLER, 1963). In this case, we can estimate relatively easily, quickly and reliably the linkage relations between embryonic or chlorophyll lethal alleles and any marker gene (for *Arabidopsis* see, f.i., RÉDEI, 1965). Provided we have these markers in each linkage group, we can estimate the appurtenance of various

embryonic and chlorophyll lethal genes into linkage groups, and its distance from the marked locus, respectively.

Our method is based on the MÜLLER's "Embryonentest". On the stigmata of the marker $\frac{a}{a}\frac{ch^+}{ch^+}$ pollen of a plant heterozygous for the lethal mutation $\frac{a^+}{a^+}\frac{ch^+}{ch}$ is brought. In F_1 the double heterozygotes $\frac{a^+}{a}\frac{ch}{ch^+}$ are crossed with the markers.

In B_1, two externally different groups are obtained: phenotypically standard plants $\frac{a^+}{a}\frac{ch^+}{ch^+}$ and $\frac{a^+}{a}\frac{ch}{ch^+}$, and the plants with the marker phenotype $\frac{a}{a}\frac{ch^+}{ch^+}$ and $\frac{a}{a}\frac{ch}{ch^+}$.

Using the embryo test, it is possible to determine all four genotypes within both given groups:



If independent combination takes place, these genotypes occur in a 1:1:1:1 ratio, while the increased frequency of the second and third class indicates the linkage.

This method has some advantages in Arabidopsis : (1) The information about linkage is gathered from the B_1 instead of the F_2. (2) The non-analytical procedure is made analytical owing to MÜLLER's "Embryonentest". (3) In the parental generation, emasculation is not necessary provided that the marker genotype is used as mother so that all standard F_1 plants are hybrid. (4) If, on the contrary, embryonic and chlorophyll lethals will be used as markers for each group, the localization of any visible mutant recessive allele will be equally easy.

The given method was successfully used in a series of recessive chlorophyll lethal mutations. Several chlorophyll lethals were localized into the first and third linkage group.

In the next future, it is necessary to construct special genotypes enabling us to mapping particular chromosomes.

References:
HIRONO, Y., and G.P. RÉDEI: Arabid.Inf.Serv. 1, 9-10 (1964)
McKELVIE, A.D.: Arabid.Research, pp. 79-84 (1965)
MÜLLER, A.J.: Biol.Zbl. 82(2), 133-163 (1963)
RÉDEI, G.P.: Arabid.Research, pp. 210 (1965)

Variability and heritability of a developmental character in mountain populations of Arabidopsis

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In previous communications (DOBROVOLNÁ, 1968, 1969a,b; CETL and RELICHOVÁ, 1971) high heritability coefficients of some developmental characters were found by means of offspring-parent regression coefficients not only in variable natural populations but also in a uniform one.

In the last years, samplings of the seed material were undertaken in the mountains Hrubý Jeseník from the topmost to the foot positions. As yet, heritability was estimated in four population samples. For cultivation conditions see CETL, RELICHOVÁ-DOBROVOLNÁ and KRSKOVÁ, 1970. In three population samples, the offspring-parent regression coefficients, b_{OP} and $b_{O\bar{P}}$, did not differ significantly from zero;

analogously, the W coefficients were low. Only in the remaining population sample originating from the upmost locality, the estimations of heritability were high and significant. The absence of measurable heritability was connected with low variability in the character studied (Table).

Table 1: The mean number of days to appearance of the flower primordia ($\bar{x} \pm s_{\bar{x}}$) with the variability coefficient (v) and the estimates of heritability of this character given as offspring-parent regression coefficients (b_{OP}, $b_{O\bar{P}}$) and as intraclass correlation coefficients (W) in four samples of natural populations of *Arabidopsis* from the mountain Hrubý Jeseník

Locality	meters above sea-level	n_O	n_P	$b_{OP} \pm s_b$	$b_{O\bar{P}} \pm s_b$	W±s	reliability intervall	$\bar{x} \pm s_{\bar{x}}$	v(%)
Anín	650	453	27	0.06±0.04	0.10±0.02**	0.16±0.01	0.10-0.27	14.2±0.1	10.01
Karlov	720	376	24	0.00±0.02	-0.02±0.06	0.23±0.02	0.14-0.36	11.7±0.1	11.28
Krasov	525	505	36	0.61±0.05**	0.60±0.15**	0.48±0.02	0.38-0.58	11.9±0.2	19.68
Petrovka	1160	412	28	-0.01±0.05	-0.06±0.09	0.25±0.02	0.16-0.38	13.4±0.1	11.52

** Exceeds the 1 per cent point

Several hypotheses might be drawn to explain this unexpected difference in variability and heritability of population samples as to the developmental character. Most probably, however, this fact can be connected with extreme environmental conditions on the mountain localities where only unique genotypes could be successfull in founding a population.

References:

CETL, I., and J. RELICHOVÁ: Arabid.Inf.Serv. 8, 4 (1971)
CETL, I., J. RELICHOVÁ-DOBROVOLNÁ, and M. KRSKOVÁ: Arabid.Inf.Serv. 7, 35-36 (1970)
DOBROVOLNÁ, Jiřina: Arabid.Inf.Serv. 5, 15-16 (1968)
- : Arabid.Inf.Serv. 6, 20 (1969a)
- : Biologia plantarum 11 (4), 310-318 (1969b)

B. TECHNIQUES

The aseptic culture of Arabidopsis thaliana (L.) HEYNH. in liquid medium

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A method for the aseptic culture of an intact higher plant *Arabidopsis thaliana* (L.) HEYNH. in liquid medium is described.

Various methods for the aseptic culture of the small *Arabidopsis* plant have been reviewed (1). The plant was generally supported by agar, silica gel (2), "perlite" (3) or soil (4). Under these conditions, the withdrawal of culture medium or the fast equilibrium of additives with the medium during the course of experiment would be difficult. Also, under such circumstances removal of the intact root system for analysis would be problematical. This problem can be solved by liquid culture such as reported by RÉDEI and PERRY (5). Their method is not desirable if an aerial shoot system is also required for the investigation. Therefore an aseptic culture method which fulfills the requirements of using liquid medium and the development of an aerial shoot system together with a normal root system of an intact plant is reported here.

This method utilizes a thin layer of Pyrex fiberglass wool for the support of the plant while allowing for the supply of nutrients on its moistened surface during germination stages. The piece of fiberglass wool was in turn supported by a glass frame made by four 1" Pyrex glass rods (diameter 1/4"). The glass frame was formed by arranging two glass rods parallel to each other but 1/4" apart. The other two glass rods were arranged in the same manner but were placed on top and perpendicular to the bottom pair. The four contact points of the glass rods were fused by heat. The glass frame was then put on the bottom of a cylindrical flat-bottomed glass vial (diameter 1 7/16", height 4 3/4"). A circular piece of a single layer of fiberglass wool, obtained from Corning Glass Works, Corning, New York, of 1 1/4" diameter was cut out and placed on top of the glass frame.

The composition of nutrient medium in mg/liter was: glutamic acid, 20; $Ca(NO_3)_2 \cdot 4H_2O$, 48; $MgSO_4 \cdot 3H_2O$, 8.87; NH_4NO_3, 8; KNO_3, 8.4; KCl, 4.27; K_2HPO_4, 8.4; H_3BO_3, 2.86; $MnSO_4 \cdot H_2O$, 1.8; $ZnCl_2$, 0.22; $CuSO_4 \cdot 5H_2O$, 0.08; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.08; $Fe_2(SO_4)_3 \cdot 6H_2O$, 3.26; EDTA, 0.78. The nutrient medium was adjusted to pH 6.1 with HCl. Sucrose was added at 20 g/liter.

An aliquot of 5 ml nutrient medium was transferred into the glass vial. The central portion of the piece of glass wool should be above the level of nutrient medium while its peripheral area submerged. The entire piece of glass wool was then moistened with nutrient medium due to the capillary action of the fine fiberglass wool structure. The culture glass vial was covered with aluminum foil and sterilized in an autoclave at 15 lb pressure for 7 minutes.

The *Arabidopsis* seeds were surface sterilized by immersion in a mixture of 95% ethanol: 1% H_2O_2 (1:10, v/v) for 3 minutes and washed three times with sterilized distilled water.

Under aseptic conditions, a seed was transferred with a Pasteur pipette to the center of the surface of the fiberglass wool. A square piece (3" x 3") of Saran Wrap, which previously was sterilized in an autoclave, was used to seal the top of the culture vial and was secured with a rubber band. The Saran Wrap, made by the Dow Chemical Company, Midland, Michigan, was used because it is transparent and has a low transmission rate for H_2O vapor.

Immediately after planting the seed, the culture vial was maintained at 24°C and illuminated continuously with 150 f.c. from Wide-Spectrum Grolux fluorescent light tubes in an environmental growth chamber.

After 2-3 days, germination began. The primary root penetrated down through the porous fiberglass wool and eventually the whole root system grew submerged in the liquid medium. The shoot system developed above the fiberglass wool. Efflorescence started at about the 16th day. In general the plant grew as well as on 1% agar.

The described aseptic liquid culture technique provides an easy and economic assembly arrangement for the growth of an intact plant with both submerged root and aerial shoot systems. These advantages may prompt its use in the studies of physiology, agronomy and genetics.

References:
1. REDEI, G.P.: Bibliographia Genetica 21, 1-151 (1969)
2. LANGRIDGE, J.: Aust.J.Biol.Sci. 10, 243-252 (1957)
3. FEENSTRA, W.J.: Arabid.Inf.Serv. 2, 23 (1965)
4. LI, S.L., and G.P. REDEI: Arabid.Inf.Serv. 5, 58 (1968)
5. REDEI, G.P., and C.M. PERRY: Arabid.Inf.Serv. 8, 34 (1971)

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Culture aseptique d'Arabidopsis sur sable et liquide minéral "minimum"

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Une méthode de culture aseptique de plants d'*Arabidopsis thaliana* sur sable et liquide nutritif minéral "minimum" a été mise au point en vue de l'étude biochimique de mutants chlorophylliens (BOUNIAS, 1967).

1) T e c h n i q u e

a) Les cellules de cultures individuelles sont composées, comme indiqué fig. 1-a, de deux tubes de verre emboités: le premier, de 2 cm de diamètre et 4 cm de hauteur, est bouché au fond par un tampon de laine de verre et contient 6 cm^3 de sable fin et 3 cm^3 de liquide nutritif. Ce tube, qui contient la plante, est lui-même recouvert par un autre tube de 2,5 cm de diamètre et 25 à 30 cm de hauteur, bouché à l'extrémité supérieure par un tampon de coton hydrofuge. Les tubes-composés ainsi constitués sont groupés par 10 dans des portoirs en "plexiglas" usiné. Ils permettent un accès facile aux plantules et l'ensemble des cultures peut être arrosé par le bas, à l'aide d'un bac en plastique. Le bouchage des alvéoles permet en outre de trailer chaque tube séparément.

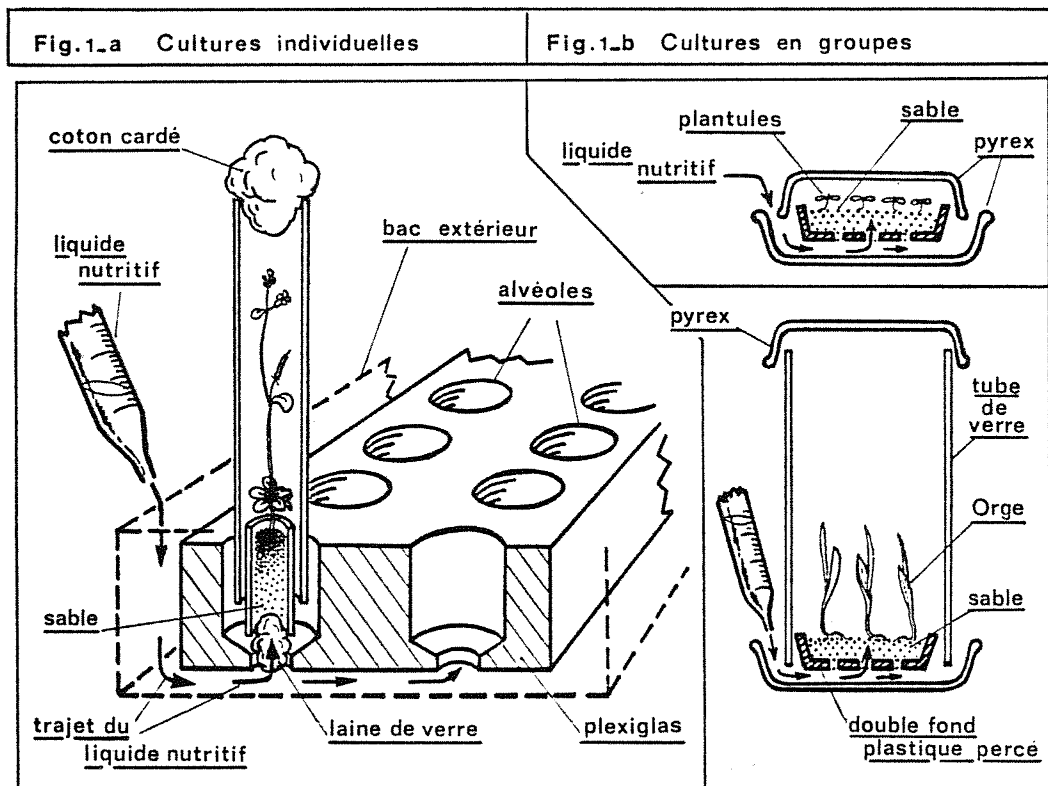

Figure 1-a: Cellules de cultures individuelles en tubes composés avec leur portoir (vue en coupe)

Figure 1-b: Cellules de cultures constituées à partir de Boites de Pétri à double fond (vue en coupe).
Le dessin du bas montre une cellule dont la hauteur a été augmentée par un tube de verre pour permettre la culture de plantes plus élevées. (*Arabidopsis* avec hampes florales ou orge)

b) Les plantes peuvent être cultivées en groupes dans des boites de Pétri inversées contenant un double fond percé, garni de sable et de liquide nutritif (par imbibition). Le prolongement en hauteur de l'enceinte de culture est obtenu à l'aide d'un tube de verre de 25 à 30 cm de long et de diamètre compris entre celui du couvercle de la boite de Pétri et celui du double fond (fig. 1-b).

Le liquide nutritif est constitué de la facon suivante:

- solution de macro-éléments de KNOP..	30 cm^3	(KNOP, 1860)
- solution d'oligo-éléments de HELLER.	1 cm^3	(HELLER, 1953)
- solution de complexe Fer-EDTA	4 cm^3	(JACOBSON, 1951)
- Eau distillée: Q.S.P.	1 litre	

2) R é s u l t a t s

Sous un éclairement de 2600 lux, avec une photopériode de 19 H de "jour" et à la température de 24°C, la floraison se produit 28 ± 2 jours après germination et le cycle complet dure environ 45 jours.

a) Les plants cultivés isolément sont de taille très homogène: le calcul montre que les hauteurs des hampes florales mesurées sur un échantillon de 40 plants pris au hasard se distribuent selon la loi normale (au seuil 5%: probabilité = 0,90) (fig. 2).

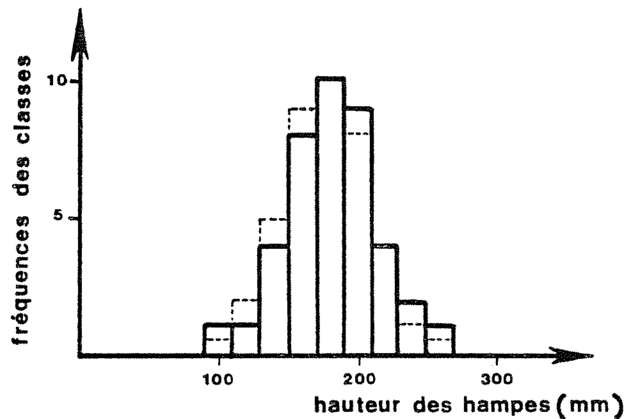

Figure 2: Distributions d'échantillonnage de la hauteur des hampes florales chez Arabidopsis. (N = 40)
Distribution observée: (———)
Distribution théorique calculée: (-----)

b) La taille maximum atteinte par les plantes cultivées en groupe est fonction inverse du numbre de plants présents par cellule de culture, et du volume de liquide nutritif disponible.

Tableau 1: Influence du nombre de plants présents dans une même cellule de culture sur leur croissance

Nbre de plants	Volume de liquide/plant	hauteur moy. des hampes	Nbre moyen de graines/plant
1 (tube)	3 cm^3	177 ± 10 mm	92 ± 20
6 (boite)	3 cm^3	167 ± 34 mm	70 ± 30
11 (boite)	1,8 cm^3	148 ± 30 mm	22 ± 4
18 (boite)	0,9 cm^3	125 ± 25 mm	-
25 (boite)	0,8 cm^3	119 ± 30 mm	-
40 (boite)	0,5 cm^3	99 ± 17 mm	8,1 ± 2,5

Ces résultats confirment les travaux de MYERSCOUGH et MARSHALL (1967). L'effet de masse semble plus sensible sur la fertilité que sur la taille des hampes: de nombreuses fleurs deviennent stériles au-delà de 15 plants par boite de Pétri. De plus, il est à noter que l'homogénéité des plants est moins bonne en culture de groupe qu'en culture individuelle.

3) C o n c l u s i o n

Cette méthode de culture sur sable, assez différente des techniques précédemment décrites (LANGRIDGE, 1957; VELEMÍNSKÝ et al., 1964) permet d'obtenir, dans de bonnes conditions d'asepsie, des plantes d'une homogénéité statistiquement vérifiée constituant un matériel de choix pour les travaux de biochimie et de physiologie. Enfin, l'emploi de tubes composés facilite l'accès aux plantes, en particulier au moment de la récolte des graines, sans nuire à la stérilité du Milieu de culture. Cette technique a été appliquée avec succès à d'autres plantes telles que l'Orge jusqu'au stade 3ème feuille (BOUNIAS, 1972).

S u m m a r y
An aseptic-culture technique on sand and "minimum" mineral medium is described for Arabidopsis. The method, utilizing compound glass tubes or modified Petri dishes, conduces to statistically homogen cultures, and allows the access to the plants during their growth.

Références:
BOUNIAS, M.: D.E.A., Université de LYON, 12 p. (1967)
- : Thèse Doctorat-ès-sciences - LYON - 250 p. (1972)
HELLER, R.: Ann.Sc.Nat., 14, 2 (1953)
JACOBSON, L.: Plant Physiol., 26, 411-413 (1951)
KNOP, W.: Landw.Vers.Stat., 2, 270-272 (1860)
LANGRIDGE, J.: Austr.J.Biol.Sci., 10, 243-252 (1957)
MYERSCOUGH, P.J., and J.K. MARSHALL: Arabid.Inf.Serv., 4, 24-25 (1967)
VELEMÍNSKÝ, J., J. SVACHULOVA, and T. GICHNER: Proc.Symp. "Induction of Mutations and the Mutation Process", Praha, 57-61 (1965)

Détermination de l'acide indolyl-3-acétique dans les rosettes de feuilles d'Arabidopsis

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La croissance rapide d'Arabidopsis, même après irradiation des graines à doses assez élevées (DALY, 1960; BOUNIAS, 1973) peut conduire à penser que certaines hormones de croissance sont particulièrement actives chez cette crucifère. Nous avons donc mis au point une microméthode permettant le dosage rapide de l'acide indolyl-3-acétique (AIA) dans les extraits de feuilles.

1) E x t r a c t i o n s
Les rosettes entières sont pulvérisées dans des broyeurs du type POTER conçus pour être directement centrifugés, en présence d'éther éthylique sans péroxydes, à raison de 100 mg de feuilles fraiches pour 2 cm^3 d'éther. Deux à trois extractions successives sont réalisées. Le volume final est ramené à 1 μl par mg de poids frais.

2) C h r o m a t o g r a p h i e s
L'acide indolyl-3 acétique migre sur couche mince de Gel de silice (plaques préfabriquées KODAK-E.C.S.-K 301 V), avec les solvants indiqués dans le tableau 1. (cuves cylindriques - chromatographie ascendante)

Tableau 1: Rf. de l'A. I. A. dans divers solvants; comparaison avec le Rf. des pigments chlorophylliens

Solvants	Rf. AIA	Rf. Pigments
1 - nButanol-ac.acétique-eau (70-15-15)	0,82	0,8 - 0,9
2 - Iso propanol-eau (85-15) - 2 migrations	0,50	0,79
3 - nPropanol-acétate d'éthyle-eau (70-20-10)	0,28	-
4 - Chloroforme - acide acétique (1-1)	0,21	0,45-0,67
5-a - Ether-Cyclohexane (5-95)	0	0,9
5-b - Ether-Cyclohexane (30-70)	0,05	0,95
6 - Ether-Chloroforme (1-1)	0	0,7 - 0,8
7 - Chloroforme	0	0,2 - 0,6

Il est donc possible de séparer l'A. I. A. des pigments chlorophylliene également contenus dans les extraits éthérés en entraînant d'abord ces derniers seuls à l'aide de solvants du type 5 à 7, ensuite l'A. I. A. à l'aide de solvants type 1 à 3 (séparations en 1 ou 2 dimensions) (fig. 1).

3) R é v é l a t i o n   c h i m i q u e   e t   d o s a g e
L'observation en lumière ultraviolette convient mal du fait de la présence d'autres composés fluorescents (pigments). Deux méthodes de révélation chimique sont donc proposées.

a) Réactif de Prochàzka (1964):

Formaldéhyde 35% .... 10 cm^3
HCl, 25% .... 10 cm^3
Ethanol .... 20 cm^3

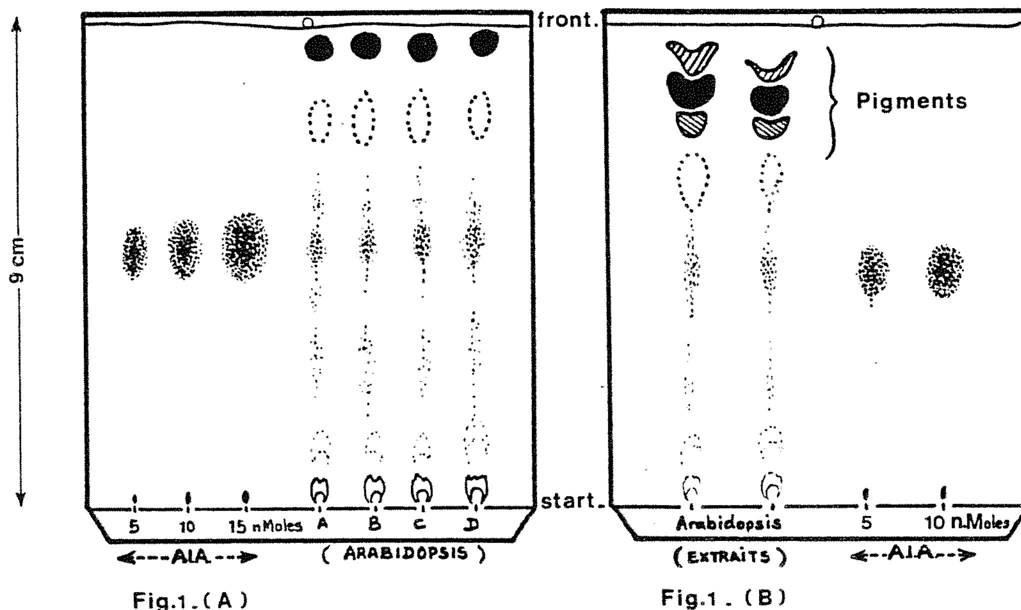

Figure 1: Séparation de l'acide indolyl-3-acétique dans un extrait éthéré de feuilles d'Arabidopsis. Réactif de Prochàzka

(A) 1º - Solvant 5, 2 migrations successives
2º - Solvant 2, 1 migration
3º - Solvant 3, 1 migration

(B) 1º - Solvant 7, 2 migrations successives
2º - Solvant 2, 2 migrations successives

Ce réactif pulvérisé sur les plaques révèle l'AIA en rouge: la bande d'absorption maximum se situe entre 490 et 510 nm

b) Réactif au Pentachlorure d'Antimoine

$Sb\ Cl_5$ .... 20% — pulvérisation, puis
CCl_4 .... 80% — chauffage à 60ºC environ

Ce réactif, relativement peu spécifique, offre cependant l'avantage d'une très grande sensibilité; le maximum d'absorption sur plaques (450 à 490 nm) est très proche du minimum d'absorption des pigments tétrapyrroliques et leurs dérivés (490 à 540 nm) ce qui facilite le dosage.

Les taches ainsi révélées sont enregistrées à l'aide d'un photomètre-densitomètre intégrateur[1]. La surface des pics est directement proportionnelle à la quantité d'AIA présente (fig. 2).

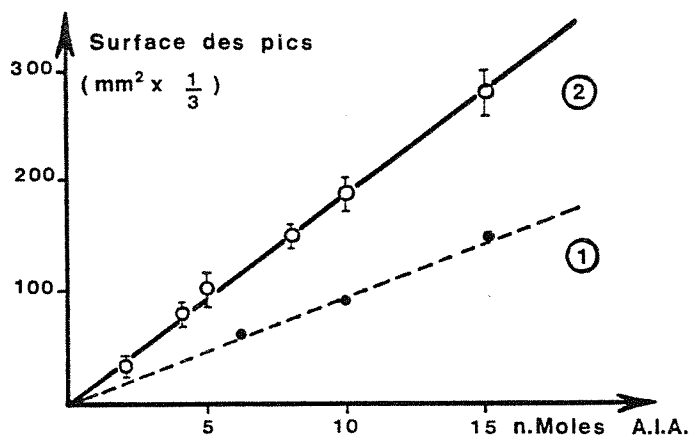

Figure 2: Courbes d'étalonnage de l'A. I. A. pur sur couche de gel de silice:

1) Réactif de Procházka - Enregistrement à 490 nm
2) Réactif au $SbCl_5$ - Enregistrement à 465 nm

(1) Chromoscan Joyce LOEBL

4) R é s u l t a t s

Cette technique, appliquée à l'Arabidopsis, a donné les résultats préliminaires suivants:

Age des plants	Poids par Rosette	N	Dosage de l'AIA
38 jours	20 ± mg	8	0,25 ± 0,12 uMole/mg de poīds frais

S u m m a r y

Indolyl-3-Acetic Acid (IAA) is quantitatively determined from crude ether-extracts of Arabidopsis rosette leaves by means of a thin layer chromatographic micro-method. IAA is first separated from the leaf pigments with two solvent-systems, then chemically revealed with formaldehyde reagent (Prochâzka) or with a highly sensitive Antimony-Pentachloride reagent. The spots are quantitatively recorded by means of a densitometer.

Références:

BOUNIAS, M.: Arabid.Inf.Serv., this issue
DALY, K.: Genetics 45, 983 (1960)
KUTACEK, M., and Z. PROCHAZKA: Coll.Int.CNRS, 123, 445-456 (1964)

C. I N F O R M A T I O N S

ANNOUNCEMENT

Annual Review of Genetics inyited me to summarize the high-lights of Arabidopsis genetics for Volume 8. In order to facilitate adequate coverage, I would much appreciate receiving reprints of your paper which have not been sent to me earlier. I would be particularly interested in papers in rare journals or in other publications of limited circulation.

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GRADUATE ASSISTANTSHIP

By the fall semester 1973 there will be an opening in my laboratory. Applications should include detailed academic records, previous experience if any, linguistic proficiency, and the name of two professors who can provide personal evaluation.

The University of Missouri offers a broad graduate program in Genetics. The stipend for the half-time research assistantship is $ 3,600 without M.S., and $ 4,200 with M.S. or after the completion of the comprehensive examination. The stipend is sufficient to cover educational and subsistence expenses.

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CORRECTION

Arabidopsis Information Service 9, 40 Table: Enzyme activity data have to be multiplied by a factor of 3.

IMPORTANT EDITORIAL CHANGE

In order to save costs and labour, from now on no further request for manuscripts and orders of AIS will be sent out anually. As indicated on the inner cover page of each volume of AIS your contribution will be expected not later than February 1, each year.

D. BIBLIOGRAPHY

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

AHNSTRÖM, G., A.T. NATARAJAN, and J. VELEMINSKÝ: Chemically induced somatic mutations in Arabidopsis. Hereditas 72, 319-322 (1972)

BALKEMA, Gijsje H.: Chimerism and diplontic selection. Ph.D. Thesis, Wageningen, The Netherlands, pp. XV + 173 (1971)

- : Diplontic drift in chimeric plants. Radiat.Bot. 12, 51-55 (1972)

BOUNIAS, M.: Etude du developpement d'Arabidopsis thaliana après irradiation γ des graines sèches. Diplome d'Etudes Approfondies. Université de Lyon, 12 p, 1967

- : Une microméthode d'analyse quantitative par chromatographie en couche mince des sucres, amino-acides, pigments et cofacteurs éthéro-solubles de plants d'Arabidopsis thaliana (L.) HEYNH. Chimie analytique 51, 76-82 (1969)

- : Etude comparée des amino-acides libres dans les rosettes de feuilles d'Arabidopsis thaliana, plants normaux et mutés. Colloque Intergroupe de Photosynthèse CEA, Cadarache 5-9 Mai 1969, 4 p, 1969

- : Etude biochimique et cytochimique de l'appareil photosynthétique chez des végétaux supérieurs normaux et mutés. Thèse Doctorat d'Etat ès-sciences, Université de Lyon, 250 p, 1972

BROCK, R.D.: Nitrous acid as a mutagenic agent for Arabidopsis thaliana. Radiat.Bot. 11, 309-311 (1971)

BROWN, J.A.M.: Distribution of ^{3}H-thymidine in Arabidopsis vegetative meristems after 5-iododeoxyuridine treatment. Amer.J.Bot. 59, 228-232 (1972)

JACOBS, M., and F. SCHWIND: Genetic control of isozymes of acid phosphatase in Arabidopsis thaliana. Plant Science Letter 1 (3), 95-104 (1973)

LEDOUX, L., R. HUART, and M. JACOBS: Fate of exogenous DNA in Arabidopsis thaliana. Translocation and integration. Eur.J.Biochem. 23, 96-108 (1971)

- , - , and - : Fate of exogenous DNA in Arabidopsis thaliana. 1. Evidence for replication and preliminary results at the biological level, in uptake of informative molecules by living cells (Edited by L. Ledoux) North Holland Publishing Co., pp 159-175, 1971

NASYROV, Yu.S., A.G. KASYANENKO, and Z.N. ABDURAHKMANOVA: Genetic regulation, structure and function of the photosynthetic apparatus. (Russ.) Reference not indicated

NEALES, T.F.: Effects of high temperature and genotype on the growth of excised roots of Arabidopsis thaliana. Aust.J.biol.Sci. 21, 217-223 (1968)

PEDERSON, D.G., and D.F. MATZINGER: Selection for fresh weight in Arabidopsis thaliana under two mating systems. Theoret.App.Genetics 42, 75-80 (1972)

RÉDEI, G.P.: A mechanism of fructose inhibition in Arabidopsis and relatives (Abstr.). Plant Physiol. 49 (Suppl.), 1972

RÉDEI, G.P., and S.B. PLURAD: A cytoplasm-targeted nuclear mutator locus in Arabidopsis (Abstr.). Genetics 71 (Suppl.) 50-51 (1972)
RÖBBELEN, G.: Untersuchungen zur genetischen Charakterisierung von induzierten phänotypischen Reversionen bei Arabidopsis-Mutanten. Z.Pflanzenzüchtg. 67, 177-196 (1972)
SVACHULOVA, Jiřina, and Vera HADACOVA: Strukturproteine und lösliche Proteine in zwei letalen, auf Saccharosemedium kultivierten Chlorophyllmutanten von Arabidopsis thaliana. Biologia Plantarum 14, 297-301 (1972)
WEILAND, Ulrike, und A.J. MÜLLER: In vitro-Kultur der Wurzeln von letalen fuska-Mutanten von Arabidopsis thaliana. Kulturpflanze 20, 151-164 (1972)

(In the press)

JACOBS, M.: Contrôle génétique des isozymes de la leucine aminopeptidase chez Arabidopsis thaliana. Bull.Soc.Roy.Bot.Belg.
KRANZ, A.R.: Monogen kontrollierte Langzeit-Transformationen der Chlorophylle in Blättern von Arabidopsis thaliana (L.) HEYNH. Z.Pflanzenphysiol.
RÉDEI, G.P.: Extra-chromosomal mutability determined by a nuclear gene locus in Arabidopsis. Mutation Res.
- : Arabidopsis thaliana (L.) HEYNH. In: Handbook of Genetics (R.C. King, Ed.) to be published in 1974.